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Abstracts

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DEFINITION OF INTERCALATED CELL SUBTYPES IN RAT COLLECTING DUCT USING ANTIBODIES AGAINST ERYTHROID BAND 3 AND RENAL VACUOLAR H^+ATPase. S. L. Alper, S. Gluck, H. F. Lodish* and D. Brown. Renal Unit, Beth Israel Hosp., Whitehead Inst., Renal Unit, Mass. Gen. Hosp., Boston, MA and Washington University and Renal Division, Jewish Hospital, St. Louis, MO.

The cellular distribution of the kidney form of the erythroid band 3 Cl^-/HCO_3^- exchanger and the kidney vacuolar H^+ATPase was examined in rat kidney collecting duct by immunocytochemical staining of adjacent semithin sections. Polyclonal antipeptide antibodies directed against three distinct regions of murine erythroid band 3 gave a similar pattern of basolateral labeling. In the medullary collecting duct (MCD), all intercalated cells were labeled, but in the cortical collecting duct and connecting segment (CCD), band 3 labeling was restricted to a subpopulation of intercalated cells. In the MCD, all intercalated cells with basolateral membrane band 3 also displayed apical membrane H^+ATPase. In the CCD, 54% of intercalated cells had apical H^+ATPase and basolateral band 3. Cells that had either basolateral or diffuse cytoplasmic staining for H^+ATPase were all band 3-negative, and accounted for 46% of the intercalated cells. In addition, occasional intercalated cells with apical H^+ATPase appeared to lack basolateral band 3. These results demonstrate the coexpression of H^+ATPase and band 3 in opposite plasma membrane domains of a subpopulation of intercalated cells that are probably the acid-secreting A-cells. All other intercalated cells were band 3-negative. These cells probably are, or at least include, the bicarbonate-secreting B-cells.

IN VITRO CHOLESTEROL (Chol) MODULATES RENAL BRUSH BORDER MEMBRANE (BBM) FLUIDITY AND PHOSPHATE (Pi) TRANSPORT. B. Baird*, P. Wilson*, and M. Levi. VAMC, U.T. Southwestern Med. Ctr., Dallas, TX.

Recent studies with dietary Pi restriction and with aging indicate a strong inverse correlation between BBM Chol content and BBM fluidity and Pi transport. To determine whether Chol directly modulates BBM fluidity and Pi transport, BBM isolated from rat superficial cortex were incubated in vitro with Chol. An 18% increase in BBM Chol content caused a marked decrease in BBM fluidity, i.e. increase in the fluorescence anisotropy of diphenylhexatriene, (r_{DPH}, 0.240 vs 0.219 in Control, $p<.01$), and a marked decrease in the initial rate of Na-Pi cotransport activity (720 vs 1994 pmole/mg/5sec in Control, $p<.01$), which is caused by a decrease in Vmax (1586 vs 4449 pmole/mg/5sec in Control, $p<.01$), and no change in Km (126 vs 136 uM in Control, p=NS). Chol had a similar effect to decrease fluidity and Pi transport in BBM isolated from the juxtamedullary cortex. Furthermore, in BBM isolated from rats fed a low Pi diet, Chol reversed the adaptive increases in BBM fluidity and Na-Pi cotransport activity. The results therefore indicate that Chol is a direct and important modulator of BBM fluidity and Na-Pi cotransport activity.

MEMBRANE HETEROGENEITY OF THE GLOMERULAR EPITHELIAL CELL. Masaaki Arakawa,* Shinichi Nishi,* Takao Saito,* Sojiro Ogino,* and Yuichiro Maruyama* (intr. by F. Marumo). Niigata Univ. Med. School, Dept. of Med.(II), Niigata, Japan.

The glomerular epithelial cell (GEC) has abundant glycocalyx on its luminal surface. But scarecely known about the basal surface glycocalyx. We studied the whole surface glycocalyx components by using phosphotangustic acid chrome (PTA-Cr) stain and enzyme digestive methods under the electron microscope. We also investigated the distribution of membrame cholesterol by digitonin decoration methods. Control Sprague-Dawley and Puromycin Aminonucleoside (PAN) nephrosis rats were fixed with 4% paraformaldehyde and embedded in glycolmethacrylate. The ultrathin sections were stained with PAT-Cr. The whole GEC surface glycocalyx became visible well. Then, before staining, the sections were treated with hyaluronidase, neuraminidase, heparitinase and chondroitinase ABC. The luminal surface stain decreased after treated with hyaluronidase, neuraminidase and heparitinase. However, the basal surface stain decreased only after chondroitinase ABC treatment. This heterogeneity was seen in both control and PAN nephrosis rats. For the study of membrane cholesterol, rats were fixed with the mixture of digitonin and fixatives and embedded in Epon 812. In control rats the foot process surface was decorated markedly with digitonin-cholesterol complex crystals, but the basal surface not. In PAN nephrosis rats, both luminal and basal surfaces were not decorated well. This membrane heterogeneity may be related to GEC attachment to negative charged GBM and the morphological transformation, i.e. loss of foot processes.

LIGHT CHAIN (LC) BINDING SITES ON HUMAN RENAL BRUSH BORDER MEMBRANES (BBM). V. Batuman, A. Dreisbach,* J. Cyran.* VA Medical Center, East Orange, NJ and UMDNJ-NJ Medical School, Newark, NJ.

We previously demonstrated specific binding of human 125-I LC to rat renal BBM. We now present data on 125-I LC binding to human kidney BBM prepared from the nephrectomy specimen of a patient who underwent surgery for renal cell carcinoma. A monomeric kappa LC isolated from the urine of a patient with IgG myeloma was used in these experiments. Binding assays were done in triplicate with 1 nM 125-I LC and increasing amounts of unlabelled LC (from 0.025 to 2.4 mM) and 0.1 mg/ml BBM protein in the medium. Scatchard analysis of data using the Ligand program revealed a Kd of 286 uM, Bmax of 171 uM at 37°C and Hill coefficient of 1.05. Specific binding defined as 125-I LC bound in the absence of competing ligand minus the amount bound in the presence of 2.4 mM excess LC was 79%. Binding parameters of the same LC to rat kidney BBM (N = 3) were similar to human kidney BBM binding with a Kd of 248 ± 51 uM (mean ± SEM), Bmax of 25.4 ± 1.6 uM, Hill coefficient of 1.00 ± 0.01 and a specific binding of 77 ± 1.5% at 37°C. These data reveal the presence of a single class of low affinity, high capacity and non-cooperative specific binding sites on human kidney proximal tubule BBM for immunoglobulin LC. These sites share similar properties with those we previously observed on rat kidney BBM for the same class of ligand. LC binding sites may function as endocytotic receptors for tubular reabsorption of filtered LC.

MECHANISM OF PROTAMINE ACTION IN INDUCTION OF TIGHT JUNCTION FORMATION IN NECTURUS GALLBLADDER. Carl J. Bentzel and Susan Richards*. Dept. of Medicine, School of Medicine, East Carolina University, Greenville, NC.

When protamine sulfate is applied to the mucosal surface of Necturus gallbladder mounted in a Ussing Chamber, transepithelial resistance doubles within 12 minutes. This decrease in electrical conductance is reversible and is associated with a change in tight junctional morphology characterized by an increase in number of intramembranous strands leading to increased junctional depth.

We have now examined the mechanism of this effect. We find no effect on epithelial cell cAMP concentration, whereas methylisobutylxanthine increases cAMP concentration 2 fold in 5 min and 3 fold in 30-40 minutes.

When the mucosal bath is rendered virtually Ca-free with 2mM EGTA, the protamine electrical response is reduced by about 50%. Prostaglandin E_1 and E_2 (10^{-8}-10^{-6}M) and cytochalasin B (10^{-6}M) reduce the response but not to the same extent. The strong positive charge carried by protamine (pI>10) may be necessary but not sufficient for the response since the protamine effect can be ablated by SO_4^{--}, and is not elicited by poly-l-lysine.

We conclude that protamine interacts with the apical cell membrane and initiates a complex series of cytoplasmic events involving Ca^{2+} entry, and modulation by prostaglandins and the cytoskeleton eventually leading to insertion of new fibrils into the tight junctional membrane domain.

CURRENTS ASSOCIATED WITH THE NA/GLUCOSE COTRANSPORTER CLONED FROM RABBIT INTESTINE. B. Birnir,* D.D.F. Loo*, H.S. Lee*, M.J. Coady*, M.A. Hediger*, and E.M. Wright. Dept. of Physiology, UCLA School of Medicine, Los Angeles, Ca. 90024.

Injection of RNA prepared from clone pMC424 into Xenopus laevis oocytes resulted in expression of the intestinal Na^+-dependent glucose cotransporter (Hediger, Coady, Ikeda & Wright, Nature 330:379-381, 1987). We measured the currents associated with the cotransporter using the two-microelectrode voltage clamp technique. Four days after injection, addition of 500 uM glucose to the bathing medium containing 100 mM Na^+ resulted in a depolarization of 25 mV,and an inward current associated with a 9% increase in conductance. The absolute value of the depolarization and the conductance increase depends on the number of transporters expressed in the oocyte membrane. Current-voltage relations were obtained from the difference in the current in the presence and absence of glucose. Current activation depended on [Na^+], [glucose] and membrane voltage. When glucose and Na^+ concentrations were kept constant, the current increased with hyperpolarizing potentials until a saturation level was reached. At a constant membrane potential, the currents exhibited Michaelis Menten behavior. At a holding potential of -90 mV, the K_m for glucose was approximately 50 uM at 100 mM NaCl, and the K_m for Na^+ was 11 mM at 500 uM glucose. The currents were abolished by phlorizin. The magnitude of the currents evoked by 1 mM D-glucose, alpha-methyl-D-glucopyranoside or D-galactose were similar, whereas L-glucose, 3-O-methyl-D-glucoside, or mannitol did not induce any measurable current. We conclude that the Na^+ dependent glucose transporter is indeed electrogenic. The results are in general agreement with glucose transport studies carried out on the rabbit intestinal epithelium and on brush border membrane vesicles.

INSULIN-LIKE GROWTH FACTOR 1 (IGF1) AND INSULIN RECEPTORS IN TOAD URINARY BLADDERS (TUB). B. Blazer-Yost,* M. Cox, and R. Furlanetto.* V.A. Med. Cntr. & Univ. Pa. Sch. Med., Phila. PA

The urinary bladder of the toad, Bufo marinus, is a well characterized in vitro model of the mammalian distal nephron. Nanomolar concentrations of both IGF1 and insulin stimulate net mucosal to serosal Na^+ flux in TUB. The present studies were designed to determine whether: 1) both IGF1 and insulin receptors exist in TUB; 2) ligand-receptor binding affinities correlate with the natriferic dose-response relationships of the two peptides and; 3) the hormone-binding subunits of the TUB receptors are similar to those of the corresponding human placental receptors. Equilibrium binding assays using radio-iodinated peptides demonstrated the presence of distinct IGF1 and insulin receptors in TUB (half maximal binding of 3-10nM). The binding affinities indicate that each peptide mediates its natriferic effect by interacting with a distinct receptor. Covalent crosslinking (disuccinimidyl suberate) of the iodinated peptides to their specific receptors, followed by electrophoretic analysis (reduced SDS-PAGE), showed that the apparent molecular weights of the TUB hormone-binding receptor subunits are 5-10kDa less than those of the corresponding placental receptor subunits. Thus, the TUB contains IGF1 and insulin receptors with peptide binding affinities and molecular weights that are very similar to those of their mammalian counterparts. Furthermore, ligand binding to IGF1 and insulin receptors appears to initiate transcellular Na^+ flux in this model "high-resistance" renal epithelium.

MYO-INOSITOL INFLUX AND EFFLUX ARE ACTIVATED BY SODIUM IN THE RAT PROXIMAL TUBULE. R. Bloom,* J. Rothrock,* and J. Dominguez, Dept. of Med, Ind. U. School of Med. and V.A. Med. Ctr., Indpls., IN.

Myo-inositol (MI) is actively reabsorbed by the rat proximal tubule (PT). Since MI is vital to signal transduction and membrane structure, information on the mechanisms that govern renal MI transport are critical to the understanding of MI homeostasis in the PT. We studied the role of extracellular (Na_o) and intracellular (Na_i) sodium on MI influx and efflux in rat PT. We found that MI influx was dependent on Na_o. In PT at 30 sec., isosmotic restitution of Na_o from low to normal (15 to 150 mM), increased influx from 32±8 to 51±18 pmoles/mg prot (mean ± S.D., n = 9, $p<0.05$), At 8 minutes, MI influx measured 239 ± 55 and 559 ± 99 for 15 mM and 150 mM Na_o respectively, $p<0.01$. MI influx at 15 mM Na_o did not saturate, whereas at 150 mM Na_o saturation occurred at 8 minutes. Influx values at 1,2,3,5, and 10 minutes confirmed the activation by Na_o, $p<0.01$. We also studied Na_o-dependent influx kinetics at 60 seconds in PT. We documented an approximate Km of 117 µM and Vmax of 51 pmoles/mg protein/minute. Since the MI carrier may be bidirectional, we tested the role of Na_i on MI efflux in PT. We measured MI efflux as the fractional efflux ratio (experimental/control) of MI in PT (FER MI). Initially, experimental and control PT were both exposed to 15 mM Na_o, FER MI = 1.01 ± 0.08. We then added 1 mM ouabain (OU) to both and FER was 0.92 ± 0.11. While both groups were exposed to OU, Na_i was allowed to accumulate only in the experimental group by restoring Na_o to 150 mM. Na_i accumulation increased FER MI to 1.97 ± 0.13, $p < 0.05$, $n = 3$. We conclude that Na activates MI influx and efflux in PT. Hence, MI net influx (influx - efflux) may be directly proportional to the gradient $Na_o >> Na_i$ in PT. We suggest that in diseases which may be associated with high Na_i, such as diabetes mellitus, MI efflux may be increased and thus, MI net influx decreased in PT.

ORGANIC OSMOLYTE ACCUMULATION IN MDCK CELLS: DIFFERENTIAL REGULATION OF POLYOLS.
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MDCK cells are known to accumulate methylamines [e.g., glycerophosphorylcholine (GPC)] and myo-inositol but not sorbitol in response to elevated medium NaCl. Since high glucose levels are known to promote sorbitol accumulation in other tissues we compared the response of the MDCK cell to high concentrations of NaCl or glucose. MDCK cells were grown to confluence (~5 days) in Dulbecco's-Modified Eagle Medium with 10% FBS (DMEM). At confluence, cells were exposed for 24 hours to either DMEM (Control), DMEM + 150 mM NaCl (+ NaCl), or DMEM + 300 mM glucose (+ Glucose). GPC, myo-inositol, and sorbitol were identified by ^{1}H NMR and quantified by biochemical assays of perchloric acid extracts.

	Control	+ NaCl	+ Glucose
GPC	21±7	54±10*	165±20*
myo-Inositol	102±22	270±37*	107±19
Sorbitol	< 10	< 10	130±23*

(nmol/mg protein; *p < 0.05 vs. Control)

In comparison to control cells, + NaCl and + Glucose cells exhibited significantly higher GPC contents. Whereas myo-inositol was increased in the + NaCl cells it was unchanged in the + Glucose cells. Furthermore, sorbitol was increased only in the + Glucose cells. Therefore, glucose like NaCl promotes an increase in both methylamine and polyol contents. However, under similar hyperosmotic conditions, different polyols can be accumulated by MDCK cells indicating differential regulation of osmolytes.

LOCALIZATION AND CHARACTERIZATION OF ENDOSOMES CONTAINING WATER CHANNELS IN THE BRATTLEBORO RAT. D. Brown, W. I. Lencer*, A. S. Verkman, P.Weyer* and D. A. Ausiello. Renal Unit, Mass. General Hospital, Boston and Cardiovascular Research Unit, UCSF, San Francisco.

The antidiuretic action of vasopressin in the renal collecting duct is believed to be mediated by the cycling of water channels between the apical membrane and the endosomal compartment. We recently confirmed this hypothesis by showing that water channels are present in endocytic vesicles isolated from collecting ducts of vasopressin-treated Brattleboro rats (Nature, 333: 268-269, 1988). In the present study, we localized these endosomes in semi-thin sections of fixed, frozen kidney medulla from animals infused intravenously with FITC-dextran. Principal cells from untreated Brattleboro rats contained few fluorescent vesicles, and the number of labeled vesicles was markedly increased by vasopressin treatment. In contrast, vasopressin had no effect on the endocytosis of FITC-dextran into intercalated cells, which internalized large amounts of the fluorophore. Fluorescent vesicles were concentrated in the subapical pole of both cell types. This method permits the same fluorescent probe to be used for correlative morphological and functional studies on endocytosed vesicles. Endosomes from the lower two-thirds of the papilla (in which intercalated cells are not found) were isolated and assayed for osmotic water and ATP-dependent proton transport. These vesicles had an osmotic water permeability coefficient of 0.03 cm/sec at 23 °C and no measurable H^+ATPase activity. These results indicate that vasopressin-induced endosomes, derived from the apical membrane of principle cells, contain water channels but do not contain an H^+ATPase. The data are consistent with the hypothesis that water channels are shuttled through the apical cytoplasm in vesicles that are not involved in degradative pathways.

ALTERNATIVE 5' EXON SPLICING OF THE PRINCIPAL KIDNEY BAND 3 mRNA PREDICTS A PROTEIN WHICH DIFFERS FROM ERYTHROID BAND 3 AT ITS AMINO TERMINUS. Frank C. Brosius III,* Harvey F. Lodish,* Seth L. Alper. Beth Israel Hospital, Boston, MA, and Whitehead Institute for Biomedical Research, Cambridge, MA.

Multiple lines of evidence suggest that the basolateral anion transporter of collecting duct alpha-intercalated cells is highly similar, but not identical, to the erythrocyte anion transporter, Band 3 (EB3). In order to determine the specific differences between the intercalated cell anion transporter ("kidney Band 3" [KB3]), and EB3, we have cloned, partially sequenced, and restriction mapped several KB3 cDNA's (none of which were full-length). We have shown that the 3'-most 4.0 kb of the ca. 4.2 kb KB3 mRNA is identical to the corresponding portion of the ca. 4.5 kb EB3 mRNA, extending from mid-exon 4 through the 3' terminal exon 20 of the Band 3 gene. S1 nuclease and Northern blot analyses indicate that the major murine KB3 mRNA contains all of exon 4 but lacks the first three exons of EB3. This predicts a KB3 protein which lacks at least the first 45 amino acids of EB3. Northern blots further indicate that the principal KB3 transcript(s) in rat also lack exons 1-3 and suggest the presence of minor KB3 mRNAs in both species which encode exons 1-3. Primer extension analyses with different primers suggest that cap sites for the major murine KB3 transcript(s) are situated 40 to 200 bp upstream from the 5' end of exon 4. Thus, murine and rat KB3 proteins likely differ from EB3 at their amino termini because of alternative 5' exon splicing of the most abundant transcripts of the Band 3 gene. This indicates the use of alternative and probably tissue-specific promoters.

GLUCOSE METABOLISM AND BRUSH BORDER MEMBRANE (BBM) LIPID COMPOSITION IN RENAL PROXIMAL TUBULE. A. Capparelli*, O.D. Jo and N. Yanagawa, Neph. Div., Sepulveda VAMC, UCLA Sch. of Med. Los Angeles, CA.

We have previously reported that stimulation of glucose metabolism through pentose cycle (PC) in renal proximal tubule (PT) by insulin (I) or phenazine methosulfate (PM) increased, while inhibition of PC by 2-deoxyglucose (DG) or 6-aminonicotinamide (AN) suppressed, BBM phosphate transport (Kidney Int. 29:161, 1986). Since BBM lipid composition is known to affect its phosphate transport and PC may interact with cellular lipid metabolism, we have examined the possible effect of PC on BBM lipid composition. Purified PT were isolated from rabbit kidneys and incubated with I (10 uU/ml), PM (10 uM), DG (10 mM) or AN (100 uM) for thirty minutes prior to isolation of brush border membrane (BBM) vesicles. Contents of cholesterol (C) and phospholipid (P) in BBM were measured by gas chromatography and organic phosphate assay, respectively, and C/P ratio were obtained as following (n=3):

Cont.	I	PM	DG	AN
0.62 ±0.03	0.54 ±0.02	0.56 ±0.04	0.71 ±0.07	0.70 ±0.01

The C/P ratio in PT homogenate showed changes parallelling those in BBM. These results suggest that PC affects lipid metabolism in PT and may modulate BBM transport through lipid modification.

DOPAMINE STIMULATION OF cAMP PRODUCTION IN CULTURED OPOSSUM KIDNEY (OK) CELLS. L. Cheng, P. Precht, C.T. Liang, D. Frank and B. Sacktor. (intr. by D. Spector) GRC, NIA, NIH, Baltimore, MD 21224

Dopamine (DA) receptor has been identified in kidney. However, the direct effect of dopamine on renal tubular function remains controversial. To determine the existence and function of DA receptor in proximal tubule of kidney, we studied the effect of dopamine receptor agonists and antagonists on cAMP production in cultured OK cells which display proximal tubule cell like properties. The stimulation of cAMP by DA is dose dependent. In the presence of IBMX, a significant increase in cAMP production was seen at 10^{-8} M DA. Maximal stimulation occurred at 10^{-5} M DA. DA (10^{-5} M) caused a 25-30 fold increase in cAMP production as compared to basal values (76.4±12.1 vs 2.7±0.3 pmol/dish). D_1 agonist, SKF 82526, also stimulated cAMP production, whereas D_2 agonist, Ly 171555, had little effect. The DA and SKF 82526 stimulated cAMP production were inhibited by DA_1 antagonist, SCH 23390. SCH 23390 (10^{-8} M) inhibited DA (10^{-5} M) stimulated cAMP production by about 70%. In contrast, D_2 antagonist, spiperone had no inhibitory effect. In addition, α or β adrenergic antagonist had no effect on DA or SKF 82526 stimulated cAMP. These results demonstrated that activation of DA_1 receptor can stimulate cAMP production. These findings also indirectly showed the existence of DA_1 receptor in OK cells.

PHOSPHATE DEPLETION ALTERS THE PHOSPHOLIPID COMPOSITION OF RENAL BRUSH BORDER MEMBRANES. Dabbagh S, Wong C,* Epley M,* Diven W,* Falsetti G,* Kocochis S,* Ellis D. Dept of Pediatrics, Children's Hospital, Pgh, PA.

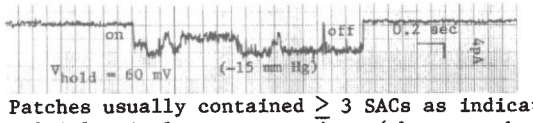
Phosphate (P) depletion, irrespective of vitamin D status, was shown to induce a taurinuria which manifests at the renal brush border membrane (BBM) and is characterized by decreased affinity of the symport and reduced peak of the overshoot of uptake by BBM vesicles. To study the effect of P depletion on membrane phospholipid (PL) composition, BBMV were prepared from animals fed the following diets for 5 weeks: 1) CON (0.7% P, 2.5µg% vit.D); 2) D^-P^- (0.1% P, 0 vit.D); 3) D^+P^- (0.1% P, 2.5µg% vit.D); 4) D^-P^+ (0.7% P, 0 vit.D). A group of rats fed D^-P^- were supplemented with 600 pmoles of calcitriol 14 hours prior to sacrifice.

There was a 40±10% reduction in phosphatidyleththanolamine (PE), phosphatidylcholine (PC) and sphingomyelin contents of BBM in all the diets ($p<0.01$). Total PL content was not affected. There was a 15% increase in palmitic acid and a 15±3% decrease in oleic, stearic and linoleic acids in the PC fraction in all diets ($p<0.05$). D^-P^- produced similar fatty acid changes in the PE and PI fractions. Acute supplementation with calcitriol normalized stearic acid but didn't affect PL content or the other fatty acids. There were significant correlations ($p<0.05$) between PC content and peak of the overshoot of taurine uptake ($r=0.471$), urinary taurine ($r=0.429$), plasma P ($r=0.679$) and 25(OH)D levels ($r=0.528$).

Thus P depletion induces changes in PL and fatty acid content of BBM which may be responsible for the tubulopathy described earlier.

IONIC CURRENTS INDUCED BY STRETCH IN CULTURED MESANGIAL CELLS. William Craelius*, Nabil El-Sherif*, Ira Kurtz and Carlos E. Palant*, Depts of Medicine, VAMC and SUNY Health Science Ctr. at Brooklyn, NY and UCLA School of Medicine, Los Angeles, CA.

The behavior of stretch-activated ion channels (SACs) was examined in cultured mesangial cells (MCs) (provided by Dr. Sharon Adler). Cell membranes were stretched by applying suction (0-40 mm Hg) to recording patch clamp pipets. Ion channels opened in response to stretch, as illustrated below:



Patches usually contained ≥ 3 SACs as indicated by multiple simultaneous openings (above patch contained 3 SACs). Open probabilities were directly related to suction level, being 0 at suctions < 10 mm Hg, and rising to 1 at suctions > 30 mm Hg. SACs activation did not exhibit voltage dependence but current amplitudes varied ohmically at negative voltages and rectified at positive voltages. Reversal potentials of SACs in different electrode solutions averaged -11.5 mV±7 (150 mM KCl) (n=12), -11.6 mV±7 (150 mM NaCl) (n=7). Conductances averaged 53 picoSiemens (n=19) Substituting Choline for K+ or Na+ abolished inward currents and with $BaCl_2$, 110 mM (n=10), no SACs were seen. These results indicate that SACs are permeable to K+ and Na+ and may represent a cellular reflex whereby MCs sense and respond to changes in glomerular capillary pressure.

GENERATION OF MONOCLONAL ANTIBODIES (MAB) TO MEMBRANE PROTEINS OF GLOMERULAR PODOCYTES AND ENDOTHELIAL CELLS. G. Dekan*, M. G. Farquhar, and A. Miettinen*. Dept. of Cell Biology, Yale Univ. School of Medicine, New Haven, CT and Dept. of Bacteriology & Immunology, Univ. of Helsinki, Helsinki, Finland.

We generated MAB to membrane proteins of podocytes and endothelial cells in order to identify new epithelial and endothelial membrane proteins and to study the possible role of the corresponding antigens (AG) in the formation of glomerular immune deposits (ID).

Detergent extracts of isolated glomeruli were subjected to Triton X-114 phase separation, the resultant detergent and aqueous phases were used to immunize mice, and MAB were prepared by standard techniques. The specificities of the IgGs were determined by immunoblotting on glomerular extracts and/or by immunoprecipitation performed on [^{125}I]-labeled glomerular or [^{35}S] cysteine-labeled renal cortical extracts. The corresponding antigens were localized by immunofluorescence, immunoperoxidase, and immunogold labeling. Several of the MAB recognized known glomerular proteins (podocalyxin, gp330). Others recognized novel epithelial and/or endothelial membrane proteins as follows:

MAB	Type	Glomerulus Epi.	Glomerulus Endo.	Prox. Tubule	Peritubular Endo.	AG
1A	IgG2b	+++	++	-	++	Podocalyxin
5A	IgG1	+++	++	-	++	Podocalyxin
26C	IgG1	+	+/-	+++	-	105 kd
14A	IgG1	+	+/-	++	-	150 kd
20B	IgG1	+	-	+++	-	gp330
27A	IgG3	++	-	-	-	103 kd
13A	IgG1	+	-	-	-	120 kd(?)
23A	IgG2b	-	++	-	++	?

When these IgGs (0.25-5 mg) were injected i.v. or i.p., most (5A, 26C, 20B, 13A and 27A) bound rapidly to glomeruli. The kinetics of ID formation was characteristic for a given antigen. Thus these MAB should prove useful for studies on the mechanism of formation of glomerular ID.

IMAGING KIDNEY CELL MEMBRANE FLUIDITY BY SPATIALLY-RESOLVED ANISOTROPY MICROSCOPY. J.A. Dix* and A.S. Verkman. CVRI, UCSF, CA.

The fluidity of cell plasma and internal membranes is sensitive to phospholipid metabolism and is likely an important modulator of cell function. Studies of membrane fluidity have been limited to spatially averaged fluorescence measurements of fluidity-sensitive probes in vesicles and whole cells. We have developed a new approach to measure membrane fluidity in intact cells by quantitative imaging microscopy. Kidney cells (MDCK and LLC-PK1) were loaded with fluorophores having fluidity-sensitive anisotropy (DPH, TMA-DPH and PROP-DPH, 5 μM, 10 min, 37°C) or eximer formation (pyrenedecanoic acid). Cells were imaged by an intensified CCD or SIT camera using an inverted epifluorescence microscope with UV optics. Pseudocolored images of anisotropy were calculated from background and sample images obtained using vertical and horizontal orientations of excitation and emission polarizers. The detection system was tested by imaging a series of thin capillary tubes containing 1 mM fluorescein in varying glycerol:water mixtures. Anisotropy images were uniform within capillaries; values (0.02-0.32) agreed with those measured by cuvette fluorometry. Cell plasma membranes stained with TMA-DPH showed a uniform anisotropy (0.29-0.32) sensitive to addition of membrane fluidizing agents (e.g. butanol). Cells stained with DPH or PROP-DPH showed fine structure in intracellular membrane fluidity, with minimum fluidity in the plasma membrane. These results establish a new methodology to measure the fluidity of intact cells in real time, providing a strategy to examine the role of fluidity in cell signalling.

IDENTIFICATION OF THE LIVER ISOFORM OF THE BETA SUBUNIT OF THE NaK-ATPase. DS Friedenberg*, J Long, AS Pollock, BC Rossier, and DG Warnock. Dept. Med., VA Med. Ctr., UCSF, San Francisco, CA., and Institute of Pharmacol., Lausanne, Suisse.

The beta subunit mRNA in brain and kidney has the same coding sequence (JBC 262: 4905, 1987). Liver beta subunit is poorly detected in total RNA with kidney cDNA probes, suggesting that another isoform may exist. We have used cDNA probes to probe adult rat kidney and liver lambda gt11 libraries, and poly(A)+ RNA. Probes were complementary to the highly conserved 3' noncoding region and the coding region. Four independent clones were isolated from 800,000 colonies screened in the liver library. Each was 1250 bp long, and identical to the kidney isoform on sequence analysis. Northern blots of poly(A)+ RNA showed the abundance of the beta subunit was 10x greater in kidney than liver, but that the abundance relative to the alpha subunit was the same for both organs.

Conclusions: 1). The coding and 3' noncoding region of the liver beta subunit mRNA is appears to be identical to the kidney and brain isoforms. 2). Message abundance correlates with enzyme activity if poly(A)+ RNA is compared rather than total RNA. 3). The relative abundance of mRNA for alpha and beta subunits is the same for liver and kidney. 4). If another beta isoform exists, its abundance is very low in mRNA from adult rats.

DEVELOPMENT OF Na^+-CHANNEL REGULATION BY GUANINE NUCLEOTIDE REGULATORY (Gi) PROTEINS IN RENAL EPITHELIAL CELLS. L. Ercolani and D.A. Ausiello. Renal Unit, Massachusetts General Hospital, Boston, MA.

The renal epithelial cell line, LLC-PK_1, has on its apical membrane an amiloride-sensitive Na^+ channel which we have shown to be regulated by pertussis-toxin sensitive G protein(s) (G_i). We have recently shown that this regulation occurs during differentiation by day 6 of culture which coincides with these cells becoming confluent and polar. Na^+ channel regulation on day 6 was correlated with the presence of new alpha subunits (αi) in apical cell membranes by using 1) antibodies to 41 kd αi subunits of G_i which immunolocalized to the apical cell membrane only on day 6 of culture; 2) a cDNA probe which hybridized to αi mRNAs which were only detected by day 4 of culture, and 3) 2-D gels of 32-P-ADP ribosylated membranes which revealed a new 41 kd protein on day 6 in addition to an increase in the content of a 41 kd protein seen only on day 2. Recent reports have demonstrated the G_i alpha subunit which regulates the potassium channel is αi-3. To determine which G_i alpha subunit may be involved in regulating the Na^+ channel, oligonucleotide probes (66 nucleotides) capable of hybridizing to specific rat 3' untranslated regions of αi-1, αi-2, and αi-3 mRNAs were constructed. Northern and slot blot analysis of poly A^+ mRNA harvested from day 1 through day 7 revealed the presence of αi-2 and αi-3 but not αi-1 mRNAs. In vitro translation of hybridization-selected mRNA from Day 6 cells produced a 41 kd protein(s) on gel electrophoresis which was recognized by G_i specific antibodies. These data are consistent with the new synthesis and apical-membrane insertion of αi-2 and /or αi-3 subunits which may regulate the epithelial Na^+ channel in polarized cells. We are currently cloning specific αi cDNAs and expressing them in LLC-PK1 cells to confirm these possibilities.

PROTEIN KINASE C (PKC) ACTIVATION HAS DISSIMILAR EFFECTS ON Na-COUPLED UPTAKES IN RENAL PROXIMAL TUBULAR CELLS (PTC) IN PRIMARY CULTURE. G. FRIEDLANDER * and C. AMIEL * (intr. by C. Le Grimellec). INSERM U 251 and Dept. Physiol., Fac. X. Bichat, Univ. Paris 7, France.

PKC is present in renal brush border membranes. PKC activators were shown, in renal epithelial cells, to open tight junctions (TJ), a maneuver which affects the activity of Na-coupled transport systems. To evaluate the influence of PKC activation on different apically located transporters, we studied the effect of phorbol myristate acetate (PMA), oleoylacetylglycerol (OAG), and diacylglycerol (DiC8) on Na-dependent uptake of phosphate (Pi), α-methyl-D-glucoside (MG), and L-alanine (Ala), and on phlorizin (Phl) binding, in primary cultures of rabbit PTC grown in serum-free medium. PMA (100 ng/ml) decreased Vmax (nmoles/mg protein /5 min) of Pi and MG uptake and the number of Phl binding sites (pmoles/mg protein), but did not change Ala uptake : (mean ± SE ; n = 3, *:$p < .01$)

	Pi	MG	Ala	Phl
Control	15.7±1.07	23.0±0.22	9.1±0.26	2.5±0.11
PMA	11.0±0.27*	19.1±0.70*	9.8±0.23	1.5±0.06*

Km (uptakes) and Kd (binding) were not affected. The effect of PMA was time- and concentration - dependent, was mimicked by OAG and DiC8, persisted in the presence of amiloride or dimethylamiloride, was potentiated by Ca ionophore A23187, and was reversed by the PKC inhibitor H7. Opening of TJ blunted subsequent PMA-induced decrease of MG uptake, but not of Pi uptake. We conclude that : i) in the proximal tubule, activation of PKC has different effects on Na-Pi, Na-MG, and Na-Ala cotransports ; ii) different pathways are involved in the observed effects.

MECHANISMS UNDERLYING VOLUME REGULATORY DECREASE BY NECTURUS GALLBLADDER EPITHELIUM. Timothy J. Furlong* and Kenneth R. Spring. NIH, NHLBI, Laboratory of Kidney and Electrolyte Metabolism., Bethesda, Maryland.

Volume regulatory decrease (VRD) by Necturus gallbladder epithelium involves the efflux of K and Cl across the basolateral membrane, a process inhibited by the addition of bumetanide to the serosal bathing solution (Larson and Spring, J.Memb.Biol. 81:219-232,1984). Quantitative light microscopic measurements of cell volume were used to show that VRD was inhibited by the addition of 5 mM $BaCl_2$ (a K channel blocker) to serosal bathing solutions. VRD was also inhibited by the serosal addition of 3 or 50μM phencyclidine (a blocker of K channels activated by N methyl D aspartate). VRD was not inhibited by the addition of 0.1mM diphenylamine-2-carboxylate (a blocker of some Cl channels) to serosal bathing solutions. VRD was not inhibited by the replacement of Cl in mucosal and serosal bathing solutions by NO_3 or SCN. However, VRD in SCN substituted Ringer was inhibited by the addition of 10μM bumetanide to the serosal bathing solution. As the bumetanide-sensitive KCl cotransporter does not accept NO_3 or SCN (Hoffmann, Biochim.Biophys.Acta. 864:1-31,1986), it does not mediate volume regulatory decrease in Necturus gallbladder epithelium. The inhibitory effects of $BaCl_2$ and phencyclidine suggest that specialized K channels are activated during VRD. As Cl channels in other cells accept both NO_3 and SCN and are inhibited by bumetanide, it is likely that they mediate VRD in Necturus gallbladder epithelium.

EXPRESSION OF A6 CELL RNA ENCODING THE AMILORIDE-SENSITIVE SODIUM CHANNEL IN *XENOPUS* OOCYTES. A. George*, O. Staub*, K. Geering*, B. Rossier*, J.-P. Kraehenbuhl*, and T. Kleyman. U of Pennsylvania, Philadelphia, PA, U of Lausanne, Lausanne, Switzerland, and Columbia U, New York, NY.

An amiloride-sensitive Na^+ channel is localized to the apical plasma membrane of high resistance Na^+ transporting epithelia such as the mammalian cortical collecting duct and the amphibian kidney cell line A6. To assist with cDNA cloning we have developed a ^{22}Na transport assay to detect functional expression of the Na^+ channel in *Xenopus laevis* oocytes microinjected with A6 cell mRNA. Mature, defolliculated *Xenopus* oocytes were injected with either H_2O or 25 ng of poly-(A)$^+$ RNA prepared from aldosterone treated A6 cells grown on collagen-coated filters. RNA-injected oocytes exhibited 15-fold greater ^{22}Na uptake than H_2O-injected oocytes when assayed 3 days after injection. Amiloride inhibited ^{22}Na uptake in RNA-injected oocytes with an IC_{50} of 60 nM (2 mM NaCl buffer); 97% inhibition occurred at 1 μM. Benzamil (10^{-7} M) inhibited transport 96% in RNA-injected oocytes, but 1 μM 5-(*N*-ethyl-*N*-isopropyl) amiloride, an analog with greater specificity for inhibiting Na^+/H^+ and Na^+/Ca^{2+} exchange at this concentration, inhibited ^{22}Na uptake only 16%. The profile of inhibition by amiloride and these analogs strongly suggests our assay specifically detects expression of the amiloride-sensitive Na^+ channel. A6 cell poly-(A)$^+$ RNA was size-fractionated by sucrose density gradient centrifugation and oocytes were injected with 10 ng of each fraction. Amiloride-sensitive ^{22}Na uptake was maximally expressed by oocytes injected with two contiguous fractions sedimenting between 18s and 28s.

We conclude that the amiloride-sensitive Na^+ channel can be functionally expressed in *Xenopus* oocytes microinjected with total and size fractionated A6 cell mRNA.

THE PHOSPHATIDYL INOSITOL SYNTHASE OF RENAL PROXIMAL TUBULE CELLS Carlos R. Galvao, James A. Shayman. University of Michigan, Ann Arbor, MI.

Phosphatidyl inositol (PI) is a precursor for an important class of phospholipids, the phosphatidyl inositol polyphosphates. The formation of PI from CDP-diglyceride (CDP-DG) and myo-inositol and the regulation of PI synthase therefore have important implications for the cellular biology of renal epithelial cells. We sought to understand the role of PI synthase by determining its subcellular localization, kinetic properties, and regulation in rabbit proximal tubule cells.

Proximal tubular cells were isolated from New Zealand white rabbits. The subcellular synthesis of PI was assessed by preincubation of cells with $^{32}PO4$ with subsequent subcellular fractionation. Labeling of PI was time-dependent and consistent with the rapid incorporation of PI into basolateral, brushborder, microsomal, and nuclear fractions. Pulse-chase labeling was consistent with the in situ formation of PI in basolateral and brushborder membranes in addition to microsomal fractions.

Microsomal, brushborder, and basolateral membranes were isolated and assessed for PI synthase activity. The apparent Kms for myo-inositol were 0.19, 0.22, and 0.21 mM and for CDP-DG were 0.15, 0.15, and 0.10 mM respectively. Moreover, PI synthase activity was inhibited by coincubation with PI (Ki 0.005 mM) without differences in inhibition in the subcellular fractions.

In conclusion, the in situ synthesis of PI occurs in several membrane fractions; the kinetic properties of PI synthase appear to be identical in each fraction; and PI synthase in proximal tubule cells is inhibited by its formation product.

INCREASE IN INTRACELLULAR cAMP INDUCES TRANSIENT CHANGES IN MEMBRANE FLUIDITY IN RENAL EPITHELIAL CELLS. Marie-Cécile Giocondi*, Gérard Friedlander* and Christian Le Grimellec. INSERM U251, Fac. Med. Xavier Bichat, Université Paris 7, Paris, France.

The effects of an increase in intracellular cAMP on the lipid order of the plasma membrane of MDCK cells in suspension has been determined from fluorescence anisotropy (r) measurement of TMA-DPH, a probe which labels specifically plasma membrane in MDCK cells. In the presence of theophylline as phosphodiesterase inhibitor, AVP, PGE2 or forskolin induced a dose-dependent increase in intracellular cAMP, and a correlated decrease in membrane lipid order. Maximal effect corresponded to a 5% decrease in anisotropy (from 0.292 $\pm$ 0.001 to 0.278 $\pm$ 0.001, N=9) and was obtained after 2-5 min following addition of the drug. Anisotropy returned to control levels within 10-15 min. Incubations with dibutyryl cAMP mimicked the effect of AVP, PGE2 or forskolin, both for the extent of the maximal response and for its transient character. The effect of either substance was independent from changes in cellular volume and as estimated from rapid dilution experiments, did not originate from a flip-flop of the probe within the membrane. These results indicate that : i) increase in intracellular cAMP content influences membrane fluidity, ii) cAMP-mediated events might be responsible for the transient character of the response.

uPA-DEPENDENT MESANGIAL CELL SHAPE CHANGE AND ADHESION LOSS IS ASSOCIATED WITH FIBRONECTIN AND LAMININ RELEASE. William F. Glass II, Robert A. Radnik, and Jeffrey I. Kreisberg. Univ. of Texas Health Science Center, Departments of Pathology and Medicine, San Antonio, Texas.

We have shown that mesangial cell shape change and adhesion loss in response to cAMP elevating agents (e.g., isoproterenol plus MIX) (IM) involves urokinase-type plasminogen activator (u-PA) located in preparations of ventral membranes plus extracellular matrix (ECM). Pretreatment of cells with anti-uPA antibody blocks shape change and adhesion loss (J. Clin. Invest., in press). Immunofluorescence revealed marked reorganization of the fibronectin present in the ECM following shape change and adhesion loss. The fibronectin was removed from large areas of the substratum and aggregated along cellular processes. We examined conditioned media from cells treated with RPMI 1640 alone and RPMI 1640 plus IM for evidence of extracellular matrix proteins involved in this process. A solid phase ELISA revealed a 4-fold increase in fibronectin release and a 2-fold increase in laminin release. No change in the type IV collagen content of the media was observed. The increase in media fibronectin followed closely the activation of uPA in the adhesion plaques. Vasopressin and anti-uPA antibody inhibited fibronectin release as well as shape change and adhesion loss. Thus, fibronectin and laminin appear to play important roles in the mechanism whereby mesangial cells lose adhesion and change shape in response to cAMP. Supported by grants DK 29787 USPHS, and The National Kidney Foundation.

A NOVEL, HIGH-AFFINITY BINDING SITE FOR 5-(N-METHYL-N-ISOBUTYL)-AMILORIDE IN RENAL PROXIMAL TUBULAR (PT) CELLS: INCREASED BINDING AFFINITY AFTER UNINEPHRECTOMY. R.D. Gunther*, E.J. Cragoe Jr.*, L.G. Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA and Merck, Sharp and Dohme Research Laboratories, West Point, PA.

Since Vmax of Na^+/H^+ exchange is increased after uninephrectomy (UNINX) we attempted to quantitate the number of antiporters on renal PT cells derived from normal and 24 hour UNINX rabbits using [^{3}H]5-(N-methyl-N-isobutyl) amiloride (MIA) as a ligand. Two specific binding sites were found: 1) a low-affinity site ($K_D \approx 50$ μM; $B_{max} \approx 10^9$ sites/cell), and 2) a high-affinity site ($K_D \approx 0.1$ μM; $B_{max} \approx 10^6$ sites/cell). Neither binding affinity corresponded with the K_i for Na^+ uptake of ≈ 10 μM. The high-capacity, low-affinity binding presumably obscured a binding site of similar affinity but lower capacity corresponding to the inhibitory site on the antiporter. Specific α_2 adrenergic receptors were demonstrated in bbm vesicles. Low concentrations of MIA (0.01-0.1 μM) increased binding of [^{3}H]rauwolscine to a clonidine-insensitive site. MIA had no effect on conductive Na^+ transport. In UNINX, V_{max} of MIA-sensitive Na^+ uptake into bbm increased and the K_D of the high-affinity MIA binding site decreased (80 nM to 40 nM). Conclusion: A high affinity MIA binding site was identified on PT cells. This site is neither the inhibitory site on the Na^+/H^+ exchanger nor the α_2 receptor nor a Na^+ channel. Parallel changes in Na^+/H^+ exchanger and high affinity MIA binding in UNINX suggest binding to an allosteric site on the Na^+/H^+ antiporter.

AN ANION EXCHANGER $Cl^-/HCO_3^-(OH^-)$, IN OSTEOBLAST(UMR-106 CELLS) IS REGULATED BY INTRACELLULAR(IC) pH AND Ca^{2+} IONS. Jacob Green*, Dean T. Yamaguchi, Charles R. Kleeman and Shmuel Muallem*, Lab. of Membrane Biology, Div. of Neph., Cedars-Sinai Med. Ctr., UCLA Sch. of Med., L.A., CA.

To study whether the osteoblast possesses a base secretory mechanism, we investigated the presence of a $Cl^-/HCO^-_3(OH^-)$ exchanger and its regulation in UMR106 cells. Exchanger activity was assessed by monitoring DIDS inhibitable changes in IC pH $[pH]_i$ using the pH sensitive dye, BCECF and by ^{36}Cl fluxes. When cells are added to a Cl^- free solution a DIDS inhibitable rise in $[pH]_i$ is observed. The exchange is reversible and electroneutral. Cl^- interacts with a single saturable extracellular site and there is simple competition between OH^- and Cl^- for binding to this site. The dependencies of both net anion exchange and Cl^- self-exchange on IC OH^- did not follow simple saturation kinetics. This suggests that the exchanger is regulated by $(OH^-)_i$. We further evaluated the effect of IC Ca^{2+} $[Ca^{2+}]_i$ on exchanger activity. A rise in $[Ca^{2+}]_i$, induced by various pathways, resulted in DIDS inhibitable Cl^- dependent decrease in $[pH]_i$. The low $[pH]_i$ in turn, stimulated the Na^+/H^+ exchanger resulting in increased $[pH]_i$. Kinetic analysis showed that a rise in $[Ca^{2+}]_i$ increased the apparent affinity for OH^-_i of both net anion and Cl^- self-exchange. Conclusions: 1) UMR-106 cells possess a $Cl^-/HCO_3^-(OH^-)$ exchanger. 2) The exchanger can be regulated by $[pH]_i$ and $[Ca^{2+}]_i$.

APICAL AND BASOLATERAL Na/H ANTIPORTERS OF MDCK CELLS. John G. Haggerty,* Robert F. Reilly,* Edward A. Adelberg,* and Carolyn W. Slayman.* (intr. by Peter S. Aronson) Yale University School of Medicine, Depts. of Human Genetics & Medicine, New Haven, CT.

In LLC-PK_1/Cl_4, an established cell line derived from pig kidney, we have recently detected two pharmacologically distinct Na/H antiporters: an apical system with a half-maximal inhibitory concentration (IC_{50}) of $1x10^{-5}$ M ethylisopropylamiloride (EIPA), and a basolateral system with an IC_{50} of c. $3x10^{-8}$ M EIPA (J.G. Haggerty, et al., Proc. Natl. Acad. Sci. in press). These measurements have now been extended to MDCK, a widely used epithelial cell line from dog kidney. MDCK cells were grown to confluence on Nuclepore filters and assayed for ouabain-sensitive ^{86}Rb uptake (Na-K pump) and acid-stimulated, EIPA-sensitive ^{22}Na uptake (Na/H antiporter) at both surfaces. Na-K pump activity was localized to the basolateral surface, indicating that the cells had developed the expected polarity. The initial rate of ^{22}Na uptake, measured at 15 mM Na, was 16.4 nmol/min·mg protein at the apical surface and 56.6 nmol/min·mg protein at the basolateral surface (av. of 2 experiments); and the IC_{50} values for EIPA were $6.7x10^{-8}$ M and $3.6x10^{-8}$ M, respectively. Thus, although the difference between apical and basolateral IC_{50} values is less pronounced for MDCK (18-fold) than for LLC-PK_1/Cl_4 (300-fold), the presence of two distinct antiporters may be a general phenomenon in epithelial cell lines.

UREMIC SERUM SUPPRESSES PROLIFERATION OF AND PROSTACYCLIN PRODUCTION IN HUMAN ENDOTHELIAL CELLS Gerhardt Hampel, Dept of Medicine, Division of Nephrology, University Hospital, Erlangen, FRG

The high incidence of vascular disorders found in dialysis patients suggests that uremia plays an important role in their pathogenesis.

In order to examine this relationship, human endothelial cells from umbilical veins were cultured in medium M199 which was supplemented either with 20% pooled serum from healthy controls or from hemodialysis patients (HD). Density was 10^4 cells per dish. Cell growth in the HD group remained static but was exponential in the control group. On day 5, cell count was 191,979 per dish in the control group and 15,729 in the HD group ($p<0.001$). If sera were exchanged, then the effect of the original serum was reversed in 48 hours.

In order to examine the effects of uremia on prostacyclin production, cells were grown in control or HD serum for 72 hours and then stimulated for 15 min at 37°C with 300µM histamine. 6-keto-$PGF_{1\alpha}$ was measured using RIA. There was significant suppression following incubation in HD serum compared to that in control serum (150.8 vs 966.2 pg/10^6 cells).

We conclude from these data that endothelial function is grossly suppressed in uremia.

HYPOTHALAMIC FACTOR (HF) INHIBITS OUABAIN-RESISTANT NA,K-ATPASE IN PRIMATE CELLS TRANSFECTED WITH A OUABAIN RESISTANCE GENE. Garner T. Haupert, Jr. and John T. Schulz.* Renal Unit, Mass. Gen. Hosp. and Dept. of Physiol., Tufts Univ. Sch. Med., Boston, MA.

The plasma membrane Na,K-ATPase (NKA) in animal cells is pharmacologically inhibited by cardiac glycosides. Endogenous (physiologic) regulation of NKA remains incompletely understood, but may be related to properties intrinsic to the enzyme and to actions of a naturally occuring circulating NKA inhibitor. A murine gene has been isolated which when transfected into ouabain (Ou)-sensitive monkey kidney fibroblasts (CV-1 cells) renders the endogenous CV-1 Na,K-pump insensitive to Ou. To determine activity of a non-peptidic, high affinity, specific NKA inhibitor isolated from hypothalamus (HF) on this Na^+-dependent, Ou-resistant pump, we tested its effects on NKA in plasma membranes of canine kidney (CKE), native CV-1, and transfected CV-1 expressing Ou resistance (OR6-S):

	ATPase Activity (nmol mg^{-1} min^{-1})		
Enzyme	Tot. Na^+-dep.	+Ou (1mM) (% Inhib)	+HF (17U/ml)
CKE	3140 ± 140	0 (100)	0 (100)
CV-1	90.6 ± 3.1	0 (100)	17.8 ± 2.7 (80.4)
OR6-S	50.8 ± 5.1	25.4 ± 2.6 (50)	7.2 ± 1.4 (85.8)

This work shows: 1) Transfected CV-1 express Ou-insensitive NKA; 2) HF potently inhibits Na^+-dependent, Ou-resistant ATPase in transfected CV-1 (OR6-S); 3) Ou may be an inadequate probe to fully characterize physiologic regulation of mammalian NKA.

THE 55 AND 17 kD POLYPEPTIDES ASSOCIATED WITH THE ANTIDIURETIC HORMONE (ADH) WATER PERMEABILITY RESPONSE ARE INTEGRAL MEMBRANE PROTEINS. H.W. Harris Jr. and C. Hosselet*. Div. of Nephrology, The Children's Hospital Boston, Mass.

We have previously identified ^{125}I-labeled polypeptides of molecular weights 55, 17, 15 and 7 kD as apical membrane constituents of the ADH-stimulated water permeability response in the toad urinary bladder (PNAS 85:1942 1988). Konieczkowski and Rudolf (J. of Pharm. and Exp. Therap. 234:515, 1985) have demonstrated that ADH increases the phosphorylation of 17 and 15.5 kD toad bladder proteins and water flow specifically decreases ^{32}P-labeling of this 15.5 kD protein. We have utilized the Triton X-114 phase separation technique (J.B.C. 256:1604, 1981) to determine whether these labeled proteins are integral membrane proteins which might span the lipid bilayer and serve as components of the ADH water channel. ADH stimulated toad bladders were ^{125}I-labeled via apical membrane iodination or ^{32}P-labeled via endogenous phosphorylation with ^{32}P-orthophosphate. Membrane fractions enriched for apical membrane and aggrephores were prepared and analyzed by SDS polyacrylamide gel electrophoresis and autoradiography. The ^{125}I-labeled 55 and 17 kD proteins are integral membrane proteins while the 15 kD is hydrophilic. The ^{32}P-labeled 17 kD protein, also an integral membrane protein, is slightly smaller than and distinct from the ^{125}I-labeled 17 kD band. The ^{32}P-labeled 15.5 kD band is a cytoplasmic hydrophilic protein. We conclude that the ^{125}I-labeled 55 and 17 kD and the ^{32}P-labeled 17 kD are integral membrane proteins which may be components of the ADH water channel.

SURFACE LECTIN BINDING FOR CHARACTERIZATION AND ISOLATION OF TWO TYPES OF MDCK-CELLS. H Holthofer* and D Schlondorff. Univ of Helsinki, Finland, and Albert Einstein College of Medicine, Bronx, NY

MDCK-cells are polarized renal tubular epithelial cells consisting of two variants (Type I and II). The variants are expressed in typical proportions in culture. To characterize them we used lectin binding to surface glycoconjugates. The FITC-coupled lectins of different specificities included WGA, RCA, WFA, DBA, HPA, and PNA.

WGA and RCA reacted with all cells, whereas PNA WFA and HPA identified a morphologically round variant consisting of up to 70% of the cells. DBA gave a bright reaction only with an elongated cell type forming small nests and accounting for an approximated 15% of cells. 15% of cells remained lectin negative. Enzyme treatment with trypsin or collagenase to obtain single cell suspension did not alter the staining patterns. In western blotting experiments, each of the lectins identified a typical pattern of membrane glycoconjugates separated on SDS-gels. To isolate the two MDCK-cell types, we used lectin coated magnetic beads (Dynalab, Oslo, Norway). Aliquots of cell suspensions containing both cell types were exposed to either PNA or DBA-coated beads. Lectin-positive cells were then isolated by the use of a magnet and cultured. After confluency the PNA-isolated cells showed enrichment from 70% to over 90%, and a simultaneous decrease of DBA positive cells from 15% to less than 5%. Conversely, the DBA isolated cells were enriched from 15% to over 40%, and showed a decrease of PNA-positive cells to less than 50%. These results show that differential lectin binding can be used to identify and enrich specific types of MDCK-cells.

RAT MESANGIAL CELLS POSSESS NA/K/CL COTRANSPORT ACTIVITY THAT IS REGULATED BY VASOACTIVE AGENTS

T. Homma*, R.L. Hoover* and R.C. Harris. Dept. of Medicine, Pediatrics and Pathology, Vanderbilt Univ., Nashville, TN.

Mesangial cells (MC) are smooth muscle-like cells that respond to a variety of vasoconstrictors and vasodilators. Because anion-cation co-transport systems regulate net salt and water and cell volume in many cells, we have characterized loop-diuretic sensitive anion-cation cotransport in cultured rat MC. In these cells, 55% of ^{86}Rb influx was insensitive to 2mM ouabain; of this component, 41±3% (n=6) was inhibited by loop diuretics ($3x10^{-4}$M furosemide (fur) or $6x10^{-6}$bumetanide (bum)). This fur/ bum sensitive ^{86}Rb influx was completely abolished by substitution of either $[Cl^-]o$ or $[Na^+]o$ with gluconate or choline, respectively. In the presence of 5mM $[K^+]o$, the titration curve for ^{86}Rb influx vs $[Na^+]o$ was hyperbolic, while for $[Cl^-]o$ it was sigmoidal, with apparent Km of 23mM for $[Na^+]o$ and 53mM for $[Cl^-]o$. Fur/bum-sensitive, $[K^+]o$ and $[Cl^-]o$ dependent $^{22}Na^+$ influx was also present in MC. In ^{86}Rb loaded MC, the rate of ^{86}Rb efflux was inhibited 47±4% (n=5) by fur or bum.

In response to the vasodilator, ANP (10^{-7}M), fur/bum sensitive ^{86}Rb influx was stimulated 39% (10.4±0.4 vs 14.5±1.8nmol/mg pro./3min; n=5; $p<0.05$) a response mimicked by 8-bromo-cGMP (10^{-4}M), while fur/bum-sensitive ^{86}Rb efflux was inhibited 27±3% ($p<0.01$; n=3) by ANP. In contrast, the vasoconstrictors AII (10^{-7}M) and AVP (10^{-7}M) inhibited fur/bum-sensitive influx by 13±3%; n=7; $p<0.05$) and 26% (n=2) respectively, while stimulating fur/bum sensitive efflux by 16 % (n=2) and 30% (n=3).

This study demonstrates that glomerular mesangial cells possess an Na/K/Cl cotransporter, which serves as a major mediator of ouabain-insensitive K^+ influx. In addition, a predominant portion of K^+ efflux is inhibitable by loop diuretics, suggesting K/Cl or Na/K/Cl cotransport. That agents known to contract or relax MC co-ordinately regulate these two transport processes suggests a possible role for these cotransporters in mediating changes in net cellular ion content necessary for regulation in cell volume during contraction or relaxation.

IDENTIFICATION AND PURIFICATION OF A RENAL AMILORIDE-BINDING PROTEIN WITH PROPERTIES OF THE NA/H EXCHANGER.

Stephen J. Huot*, Dan Cassel*, Peter Igarashi*, Edward J. Cragoe, Jr.*, Carolyn W. Slayman*, and Peter S. Aronson. Depts. of Med., Cell. & Mol. Physiol., and Human Genetics, Yale Sch. of Med., New Haven, Ct.; and Technion-Israel Inst. of Technology, Haifa, Israel.

The aim of this study was to identify and purify the Na/H exchanger from rabbit renal brush border membranes (BBM) by use of affinity chromatography. Membranes were solubilized with 0.6% Triton X-100, and equilibrated with an affinity matrix consisting of the potent amiloride analogue A35 (5-N-[3-aminophenyl]amiloride) covalently coupled to CL Sepharose 4B beads through a triglycine spacer arm. After a one hour incubation, the matrix was washed extensively with buffer and then sequentially eluted with buffer, buffer containing 5 mM amiloride, and 1% SDS. Eluates were concentrated and subjected to SDS-PAGE. The silver-stained gel revealed a 25 kD protein that was not visible in the initial solubilized brush border membrane extract, was not eluted from the affinity matrix by buffer alone, but was eluted with 5 mM amiloride. A subsequent elution with 1% SDS did not release any more of the 25 kD protein, indicating that it had been completely eluted from the affinity matrix by amiloride. The presence of 5 mM amiloride during equilibration of the solubilized brush border extract with the affinity matrix completely blocked adsorption of the 25 kD protein. The abundance of the 25 kD amiloride-binding protein per mg cortical BBM outer medullary BBM, and cortical basolateral membranes correlated closely with specific activity of Na/H exchange (measured as amiloride-sensitive, pH gradient-stimulated Na uptake) in these preparations (c-BBM > om-BBM > BLM). We then used adsorption of the 25 kD protein to the affinity matrix as an assay for the amiloride-analogue specificity of its binding site. The adsorption of the 25 kD protein to the affinity matrix was blocked by increasing concentrations of amiloride and its analogues with the rank order methylisobutylamiloride > amiloride > benzamil, which was identical to that for inhibition of Na/H exchange activity in transport assays. These findings strongly suggest that the 25 kD amiloride-binding protein is a structural component of the rabbit renal Na/H exchanger.

MULTIDRUG TRANSPORT BY KIDNEY DERIVED CELL LINES.

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We studied transepithelial transport of ^3H-labeled hydrophobic cationic cytotoxic drugs by MDCK epithelia grown on filters. Basal (B) to apical (A) flux of 100nM vinblastine (Vbl) was over six times A→B flux. Addition of 20μM unlabeled Vbl, reduced B→A and increased A→B flux of ^3H-Vbl, a pattern expected if there is a saturable Vbl extruding pump in the apical plasma membrane. The B→A flux of low concentrations of daunomycin, vincristine, and actinomycin-D exceeded A→B flux. At 20μM, these agents inhibited transport of Vbl, suggesting a common transporter for all these alkaloids. Vbl transport was also inhibited by 20μM verapamil. The organic cation transporter of the proximal tubule is not involved because Vbl transport was not affected by 3mM tetramethyl- or tetraethylNH_4. Active B→A transport was also observed in epithelia formed by LLC-PK_1 and by OK cells. The competitive substrates and inhibition by verapamil are characteristic of the multidrug resistance (mdr1) gene product, P-glycoprotein. It is an ATP consuming plasma membrane pump that is important because it extrudes cytotoxic chemotherapeutic alkaloids from resistant cancer cells and is often found in renal cancers. P-glycoprotein has been immunolocalized to the apical membrane of the proximal tubule, where it is assumed to function in the excretion of xenobiotics by the kidney. The transport pattern we observed is that predicted to result from the function of P-glycoprotein in the apical plasma membrane. The system described here facilitates study of the function of a transporter that resembles or is P-glycoprotein in its epithelial setting.

EFFECT OF OSMOTIC GRADIENT ON LUMINAL MEMBRANE RESPONSES OF ADH-TREATED TOAD URINARY BLADDER.

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Aggrephores fused with and particle aggregates in flat areas of granular cell luminal membrane are part of the mechanism through which transtissue water flow is increased by ADH. Though the incidence of each of these morphologic sequelae to ADH action is greater in bladders treated in the complete absence than in the presence of a transmural osmotic gradient, we questioned whether this would also hold for tissues with gradients of different magnitudes. Accordingly, we studied ADH-treated (20mU/ml) paired bladders (n=6) in which transmural gradient was either 100 or 200 mOsm. Morphologic analysis of tissues fixed when water flow achieved maximum, revealed that while fused aggrephores were twice as numerous with the 100 vs. 200 mOsm gradient, aggregate frequency in flat membrane areas was not detectably different. Though at the time of fixation water flow was greater in bladders with the steeper gradient, osmotic water permeability (i.e., water flow normalized for gradient) was comparable to that for tissues with the less steep gradient. These findings are not predicted from non-gradient vs. gradient studies. The data suggest that factors controlling membrane fusion of aggrephores are not identical to those regulating aggregate appearance in membrane areas away from fusion sites. The frequency of the former (fused aggrephores) appears to be inversely influenced by water flow volume, while the frequency of the latter (flat area aggregates) appears directly related to water permeability.

ALTERED BIOPHYSICAL PROPERTIES OF RENAL BRUSH BORDER (BBM) AND BASOLATERAL MEMBRANE (BLM) IN RATS WITH DIABETES MELLITUS (DM). G.J. Kaloyanides, C. Josepovitz,* C. Boos* and L. Ramsammy*. Dept. of Med., SUNY-Stony Brook, and Northport, NY.

We have previously reported that renal cortical phospholipids of DM rats contain a reduced amount of arachidonic acid (AA). We postulated that this reduction of AA would result in alterations of the biophysical properties of renal plasma membranes. To test this hypothesis we compared BBM and BLM prepared from control and DM rats in terms of their 1) fatty acid composition, 2) membrane microviscosity as measured by fluorescence polarization (rs) of diphenylhexatriene (DPH), and 3) glycerol permeability.

	BBM	BLM	BBM	BLM	BBM	BLM
	% AA		rs (37°C)		Glycerol Perm.	
Control	31.7	30.0	.161	.111	.197	.237
DM	24.3	23.7	.194	.210	.161	.154
p<	.001	.001	.07	.001	.001	.001

The results indicate that both BBM and BLM of DM rats contain approximately 25% less AA than age matched non-DM rats. The microviscosity of BBM and BLM from DM rats is higher than the corresponding membranes from non-DM rats. Glycerol permeability (37°C) is decreased approximately 20% and 36% in DM BBM and BLM, respectively.

We conclude that DM in the rat causes increased microviscosity and decreased non-electrolyte permeability of BBM and BLM. These changes can be explained by the alteration in the fatty acid composition of these membranes.

BIOCHEMICAL ANALYSIS OF SULFATED MACROMOLECULES SYNTHESIZED BY CULTURED GLOMERULAR EPITHELIAL CELLS (GEC). BS Kasinath, AK Singh, Rush Medical Collge, Chicago, IL.

Sulfated proteoglycans and glycoproteins are thought to be involved in permselectivity characteristics of the glomerular basement membrane. The purpose of this study was to characterize the sulfated macromolecules secreted by the GEC. Following incubation of GEC with 200 uCi/ml of $^{35}SO_4$ for 72 hrs, media and cell layers were extracted with 4M guanidinium HCl in the presence of protease inhibitors. The SO_4 labelled macromolecules were separated by G-25 chromatography. Pooled macromolecules from media and cell extracts were subjected to nitrous acid treatment and rechromatography on G-25. Major part of the macromolecules was oxidized with release of free label. DEAE sephacel chromatography (0.1 to 1.5M NaCl linear gradient) of the sulfated macromolecules yielded a single peak at 0.45M NaCl in both media and cell extracts. Following nitrous acid oxidation and rechromatography on G-25, the peak disappeared with release of free label. Confluent layers of GEC grown on millipore membranes showed SO_4 uptake from both the apical and basolateral membranes; macromolecules could be recovered from media individually bathing the apical and basolateral membranes. We conclude that most of the sulfated macromolecules synthesized by the GEC are glycosaminoglycans, the predominant form being heparan sulfate. Furthermore, the GEC are capable of sulfate uptake and secretion of sulfated macromolecules from both the basolateral and apical membranes.

PURIFICATION AND CHARACTERIZATION OF A BAND 3-LIKE PROTEIN FROM RABBIT RENAL BRUSH BORDER MEMBRANES (BBM). L Karniski and M Jennings*. U of IA and VAMC, IA City, IA and U of TX, Galveston, TX.

A protein immunologically related to the erythrocyte Cl^-/HCO_3^- exchange protein (band 3) has been identified immunocytochemically in the collecting duct but not in other nephron segments. We used a monoclonal antibody to the membrane domain of human erythrocyte band 3 to probe BBM's by immunoblot analysis for a band 3-related protein. Immunoblots of BBM's demonstrate that a single band with a molecular mass of 43 kDa binds the band 3 antibody. This 43 kDa protein was partially purified and then eluted from SDS-polyacrylamide gels to produce a sensitive and specific guinea pig antisera. The antisera to the 43 kDa protein, but not control antisera, cross-reacts with purified human erythrocyte band 3, confirming the immunologic similarity between the two proteins. Using the antisera as a marker the BBM immunoreactive protein was purified and isolated by gel filtration chromatography. Further analysis shows the 43 kDa band to be a glycoprotein as demonstrated by its ability to bind concalavin A and wheat germ agglutinin. In addition, in the absence of dithiothreitol, the immunoreactive protein appears to migrate as a dimer on SDS-PAGE suggesting it contains at least one sulfhydryl residue. Finally, the overall amino acid composition (mol %) of the 43 kDa protein is very similar to that of band 3. Both contain 46% hydrophobic nonpolar amino acids implying that, like band 3, the 43 kDa band is an integral membrane protein. This 43 kDa band 3-like protein may represent one of the renal brush border membrane chloride transporters.

A β_1-INTEGRIN RECEPTOR FOR FIBRONECTIN (FNR) IN HUMAN KIDNEY GLOMERULI. D. Kerjaschki, P.P. Ojha, M. Susani, R. Horvat, and R. Pytela. Univ. Vienna, Dept. Pathol., Vienna, Austria; and Basel Inst. Immunol., Basel, Switzerland. (Intr. by G. Giebisch).

The FNR is a transmembrane heterodimeric glycoprotein which shares a common β_1-chain with other members of the integrin family of adhesive receptors. We have enriched membrane proteins of human glomeruli, from which two proteins (MWs 120 kD and 140 kD) bound to a fibronectin-column and were released specifically by the synthetic peptide Arg-Gly-Asp-Ser. These molecules were labeled in immune-overlays of glomerular proteins by an antibody raised against human placentaFNR which labeled in normal human kidneys the mesangia and the peripheral capillaries by immunoperoxidase. The FNR was found concentrated on the cell membranes of mesangial-, epithelial- and endothelial cells which are in contact with the mesangial matrix or with ther GBM by quantitative immunogold electron microscopy. In kidney biopsies of patients with various glomerular diseases (including membranous glomerulonephritis) the distribution was similar to that in normal glomeruli. these findings indicate that a β_1-integrin related FNR is present in membranes of glomerular cells which face the matrix, both in normal and in diseased human glomeruli.

CELL SURFACE EXPRESSION OF THE AMILORIDE-SENSITIVE Na CHANNEL IN A6 CELLS GROWN ON PLASTIC OR POROUS SUPPORT. STUDIES USING AN ANTI-IDIOTYPIC ANTIBODY. T.R. Kleyman, B. Rossier,* B.F. Erlanger,* and J.P. Kraehenbuhl.* Columbia U., N.Y. and Univ. of Lausanne, Epalinges, Switzerland.

A6 cells express an amiloride-sensitive Na channel restricted to the apical plasma membrane. Monoclonal antibodies which recognize amiloride binding sites were raised by an anti-idiotypic approach. These antibodies were used to examine the expression of the channel at the apical plasma membrane of A6 cells grown on a porous (collagen coated polycarbonate filters) or on a plastic support. Apical and basolateral (filter only) plasma membrane proteins were specifically radio-iodinated, immunoprecipitated with anti-idiotypic or anti-amiloride (control) antibodies, and then analyzed by SDS-PAGE and autoradiography. Immunoprecipitible proteins were found only at the apical plasma membrane and the amount present was markedly reduced in A6 cells grown on plastic.

The total cellular pool of channels was estimated by specific [^{3}H]benzamil binding. The number of channels was similar in cells grown on plastic (7 ± 3 pmol/mg protein) or filters (8 ± 3 pmol/ mg protein) (n=2). These data suggest that the cellular pool of channels is similar in these two growth conditions; however, expression at the apical plasma membrane is increased in cells grown on porous support.

ON THE MECHANISM OF CELL VOLUME REGULATION IN ISOLATED PERFUSED MOUSE PROXIMAL STRAIGHT TUBULES F. Lang and H. Voelkl (intr. by G. Giebisch). Univ. of Innsbruck, Dept. of Physiology, Austria.

Experiments have been performed in isolated perfused mouse proximal straight tubules to elucidate the mechanism of volume regulatory decrease (VRD). Reduction of bath osmolarity by 80 mOsm/L (omission of mannitol) leads to a sustained depolarization of the cell membranes from -65 ± 1 mV to -57 ± 1 mV, an increase of the bicarbonate- selectivity from 0.11 ± 0.02 to 0.20 ± 0.03, a decrease of the potassium- selectivity from 0.69 ± 0.02 to 0.43 ± 0.04 and an increase of cell volume, followed by almost complete VRD. VRD is impaired by either, barium, acetazolamide, amiloride or lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) in the bath, by increase of bath potassium concentration to 30 or 40 mmol/l, replacement of bath sodium with choline or omission of bicarbonate and CO2. While amiloride and barium reduce the potassium conductance and thus depolarize the peritubular cell membrane to -28 ± 2 mV and -46 ± 1 mV, resp., NDGA hyperpolarizes the cell membrane to -69 ± 4 mV. VRD is unaffected by either, chloride channel blocker NPPB or 20 minutes preperfusion with chloride free perfusates (replaced with gluconate). Furthermore, an increase of bath potassium concentration to 20 mmol/l does not affect VRD despite a depolarization of the cell membrane to -40 ± 1 mV, which is below the equilibrium potential for chloride conductance or (3:1)-bicarbonate-sodium-cotransport. In conclusion, VRD in proximal straight tubules depends on potassium, bicarbonate and sodium, but not chloride, and is probably triggered by leucotrienes.

PURIFICATION AND RECONSTITUTION OF RENAL AND TRACHEAL CHLORIDE CHANNELS

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Chloride channels mediate epithelial absorption and secretion of NaCl. Their conductance is increased by cAMP, a step that is defective in cystic fibrosis. We identified inhibitors of the chloride conductance of bovine kidney microsomes, the most potent of which was an indanyl oxyacetic acid, IAA-94 with an I.C.$_{50}$ of 1 μM. Scatchard analysis of ^{3}H-IAA-94 binding showed a high affinity binding site with a K_d of 0.6 μM. The rank order of potency for transport inhibition agreed well with displacement of ^{3}H-IAA-94 when a number of inhibitors were used, suggesting that ^{3}H-IAA-94 was binding to the Cl channel. An IAA affinity column depleted solubilized kidney and tracheal vesicles of 50 % of their ^{3}H-IAA-94 binding sites. Specific elution of the loaded resin with IAA-94 yielded proteins from both sources with molecular weights of 240, 97, 64, 40 and 27 kDa. When the purified proteins were reconstituted into planar lipid bilayers only Cl selective channels were seen. These channels were of 3 types with single channel conductances of 25, 40 and 80 pS (in 150 mM KCl). The 40 pS channel rectified, while the others had a linear I-V relationship.

ROLE OF DEXAMETHASONE IN PROTEOGLYCAN SYNTHESIS BY PROXIMAL TUBULE CELLS IN CULTURE. B.Lelongt, P. Ronco, B. Baudouin, A.Vandewalle and P. Verroust. INSERM U64 & U246, Paris & Saclay, France. (Intr. by T. Anagnostopoulos).

To investigate the properties and distribution of proteoglycans (PGs) in confluent primary cultures of rabbit proximal tubule cells, PGs were labeled with [^{35}S] sulfate, extracted with 4 M GuCl, isolated from medium (M) and cell layers (C) by ion exchange chromatography and characterized by sepharose CL6B chromatography before and after treatment with chondroitinase ABC or nitrous acid. When cells were grown in DMEM + 1 % fetal calf serum (FCS) PGs were composed of 75 % heparan sulfate (HS) and 25 % chondroitin sulfate (CS). By contrast, cells grown in serum-free defined medium (DM) [basal medium supplemented with insulin (I) and dexamethasone 5x10^{-8}M (Dex)] synthetized PGs composed only of HS. In addition the C/M PGs ratio was increased 3 fold and the Kav values for both PGs and glycosaminoglycans (GAGs) were higher. (PGs Kav : C 0.16 vs 0.13, M 0.20 vs 0.17 ; GAGs Kav : C 0.5 vs 0.34, M 0.57 vs 0.38). These observations were associated with distinct ultrastructural localization (using ruthenium red) of PGs : FCS-PGs were expressed both in the apical domain and along a continuous basement membrane like material ; DM-PGs were almost exclusively located in a continuous layer of basal membrane. Synthesis of PGs and GAGs in C and M and ultrastructural localization of ruthenium red described in cells grown in DM were not observed in cells grown in basal medium supplemented or not with I, but were induced by addition of Dex only. These observations show that Dex permits basal membrane formation in association with increased synthesis of PGs containing only HS. They are in keeping with our previous studies showing, in the same culture conditions, a high degree of cell differentiation assessed by the presence of microvilli, sorting of hydrolases and hormonal response.

RENAL K ADAPTATION: EFFECT OF BASOLATERAL [K] ON NA,K-ATPASE. Margery A. Manuli* and Isidore Edelman* (intr. by LI Kleinman). Dept. of Biochem & Molec Biophysics, Columbia Univ., NY.

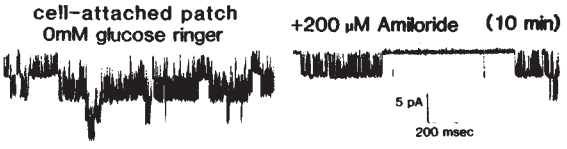
In animals, increase in distal nephron Na,K-ATPase activity is significant in adaptation to high dietary K. This response is only in part mediated by aldosterone. The MDCK cell line was used to assess the role of high external [K] in cellular adaptation independent of hormone secretion, regional hemodynamics or tubular flow. Enzyme activity (nmoles P_i/mg protein/hr) was measured in plate-grown cells adapted for 24 hrs to 5 or 7.5 mM K on both sides. Active ion transport (ouabain-sensitive ^{86}Rb uptake) and pump abundance (^{3}H-ouabain binding) were measured in filter-grown cells adapted for 24 hrs to 5 or 7.5 mM K basolaterally and 5mM K apically. Units were ul cleared/mg^2 protein/min and # molecules bound/mm^2 x 10^{11}, respectively. Cells adapted to 7.5mM K basolaterally showed greater enzyme activity, active ion transport, and enzyme abundance in the basolateral membrane. Means ± sem are shown in the table; n = # of pairs; * $p<0.05$.

	Basolateral [K] 5 mM	7.5 mM
NA,K-ATPase activity;n=7	1057±92	1495±137*
Ouabain sens ^{86}Rb uptake;n=9	1.1±0.3	1.7±0.3*
^{3}H-ouabain binding;n=4	1.3±0.6	2.4±0.7*

We conclude that high basolateral [K] alone, only slightly above normal serum [K], increases tubular Na,K-ATPase activity, pump-dependent ion transport and pump abundance. The results suggest that basolateral [K] may have regulatory importance in renal adaptation to high dietary K, independent of hormonal or luminal factors.

ACTIONS OF DIURETICS ON AN ATP-SENSITIVE K^+ CHANNEL IN PANCREATIC B-CELLS. Stanley Misler* and Kevin Gillis* (Intr. by E. Simon), Jewish Hospital, St. Louis, MO.

Hydrochlorothiazide, (HCTZ) and furosemide, (FUR) depress, while amiloride (AMIL) enhances glucose-induced insulin secretion. Their loci of action might be the voltage-independent ATP-sensitive K^+ channel, [K+(ATP)], in islet B cell: (1) this channel is the major contributor to resting K^+ permeability in islet cell; its closure during metabolism precedes Ca^{2+} dependent electrical activity and insulin secretion; (2) the hyperglycemic vasodilator diazoxide (DZ), which structurally resembles HCTZ and FUR, opens this channel; and (3) cell acidification (e.g. by transient exposure to a salt of a membrane permeant weak acid), which depolarizes the cell, closes this channel. By patch clamping normal rat B cells, we have now shown that acute bath application of HCTZ or FUR (up to 1 mM) does not affect K^+(ATP) activity even in those cell attached patches responsive to acute bath application of DZ (at 100-200 μM). (By reducing K^+_i longterm, HCTZ and FUR might inhibit glycolysis and reduce glucose-induced channel closure.) AMIL (at 100-200 μM) reduces K^+(ATP) activity 2-4 fold in the cell attached patch, but has no effect on the excised patch. AMIL may operate indirectly to reduce pH_i by blocking Na^+_o-H^+_i exchange.



AGE-DEPENDENT CHANGES OF RENAL BRUSHBORDER MEMBRANE (BBM) FLUIDITY AND LIPID COMPOSITION. Marvin S. Medow, Steven M. Schwarz*, Marc D. Danziger* and Leonard J. Newman*, Dept. of Pediatrics, New York Medical College, Valhalla, New York

We have demonstrated marked postnatal increases in rat renal BBM amino acid transport, with enhancement of both initial Na^+-solute uptake velocity and maximum overshoot height. These functional changes are temporally associated with decreases in DPH-determined membrane fluidity; and, a direct correlation is noted between overshoot height and fluorescence anisotropy, r (reciprocal of fluidity). To establish lipid-dependency of these age-related BBM physical changes, DPH anisotropy of liposomes prepared from extracted BBM lipid demonstrate markedly reduced lipid fluidity (increased r) occurring at the time of weaning (*$p<0.005$):

	7-14 day	21 day-adult
r @ 25°C (± SE)	.188±.007	.218±.009*
r @ 37°C (± SE)	.155±.010	.182±.009*

To determine origins of these fluidity differences BBM phospholipids (PL) were separated by HPLC, fatty acids (FA) and cholesterol (C) were quantitated by GLC (7 day vs adult). Postnatal lipid changes included increases in C/PL molar ratio, a major fluidity determinant (.61 vs .90, $p<0.05$) and in degree of relative FA saturation ($p<0.05$). Total BBM PL decreased with age, and significant increases in PE (12.6 vs 25.6% total PL) and decreases in PC (36.5 vs 12.8%) were noted. CONCLUSIONS: 1) These data establish lipid-dependency of postnatal decreases in renal BBM fluidity; 2) Decreasing fluidity is a consequence of increases in both C/PL ratio and saturation of membrane FA. SPECULATION: Age-related BBM fluidity decreases may be involved in maturation of renal transport function.

MODULATION OF Na^+/H^+ EXCHANGE IN RENAL BRUSH BORDER MEMBRANE (BBM) BY GTP-BINDING (G) PROTEIN. G. Morduchowicz*, D. Sheikhamad and N. Yanagawa, Neph. Div., Sepulveda VAMC, UCLA Sch. of Med., Los Angeles, CA.

G protein has been suggested to modulate ion transport. We have examined if G protein modulates BBM Na^+/H^+ exchange. BBM vesicles were isolated from rabbit kidneys and ^{22}Na uptake measured by rapid filtration procedure. Preincubation of BBM vesicles with pertussis toxin (PT) caused a dose-dependent inhibition on BBM Na^+ uptake (nmol/mg/5sec) (n=7).

	PT (ng/ml)				
Control	1	10	100	500	1000
3.73	3.22	2.98	2.93	2.68	2.71
± 0.25	± 0.20	±0.19	±0.21	±0.19	±0.10

In contrast, addition of nonhydrolyzable GTP analogue, Gpp(NH)p, caused a dose-dependent increase in BBM Na^+ uptake (nmol/mg/5sec) (n=7).

	Gpp(NH)p (M)		
Control	10^{-6}	10^{-5}	10^{-4}
2.98	3.33	3.88	4.46
±0.15	±0.18	±0.30	±0.28

The effects of PT and Gpp(NH)p on BBM Na^+ uptake required outwardly directed pH-gradient and were abolished by amiloride, suggesting the involvement of Na^+/H^+ exchange. Both PT and Gpp(NH)p did not affect BBM glucose uptake. These results suggest the modulation of BBM Na^+/H^+ exchange by G protein.

RECEPTORS FOR EXTRACELLULAR MATRIX COMPONENTS (EMC) IN HUMAN MESANGIAL CELLS (MC). NS Nahman Jr* and FG Cosio. Department of Medicine, Ohio State University, Columbus OH.

We demonstrated that the EMC fibronectin (FN) mediates the uptake of certain antigens and immune complexes by the glomerulus (J.Clin.Invest.80:1270,1987). The present study assessed the presence of receptors for FN and other EMC in human glomerular cells. Receptors for EMC belong to a family of at least 5 proteins (very late antigen or VLA 1 to 5) that shares a common β subunit (Mr 130Kd) linked, noncovalently, to variable α subunits (α1 to α5, Mrs 135 to 210Kd). VLA-2 is a receptor for collagen. VLA-3 is a receptor for FN, laminin and collagen. VLA-5 is a receptor for FN. EMC receptors modulate cell function. Thus, binding of FN to VLA-3 enhances Fc and complement receptor function in phagocytic cells. Cryostat sections of kidney and MC in culture were stained, by immunoperoxidase, using monoclonal antibodies anti-β subunit (A1A5), anti-α1 subunit (TS2/7), anti-α2 subunit (12F1), anti-α3 subunit (J143) and anti-α4 subunit (B5G10). No anti-α5 subunit antibody is available. The β subunit was present in the glomerular mesangium, the basal aspect of all tubular cells and the interstitium. In culture, MC membranes were positive for the β subunit. VLA-2 was weakly positive in the mesangium and stained the base of only distal tubular cells. Only a subpopulation of cultured MC (<5%) was positive for VLA-2. In tissue sections, VLA-3 was strongly positive in the glomerular mesangium and on both sides of the GBM. Glomerular epithelial, endothelial and tubular cells were negative for VLA-3. Cultured MC membranes were strongly positive for VLA-3. VLA-1 and VLA-4 were not present in kidney or MC. Summary, in the kidney, VLA-2 and VLA-3 are present in specific glomerular and tubular locations. MC express both VLA-2 and VLA-3 although the latter is predominant. Conclusion: membrane receptors for EMC may be important modulators of MC function.

ALDOSTERONE-INDUCED AMPLIFICATION OF GOLGI CISTERNAE PRECEDES BASOLATERAL MEMBRANE AMPLIFICATION OF CORTICAL COLLECTING DUCT (CCD). R.G. O'Neil, S.T. Vu, J.M. Reid, and L.T. Garretson. U. Texas Med. Sch., Houston, TX 77225.

The actions of aldosterone (ALDO) on fine structure of CCD were assessed in rabbits maintained on a high Na, low K diet for 2 weeks prior to bolus injection of ALDO (10 μg/kg, i.v.) and continuous ALDO infusion (70 μg/kg/day via osmotic minipumps, s.c.) for 24 or 48 hrs. Kidneys were perfusion-fixed in situ with modified Karnovsky's and prepared for electron microscopy. From cross-sections of principal cells from CCD both the basolateral membrane and Golgi cisternae areas were quantified stereologically. After 24 and 48 hrs of treatment, the basolateral membrane area increased to 117 $\pm$ 9% (n=9, n.s.) and 140 $\pm$ 15% (n=5, P<0.05) of control, respectively; whereas the Golgi area increased to 178 $\pm$ 27% (n=4, P<0.05) and 254 $\pm$ 43% (n=5, P<0.05) of control, respectively. Previously, we demonstrated that the Vmax Na-K-ATPase activity of the CCD (permeabilized cells) increased 193% (24 hrs) and 230% (48 hrs) after similar ALDO treatment (AJP 254:F698, 1988). It is concluded that the ALDO-induced increase in Na-K-ATPase activity and the activation and enlargement of Golgi cisternae observed after only 24 hrs, reflect the initial stimulation in synthesis of the Na-K-ATPase and its processing within the Golgi apparatus. These events precede the final insertion of transport vesicles into, and subsequent amplification of, the basolateral membrane observed at 48 hrs.

β-ADRENERGIC RECEPTOR MEDIATED REGULATION OF cAMP GENERATION IN RENAL MICROVASCULAR SMOOTH MUSCLE CELLS (SMC) IN CULTURE. T. Nguyen*, A. Chaudhari, A. Pedram*, M.A. Kirschenbaum. Nephrology Section, VA Medical Center and UCI-LB Medical Program, Long Beach, CA.

Previous studies have demonstrated that freshly dissected renal preglomerular microvessels (RMV) respond to β-adrenergic agonists. Since cAMP is thought to act as a second messenger in the regulation of SMC contractility, we have examined, whether isoproterenol (IP), a β-adrenergic agonist, has the ability to stimulate cAMP generation in cultured SMC derived from RMV. Freshly isolated rabbit RMV (interlobular arteries and afferent arterioles) were isolated using methods developed in this laboratory. Primary cultures of SMC were derived from these RMV and confluent monolayers were utilized after 3-4 subpassages. SMC were preincubated with IBMX (1 mM, a phosphodiesterase inhibitor), or IBMX+aspirin (1 mM, a cyclooxygenase inhibitor) for 10 min to prevent cAMP degradation and *de novo* prostanoid generation during the subsequent incubation with IP, epinephrine (EPI) and norepinephrine (NE), all 10^{-8}-10^{-4} M, for 5 min at 37° C. Following acid lysis of SMC, cAMP was determined by specific RIA. In the absence of agonists, cAMP production was 42±3 pmol/mg prot/15 min. A dose-dependent increase in cAMP formation was seen with the 3 agonists used with the following hierarchy: IP>EPI>NE. Propranolol (β_1-β_2 blocker) or metoprolol (β_1-blocker), added in 10 μM concentration, was capable of inhibiting IP-induced cAMP formation. These data strongly suggest the presence of β-adrenergic receptors in cultured SMC and suggest a role of these receptors in the regulation of cAMP generation in these cells.

SINGLE ION POTASSIUM CHANNELS IN RAT GLOMERULAR VISCERAL EPITHELIAL CELLS: A PATCH CLAMP STUDY. JM Orlowski*, BS Kasinath. Rush Medical College, Chicago, IL.

Glomerular epithelial cells (GEC) in cloned culture and in isolated whole decapsulated glomeruli were studied via patch clamp technique to evaluate similarities in their membrane channel characteristics. Having established the viability of GEC in isolated glomeruli by measurement of resting potentials, the cells were patch clamped. In addition a well characterized confluent layer of cloned GEC were patch clamped. Patch pipettes tapered to 0.5 u M were used to apply depolarizing voltage stepwise from -110mV to +110mV to the cell surface membrane in a "cell-attached" patch; seals of 10-50 Gigohms were achieved. The bath of NaCl Ringer's solution contained 4.74mM of KCl. The pipette contained a solution of 150mM KCl. Permeability ratios (PK/PNa) and reversal potentials were extrapolated with different concentrations of K+ in the pipette. Current-Voltage curves were linear over the voltage range studied. The slope conductances were found to be 36.4pS and 33.6pS and the reversal potentials were 15mV and 19mV (pipette voltages) in the cultured GEC and isolated glomerulus GEC preparation respectively. The frequency of channel opening increased with hyperpolarization. We therefore describe transport sites consistent with single ion channels for cell membrane transfer of potassium in the GEC. Our results reveal that the cultured GEC is a valid model for the study of potassium channels which were demonstrated in the in situ preparation.

IDENTIFICATION AND CHARACTERIZATION OF A cDNA ENCODING THE CORE PROTEIN OF HEPARAN SULFATE PROTEOGLYCANS FROM THE RAT GLOMERULAR BASEMENT MEMBRANE.

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Heparan sulfate proteoglycans (HSPG) are essential components of the glomerular basement membrane (GBM). Our goal has been to study the structure and function of these HSPG in the normal glomerulus and the changes they undergo in kidney disease. HSPG were isolated from rat GBM by extraction with guanidine-HCl and ion exchange chromatography. Chemical deglycosylation of the HSPG using trifluoromethane sulfonic acid yields a 130 kd core protein , which is recognized on immunoblots by a polyclonal anti-HSPG previously characterized in this laboratory [Stow, J.L., Sawada, H., and Farquhar, M.G. (1985) Proc. Natl. Acad. Sci.: 82, 3296-3300].

We have used this antiserum to screen a rat kidney cDNA expression library in λgt11 and have identified a recombinant containing a cDNA insert of 415 bp. In order to verify that this cDNA clone was specific for the HSPG from the GBM, we affinity-purified the polyclonal antiserum on the cDNA-encoded fusion protein. Using this affinity-purified antibody, we obtained GBM staining by immunofluorescence on kidney sections and binding to HSPG purified from GBM on dot blots.

DNA sequence analysis of the recombinant clone predicts an amino acid sequence containing cysteine-rich domains. Comparison of this sequence with the NBRF protein database using the FASTP program reveals a striking similarity with the cysteine-rich domains of the LDL-receptor (51% identity) and a similar region of C9 component of complement (39% identity). By RNA blot analysis, a single mRNA greater than 10 kb is detected in rat kidney, suggesting that the HSPG core protein is made as a large precursor. Work is underway to obtain the full-length cDNA sequence, and to study the biosynthesis of these HSPG.

MECHANISM OF DECREASED MICROVISCOSITY OF RENAL BRUSH BORDER (BBM) AND BASOLATERAL MEMBRANE (BLM) IN RATS WITH DIABETES MELLITUS (DM) L. Ramsammy,* C. Josepovitz,* B. Haynes,* and G.J. Kaloyanides. Dept. of Med., SUNY-Stony Brook, and VAMC, Northport, NY.

We have observed decreased arachidonic (AA) content, increased microviscosity and decreased non-electrolyte permeability in renal BBM and BLM from DM rats. We sought to evaluate the role of decreased AA in the alteration of the membrane biophysical properties. Since membrane microviscosity, measured by fluorescence polarization and expressed as steady state anisotropy (r̄s), is influenced by 1) fatty acids (FA) composition, 2) cholesterol, 3) lipid:protein ratio and 4) phospholipids (PL), we determined the composition of BBM and BLM from DM and non-DM kidneys. We observed no differences in cholesterol content between control and DM BBM (478 vs 490 nmol/mg prot) or BLM (341 vs 353). Similarly no differences were observed in total or in individual PLs. To evaluate the role of FA in the biophysical properties of the membrane, proteins and cholesterol were sequentially removed from the membranes and the r̄s measured at 30°C.

	Intact Membranes		-protein		-cholesterol	
	BBM	BLM	BBM	BLM	BBM	BLM
Control	.216	.162	.187	.125	.096	.086
DM	.254	.274	.210	.219	.129	.132
p<	.05	.001	0.2	.001	.02	.001

The data show that protein is a minor and cholesterol is a major determinant of membrane r̄s but neither explains the difference in r̄s between DM and non-DM rats. We conclude that the alterations in the microviscosity of DM membranes results from a change in FA composition.

ROLE OF PROTEIN KINASE C ON THE DEVELOPMENT OF TIGHT JUNCTIONS IN RENAL CELLS. C.A. Rabito and B. Ellis. Massachusetts General Hospital,Harvard Medical School, Boston, MA.

Protein kinase C (PKC) has been implicated in several molecular processes leading to cellular differentiation and gene expression. To study the role of PKC on the development of the epithelial membranes we analyzed the effect of an activator (phorbol ester 12-myristate 13-acetate,TPA) and an inhibitor(1-(5-Isoquinolinesulfonyl)-2-methyl-piperazine, H7) of PKC, on the development of the tight junctions,PKC activity,and 3H-TPA binding during the reorganization of dispersed LLC-PK1A and LLC-PK1B4 cells into epithelial membranes.TPA (1 uM), increases the transepithelial electrical resistance(TER) recorded during the reorganization of LLC-PK1A cells.In LLC-PK1B4 and MDCK4 cells, however,TPA has an inhibitory effect. TPA has no effect once TER has reached steady-state values. The response to TPA, however, was restored after a transitory opening of the tight junction following calcium removal. The inactive phorbol ester 4o -phorbol 12,13-didecanoate has no effect on TER. The stimulation of TER produced by TPA is inhibited by 100uM H7 but not by a similar concentration of H1004(N-(2-guanidinoethyl)-5-isoquinolinesulfonamide) an inhibitor of protein kinase A. PKC activity in LLC-PK1A and LLC-PK1B4 cells is high initially and decreases during culture. This down-regulation is increased by TPA and is more evident in the cytosolic than in the membrane fraction of the enzyme. 3H-TPA binding parallel the changes in TPA activity. These results suggest that PKC may regulate the development of the tight junctions during the reorganization of the epithelial membranes.

THE GENOMIC CLONE G-21 ENCODES THE HUMAN 5-HT1A RECEPTOR. John R. Raymond*, Annick Fargin*, Martin J. Lohse*, Brian K. Kobilka*, Marc G. Caron* and Robert J. Lefkowitz* (intr. by Vincent W. Dennis). Duke University Depts. of Medicine (Nephrology & Cardiology), Cell Biology and Biochemistry.

We have previously isolated a human genomic clone, G-21, by cross-hybridization with a full length β2-adrenergic receptor probe (Nature 329:75-79, 1987). Northern blot analysis revealed high levels of G-21 gene expression in lymphatic tissues and kidney. We now report that the protein product encoded by G-21 is the 5-HT1A receptor.

Transfection of COS-7 (monkey kidney) cells with G-21 DNA resulted in: a) transcription of a G-21 specific mRNA, indicated by high stringency Northern blotting; b) expression of a new membrane protein, indicated by immunoblotting with antisera raised against a 26 amino acid sequence encoded by G-21; and c) the acquisition of new specific 5-HT1A ligand binding properties. The binding of the 5-HT1A agonist [^{3}H]8-OH-DPAT was saturable and specific (>90% displacement by 10 μM 5-HT) in G-21 transfected cell membranes. There was no specific [^{3}H]8-OH-DPAT binding in nontransfected, plasmid-transfected, or α2-adrenergic receptor-transfected cell membranes. Two classes of sites with affinities of ≈0.1 & 14.5 nM were demonstrated by nonlinear least-squares regression analysis. Transfected cells expressed ≈14±3 pmol of binding sites/mg protein. Typical 5-HT1A pharmacology was obtained in competition studies with agonists [8-OH-DPAT (KD≈0.1 & 11nM)> ipsapirone (≈0.2 & 29nM)> 5-HT (≈0.3 & 68nM)> buspirone (≈4 & 95 nM)» dopamine> histamine ≈ epinephrine] and antagonists [spiroxatrine (≈2.5nM)> spiperone (≈46nM)> mesulergine (≈390nM)> ketanserin (≈2500nM)]. Binding properties of [^{3}H]8-OH-DPAT were sensitive to guanine nucleotides in a dose-dependent fashion, [GTPγS (IC50≈ 100nM)> GTP (≈200nM)> GDP (≈2μM)> GMP (≈80μM) » ATP], confirming that the 5-HT1A binding site is G-protein linked. Finally, the identity of the new membrane protein as the 5-HT1A receptor was confirmed by specific photoaffinity labeling and quantitative immunoprecipitation of a 72 KD band by an affinity-purified antipeptide IgG fraction directed against a 26 amino acid sequence encoded by G-21.

The isolation, identification and expression of the gene encoding the human 5-HT1A receptor provide powerful tools required to elucidate the role of 5-HT in renal function, immunomodulation and vasoregulation.

NA/H EXCHANGE ACTIVITY IN APICAL BRUSH BORDER MEMBRANE VESICLES FROM WILD TYPE AND MUTANT LLC-PK_1 CELLS. Robert F. Reilly*, John G. Haggerty*, Peter S. Aronson, Edward A. Adelberg*, and Carolyn W. Slayman*. Depts. of Human Genetics, Medicine, and Cellular & Molecular Physiology, Yale University School of Medicine, New Haven, Ct.

We have previously isolated a mutant line of LLC-PK_1 cells with elevated Na/H exchange activity at the apical surface (J. G. Haggerty et al., Am. J. Physiol., in press). The present study was undertaken to test whether this elevated activity results from an intrinsic alteration in the Na/H exchanger at the level of the apical membrane. Apical membrane vesicles were prepared from the parent cells (LLC-PK_1/Cl_4) and the mutant (PKE20) by magnesium aggregation and differential centrifugation. The apical brush border marker γ-glutamyl transpeptidase was comparably enriched in both preparations (9.3 ± 1.1 fold in Cl_4; 10.1 ± 0.5 fold in PKE20), with little contamination by the basolateral marker, Na,K-ATPase (0.96 ± 0.2, and 1.3 ± 0.1 fold enrichment, respectively). In both cases, ^{22}Na influx was stimulated by an inside-acid pH gradient, inhibited by amiloride, and unaffected by valinomycin with $K_{in}=K_{out}$, indicating that it was mediated by Na/H exchange. Two significant differences were observed between parent and mutant vesicles: (1) The V_{max} for acid-stimulated Na influx was 3-6 fold greater in PKE20 than in Cl_4, and (2) the IC_{50} for inhibition by ethylisopropyl-amiloride was ca. 80 fold greater in PKE20 (3 μM) than in Cl_4 (0.04 μM). By contrast, no significant change was observed in Na-glucose or Na-glutamate cotransport, confirming the specificity of the mutation. We conclude that the elevated Na/H exchange activity in PKE20 results from a specific alteration in the Na/H exchanger at the level of the apical membrane.

BIOGENESIS OF PODOCALYXIN -- THE MAJOR GLOMERULAR EPITHELIAL SIALOGLYCOPROTEIN -- IN THE NEWBORN RAT KIDNEY. E. Schnabel, G. Dekan, A. Miettinen, and M.G. Farquhar. Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut.

The appearance and distribution of podocalyxin during glomerular development was determined using specific monoclonal and affinity-purified polyclonal antibodies. Kidneys from 2 d old rats were perfusion-fixed and processed for immunofluorescence labelling on semithin frozen sections or immunogold labelling on ultrathin frozen sections using the techniques of Tokuyasu.

Podocalyxin first appeared on the visceral epithelial cells in the late S-shaped body stage, where it was found along their apical surfaces facing the developing Bowman's space above the level of the zonulae occludentes that connect the cells at this stage. In the early capillary loop stage, when the urinary spaces open and the junctions migrate from the apex to the base of the cells, labelling extended along the lateral plasmalemma above the migrating junctions. In the maturing glomerulus with the beginning of foot process formation, the zonulae occludentes become fragmented into maculae which give way to developing slit diaphragms. At this stage podocalyxin is found along all open surfaces of the visceral epithelial cells exposed to Bowman's space, i.e. above the occluding junctions or slit membranes. No labelling was found below. In ultrathin frozen sections, podocalyxin could also be detected intracellularly in small vesicles in the Golgi region and in carrier vesicles presumably en route to the cell surface. With the more sensitive immunoperoxidase method, podocalyxin was found throughout the entire exocytic pathway (Golgi stacks, rough ER and perinuclear cisternae).

We conclude, that 1) podocalyxin is synthesized at a high rate in the differentiating podocyte; 2) its distribution is restricted to the plasmalemmal domain facing the urinary spaces; 3) its time of appearance and distribution are identical to that reported earlier for the epithelial polyanion; and 4) its appearance is closely coupled to the development of the foot processes and filtration slits.

PROTEOGLYCAN SYNTHESIS BY SV40 TRANSFORMED PROXIMAL TUBULE CELLS IN CULTURE. P. Ronco, B. Lelongt, M. Geniteau, B. Baudouin, R.Cassingena, P.Verroust and A.Vandewalle. INSERM U64 & U246 Paris & Saclay, France.(Intr.by T.Anagnostopoulos).

The distribution and synthesis of proteoglycans (PGs) were studied in primary culture cells (PC) and in an established cell line, derived from PC after infection with wild type SV40 virus. PG from confluent cells grown in serum free hormonally defined medium were labeled with (^{35}S) sulfate, extracted with 4M GuCl and isolated from medium and cell layers by ion exchange chromatography. Glycosaminoglycan chains (GAGs) were released by alkaline treatment in sodium borohydride and both PGs and GAGs were characterized by sepharose CL6B chromatography before and after treatment with chondroitinase ABC and nitrous acid. EM histological labeling with ruthenium red showed that in both PC and SV40 infected cells PGs were detected along cell membranes, but essentially located within the basal matrix. The degree of sulfatation was identical in PC and SV40 infected cells, but the cell to medium PGs ratio was reduced by 2 fold in SV40 cells. PGs synthetized by PC contained only heparan sulfate (HS) and eluted as a single peak with a Kav of 0.16 whereas PGs synthetized by SV40 cells contained only chondroitin sulfate (CS) and eluted as 2 distinct peaks with Kav values of 0.21 and 0.39. Kav values of GAG chains isolated from PC and SV40 cells were respectively 0.50 and 0.63. Similar results were obtained for the PGs and GAGs extracted from culture media. In conclusion, the establishment of long term cell line by SV40 transformation is associated with drastic modification of PG's synthesis : i) the amount of PGs and GAGs synthetized is decreased; ii) PGs and GAGs have lower molecular weight; and iii) they contain CS instead of HS.

IDENTIFICATION AND PURIFICATION OF THE MAJOR CONCANAVALIN A-BINDING PROTEIN OF RENAL BRUSH BORDER MEMBRANES. H. See*, J.H.M. Charuk* and R.A.F. Reithmeier* (Introduced by M. Silverman), MRC Group in Membrane Biology, Department of Medicine and Department of Biochemistry, University of Toronto, Toronto, Ontario.

The glycoprotein components of brush border membranes (BBM) purified from porcine renal cortex were identified on SDS polyacrylamide gels following lectin blotting and endoglycosidase treatments. The major concanavalin A-binding protein had a molecular weight of 130,000 and also bound wheat germ agglutinin and Ricinus lectin. The protein, when deglycosylated with glycopeptidase F, had a molecular weight of 100,000. It was partially sensitive to endo-glycosidase H, showing that it contained both high mannose and complex carbohydrate chains. A similar protein was also identified in dog (130 kD) and rabbit (120 kD) BBM. The protein bound tightly to concanavalin A Sepharose and could be purified using this resin. Polyclonal antibodies were raised against this protein and were used to localize it to the BBM and in a kidney epithelial LLC-PK_1 cell line. The protein also bound specifically to a SITS-affinity column, making it a candidate for a renal anion transporter.

METABOLIC HANDLING OF HEYMANN NEPHRITIS RELATED IMMUNE COMPLEXES FORMED ON THE SURFACE OF GLOMERULAR EPITHELIAL CELLS. A.K. Singh, B.S. Kasinath, and E.J. Lewis. Rush Medical College, Chicago, IL.

Heymann nephritis (HN) a rat model of membranous glomerulopathy is distinguished by the presence of immune deposits in the lamina rara externa. It is conjectured that these deposits first form on the surface of the glomerular epithelial cell (GEC). This work was undertaken to establish a cellular model of the HN lesion utilizing cultured GEC and to study the metabolic handling of the surface complexes. Two antibodies, anti-Fx1A and anti-gp70 were compared. The first is multispecific and biologically strong in producing HN in rats; the second is monospecific and biologically weak. Immune complexes were formed on the surface of GEC by incubating with radiolabelled and gold conjugated antibodies. Fate of immune complexes was followed by immunofluorescence, gamma counting, gel filtration, SDS-PAGE and electron microscopy. While anti-gp70 complexes were internalized rapidly from the surface of GEC (2 hr), anti-Fx1A complexes cleared at 4 times slower rate. Anti-gp70 complexes were catabolized to some extent (58% in 1 hr). In contrast anti-Fx1A complexes were left undigested by the GEC. No immune complexes were detected extracellularly with either antibodies. We conclude 1) the lesion of HN can be duplicated at the cellular level, 2) surface complexes involving multiple antigens (Fx1A) are internalized much slower by GEC, and 3) non-internalized immune complexes stay attached to the GEC surface.

G-PROTEIN REGULATION OF A CATION CHANNEL IN INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. B. Stanton, D. Ausiello and D. Light*. Dept. of Physiol., Dartmouth Med. Sch., Hanover, NH and Mass. Gen. Hospital, Boston, MA.

G-proteins transduce receptor-mediated signals for a number of effector systems. In particular, G-proteins directly couple receptors to K^+ and Ca^{++} channels in myocytes and also directly regulate channel activity. In polarized epithelial cells, G-proteins are coupled with cell-surface receptors in the basolateral membrane whereas G-proteins located in the apical membrane are not linked with receptors, but may modulate ion channels. We conducted experiments to determine if a G-protein directly gates a cation channel in the apical membrane of rat IMCD cells. Patch clamp studies were conducted on inside-out patches of IMCD cells in primary culture. Previous studies showed that the cation channel is inhibited by amiloride and mediates electrogenic Na^+ uptake (Light, et al. J. Gen. Physiol., 90:28, 1987). GTPγS (10^{-4} M) in the bath increased the single channel open probability (Po) from 0.18 to 0.45 (n=4; P<0.05) in the absence of any other channel agonist. In contrast, GDPβS (10^{-4} M) reduced Po from 0.53 to 0.39 (n=4; P<0.05). Pertussis toxin (PTX: 250 ng/ml) inactivated the channel: Po fell from 0.37 to 0.05 (n=6; P<0.05). GTP (10^{-4} M) failed to activate the cation channel in PTX treated patches (n=2). Conclusion. These experiments indicate that a PTX-sensitive, GTPγS activated G-protein in the apical membrane directly gates the cation channel. The regulation of the cation channel by the G-protein is not dependent on receptor coupled G-proteins in the basolateral membrane, but may be subject to regulation through the pathways involved in signal transduction.

LOCATION AND CONCENTRATION OF SULFHYDRYL (SH) GROUPS INVOLVED IN RENAL BRUSH BORDER MEMBRANE (BBM) PHOSPHATE (Pi) TRANSPORT. Gregory B. Sorenson*, Mahmoud Loghman-Adham. Dept. of Pediatrics, Univ. of Utah, Salt Lake City, UT.

To determine the location of SH groups involved in BBM vesicle (BBMV) Pi transport, we compared the effects of 2 closely related SH reagents: p-chloromercuribenzoate (PCMB) which freely penetrates the membranes and p-chloromercuriphenylsulfonate (PCMBS) which penetrates membranes very slowly, on Na^+-Pi cotransport. BBMV were prepared from rat renal cortex by a Ca^{++}-precipitation method and transport measured by a rapid filtration method. Both reagents produced a similar degree of transport inhibition (IC 50=275 and 292uM for PCMB and PCMBS respectively, at a BBMV protein concentration of 7 mg/ml) as well as a dose-dependent reduction of total BBM SH content. There was a significant correlation between the Na^+-Pi cotransport and the SH content of the same BBMV (r=0.96, p<0.05).

$HgCl_2$ which freely penetrates membranes, also significantly inhibited Na^+-Pi cotransport. Exposure of BBMV to 2mM dithiobisnitrobenzoic acid (DTNB) or N-ethylmalei-mide (NEM) - 2 SH reagents without effect on Pi transport - prior to the addition of $HgCl_2$, had an additive effect on transport inhibition (+15% for DTNB and +30% for NEM), indicating that only a portion of SH groups are involved in Na^+-Pi cotransport.

SH groups involved in Na^+-Pi cotransport are exposed on the outer surface of BBM and are entirely accessible by polar SH reagents.

THE CORE PROTEIN OF THE BASEMENT MEMBRANE HEPARAN SULFATE PROTEOGLYCAN (BMHSPG) IS SECRETED BASALLY FROM MDCK CELLS TREATED WITH *B*-D-XYLOSIDE. Jennifer L. Stow* and Marilyn G. Farquhar. Dept. of Cell Biology, Yale University School of Medicine, New Haven, CT.

The synthesis and correct delivery of BMHSPG to the basally-located basement membrane is an important function of glomerular and tubular epithelial cells. We have previously shown that BMHSPG is sorted in a low pH compartment and secreted mainly basally from MDCK cells grown on filters (Caplan et al. Nature 329: 632, 1987). In order to determine whether sorting is a function of the core protein or of the glycosaminoglycan (GAG) chains of the BMHSPG, we followed the secretion of BMHSPG synthesized in MDCK cells grown on Transwell filter chambers in the presence of xyloside. Xyloside acts as an acceptor for GAG addition and can be used to generate free GAG and partially glycosylated core proteins. In controls 90% of the GAG secreted were heparan sulfate, and 70% of the GAG were secreted basally. In cells treated with 2-5 mM xyloside, there was a five fold increase in the total [^{35}S]sulfate-GAG secreted of which 82% were now chondroitin sulfate, and both GAG (heparan and chondroitin sulfate) were secreted equally into the basal and apical media. Thus free GAG chains are not sorted and are secreted both apically and basally. A polyclonal antibody, specific for the core protein, was used to immunoprecipitate [^{35}S]cysteine-labeled BMHSPG. In controls only the mature, fully-glycosylated BMHSPG was immunoprecipitated from the medium (80% basal, 20% apical); the cell extract also contained precursor core protein bands at 450 and 270 kd. In cells treated with higher concentrations (5mM) of xyloside, ~65% of the BMHSPG was secreted as a 320 kd form, lacking [^{35}S]sulfate-GAG, which appeared to be correctly sorted (80% basal, 20% apical). A small amount of this 320kd form was also found in extracts of the xyloside-treated cells. It appears that sulfated GAG are not required for correct sorting and basal secretion.of the BMHSPG We conclude that - 1) BMHSPG lacking GAG is still secreted by MDCK cells and, 2) the sorting signal for delivery of the BM HSPG to the basal cell surface resides in the structure of the core protein itself rather than in the GAG chains.

MEMBRANE STRETCH: A PHYSIOLOGICAL STIMULATOR OF Ca^{2+}-ACTIVATED K^+ CHANNELS IN MEDULLARY THICK ASCENDING LIMB CELLS. Junichi Taniguchi and William B. Guggino. The Johns Hopkins Univ. School of Med., Dept. of Physiology, Baltimore, MD.

Ca^{+2}-activated (maxi) K^+ channels have been reported in the apical cell membrane of medullary thick ascending limb cells. However, their functional role is not clear, because the activity of this channel is very low at the resting membrane voltage. In this study using the patch clamp technique, we found that membrane stretch induced by applying negative pressure of 33±5 cm H_2O to a cell-attached patch increased fractional open probability (NxP_o) from 0.3±0.2% to 29.9±7.6% (n=12). The activity returned to control upon releasing the negative pressure. Reduction of extracellular osmolality by 73 mOsm also activated K^+ channels in cell attached patches to NxP_o = 43.8±12.2%, (n=8). Removal of Ca^{2+} from the pipette inhibited stretch activation and removal of Ca^{2+} from the bathing solution inhibited activation induced osmotically. Thus, extracellular Ca^{2+} was necessary for activation. Stretch activated K^+ channels were shown to be Ca^{+2}-activated K^+ channels, by their conductances (151±8pS, n=4 and 146±7 pS, n=5,), by Ca^{2+} activation and by the observation that Ca^{+2}-activated K^+ channels were always in the same patch after excision. Thus, our findings in medullary thick ascending limb cells suggest that apical membrane stretch caused by swelling or possibly by changes in tubular flow enhances Ca^{2+} entry thereby stimulating maxi K^+ channel activity.

INVOLVEMENT OF CYTOSKELETAL PROTEINS IN MAINTENANCE OF LIPID ASYMMETRY OF RENAL BRUSH BORDER MEMBRANES. Catherine Vénien* and Christian Le Grimellec. INSERM U251, Fac. Med. Xavier Bichat, Univ. Paris 7, Paris, France.

The topological distribution of phospholipids of kidney brush border membranes (BBM) is highly asymmetric: amino-containing species, phosphatidylethanolamine (PE) and phosphatidylserine (PS) are primarily localized in the inner membrane leaflet whereas sphingomyelin (SM) accounts for more than 75% of the phospholipids present in the outer leaflet (BBA, in press). Involvement of energy-dependent processes and cytoskeletal proteins in maintenance of the asymmetry was assessed by 14 hr incubations at 37°C in absence of energy supply and by treatment of BBM with either diamide 5mM which crosslinked proteins or dihydrocytochalasin B. Phospholipid asymmetry, as judged by the use of sphingomyelinase, phospholipases A2 and trinitrobenzene sulfonate was fully maintained in control BBM incubated 14 hr at 37°C. Upon diamide or dihydrocytochalasin treatment a partial loss in asymmetry that primarily concerned aminophospholipids was observed. Our results strongly suggest that cytoskeletal proteins, via interactions with aminophospholipids stabilize the lipid bilayer of BBM. They also suggest that the low transbilayer migration rate of SM plays an important role in the maintenance of the lipid asymmetry.

ESTABLISHMENT OF SV40 TRANSFORMED RENAL CELL LINES WHICH DISPLAY FUNCTIONS OF DISTAL TUBULE CELLS. A. Vandewalle, B. Lelongt, M. Geniteau, B. Baudouin, R. Cassingena, G. Friedlander, P. Verroust and P. Ronco. INSERM U64 & U246, Paris & Saclay, France. (Intr. by T. Anagnostopoulos).

Two transformed cell lines (RCSV2 and RCSV3) were obtained from isolated cortical cells of rabbit kidney grown in serum-free defined medium (DM) and infected with the wild type SV40 virus (m.o.i. 100 p.f.u./cell). After 30 passages in DM, both cell lines were able to form domes and were sensitive to several hormones or compounds including vasopressin (AVP 10^{-6}M), isoproterenol (ISO 10^{-6}M), calcitonin (CT 100 pg/ml), parathormone (PTH 10^{-7}M) and forskolin (FK 10^{-5}M) as shown by their cAMP content (pmol/5 min/mg protein) :

	Basal	AVP	ISO	CT	PTH	FK
RCSV2	12.8	12.0	75.6**	124.2**	74.8**	193.3**
RCSV3	15.6	51.2*	169.5**	38.4**	18.0	233.1**

(mean of 6 to 17 determinations.*p<0.01,**p<0.001 vs B.)

In addition the two cell lines exhibited sodium dependent transport for phosphate, but lacked several proximal characteristics : there was very low (RCSV2) or no (RCSV3) α-methyl glucose uptake ; hydrolase activities were low and cells exhibited a smooth apical membrane surface. Finally the high rate of Na^+ induced H^+ efflux observed on BCECF acid-loaded cells may be related to the rapid cell growth observed which in turn may be of significance to account for the selection of distal cells from an initial population containing mainly proximal cells. In conclusion we suggest that the SV40 infected cells, mainly sensitive to CT for RCSV2 and ISO for RCSV3, could be derived from the bright and granular portions of the distal tubule respectively.

CO-RECONSTITUTION OF TRYPSINIZED SOLUBILIZED BRUSH BORDER MEMBRANE PROTEINS WITH SMALL MOLECULAR WEIGHT PEPTIDES RESTORES THE INHIBITORY EFFECT OF PKA ON THE NA^+-H^+ EXCHANGER. E. J. Weinman, S. Shenolikar, and W. Dubinsky. Univ. of Texas Medical School, Houston, Texas.

cAMP mediated protein phosphorylation (PKA) of solubilized renal brush border membrane proteins inhibits the activity of the Na^+-H^+ exchanger as assayed after the proteins are reconstituted into artificial lipid vesicles. To explore the mechanism of this response, studies were performed using detergent solubilized rabbit renal brush border membrane proteins ; trypsin to cleave the recognition sequence of PKA; and Superose-12 FPLC to separate the solubilized proteins by size. PKA inhibited the activity of the Na^+-H^+ exchanger in reconstituted proteoliposomes by 36±6%; an effect which was blocked by limited trypsin digestion prior to the PKA mediated phosphorylation reaction (% Change = -4 ± 10%).

The solubilized membrane proteins fraction eluting at V/V_e = .7 was co-reconstituted with trypsinized proteins. This fraction did not affect basal rates of Na^+-H^+ exchange activity but did restore the inhibitory response to PKA (% Decrease = 35 ± 5%). SDS-PAGE autoradiography indicated that the fraction contained only a limited number of PKA substrates.

These results suggest that the Na^+-H^+ exchanger has a small molecular weight regulatory component which may be the target for cAMP dependent protein kinase action. The ability to co-reconstitution components of the transporter provides an experimental approach toward elucidation of the structure and function of the Na^+-H^+ exchanger.

EGF AND COLLAGEN MATRIX ENHANCE GROWTH AND FUNCTION OF CANINE PROXIMAL TUBULE (CPT) CELLS WITHOUT SERUM SUPPLEMENTATION. Jerry V. White and Delmar R. Finco. Univ. of Georgia, College Vet. Med., Dept. of Physiol. and Pharmacol., Athens, Georgia.

To attain acceptable growth, CPT primary cell cultures are usually initiated on plastic or collagen surfaces in serum supplemented media and then maintained in hormonally defined media consisting of DMEM:Hams F-12 supplemented with hydrocortisone, insulin, transferrin, and NaSe (DF-4A). In an attempt to obviate this need for initial exposure to serum and its associated complications, a serum free CPT cell culture system (SF) was designed and tested against a traditional serum supplemented system (SS). CPT were isolated and plated on plastic with 10% serum supplemented DMEM:Hams F-12 or on collagen matrix with DF-4A and EGF (1 nM). After 72 hours, serum and EGF were removed and both culture systems were maintained in fresh DF-4A for the next 10 days. There was no significant difference in rate of cell growth between the 2 culture systems. SF and SS cultures formed monolayers by day 6, and cell numbers reached a plateau by day 10. Alpha-methyl glucoside (AMG) transport, PTH-stimulated cAMP production, γ-glutamyl transpeptidase (GTP) activity and O_2 consumption (QO_2) per mg protein were assessed in both systems and in freshly isolated proximal tubule (FIPT) suspensions.

	AMG (nmol)		cAMP (pmol)		GTP (mU)			QO_2(nmol/min)	
	5 min	15 min	10^{-6}M PTH	10^{-8}M PTH	0	day 7	12	day 0	12
FIPT	-	-	-	-	2117	-	-	33	-
SS	1.9*	5.6*	123.7*	50.6*		637*	317*		9.6*
SF	4.0	7.9	226.2	76.4		1174	599		13.4

* significant increase above corresponding SS value ($p<0.01$)

Scanning and transmission electron microscopy demonstrated an increased brush border and organelle density in cells of SF versus SS cultures. We conclude that EGF and collagen matrix potentiate excellent growth of canine proximal tubule cells obviating the need for serum supplementation and its inherent complications. The morphology and improved function of SF over SS cultures more closely mimics in vivo proximal tubule cell physiology.

SOLUBILIZATION, ISOLATION AND RECONSTITUTION OF THE CLATHRIN-COATED VESICLE (CCV) CHLORIDE (Cl) TRANSPORTER. X.-S. Xie* and D.K. Stone, UT Southwestern Med Ctr, Dallas, TX.

Acidification of endomembrane compartments, including CCV, is achieved by the parallel operation of an electrogenic proton pump with an electrogenic Cl transporter. The latter serves to facilitate co-ion (Cl) movement to dissipate the interior positive potential generated by the pump, and hence allow for net ΔpH formation. We have previously reported that proton pumping catalyzed by the purified, reconstituted proton pump is absolutely dependent upon K loading of the proteoliposomes and valinomycin, indicating that the Cl transporter had been functionally dissociated from the proton pump. We have now achieved the simultaneous solubilization (0.75% $C_{12}E_9$) and co-reconstitution (cholate dilution/freeze-thaw) of the CCV proton pump and Cl transporter; reconstituted proton pumping of this preparation is not valinomycin-dependent. The Cl transport activity has been partially resolved by means of hydroxylapatite chromatography and glycerol gradient centrifugation. Resultant fractions are devoid of proton pumping activity, but when coreconstituted with the purified pump, allow for valinomycin-independent proteoliposome acidification. The transport activity is specific for Cl; gluconate will not allow for acidification. In addition, the Cl transport activity is heat labile and trypsin sensitive. Pretreatment of the Cl transporter with NEM prior to coreconstitution restores valinomycin dependency to proton pumping, indicating that the transporter is NEM sensitive.

ALTERATIONS IN MEMBRANE NAK-ATPASE IN ADULT HUMAN POLYCYSTIC KIDNEY DISEASE (APKD) EPITHELIA. P.D. Wilson*, P.A. Gabow+, K. Palla*, J.T. Norman@. Dept. Physiol UMDNJ-RWJ Med.Sch., Piscataway, NJ*, Denver Gen. Hosp. Co.+; UCLA, CA@.

Normal renal tubule function is dependent on polarized insertion of membrane proteins into specific plasmalemma domains. NaKATPase is in basolateral membranes of normal renal tubules. We have previously reported that APKD epithelia have abnormalities of enhanced growth; basement membrane changes; loss of apical microvilli; unique lectin binding; loss of adenylate cyclase response to hormones and loss of γ-glutamyl transpeptidase activity. Fresh, frozen and fixed kidney sections and individually isolated and cultured defined renal tubule segments and APKD cyst epithelia were compared. In some non-end-stage APKD, NaKATPase specific activity was significantly greater than in any normal tubules, and Northern analysis showed enhanced expression of mRNA of the α and β subunits of NaK-ATPase. Using both enzyme cytochemistry and a polyclonal antibody to the α-subunit, >90% NaK ATPase localized in the apical plasma membrane of APKD epithelia in vivo and in vitro. Other normally basolateral (band 3) and apical (α95kD and Heymann nephritis) antigens showed unaltered localization. APKD NaKATPase colocalized with actin but not with the other cytoskeletal proteins tubulin or keratin, and was correlated with enhanced synthesis of a 45kD protein as shown by SDS-PAGE. We conclude that APKD cyst epithelia show reversed NaKATPase polarity which could contribute to abnormal ion and fluid movement.

EFFECT OF THYROID HORMONE ON Na^+-H^+ EXCHANGE AND INTRACELLULAR PH (pHi) IN CULTURED OPOSSUM KIDNEY (OK) CELLS. K. Yonemura, L. Cheng, J.L. Kinsella and B. Sacktor. (intr. by D. Spector) GRC, NIA, NIH, Baltimore, MD 21224

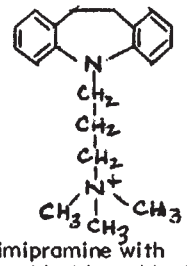
Previous studies showed that Na^+-H^+ exchange activity in renal brush border membrane vesicles is increased in the hyperthyroid rat. It is not clear whether this is due to an indirect effect on proximal tubular cells by alterations in renal hemodynamics or to a direct effect on the cells. OK cells, a cell line with proximal tubule cell properties, were used to elucidate the direct effect. In the confluent culture, T_3 did not alter cell numbers, protein and DNA. T_3 stimulated amiloride-sensitive Na^+ uptake by 60% with an E_{5_0} of 10^{-} M. T_3 increased the Vmax from 123±22 to 157±24 nmol·mg^{-1}min^{-1} but not the Km for Na^+ (21.9 vs 22.0mM). The stimulatory effect of T_3 was correlated to T_3 occupancy ($K_{0.5}$=1.1x10^{-9} M) of nuclear receptors. pHi studies using pH-sensitive dye (BCECF) showed that the after acid loading steady-state pHi (6.08 vs 6.11) and buffering capacity (58.9 vs 67.4 mM·pH^{-1}) were not different between control and T_3-treated and that pHi recovery following acid load was more rapid in T_3-treated cells (0.08±0.05 vs 0.16±0.06 pH·min^{-1}). We concluded that thyroid hormone can stimulate Na^+-H^+ exchange activity directly in cultured renal tubular cells without apparent changes in steady-state pHi, cellular hypertrophy and hyperplasia, and that its action may be initiated by binding thyroid hormone to the nuclear receptors.

GLUCOCORTICOID (GCD) BLOCK AND REVERSE THE INCREASE OF THE Na^+/Pi COTRANSPORT ACROSS THE RENAL CORTICAL BRUSH BORDER MEMBRANE (BBM) BY TRIIODOTHYRONINE (T_3). A.N.K. Yusufi, H. Moltaji *, and T.P. Dousa, Nephrol. Res. Unit, Mayo Clinic, Rochester, MN

Administration of T_3 was shown to increase the rate of Na^+/Pi cotransport in the rat renal BBM vesicles (BBMV), whereas GCD exhibited an opposite effect. We now studied whether GCD can counteract the stimulatory effect of T_3 and by which mechanism with use of ^{14}C-PFA, a probe for Na^+/Pi cotransporter. Administration (2 x 1 mg/kg; i.m.) of dexamethasone (DEX) to TPTX d rats completely blocked both the increases in rate of Na^+/Pi cotransport (Δ + 49%) and in Na^+-dependent binding of ^{14}C-PFA (Δ + 38%) by BBMV, which were elicited by treatment with T_3 (1 mg/kg, i.p.). Also, administration of DEX to rats pretreated with T_3 reduced both the rate of Na^+/Pi cotransport and the the extent of Na^+-dependent ^{14}C-PFA binding by BBMV to the control levels. Na^+-dependent transport of D-^{3}H-glucose and Na^+-dependent binding of ^{3}H-phlorizin in the same BBMV were not influenced by either T_3 or DEX administration. Conclusion: These results show that GCD can block and reverse the stimulatory effect of T_3 on Na^+/Pi cotransport across BBM. We suggest that GCD counteract the stimulatory effect of T_3 on Na^+/Pi cotransport either by inhibiting biosynthesis of new Na^+/Pi cotransporters and/or by blocking insertion of Na^+/Pi cotransporters into BBM enhanced by T_3 treatment.

THE MECHANISM FOR THE CHROMATOGRAPHIC SEPARATION OF INOSITOL POLYPHOSPHATES BY ION-PAIR CHROMATOGRAPHY. Florence S. Barcelon*, James A. Shayman, University of Michigan, Ann Arbor, MI.

Ion-pair chromatography has proven to be a useful method for the isocratic separation of inositol phosphates and their isomers. We investigated the chromatographic mechanism of action of ion-pairing agents by synthesizing an optically active quarternary amine, N-methyl imipramine (MIP).



MIP was synthesized by refluxing imipramine with gaseous methyl bromide in methylene chloride and by its conversion to its phosphate salt in the presence of silver oxide. Both the structure and purity of the amine were assessed by 1H NMR. MIP, like tetrabutyl ammonium phosphate, was capable of resolving inositol phosphates under isocratic, reverse phase conditions (21% acetonitrile, 0.03M KH_2PO_4, pH 3.5). Retention was dependent on pH and solvent polarity. The biphenyl heptane moiety of MIP was monitored by UV at 298 nm. By preequilibrating the column with either MIP or tetrabutyl ammonium phosphate and then eluting inositol phosphates with a substituted counterion, the following observations were made: (1) Ion-pairing agents freely substitute with one another; (2) inositol phosphates do not appear to elute bound to a counterion; and (3) MIP bound to the C18 column rapidly elutes when counterion is withheld from the mobile phase.

These observations support the use of MIP as a potentially valuable ion-pairing agent and strongly support the "dynamic exchange" model of ion-pair chromatography.

DIFFERENTIAL EFFECTS OF PHORBOL ESTER ON PROSTAGLANDIN E_2 (PGE_2) AND BRADYKININ (BK) INDUCED ELEVATION IN CYTOSOLIC FREE Ca^{2+} CONCENTRATION, [Ca_i^{2+}], IN $LLCPK_1$ CELLS. A. Aboolian* and E.P. Nord. Division of Nephrology, HSC, SUNY at Stony Brook, NY.

PGE_2 and BK activation of the phosphoinositol (PI) transduction pathway in $LLCPK_1$ cells and modulation of this process by the protein kinase C (PKC)-activating phorbol ester, phorbol-12,13-myristate (PMA) was studied by monitoring changes in [Ca_i^{2+}], with the Ca^{2+}-sensitive fluorescent probe, FURA-2. Acute exposure of cells to PGE_2 or BK evoked a prompt transient increment in [Ca_i^{2+}] that peaked at 2-2.5 fold the resting value within 10 sec, approached basal levels within 90 sec, and was independent of extracellular Ca^{2+} concentration ([Ca_o^{2+}]). The $K_{0.5}$ for the process for PGE_2 and BK was 8 x 10^{-7}M and 3 x 10^{-10}M, respectively. Sequential challenge of cells with PGE_2 and BK, in either order, and independent of [Ca_o^{2+}], evoked two Ca^{2+} transients, suggesting that each agonist was exclusively recognized by a specific receptor. Brief (2 min) incubation of cells with PMA (10^{-8}M) completely blocked the PGE_2 (10^{-5}M) -evoked Ca^{2+} transient. In contrast, longer (10 min) incubation of cells with PMA, even at 10^{-4}M, blunted the BK (10^{-9}M) -evoked Ca^{2+} transient by 50%. Neither 4α- nor 4β-phorbol, which lack the ability to activate PKC, were effective in this regard. Conclusions: 1. The $LLCPK_1$ cell possesses receptors for PGE_2 and BK. 2. PGE_2 and BK receptors are linked to the PI transduction pathway. 3. The PGE_2 and BK receptors differ in their response to PMA. 4. In addition to PKC, other mechanism(s) modulate the activity of the BK receptor in $LLCPK_1$ cells.

CONTROL OF PHOSPHOLIPASE C (PLC) ACTIVITY IN CULTURED RAT INNER MEDULLARY COLLECTING TUBULE (RIMCT) CELLS. T. Berl, A. Strasheim,* and I. Teitelbaum. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

In RIMCT cells, EGF (E, 0.1 µM) increases inositol trisphosphate (IP_3) release from 720±91 to 2228±84 cpm (n=6, p<.001). Incubation with pertussis toxin (PT, 100 ng/ml, 16 hr) prevents E-stimulated IP_3 release, 737±100 (n=6, NS vs basal). As was the case for PLA_2 cAMP, whether added exogenously or stimulated endogenously, inhibits PLC (n=4). *p<.001 vs E

Control	E	E+ClPheScAMP	E+AVP	E+forskolin
975±102*	2371±63	774±108*	1024±158*	868±57*

Altering extracellular Ca (Ca_E) has no effect as E-stimulated IP_3 release is 2228±84 in the absence of Ca_E, 2124±72 at 1 mM and 2202±89 at 2 mM Ca_E (n=6, NS). Similarly, decreasing intracellular Ca (Ca_I) with 1 mM EGTA has no effect on either basal, 834±69 vs 655±74, or E-stimulated PLC, 2227±132 vs 2206±114 (n=4, NS for both). Increasing Ca_I with 0.1 µM ionomycin (at 1 mM Ca_E) has no effect on basal IP_3, 975±102 vs 948±158 (n=6, NS), but markedly enhances E-stimulated IP_3 release, 2283±74 vs 3723±138 (n=8, p<.001). Activation of protein kinase C (PKC) by the phorbol ester PMA prevents E-stimulation of PLC, 2338±79 vs 894±179 (n=4, p<.001), but no inhibition is seen with 4-α-phorbol which does not activate PKC. Pretreatment with the PKC inhibitor H7 prevents inhibition by PMA. We conclude that E-stimulated PLC activity is 1) transduced by a PT-inhibitable G protein, 2) inhibited by cAMP, 3) insensitive to alterations in Ca_E, 4) insensitive to decreasing Ca_I, 5) enhanced by increasing Ca_I, and 6) inhibited by activation of PKC.

VASOPRESSIN INCREASES THE EXPRESSION OF THE EARLY GROWTH RESPONSE GENE, *Egr-1*, IN CULTURED MESANGIAL CELLS. Joseph V. Bonventre, Sara Sullivan*, Vikas P. Sukhatme, and Andre J. Ouellette*. Medical and Surgical Services, Mass. General Hospital, Harvard Medical School. Howard Hughes Medical Inst., Univ. of Chicago.

Vasopressin is mitogenic for renal mesangial cells. We have previously characterized the vasopressin-induced activation of phospholipase C with resultant increases in cytosolic free [Ca^{2+}] and protein kinase C activation. In order to better understand the mechanisms of coupling of intracellular signals to mitogenesis, we examined the effects of vasopressin, Ca^{2+}, and phorbol esters on expression of a recently cloned early growth response gene, *Egr-1*. *Egr-1* is a "zinc-finger" encoding gene whose structure suggests an ability to regulate the expression of distal genes involved in the mitogenic response. Vasopressin (100nM) rapidly increases the level of mRNA of this gene as well as another growth related gene, *c-fos*. Phorbol myristate acetate (100nM), which stimulates protein kinase C, also increases expression of *Egr-1*. In order to establish the Ca^{2+} dependency of gene expression, cells were rendered permeable with digitonin and exposed to varied concentrations of Ca^{2+} in a high K^+ buffer at pH 7.05 (to mimic the intracellular milieu). Ca^{2+} concentration was measured directly with a Ca^{2+} electrode. Under these conditions there was a Ca^{2+} dependent expression of the *Egr-1* gene.

In conclusion, vasopressin increases accumulation of an early growth response gene, *Egr-1*. This likely represents increased transcriptional rates modulated by both the stimulation of protein kinase C and changes in cytosolic free Ca^{2+} that are induced by vasopressin.

cGMP DECREASES ALDOSTERONE RECEPTOR (AR) BINDING IN INTACT CELLS. M. Cooper,* C.P. Bastl and G. Schulman, Temple Univ., Philadelphia, PA.

The factors that regulate AR binding in intact cells are unknown. Cyclic nucleotides (CN) influence hormone binding to other steroid receptors, an effect which may be mediated by cyclic nucleotide dependent protein kinases (PK). To study the effect of CN on AR binding, distal colon epithelial cells from adrenalectomized rats were incubated with ^{3}H-Aldosterone (^{3}H-A) and dibutyryl cGMP for 15 min at 30°C. RU28362 was added to prevent ^{3}H-A binding to glucocorticoid receptors (GR). Specific ^{3}H-A binding, measured in cytosol, was maximal and stable at 15-30 min.

[cGMP]	0	1nM	10nM	100nM	10uM
AR binding (fmoles/mg pro)	151± 10	124± 15	74± 12*	102± 14**	123± 15

*p<.01; **p<.05 compared to control

cGMP markedly inhibited AR binding. Inhibition was maximal at 10nM cGMP. Higher [cGMP] were less inhibitory perhaps because high [cGMP] also stimulate cAMP dependent PK. This could not be tested because dibutyryl cAMP was toxic to the cells. cGMP or cAMP added directly to cytosol did not affect AR binding implying that membrane bound factors, most likely cGMP dependent PKs, mediate this effect. Ten fold higher [cGMP] were needed to equivalently inhibit GR binding suggesting increased AR sensitivity to this effect.

[cGMP]		0	1nM	10nM	100nM
% inhibition binding	AR	0	9±8	51±8	32±9
	GR	0	-	24±6*	45±9

*p<.025 AR vs GR

Thus physiologic levels of cGMP decrease AR binding suggesting that cyclic nucleotides regulate AR binding in intact aldosterone target cells.

A LYSO-PHOSPHATIDIC ACID-DIRECTED ACYL TRANSFERASE IN HUMAN MESANGIAL CELLS: STIMULATION BY INTERLEUKIN 1. S.L. Bursten, W.E. Harris,* & D.H. Lovett. Seattle V.A. Hospital,Dept.of Medicine,Seattle, WA. San Francisco V.A. Hospital,Dept. of Medicine, San Francisco, CA.

Phosphatidic acid (PA) and interleukin-1 (IL-1) are mesangial cytokines;IL-1 may play an important role in glomerular inflammation. Early cellular signalling mechanisms of these cytokines are not well understood, although some evidence suggests a role for changes in phospholipid metabolism independent of the PI cycle. Acyl transferases (AT) are specific phospholipid-directed enzymes which modulate the fatty acid composition of membrane phospholipids and may participate in the cellular response to certain cytokines. We utilized a fluorescent 18:4 conjugated fatty acid, cis-parinaric acid (cis-PnA), to quantitate the activities of AT in HMC using differential kinetics of fluorescence depolarization (Harris & Stahl;1984).Incorporation of cis-PnA into HMC microsomal phospholipids indicates the existence of several specific AT. HPLC analysis demonstrates that >90% of cis-PnA is incorporated into PA fractions. This event is dependent upon the presence of ATP & Mg, which is typical of AT. Addition of lyso-PA serves as a potent stimulus of activity. AT activity is dose-dependently stimulated by addition of IL-1 in physiologic concentrations (0.1-20 U/ml) within 60-90 sec., with the cis-PnA exclusively incorporated into PA. This PA pool is rapidly converted to diacylglycerol (DAG). No IL-1 mediated cis-PnA incorporation into PI or PC is observed. IL-1 appears to utilize a unique pathway through PA for rapid production of DAG, a critical secondary cellular messenger.

LATHANUM AND PHORBOL ESTERS ACT SYNERGISTICALLY TO ELIMINATE BRADYKININ (BK) INDUCED Ca^{2+} TRANSIENTS IN MDCK CELLS. D.W. Coyne, M. Mordhorst*, and A.R. Morrison. Departments of Internal Medicine and Pharmacology, Washington University School of Medicine, St. Louis, MO.

We have shown that stimulation of MDCK cells with BK leads to a rapid and transient rise in intracellular Ca^{2+}. PMA, through Protein kinase C, inhibits this transient by about 50%. To study the mechanism of the PMA insensitive portion of the Ca^{2+} transients, we used La^{3+}, which is capable of blocking Ca^{2+} channels, and the Na^+/Ca^{2+} exchanger. MDCK cells were loaded with Fura-2 and suspended in 140 mM Na^+, 10 mM HEPES, 5 mM Glucose, 5 mM KCl, 1 mM CaCl, 1 mM $MgCl_2$, pH 7.4 buffer. 10^{-7} BK increased [Ca^{2+}]i by 1162 nm. 10^{-7} PMA reduced this to 465 nM, and the transient was complete in 49.4 sec. vs. 74.4 sec for the control. In 1 mM La^{3+}, the BK induced rise in [Ca^{2+}]i was 510 nM, and was not complete until 91.4 sec. The characteristics of the Ca^{2+} upstroke were quite different, with the PMA inhibited transient beginning immediately after BK addition and peaking within 10 sec. The La^{3+} inhibited transient had a 1-3 sec. delay in onset and peaked at 28 sec. 10^{-7} PMA and 1 mM La^{3+} combined reduced the Ca^{2+} transient to 77 nM, which was delayed in onset and peaked after 25 sec. Nitrendipine 50 μM does not affect BK induced transients. To exclude the Na^+/Ca^{2+} exchanger as the transporter inhibited by La^{3+}, we compared BK induced Ca^{2+} transient in 140 mM NaCl buffer, to 140 mM Choline Cl, and found only a 5% reduction in the peak Ca^{2+} in the choline buffer, in spite of the reduction in intracellular pH that accompanies Na^+ removal. We conclude that the BK induced Ca^{2+} transient in MDCK cells consists of two distinct mechanisms. One is PMA inhibited and probably involves IP_3 release of Ca^{2+} from intracellular stores. The other is most likely a receptor or nitrendipine insensitive voltage gated Ca^{2+} channel and is inhibited by La^{3+}.

EXTRACELLULAR MATRIX (ECM) REGULATES PROLIFERATION AND PHOSPHOLIPID TURNOVER IN GLOMERULAR EPITHELIAL CELLS (GEC). A.V. Cybulsky, J.V. Bonventre, R.J. Quigg, J. Badalamenti*, D.J. Salant. Royal Victoria Hosp., McGill Univ., Montreal, PQ, Boston Univ. Med. Ctr. and Mass. Gen. Hosp., Harvard Med. Sch., Boston, MA.

To understand how growth and differentiation might be regulated in GEC in vivo, we studied the effects of growth factors and ECM on proliferation and membrane phospholipid turnover in cultured rat GEC. Proliferation was measured by 24h incorporation of ^{3}H-thymidine, and in some experiments was confirmed by cell counts. In serum-containing medium, GEC proliferated when plated onto type I (3154±585cpm) or type IV collagen matrices (3478±884cpm), but not on plastic substratum (454±160cpm) or laminin. Proliferation on type I collagen was inhibited by serum deprivation (72h), and was restimulated two-fold above serum-deprived cells by epidermal growth factor (EGF, 1-100ng/ml) or insulin (8µg/ml), as well as five-fold by serum. These actions of serum and EGF were inhibited >50% by phorbol myristate acetate (PMA, 30 and 300nM) and oleoylacetylglycerol (100µg/ml). Turnover of phospholipids was measured in GEC labelled with ^{3}H-arachidonic acid or ^{3}H-inositol. In GEC plated onto plastic, 1,2-diacylglycerol (DAG) constituted 0.43±0.06% of total radiolabelled lipids (at 2h), and inositol trisphosphate (IP_3) was 208±85 cpm/10^5cells. Plating of GEC onto type I collagen in the absence of growth factors increased DAG (3.78±0.46%) and IP_3 (1650±175cpm). DAG also increased in GEC plated onto type IV collagen. Addition of EGF, insulin, serum or serum+PMA to GEC on collagen did not further increase DAG. Serum also did not increase DAG in cells on plastic. Thus, collagen matrix is required for serum-, EGF- and insulin-mediated proliferation of GEC. Growth factor-induced proliferation is not associated with increased levels of DAG. In contrast, collagen activates a phosphatidylinositol-directed phospholipase C, resulting in elevated DAG, and probable activation of protein kinase C. The latter inhibits GEC proliferation. Through this phospholipase C pathway, ECM may promote GEC differentiation.

PROPERTIES OF PLASMA MEMBRANE CALCIUM INFLUX PATHWAYS IN LLC-PK_1 CELLS. J.A. Davis*, M. Abarzua* and J.M. Weinberg. V.A. Med. Ctr. and Univ. of Michigan, Ann Arbor.

To establish a model for studying plasma membrane Ca-flux pathways in intact tubule cells, LLC-PK_1 cells growing as monolayers on coverslips were loaded for 60' with fura-2 in a Krebs-Henseleit type medium containing either 1.3 mM Ca or in nominally Ca-free medium (5-15 µM Ca) then were washed and treated with agonists known to increase cytosolic free Ca (Ca_f) - 10 µM adenosine (ADO), 10 µM ATP or 1 µM arginine vasopressin (AVP) followed by acute addition of 1.3 mM Ca (N=4-8). In Ca-containing medium each agonist gave a Ca_f transient that increased Ca_f from basal levels of < 100 nM to > 300 nM ($p<0.01$). Subsequent addition of 1.3 mM Ca following the Ca_f transient did not affect Ca_f. In Ca-free medium, the initial responses to each agonist were severely blunted or absent, however, addition of 1.3 mM Ca after the agonist uniformly gave a strong transient (Ca_f > 200 nM, $p < 0.01$). Only small and inconsistent changes of Ca_f were seen in Ca-free medium after 1.3 mM Ca in the absence of prior agonist. An ADO receptor antagonist, cyclopentyl dipropylxanthine, blocked the response to exogenous Ca irrespective of whether it was added before or after the adenosine indicating a requirement for ongoing receptor occupancy. Neither verapamil (50 µM) nor La (50 µM) blocked the responses to exogenous Ca. KCl (50 mM) did not affect Ca_f. The agonist-stimulated Ca pool of cells in Ca-free medium could be fully replaced by 0.65 mM or 1.3 mM Ca but not by 0.13 mM Ca within a minute prior to agonist addition. These data provide evidence for receptor-operated Ca channels and establish a versatile model for studying them.

DISTINCT PATHWAYS REGULATE PDGF B/c-sis TRANSCRIPTION IN RENAL MICROVASCULAR ENDOTHELIAL CELLS. TO Daniel and Zhou Fen*, Division of Nephrology, Vanderbilt University, Nashville, TN

Production of PDGF by resident and immigrant cells in the kidney may be implicated in renal pathological responses including proliferation of mesangial cells and fibroblasts, increased production of extracellular matrix components and chemoattraction of leukocytes. The biochemical processes regulating expression of PDGF gene products have not been delineated. We have previously shown that cultured human renal microvascular endothelial cells (HRMEC) express PDGF B/c-sis mRNA, correlating with release of PDGF activity into their media; c-sis mRNA levels are induced by phorbol myristate acetate (PMA), thrombin (T) or transforming growth factor (TGF), while agents that elevate cAMP levels lower c-sis levels (J Biol Chem 261:9579 and 262:11893). In the current experiments, we show that T, TGF and PMA increase transcription of the c-sis gene 3-4 fold, as assayed by a transcription run-off hybridization assay. These agents do not prolong the half-life of this message. Elevation of cAMP with forskolin lowers c-sis mRNA levels through inhibition of transcription that dominates over induction by the other agents; forskolin does not affect mRNA stability. Moreover, the induction of c-sis expression by TGF is mediated through mechanisms that are independent of phorbol-sensitive kinase C: as 1) TGF induction is unaffected by PMA down-regulation of kinase C, 2) maximal doses of PMA and TGF induce additive induction, and 3) TGF induction persists with the kinase C inhibitor, staurosporine, at doses that ablate basal and PMA-induced expression. Thus, basal HRMEC transcription appears supported by basal kinase C activity in HRMEC, while induction may be mediated through either increased kinase C activity or a separate TGF -induced pathway. Forskolin inhibits c-sis transcription, possibly through a cAMP responsive negative regulatory element.

These studies delineate specific pathways through which agents induce and suppress HRMEC PDGF B/c-sis transcription. Similar transduction systems likely affect expression of c-sis gene products by transformed tumor cells with unregulated proliferation coincident with c-sis expression.

ACTIVATION OF GLOMERULAR PROTEIN KINASE C (PKC) IN DIABETIC RATS DESPITE REDUCED INOSITOL CONTENT: POSSIBLE ROLE FOR GLUCOSE. Frederick R. DeRubertis and Patricia A Craven*, Department of Medicine, VA Medical Center, Pittsburgh, PA 15240.

Some of the complications of diabetes may be related to decreased tissue inositol with consequent defects in phosphoinositide metabolism and activation of PKC. In the present study we examined glomerular inositol content (I) in 1 - 2 week streptozotocin diabetic rats and assessed the impact of altered I on the state of activation of PKC. Compared to controls (C), I was reduced 58% in 1 - 2 week diabetic rats. Despite the reduction in I, PKC was activated in glomeruli from diabetic rats as assessed by an increase in the percentage of enzyme activity associated with the particulate cell fraction (C, 12+2; Diabetic 45+4%), while total PKC did not differ. Treatment of diabetic rats with insulin to achieve near euglycemia prevented the fall in both percent soluble PKC and in I. Treatment of diabetic rats with sorbinil also prevented the fall in I but had no effect on PKC or on plasma glucose. Incubation of glomeruli from C with either the phorbol ester, TPA or glucose (100-1000mg/dl) resulted in a progressive increase in the percentage of PKC associated with the particulate fraction, whereas 1000 mg/dl deoxyglucose had no effect. Glucose induced increases in particulate PKC were associated with an increase in production of labeled diacylglycerol, an endogenous activator of PKC. These results demonstrate a dissociation between reduced I and the state of activation of PKC in diabetic glomeruli. They support a role for hyperglycemia per se in the mediation of the activation of PKC seen in glomeruli from diabetic rats.

BRADYKININ (BK) INHIBITS VASOPRESSIN (VP)-STIMULATED cAMP FORMATION BY ACTIVATING PROTEIN KINASE C (PKC) IN CULTURED COLLECTING TUBULAR CELLS. Bradley S. Dixon*, Ruth Breckon*, John Fortune*, Franz Simon*, and Robert J. Anderson. V. A. Med Ctr. and U. of Colo., Denver, Colo.

BK inhibits VP-stimulated water transport in cortical collecting tubular (CCT) cells. The biochemical mechanism of this effect was explored using primary cultures of rabbit CCT cells. Following exposure to BK there is a rapid release of calcium from intracellular stores and an increase in *sn* -1,2-diacylglycerol levels, consistent with activation of phospholipase C. In addition, BK produces a dose dependant 46 % inhibition of VP-stimulated cAMP formation. The dose response curve for the BK-stimulated calcium release and inhibition of VP-stimulated cAMP formation are virtually the same with a half maximal BK concentration of 3 nM. Preincubation with pertussis toxin does not prevent either the BK-mediated increase in intracellular calcium or inhibition of VP-stimulated cAMP suggesting that the effects of BK are not mediated by the inhibitory guanine nucleotide regulatory protein (Gi). Pretreatment with mepacrine, which blocks the BK-mediated increase in PGE_2 production, also does not prevent the inhibition of cAMP formation. Since activation of PKC can inhibit VP-stimulated adenylate cyclase activity, we explored the role of PKC in the BK-mediated inhibition of vasopressin-stimulated cAMP formation. BK activates PKC producing a 5-fold increase in membrane bound PKC. In addition, pretreatment with the PKC inhibitors, H-7 and staurosporine both reverse the BK-mediated inhibition of VP-stimulated cAMP accumulation. Since BK also inhibits isoproterenol-stimulated cAMP formation but does not inhibit either basal-, forskolin- or cholera toxin-stimulated cAMP accumulation, the site of this inhibition appears to involve the hormone receptor or coupling of the receptor to the stimulatory guanine nucleotide regulatory subunit (Gs). The results demonstrate that BK stimulates phospholipase C leading to activation of PKC which then inhibits VP-stimulated cAMP production at the level of the hormone receptor or coupling of the receptor to Gs in cultured CCT cells.

ENDOTHELIN INCREASES CYTOSOLIC CALCIUM ($[Ca^{2+}]_i$) AND pH (pH_i) IN RAT GLOMERULAR MESANGIAL CELLS. Michael J. Dunn, Shiang Wann*, Paolo Mene'* and Yuichi Nakazato*. Depts. of Med. and Physiol., Case Western Reserve Univ. and University Hospitals, Cleveland, Ohio.

Endothelin, a potent vasoconstrictor and mitogenic peptide, may activate signal transduction pathways dependent on phospholipase C. We evaluated endothelin-induced changes of $[Ca^{2+}]_i$ and pH_i in cultured rat glomerular mesangial cells. $[Ca^{2+}]_i$ and pH_i were measured spectrofluorometrically with fura 2 and BCECF. Endothelin, 0.1 pM to 0.1 nM, doubled $[Ca^{2+}]_i$ with a slow rise and sustained elevation, unaffected by Ca channel blockade. Higher concentrations of endothelin, 1 nM to 0.1 uM, rapidly (secs) and dose-dependently increased $[Ca^{2+}]_i$ 5-fold over basal values with sustained increments (10 min). Zero Ca-EGTA solutions, but not Ca channel blockade, eliminated the sustained but not the spike elevations of $[Ca^{2+}]_i$. These results are consistent with 2 types of Ca increments, intracellular mobilization of Ca into cytosol (spike) and enhanced Ca influx (sustained). Since augmented Na-H exchange usually precedes cellular proliferation, we studied the effects of endothelin on pH_i. Endothelin, 0.1 nM to 0.1 uM, dose-dependently raised pH_i 0.1-0.2 pH units with sustained (12 min) increments. Transient acidification preceded this alkalinization. The increases of pH_i, induced by endothelin, were eliminated in Na-free media or by amiloride. We conclude that endothelin stimulates phospholipase C thereby increasing $[Ca^{2+}]_i$ and Na-H exchange with resultant mesangial proliferation and contraction.

INSULIN LIKE GROWTH FACTOR-1 (IGF-1) IS A PROGRESSION FACTOR FOR HUMAN MESANGIAL CELLS IN VITRO. Toshio Doi*,F. Conti*,L.Striker*,S. Elliott*, G. Striker*. Renal Cell Biology Group, NIDDK, NIH, Bethesda, MD 20892, U.S.A.

Mesangial cell hyperplasia is a feature of several human glomerular diseases. The signal(s) for this change are unknown. We have previously shown that mouse mesangial cells not only have an IGF-1 receptor, but they synthesize this hormone constitutively. Further we showed that the glomeruli of mice transgenic for IGF-1 responded to this hormone in vivo. Therefore we extended these studies to human glomerular cells and determined the site of action of IGF-1 on the cell cycle. We found that human mesangial cells in vitro possessed an IGF-1 receptor consisting of alpha and beta subunits (Mr 130k and 90k respectively). Fifty percent inhibition of IGF-1 binding required $1X10^{-9}$ M IGF-1, $1X10^{-6}$M insulin and $1X10^{-7}$M multiplication stimulating activity. Analysis of binding by the method of Scatchard revealed one type of IGF-1 receptor with a kd=$1.35X10^{-9}$M, with $1.04X10^5$ receptors/cell. Binding studies on intact human glomeruli revealed similar specificity (kd=$1.12X10^{-9}$M) and there were $7.17X10^7$ receptors/glomerulus. Cells pretreated with platelet derived growth factor (PDGF) followed by IGF-1 had a rapid, 3-5 fold, increase in the ^{3}H-thymidine response, which was abolished by anti-PDGF antibody. The response of cells pretreated with IGF-1 followed by PDGF was no different than cells exposed to PDGF alone. Finally, PDGF increased protein and RNA synthesis, and this was not enhanced by added IGF-1. Thus human mesangial cells, and whole glomeruli, possess IGF-1 specific receptors and IGF-1 was found to act as a progression factor.

INTRACELLULAR CALCIUM MODULATES Na-DEPENDENT PHOSPHATE (Pi) UPTAKE AND ADAPTATION TO PHOSPHATE DEPRIVATION (P-). Brigitte Escoubet*, Marie-Christine Garestier*, and Claude Amiel* (intr. by Christian Le Grimellec). INSERM U.251 and Université Paris 7, France.

MDCK cells express a Na-dependent Pi uptake which adapts to (P-) through a 2-fold increase in Vmax. We investigated the effect of changes in intracellular calcium on Pi uptake and on the adaptation to (P-) (incubation in P-free medium).

Incubation of the cells in calcium-free medium (Ca-): 1) stimulated Pi uptake after 1h and up to 15h (2-fold), through a decrease in Km (-60%); 2) potentiated the adaptation to (P-) with a 10-fold increase in Pi uptake through a 2-fold increase in Vmax and a 70% decrease in Km. (Ca-) effects were dependent on protein synthesis and gene transcription, as they were inhibited by cycloheximide and 3-deoxyadenosine. TMB8, which decreases intracellular Ca, had an effect similar to (Ca-).

Increasing extracellular calcium to 4 mM, did not change basal Pi uptake but reduced 15h adaptation to (P-) by 25% ($p<.05$). Increasing intracellular calcium with the ionophore A 23187 (10-5 M) decreased basal Pi uptake (-50%) and abolished the effect of (Ca-), (P-), and TMB8 on Pi uptake. The effects of A 23187 were not inhibited by trifluoperazine, suggesting that calmodulin activation was not involved.

We conclude that Ca 1) is a cellular messenger instrumental in the modulation of Pi uptake by kidney cells 2) act as a cellular signal in the adaptation to (P-) of Pi uptake, prior to the induction of gene transcription.

ROLE OF cAMP ON DOPAMINE-1 (DA-1) RECEPTOR REGULATED Na^+-H^+ ANTIPORT IN RENAL TUBULAR BRUSH BORDER MEMBRANE VESICLES (BBMV). Christian C. Felder*, Todd Campbell*, and Pedro A. Jose. Georgetown University Medical Center, Department of Pediatrics, Washington, DC

We have reported that the DA-1 agonist fenoldopam (F) stimulated adenylate cyclase (AC) activity in renal tubular membranes and inhibited Na^+-H^+ antiport activity in BBMV (Kidney Int 33:263,1988). These effects of F could be blocked by DA-1 antagonist SCH 23390 indicating action at the DA-1 receptor. To determine the role of AC in the actions of F on Na^+-H^+ in BBMV, we measured the 10 sec uptake of $^{22}Na^+$ in BBMV under a pH gradient (pHi=5.5, pHo=7.5) and varying Na^+ gradient (2-35 mM) in the presence or absence of dideoxyadenosine (DDA), an inhibitor of AC activity. The drugs were preincubated with renal cortical minceate before BBMV preparation by $MnCl_2$ precipitation. DDA (10^{-4}M) inhibited basal (n=3) and F (10^{-4}M) (n=3) stimulated AC activity in renal tubular membranes. F (10^{-4}M) alone decreased amiloride (1 mM) sensitive V_{max} (nmol/mg protein/min) from 141±14 (n=6) to 79±11 (n=6). DDA (10^{-4}M) alone did not affect V_{max} (140±20, n=6) but did block the inhibitory effect of F (150±20, n=6, p<.05 ANOVA, Scheffe's test). Control K_m was not different from that noted with F, DDA, or the combination of DDA and F (10±1,13±6.8±2, and 12±2 mM respectively). The isoquinolone, H4 ($5x10^{-5}$M) which inhibits cAMP dependent protein kinase A activity also blocked the inhibitory effect of F on amiloride sensitive $^{22}Na^+$uptake (n=4). The isoquinolone H7, ($5x10^{-5}$M) which inhibits both cAMP dependent protein kinase A and protein kinase C also blocked the inhibitory effect of F (n=3). The direct addition of F (n=3) to BBMV also inhibited amiloride sensitive $^{22}Na^+$uptake by a non competitive mechanism similar to the studies where the drugs were preincubated with renal tissue before BBMV preparation. In contrast to the above studies, neither DDA (n=3), H4 (n=3), nor H7 (n=3) reversed the inhibitory effect of F on amiloride sensitive $^{22}Na^+$uptake. **Conclusion:** DA-1 receptors inhibit Na^+-H^+ antiport activity in BBMV by a non-competitive mechanism. This is due in part to the stimulation of AC activity; cAMP dependent protein kinase may be involved.

AVP STIMULATION OF Na-H, Na-DEPENDENT AND INDEPENDENT Cl-HCO_3 EXCHANGE IN RAT MESANGIAL CELLS. M.B. Ganz, G. Boyarsky*, R.B. Sterzel, & W.F. Boron. Yale Univ. Sch. of Med., New Haven, CT.

Application of arginine vasopressin (AVP) to mesangial cells (MCs) in culture (passage 2-5) causes a rise of intracellular pH (pH_i) in the absence of HCO_3^-, due to stimulation of Na-H exchange. However, AVP causes a pH_i decrease in the presence of HCO_3^-. To determine whether this effect is due to modulation of either of the two MC HCO_3^- transporters, we used BCECF to monitor the recovery of pH_i from acid and alkali loads. Serum-starved MC's were grown on glass cover slips and studied at 37° in 5% CO_2/25 mM HCO_3^- at pH 7.4. After an NH_4^+-induced acid load, the rate of pH_i recovery (dpH_i/dt) in the presence of 50μM EIPA (caused by Na-dependent Cl-HCO_3 exchange) was increased 74% (n=14) by 10^{-7}M AVP. When cells were acid loaded in 50μM DIDS, the dpH_i/dt (caused by Na-H exchange) was increased by 48% (14) by AVP. Cl^- removal caused a rapid pH_i increase (caused by Cl-HCO_3 exchange), the rate of which was reduced 94% by DIDS but unaffected by Na^+ removal. AVP increased this dpH_i/dt by 168% (14). After an alkaline load induced by switching from 10% CO_2/50 HCO_3 to 5% CO_2, there was a rapid pH_i fall (caused by Cl-HCO_3 exchange) that was blocked by DIDS or Cl^- removal, but independent of Na^+. AVP stimulated the rate of this pH_i fall by 126% (12). In summary, AVP stimulates both acid extrusion (Na-H and Na-dep. Cl-HCO_3 exchange) and acid loading (Cl-HCO_3 exchange). Thus, activation of multiple transporters by growth factors increases the efficiency of pH_i regulation, possibly anticipating increased cellular metabolism.

ANGIOTENSIN II (AII) STIMULATES MESANGIAL CELL GROWTH THROUGH PHOSPHOINOSITIDE (PI) CASCADE. Y.Fujiwara*, T.Takama*, S.Shin*, S.Ochi*, M.Fukunaga*, Y.Orita* and T.Kamada*. Osaka Univ. Med. Sch., Osaka, Japan. (intr. by J.S.Handler)

To evaluate a possible mitogenic effect of AII as well as the underlying mechanisms, we examined the effect of AII on ^3H-thymidine incorporation into DNA (TdI) and metabolism of inositol phosphates (IPs) in cultured rat mesangial cells (MCs). TdI was measured in quiescent MCs maintained in serum free medium for 5 days. IPs were analyzed by our HPLC method (BBRC, 142:70, 1987) in ^3H-inositol labeled MCs. AII increased TdI in the presence of, but not in the absence of, either insulin (5 μg/ml) or 10 % plasma derived serum. Within 5 sec, AII increased inositol 1,3,4,5-tetrakisphosphate (IP_4), a phosphorylated product of inositol 1,4,5-trisphosphate (IP_3) and a possible activator for calcium influx, as well as IP_3, followed after 10 sec by an increase in inositol 1,3,4-trisphosphate, a dephosphorylated product of IP_4. These effects of AII on TdI and IPs were dose dependent and completely inhibited by a 2 order-excess of saralasin. Pertussis toxin (PT) (1-10 ng/ml) inhibited AII-induced increases in both IPs and TdI, whereas PT alone had no effect on TdI. These results suggest that: 1) AII is a competence-type mitogen for MCs, 2) AII stimulates the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C that is coupled to a PT-sensitive G protein, generating IP_3, and then IP_4, both of which may be active messengers, and, 3) This receptor-coupled initial effect on PI cascade is required for the mitogenic activity of AII.

INFLUENCE OF 2',5'-DIDEOXYADENOSINE ON THE EFFECTS OF PARATHYROID HORMONE IN OK CELLS. J. Garcia*, C. McConkey*, C.R. Betts*, D. Montani* and K.J. Martin. Renal Division, Washington University School of Medicine, St. Louis, MO.

Recent studies in our laboratory have shown that the effects of parathyroid hormone (PTH) are closely related to the c-AMP/protein kinase-A systems in OK cells. It has been suggested that PTH receptors are also coupled to hydrolysis of polyphosphoinositides, activation of protein kinase-C and mobilization of intracellular calcium. Support for the role of the alternate second messenger mechanism has been obtained from studies using the adenylate cyclase inhibitor 2',5'-dideoxyadenosine (2',5'-DDA) which inhibits c-AMP formation by interaction with intracellular adenosine receptors (P receptors). Thus, it has been shown that marked inhibition of c-AMP response to PTH was not associated with a significant alteration in the effects of PTH on phosphate transport. The present studies examine the effects of PTH on c-AMP formation, protein kinase-A activity and phosphate transport in the presence of 2',5'-DDA. In the presence of 0.1 mM 2',5'-DDA, the c-AMP response to PTH (100 nM) was markedly reduced from 464 ± 42 to 74 ± 13 pmoles/culture. The effect of PTH (100 nM) on phosphate uptake, however, was inhibited by only 20%. Examination of the activity of protein kinase-A, revealed that the diminished quantity of c-AMP generated was sufficient to achieve 80% saturation of c-AMP dependent protein kinase. Thus, in spite of a marked reduction in total cellular c-AMP, the effects of PTH on phosphate transport correlated well with the activity of protein kinase-A. These data indicate that small increases in c-AMP may be sufficient to achieve significant saturation of protein kinase-A and emphasize the importance of protein kinase-A activity in mediating the effects of PTH on phosphate transport in OK cells.

NH_4Cl INHIBITS THE RATE OF PROTEIN DEGRADATION AND DECREASES THE ACTIVITY OF CATHEPSIN B AND L IN MONKEY KIDNEY PROXIMAL TUBULE CELLS (JTC CELLS). K. Golchini* and I. Kurtz. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

We have previously reported that NH_4Cl causes JTC cells to hypertrophy without an increase in DNA synthesis. The hypertrophic response was in part due to an increase in the rate of protein synthesis associated with an increase in total RNA. The present study was designed to determine whether NH_4Cl also affects the protein degradation rate (PDR) and the activity of the lysosomal enzymes, cathepsin B and L in JTC cells. PDR was measured using ^3H-leucine release (measured over 3 hrs). Cathepsin B and L were measured fluorometrically. The control PDR was 2.9 ± 0.1 %/hr. NH_4Cl (0-20mM) caused a dose-dependent decrease in PDR. 20mM NH_4Cl (72 hrs exposure) decreased PDR significantly to 2.1 ± 0.1 %/hr, $p<0.001$. The activities of the lysosomal enzymes cathepsin B and L were 0.46 ± 0.002 and 0.57 ± 0.002 pmol/min/cell, respectively. NH_4Cl (0-20mM) decreased the activity of both enzymes in a dose-dependent fashion. 20mM NH_4Cl (72 hrs exposure) decreased the cathepsin B activity to 0.15 ± 0.001 pmol/min/cell, $p < 0.001$, and the cathepsin L activity was decreased to 0.29 ± 0.002 pmol/min/cell, $p < 0.001$. Conclusions: 1) NH_4Cl causes hypertrophy of monkey kidney proximal tubule cells by increasing the protein production rate and by decreasing the protein degradation rate 2) NH_4Cl decreases the activity of the lysosomal enzymes cathepsin B and L 3) the decrease in protein degradation is in part due to an inhibition of lysosomal enzyme activity.

PHORBOL 12-MYRISTATE 13-ACETATE (PMA) INHIBITS ACIDIFICATION BY TURTLE URINARY BLADDER. Mark Graber and Philip Devine.* VAMC Northport, and SUNY at Stony Brook, NY.

PMA has been shown by others to stimulate bicarbonate secretion by the turtle bladder. The effects of PMA on acid secretion are unknown, and we therefore studied electrogenic acid secretion in ouabain-treated bladders. In HCO3-free buffer 0.2 uM mucosal PMA depressed baseline acdification 56% (from 8.7 ± 1.6 to 4.9 ± 0.8 uA, n=9), and 0.5 mM acetazolamide further reduced transport to 1.2 ± 0.4 uA. Sodium azide, which reverses ACZL inhibition, had no effect on the PMA effect. PMA also acidified cell pH in the CA cells by 0.14 ± 0.04 units vs a 0.04 ± 0.04 fall in time controls, and caused a rounding-up of CA cells, with a reduction in the planar apical surface area by 18%, as judged by DI-O-C5 fluorescence. PMA pretreatment abolished the stimulation of acid secretion induced by HCO3/CO2.

We conclude that PMA blocks CO2 stimulation and inhibits acid secretion by an azide-insensitive mechanism distinct from that of ACZL. The inhibition acidifies the CA cell and is associated with a substantial change of CA cell morphology. Kinase C-mediated phosphorylation may be a central event in the transition from the secretion of acid to the secretion of base.

SYNCYTIAL ORGANIZATION OF CULTURED MESANGIAL CELLS (MC) AND ENDOTHELIAL-MESANGIAL INTERACTIONS. M.S. Goligorsky. SUNY at Stony Brook, NY 11794-8152.

To examine the cooperativity of MC contraction, cultured MC (passages 5-12 obtained from Dr.M.Dunn) were studied using microfluorometry of fura-2-loaded cells and microinjection (MI) of lucifer yellow. K^+-depolarization resulted in an immediate Ca^{2+}_i transients and cell contraction. K^+-depolarization of a distant MC (MI of KCl to the microenvironment of MC remote from the cell of observation) produced similar Ca^{2+}_i transients and contractile responses. MI of lucifer yellow to a MC resulted in a prompt transfer of this gap junction permeant dye to the adjacent cells. Pretreatment of MC with 0.5mM H_2O_2 caused uncoupling of gap junctions and inactivation of voltage-sensitive Ca^{2+} channels (VSCC). Co-culture of endothelial cells (EC) and MC was used to examine a possible EC conditioning of MC behavior. Exposure to endotoxin did not affect VSCC in MC, but resulted in their inactivation when MC were co-cultured with EC. The possible role of secreted free radical species in EC conditioning of MC response to depolarization was supported by the results of 3 sets of experiments: 1) EC responded to endotoxin with oxidative burst; 2) in isolated MC, H_2O_2 abolished K^+-depolarization-induced Ca^{2+}_i transients and contraction; 3) pretreatment of EC-MC co-cultures with catalase prevented endotoxin-induced inactivation of VSCC. The results demonstrate that 1) MC form a syncytium capable of propagating the depolarizing signals along the monolayer and 2) the syncytial organization and contractility of MC can be modulated by secretory products of activated EC (e.g. following treatment with endotoxin).

TUMOR NECROSIS FACTOR INHIBITS VASOPRESSIN-RESPONSIVE ADENYLATE CYCLASE IN CULTURED KIDNEY EPITHELIAL CELLS. Gloria E. Gutierrez,* Meyer D. Lifschitz, Peter Smolens, Gregory R. Mundy,* and Michael S. Katz.* UTHSCSA and VA Hospital, San Antonio, Texas.

Tumor necrosis factor (TNF), which mediates endotoxic shock, has been shown to modulate the effect of a number of systemic hormones. The biological function of TNF in kidney is yet to be determined. We have investigated the effects of TNF on vasopressin (VP)-sensitive adenylate cyclase (AC) in the kidney epithelial cell line LLC-PK_1. AC response to VP was assessed in confluent cells after 48 hours pre-incubation with human recombinant TNF. AC activity was measured in cell membranes as the conversion of [α-^{32}P] ATP to [^{32}P] cyclic AMP and also in intact cells as the conversion of [^{3}H] ATP to [^{3}H] cyclic AMP following cellular incorporation of [^{3}H] adenine. Increasing concentrations of TNF (10^{-12}M - 10^{-6}M) progressively inhibited VP (10^{-7}M)-stimulated AC, with maximal inhibition (57% in membranes and 40% in intact cells) at 10^{-7}M TNF. In LLC-PK_1 cell membranes, nonreceptor-mediated activation of AC by fluoride anion (5mM), GTP (10^{-4}M), the GTP analog 5'-guanylyl imidodiphosphate (10^{-4}M), and forskolin (10^{-4}M) was inhibited 70, 61, 61, and 50%, respectively, by TNF (10^{-7}M). The density of LLC-PK_1 cells at confluence was unchanged by treatment with TNF. The results indicate that TNF impairs VP-responsive AC of kidney epithelial cells by actions involving one or more non-receptor components of AC. These studies also suggest that TNF may modulate kidney tubule functions coupled to hormone-sensitive AC.

EPOXYEICOSATRIENOIC ACIDS (EET'S) ARE MITOGENS FOR CULTURED RAT MESANGIAL CELLS (CRMC) RC Harris, T. Homma*, J. Falck*, KF Badr and J. Capdevila*. Vanderbilt Univ. Nashville, TN and UTHSC, Dallas, TX.

Arachidonic acid (AA) is metabolized by a cytochrome P450 NADPH-dependent epoxygenase to four regio isomeric EET's. Recent studies have indicated that vascular endothelial cells produce P450 AA metabolites, and activated platelets synthesize 14,15 EET (PNAS 84:6990). Although EET's influence on vascular tone is known, no studies have examined whether these compounds mediate other aspects of vascular or glomerular injury. Because mesangial cell proliferation occurs with a variety of glomerular insults, we studied whether EET's are mitogenic for CRMC. One day after administration of 14,15 EET, CRMC demonstrated significant increases in ^{3}H-thymidine (^{3}H-thy) incorporation (10^{-7}M: 14,15 EET:120±7% increase; n=5; p<.025; 10^{-6}M:145±10%; n=17; p<0.0005). Persistent stimulation of ^{3}H-thy incorporation in the presence of indomethacin (143±9%; n=4; p<0.05) ruled out a mitogenic effect 2° to inhibition of cyclooxygenase products. In addition to ^{3}H-thy stimulation, 14,15 EET stimulated increases in cell number in CRMC grown in 0.4% FCS: 137±13% (n=6;p<0.025) by 72 hours. All four EET's increased ^{3}H-thy in CRMC by 1.3-1.7x (n=8-10). To investigate the mechanism of action of these compounds, we incubated CRMC for 72 hours with ^{3}H-myo-inositol and measured phosphoinositide hydrolysis, using anion exchange chromatography. No significant stimulation of IP_3 generation was detected. 14,15 EET slowly increased intracellular pH, monitored in BCECF loaded CRMC in HCO_3 free buffer: from 6.97±0.05 to 7.17±0.04. (n=7), This effect did not occur in the absence of extracellular Na^+ and was blunted by 500 µm amiloride.

Thus, these results demonstrate that epoxygenase products stimulate mitogenesis and Na^+-H^+ exchange in CRMC. These effects do not appear to be mediated via phospholipase C activation. These observations extend the potential biologic roles of cP450 AA metabolites to include stimulation of cell proliferation and suggest a role for these compounds in vascular and renal injury.

REGULATION OF SUSTAINED INOSITOL PHOSPHOLIPID TURNOVER DURING T CELL ACTIVATION. Sharon Inokuchi* and John Imboden*, (intr. by F.C. Rector, Jr.) Univ of CA & VAMC, San Francisco, CA.

Stimulation of the T cell line, Jurkat, with monoclonal antibodies to its antigen receptor (AR) leads to sustained generation of inositol trisphosphate (IP_3). To determine how the phospholipid precursors of IP_3 are regulated during this response, we measured the rate of ^{32}P incorporation into the inositol phospholipids of intact cells under non-equilibrium conditions and mass changes by equilibrium labeling with ^{3}H-myoinositol. Following stimulation of the AR, ^{3}H-phosphatidylinositol bisphosphate (PIP_2) fell from a baseline of 684±36 cpm to 245±15 cpm in 60s and remained at this level for >20min. An increased rate of ^{32}P incorporation into PIP_2, however, was sustained for >20min. There was a transient (<5min) 40% decrease in ^{3}H-phosphatidylinositol phosphate (PIP) with a concomitant decrease in ^{32}P-PIP. ^{3}H-phosphatidylinositol (PI) remained constant but, after a 5min lag, there was a substantial, sustained increase in ^{32}P-PI. Stimulation of the AR led to an enhanced rate of increase in ^{32}P/^{3}H ratio for >20min in PIP_2 and PI but had no effect on the ratio in PIP. The ratios in unstimulated and stimulated cells at 20min were, respectively: 0.02 and 0.43 (PI); 1.13 and 1.16 (PIP); 4.1 and 16.4 (PIP_2). We conclude that, in Jurkat, sustained hydrolysis of PIP_2 occurs, paradoxically, without a detectable increase in the turnover of PIP and without depletion of PIP mass. We propose that stimulation of the AR activates an alternative pathway of PIP_2 production that uses closely coupled PI and PIP kinases and a small but highly labile pool of PIP.

SPHINGOSINE REVERSES PROTEIN KINASE C INHIBITION OF CELL GROWTH. Chou-Long Huang*, Jason Shey*, and Harlan E. Ives. Univ. of Cal., SF, CA.

Phorbol myristate acetate (PMA) is a potent growth inhibitor of cultured vascular smooth muscle cells (VSM) when added to cultures 4-6 h after a mitogen like thrombin. In the present study, we further explored the role of PKC in this growth inhibition by using sphingosine as an antogonist of PKC activity. Sphingosine inhibited PKC enzyme activity extracted from VSM cells in a dose-dependent fashion (12%, 33% and 67% inhibition of histone phosphorylation at 40, 100 and 200 µM respectively). In intact VSM cells, sphingosine, when added with PMA at 4 h after thrombin addition, reversed the inhibition of DNA synthesis caused by PMA.

	^{3}H-thymidine incorp., cpm/dish
control	15,800 ± 562
sphingosine (10 µM)	17,340 ± 1,138
thrombin (Thr, 1 U/ml)	109,944 ± 5,957
Thr+PMA (10 ng/ml)	22,429 ± 1,223
Thr+PMA+sphingosine (1 µM)	31,336 ± 1,102
Thr+PMA+sphingosine (3 µM)	60,334 ± 2,312
Thr+PMA+sphingosine (10 µM)	97,333 ± 2,627

This effect of sphingosine appears to result from a specific antagonism of PKC since sphingosine alone did not affect DNA synthesis and the inactive analogs, N-acetylsphingosine and C11-sphingosine (10µM), did not reverse growth inhibition caused by PMA. Unlike sphingosine, the non-specific protein kinase inhibitor H7 failed to reverse growth inhibition caused by PMA. In conclusion, 1) inhibition of cell growth by PMA at a late phase involves activation of PKC; 2) sphingosine is a specific inhibitor of PKC in vascular smooth muscle.

SPHINGOSINE DIFFERENTIALLY BLOCKS ACTIVATION OF THE Na^+/H^+ EXCHANGER BY PHORBOL ESTERS AND GROWTH FACTORS. Harlan E. Ives, Chou-Long Huang*, and John Lowe*. Univ. Cal., SF, CA.

Sphingosine is a naturally occurring lipid antagonist of protein kinase C. To study the effect of sphingosine on activation of Na^+/H^+ exchange in vascular smooth muscle cells, pH_i was measured using BCECF. Pure synthetic D(+)-erythro-sphingosine raised pH_i in a dose dependent manner (from 7.00 ± 0.04 to 7.23 ± 0.09 over 10 min. for 10 µM sphingosine). This alkalinization is not due to Na^+/H^+ exchange (no effect of dimethylamiloride (50 µM) or 0 Na^+ solution). During the subsequent 24h continuous exposure to sphingosine (10µM), cell pH gradually returned to baseline. N-acetyl sphingosine, in which the NH_3 group of sphingosine is blocked, showed no alkalinization. Activation of Na^+/H^+ exchange (Δ pH/min x 10^{-4}) by PMA (20 ng/ml), thrombin (0.4 U/ml), PDGF (5 ng/ml) and osmotic shrinkage (50 mosm sucrose) was measured in cells incubated with sphingosine (10 µM) for 24 h.

	Control	Sphingosine	%Inhibition
PMA	99 ± 9	23 ± 2	77
Thrombin	227 ± 7	151 ± 17	33
PDGF	129 ± 15	100 ± 17	22
Sucrose	134 ± 34	127 ± 15 (NS)	5

Sphingosine almost totally blocked activation of Na^+/H^+ exchange by PMA but did not block activation by sucrose, which works independently of protein kinase C. Interestingly, activation of Na^+/H^+ exchange by thrombin and PDGF was only partially blocked, indicating that these hormones stimulate Na^+/H^+ exchange by both kinase C-dependent and -independent mechanisms.

AMINO ACID REGULATION OF VASOPRESSIN SIGNAL TRANSDUCTION IN LLC-PK CELLS. Margaret Kaehny*, Bradley S. Dixon*, Carolyn Burke*, Ruth Breckon*, Mark A. Dillingham and, Robert J. Anderson. Denver VA and Univ. Colo. Hlth. Sci. Ctr., Denver, Colorado.

Amino acids regulate several cellular processes. Recently, histidine has been postulated to modulate the hydroosmotic effect of vasopressin (VP) in anuran membranes. We therefore examined the effect of histidine on adenylate cyclase activity (ACA) in membranes from VP-responsive LLC-PK_1 cells. Histidine exerts a significant, dose-dependent effect to potentiate VP-stimulated ACA. To delineate the site of this stimulation, the effect of histidine on hormone receptor independent regulation of ACA was measured. Histidine significantly increases basal and potentiates GTP- and forskolin-stimulated ACA. To determine the molecular feature responsible for histidine stimulation, we examined the effect of alanine (the histidine core) and imidazole (the histidine side chain) on ACA. Imidazole but not alanine increases basal, VP- and forskolin-stimulated ACA. To examine if other amino acids regulate VP-stimulated ACA, amino acids with differing side chain characteristics were studied. Phenylalanine but not glutamic acid or arginine potentiate VP-stimulated ACA. These results establish that selected amino acids can modulate the VP signal transduction system. Histidine enhancement of VP-stimulated ACA is due to an imidazole ring interacting directly with the catalytic subunit. Since phenylalanine also enhances VP-stimulated ACA, potentiation of VP may involve amino acids with aromatic as well as imidazole side chain ring structures.

INTRACELLULAR CALCIUM, BUT NOT PROTEIN KINASE C ACTIVATION, IS NECESSARY FOR INTERFERON-GAMMA INDUCTION OF HLA-DR ON U937 CELLS. J Klein*, T Schepers*, G Sonnenfeld*, W Dean*, P Feldhoff*, and K McLeish. Depts. of Medicine, Biochemistry, and Microbiology and Immunology, Univ. of Louisville and VAMC, Louisville, KY.

Interferon-gamma (IFN) regulates HLA-DR antigen expression on immune cells and amplifies the allogeneic response. IFN increases the cystolic calcium concentration ($[Ca^{2+}]i$) and HLA-DR expression of U937 cells. The present study examined the hypothesis that intracellular Ca^{2+} redistribution and activation of the Ca^{2+}-dependent enzymes protein kinase C (PKC) and calmodulin (CaM) are required for IFN regulation of HLA-DR. HLA-DR expression was measured by flow cytometry on U937 cells exposed to IFN (100 U/ml) in the presence or absence of TMB-8 (75 μM), H-7 (20 μM), or W-7 (10 μM). Inhibition of intracellular Ca^{2+} redistribution with TMB-8 prevented IFN-enhanced expression of HLA-DR. Blockade of CaM with W-7 or PKC with H-7 did not alter IFN-enhanced expression of HLA-DR. U937 cells were also exposed to the Ca^{2+} ionophore A23187 or the PKC activator phorbol myristate acetate (PMA) with subsequent HLA-DR measurement. Neither A23187 nor PMA altered HLA-DR expression. The pattern of protein phosphorylation caused by IFN differed from that of PMA, as determined by two dimensional gel electrophoresis. We conclude that IFN induces HLA-DR expression on U937 cells by a signal transduction pathway that requires the redistribution of intracellular Ca^{2+}, but an increase in $[Ca^{2+}]i$ is not sufficient to induce HLA-DR expression. This pathway does not require the activation of either PKC or CaM.

1,25-DIHYDROXYCHOLECALCIFEROL (1,25) INDUCED ACTIVATION OF DIACYLGLYCEROL (DAG) AND INOSITOL TRISPHOSPHATE (IP_3) PRODUCTION IN OSTEOBLAST-LIKE CELLS. Yee S. Kim,* Roberto Civitelli,* and Keith A. Hruska, Renal Division, Jewish Hospital, St. Louis, Missouri.

Since 1,25 increases cytoplasmic calcium (Ca^{2+}_i) during differentiation of HL-60 cells to a monocytic phenotype, we questioned whether target cells of the Ca^{2+} metabolism system express similar responses. Human osteoblasts and ROS 17/2.8, an osteogenic sarcoma cell line with an osteoblastic phenotype, were responsive to 1,25. In 17/2.8 cells, 1,25 produced dose-dependent increases in Ca^{2+}_i. At high doses, the effect of 1,25 was an immediate transient elevation of Ca^{2+}_i due to an increase in both Ca^{2+} entry and release from intracellular stores. At these doses, 1,25 also increased IP_3 and DAG production indicating activation of phospholipase C. At lower physiologic doses, the 1,25 effect on Ca^{2+}_i was slower to develop and dependent upon extracellular Ca^{2+}. Phorbol esters produced elevations of Ca^{2+}_i and inhibited the effect of 1,25 implicating DAG in the effects of 1,25 on Ca^{2+}_i. In addition, the increase in DAG was only partially accounted for by phosphatidylinositol bisphosphate hydrolysis. These results demonstrate that 1,25, at high doses, can immediately activate phospholipase C and increase Ca^{2+}_i through a nongenomic action of the steroid. They also suggest that regulation of DAG metabolism may be related to the hormone's effects on phosphatidylcholine metabolism, and that this mechanism may participate in regulation of cell Ca^{2+} by 1,25.

PLATELET ACTIVATING FACTOR INCREASES CYTOSOLIC FREE CALCIUM CONCENTRATION IN INNER MEDULLARY COLLECTING DUCT CELLS. J.A. Kraut, A. Aboolian* and E.P. Nord. Divisions of Nephrology, Wadsworth V.A. Medical Center, UCLA School of Medicine, Los Angeles, CA, and HSC, SUNY at Stony Brook, NY.

Cells present in the renal medulla have been shown to produce bioactive platelet activating factor (PAF). If PAF functions as an autacoid in this region of the kidney, it might affect the function of tubular segments including the inner medullary collecting duct (IMCD). In other cell types, the action of PAF has been thought to be mediated in part by changes in cytosolic free Ca^{2+} concentration, $[Ca^{2+}_i]$. Therefore, changes in $[Ca^{2+}_i]$ were monitored in a primary culture of IMCD cells loaded with the Ca^{2+}-sensitive fluorescent dye, FURA-2. In a Ca^{2+}-replete medium basal $[Ca^{2+}_i]$ was 137 ± 12 nM (n=5). Exposure of cells to 10^{-5} M PAF resulted in a rapid and sustained increase in $[Ca^{2+}_i]$ to 507 ± 124 nM (n=4). When Ca^{2+} was deleted from the bathing medium, no Ca^{2+} increment was observed on challenge with PAF. In contrast to PAF, arginine vasopressin (AVP), prostaglandin E_2 (PGE_2) and angiotensin II (Ang II) were without effect. Conclusions: 1. IMCD cells respond to PAF with a rise in $[Ca^{2+}_i]$. 2. The rise in $[Ca^{2+}_i]$ derives primarily from an extracellular source. Whether the rise in $[Ca^{2+}_i]$ is linked to cellular functions, e.g. transport events, remains to be determined.

ROLE OF PROTEIN KINASE C IN VASOACTIVE HORMONE INDUCED CHLORIDE CHANNEL ACTIVATION.
S. Kremer*, S. Sridhara*, and K. Skorecki. University of Toronto, and Toronto General Hospital, Toronto CANADA.

We have previously reported that vasopressin(VP) activates chloride channels, leading to depolarization(dep) of glomerular mesangial cells (MC) via both calcium(Ca) dependent and Ca independent pathways. The Ca independent pathways were however not defined. Using the potential sensitive fluorescent dye Bis-oxonol, we determined that the protein kinase C(PKC) activator PMA induced cellular dep in the absence of a Ca signal, as determined using the Ca sensitive fluorescent probe Indo-1. Dep could be detected at 0.05ng/ml PMA with a maximal response occurring at 1-10ng/ml PMA. Extracellular ion substitutions revealed that the dep was mediated by chloride channel activation. Down-regulation of PKC with 0.3µg/ml PMA for 24hrs, abolished the dep response to PMA. The response to VP was attenuated, however still present under these conditions. When the Ca signal to VP was also frustrated by prior depletion of Ca stores in the PKC down-regulated cells, VP was still able to partially dep MC. Addition of exogenous PGE_2 also resulted in a rise in cytosolic Ca and dep. The dep response to PMA and VP however were not secondary to the stimulation of prostaglandin (PG) synthesis, as PMA by itself failed to induce PG production, and 2.5µM indomethacin which completely abolished VP induced PG synthesis, did not eliminate the dep response to VP. We conclude that: 1) Stimulation of PKC or a rise in Ca are each sufficient to activate chloride channels in MC; 2) a dep response to VP can occur independently of both these signals and of PG release, suggesting another mechanism is operative in chloride channel activation.

EFFECT OF AGE ON mRNA LEVELS OF GTP-BINDING PROTEINS IN RAT RENAL CELLS. C.T. Liang, J. Barnes, L. Fabijanski, M. Levine*, and B. Sacktor. GRC, NIA, NIH, Baltimore, MD 21224; *Johns Hopkins University, Baltimore, MD 21205 (intr. by D. Spector)

The response of renal adenylate cyclase to PTH is blunted in old rats. One of the causes of this defect is the decrease of stimulatory (Gs) and inhibitory (Gi) GTP-binding proteins. We have examined the steady state mRNA levels of Gs and Gi. Renal cortical RNA samples were prepared from adult (6 mo) and old (24 mo) rats by guanidinium/CsCl method and 8µg of RNA was applied to the dot blot. Purity of RNA preparations was monitored by OD at 260 nm and 280 nm, and no difference was found. Recoveries of renal RNA and mRNA were not significantly different. Rat cDNA was used to probe αs and αi. After hybridization message levels were quantitated by densitometry. Actin mRNA and Poly(A)$^+$RNA were also probed. Relative abundance of mRNA was expressed as the OD ratio of the specific message vs poly(A)$^+$RNA.

	mRNA(OD)				Relative Abundancy		
	poly(A)$^+$	αs	αi	Actin	αs	α	Actin
6mo	0.28	0.31	0.22	0.65	1.23	0.84	2.56
	±0.03	±0.02	±0.02	±0.04	±0.09	±0.04	±0.13
24mo	0.38	0.28	0.38	0.88	0.83	1.08	2.77
	±0.04	±0.02	±0.03	±0.04	±0.06	±0.06	±0.21
	p<0.05	N.S.	p<0.01	p<0.01	p<0.01	p.<0.01	N.S.

Our findings suggest that (1)total mRNA and mRNA of αi and actin increase in the old rat whereas αs does not change with age, (2)the relative abundance of αs decreases and αi increases in the old rat with no apparent change in actin.

THE UNIQUE NATURE OF COMPENSATORY RENAL GROWTH: EARLY PRIMARY-RESPONSE GENE EXPRESSION IN REGENERATIVE HYPERPLASIA VS COMPENSATORY HYPERTROPHY.
D.A.Kujubu*, J.T.Norman, H.R.Herschman*, and L.G. Fine. Departments of Medicine and Biological Chemistry, UCLA School of Medicine, Los Angeles, CA.

The induction of a family of seven primary response genes (i.e. not dependent upon new protein synthesis) occurs within minutes after stimulation of quiescent 3T3 cells by phorbol esters and growth factors. This family includes c-fos and egr-1 [Sukhatme, etal. Cell 53:37 (1988)]. A similar pattern of gene expression is seen in PC-12 pheochromocytoma cells induced to differentiate by NGF [Kujubu, et al. Oncogene 1:257 (1987)], suggesting that a common set of activating signals occur in different forms of cell growth. To determine whether the same "activation" process occurs in renal hypertrophy, we measured mRNA levels in mice subjected to uninephrectomy (UNX) or sham operation. Regenerative renal hyperplasia was induced by intraperitoneal folic acid (FA) injection with vehicle as control. Northern blots revealed induction of these genes by FA with elevated mRNA levels persisting for up to 24 to 48 hours. UNX and sham operation demonstrated a slight and very transient elevation of mRNA levels with a prompt return to basal levels by 60 minutes.

Conclusion: Cell enlargement in UNX is not characterized by the same pattern of gene expression as occurs in renal regeneration. These two forms of cell growth appear to be fundamentally different.

MECHANISM OF cAMP MEDIATED INHIBITION OF PROSTAGLANDIN (PG) PRODUCTION IN GLOMERULAR MESANGIAL CELLS (M). B. Margolis, N. Hack*, S. Kremer*, P. Clayman*, K.L. Skorecki. Univ. of Toronto, Canada.

cAMP is known to inhibit PG production in M and other cells, but the mechanism is undefined. We have previously shown that vasopressin (V) and calcium ionophore (I) stimulate phospholipase A_2 (PLA_2) in M, and epidermal growth factor (E) is synergistic. Therefore, we studied the effect of forskolin (F) on phospholipase signalling in rat M in culture. M were labelled with ^{14}C-arachidonic acid (AA) and percent of labelled lipids released as free fatty acid (FFA) and diacylglycerol (DAG) measured.

	basal	V(100nM)	V+E(10nM)
FFA:control	0.34±.02	1.43±.07	2.56±24+#
F(100uM)	0.44±.03	0.66±.03	0.85±.03*
DAG:control	0.88±.05	1.45±.05	1.38±.08
F(100uM)	0.98±.06	1.42±.04	1.54±.15

mean±SE; p<.05 *vs.control; +vs.basal; #vs.V

F similarly inhibited I and I+E mediated AA release. Dual-labelling with ^3H-glycerol and ^{14}C-AA confirmed significant inhibition by F of V+E stimulated lysophosphoinositol production but not of phosphatidic acid. Dideoxy-F which does not stimulate cAMP, failed to inhibit AA release.

These results indicate that F, most probably working through cAMP, inhibits activation of phospholipase A_2 without affecting phospholipase C.

ENDOTHELIN ACTION ON VASCULAR SMOOTH MUSCLE INVOLVES INOSITOL TRISPHOSHATE AND CALCIUM MOBILIZATION. P.A. Marsden*, N.R. Danthuluri*, B.M. Brenner, T.A. Brock*, B.J. Ballermann. Brigham and Women's Hospital, Boston, MA.

Cultured endothelial cells release a potent vasoconstrictor peptide, endothelin (EN)(Nature 332;411). We found that cumulative addition of synthetic EN to isolated rabbit aortic rings elicited a concentration-dependent increase in contractile tension which was endothelium-independent (EC_{50} value 1.01±0.09 nM, mean ± SE, n=5). In cultured rabbit vascular smooth muscle cells (VSMC) loaded with fura 2, EN induced a concentration-dependent increase in $[Ca^{2+}]_i$ which was not dependent on extracellular Ca^{2+}(0.1 nM threshold, EC_{50} 1.8 nM). In addition, EN stimulated $^{45}Ca^{2+}$ efflux from preloaded VSMC in the presence and absence of extracellular Ca^{2+}, as well as stimulating $^{45}Ca^{2+}$ influx in a concentration-dependent manner over the range 0.01 to 100 nM. Measurement of inositol polyphosphates in [^{3}H]-myoinositol-labelled VSMC showed that EN induced rapid (15 sec), transient formation of IP_3. Unlabelled EN inhibited [^{125}I]-EN binding to VSMC in a concentration-dependent manner (50% inhibition of total binding at 0.40±0.12 nM, mean ± SE, n=3). Binding was due to inhibited by other vasoactive hormones or calcium channel ligands, suggesting cell surface receptors specific for EN.

We conclude that one of the initial membrane events in the action of EN is to induce phospholipase C-stimulated PIP_2 hydrolysis and that this signalling mechanism is initiated by EN/receptor interaction at the plasma membrane.

CHARACTERIZATION OF THE LTB_4 AND FMLP RECEPTOR-G PROTEIN INTERACTION IN HL60 CELLS. Kenneth R. McLeish, P. Gierschik, K.-H. Jakobs, Department of Medicine, University of Louisville, Louisville, KY and Department of Pharmacology, University of Heidelberg, Heidelberg, FRG.

Although FMLP and LTB_4 evoke similar functional and biochemical responses in neutrophils, there are significant qualitative and quantitative differences in these responses. To determine if these differences are produced by the receptor-G protein interaction, the characteristics of the interaction of LTB_4 and FMLP receptors with their G proteins were compared in membranes isolated from differentiated human HL60 cells. FMLP and LTB_4 stimulated GTP-GDP exchange, as measured by GTP[S] binding, and GTP hydrolysis in a dose-dependent manner. The maximal response in each assay was similar for the two compounds, but the maximal response to FMLP occurred at a 10-fold higher concentration than for LTB_4. Pertussis toxin and cholera toxin inhibited GTP[S] binding and GTPase activity stimulated by either FMLP or LTB_4. Optimal concentrations of FMLP and LTB_4 produced an additive effect in both assays. This additive effect was not abolished by inactivation of up to 80% of G protein activity by N-ethylmaleimide. We conclude that the disparity in responses to FMLP and LTB_4 are not due to differences in G protein activation or to differences in the rate of inactivation. However, FMLP and LTB_4 receptors may be coupled to unique G proteins or to separate, non-communicating pools of the same G protein. Therefore, differences in the receptor-G protein interaction may account for the disparate cellular responses to FMLP and LTB_4.

EVIDENCE FOR VOLTAGE-GATED CALCIUM CHANNEL CURRENT IN RAT MESANGIAL CELLS IN CULTURE. H Matsunaga*, H Chang*, T Okuda*, S Uchida* and K Kurokawa. IVth Dept int Med, Univ Tokyo Sch Med, Tokyo.

Voltage-gated calcium (Ca) channels are of critical importance for cellular signal transduction in a variety of cells. In the present study, Ca channel currents were studied using whole-cell patch-clamp techniques in rat mesangial cells in early passages of culture. Ca channel current was dissected from the net current by dialyzing the cells with CsCl solution and using $BaCl_2$ (110 mM) as charge carrier. The Ca channel identified was a transient type which is similar to "T-type" Ca channels found in excitable cells. This Ca channel current was found to be activated by a relatively large depolarization when cells were held at a large negative holding potential and it was inactivated within 500-1,000 msec. The threshold potential was high (-10-0 mV) and peak inward Ba current of about 110 pA was obtained at membrane potential of about +20 mV. Time course of activation and inactivation was voltage-dependent and became faster when the amplitude of depolarization was increased. The curves for steady-state activation and inactivation overlapped in the voltage range of -10 to +10 mV, suggesting this channel is activated in this voltage range at unstimulated basal state. Interestingly, this Ca channel was detected in mesangial cells in early passages of culture, but not following multiple passages.

THROMBIN-INDUCED CASCADE OF ENDOTHELIAL CELL ACTIVATION AND RETRACTION. D.Menton*, A.Laszlo*, and M.Goligorsky. Washington Univ. of St. Louis, MO 63110 and SUNY at Stony Brook, NY 11794-8152

It has been recently appreciated that endothelial cells (EC) actively participate in macromolecular transport at the level of microvasculature. Thrombin enhances the permeability of EC layers. We studied thrombin-induced changes in Ca^{2+}_i (microfluorometry of fura-2-loaded cells), cell topography (scanning EM), and cytoskeleton in EC. Thrombin caused an initial and sustained phase of an increase in Ca^{2+}_i. Pretreatment with pertussis toxin prevented both initial and sustained phases of the Ca^{2+}_i response. Sustained phase of the thrombin effect required extracellular Ca^{2+}. Pretreatment of EC with indomethacin protracted sustained phase, while a lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA) curtailed it. The sustained phase could be restored by the addition of 15-HETE (which is synthetized by thrombin-stimulated EC; Circ,72: 708,1985), but not by 15-HPETE or LTD_4 to the NDGA-treated EC exposed to thrombin in the presense of extracellular Ca^{2+}. Thrombin caused a marked retraction of EC resulting in a partial denudation of the membrane. This was paralleled by the redistribution of actin and tubulin. Pretreatment of the EC with NDGA blunted the thrombin-induced retraction of EC. We conclude that the initial effect of thrombin is exerted via pertussis toxin-sensitive G protein with the subsequent mobilization of intracellular Ca^{2+}. This initial Ca^{2+}_i transient triggers the lipoxygenase cascade with the formation of a product(s) (probably, 15-HETE) which stimulates Ca^{2+} influx and induces EC retraction.

COMPARISON OF CYCLIC AMP PATHWAYS IN HUMAN AND OPOSSUM PROXIMAL RENAL CELLS.
J.P.Middleton, J.J.Onorato, C.B. Dunham, D.A. Sens, V.W. Dennis. Duke University Medical Center, Durham, NC, and Medical University of S. C., Charleston, SC.

We have shown previously that in contrast to opossum kidney (OK) cells, sodium-dependent phosphate uptake in cultured human proximal cells is not inhibited by PTH stimulation of adenylate cyclase. The present studies compare the integrity of the cyclic AMP pathways in human and OK cells. In both cell lines, the threshold concentration for increased cyclic AMP was 10 nM PTH. Photoaffinity labeling with 8-azido-32P-cAMP identified a 48kDa band (R1) in both cell types and an additional 53kDa band (R2) in human cells. Dose-related displacement of 8-azido-32P-cAMP occurred with PTH at a threshold of 1 nM in both cell lines. Maximal activation of protein kinase (PK) was similar in both cells with the PK activity ratio increasing from 0.34±0.02 to 0.45±0.04* in OK and from 0.35±0.02 to 0.55±0.03* in human cells (*P<0.05 vs control). Sodium/proton exchange and phosphate uptake were inhibited by PTH in OK but not in human cells. We conclude that the lack of effect of PTH on human cells in vitro 1) arises from a factor beyond protein kinase, and 2) indicates the participation of an additional component that is common to both phosphate and sodium-proton transport.

ABNORMAL GROWTH OF RENAL FIBROBLASTS DERIVED FROM KIDNEYS WITH INTERSTITIAL FIBROSIS.
Gerhard A. Müller, Teut Risler, Adalbert Bohle and Peter Rodemann*: (intr. by H.G. Rennke). Medical University Clinics, Dept. of Pathology, Tübingen and Developmental Biology University of Bielefeld, FRG.

Renal interstitial fibrosis is a major cause for uremia. Fibroblasts from normal kidneys (NKF-cells) and from kidneys with interstitial fibrosis (FKIF-cells) were established from kidney biopsies. The amount of fibroblasts in primary cultures derived from kidneys with interstitial fibrosis was increased by a factor of 5 - 10 as compared to normal controls. Clonal culture techniques and growth kinetic experiments revealed that FKIF-cells showed abnormal proliferation capacities and reduced doubling times as signs of hyperproliferative growth. This hyperproliferation can be inhibited by the admission of mitomycin C (MMC). MMC (2×10^{-6} M) induces irreversible, nondividing FKIF-cells. As analysed by 2-dimensional gelelectrophoresis of medium supernatants proliferating FKIF-cells are supposed to secret factors into the culture medium inducing hyperproliferation of normal dermal fibroblasts.

ALTERATION OF GUANINE NUCLEOTIDE BINDING AND HYDROLYSIS BY TWO POINT MUTATIONS IN Gsα.
R.T. Miller*, S.B. Masters*, K.A. Sullivan*, and H.R. Bourne*. (intr. by R.J. Alpern). Dept. Medicine, UTSW Med. Ctr. Dallas, and Dept. Pharmacology, UCSF, San Francisco.

Two point mutations were made in αs at codons 49 and 227. Gly 49 was changed to valine (G49 V), and Gln 227 was changed to leucine (Q227L). These codons correspond to 12 and 61 in p21ras, sites where multiple amino acid substitutions produce constitutively activate proteins. The mutant cDNA's were expressed in S49 cyc- cells where no endogenous αs exists. Basal adenylyl cyclase (AC) activity of G49V was slightly increased over wild type (WT), but stimulation by GTP and isoproterenol (Iso), nonhydrolyzable GTP analogs, and AlF4- was markedly reduced. GTPase activity, as determined in membranes by the addition of propranolol with (32P)ATP following prior activation with GTP and Iso, was reduced. In contrast, Q227L showed a marked increase in basal AC activity in whole cells and membranes with a small stimulation by GTP and Iso. GTPase activity was also reduced in Q227L compared to WT. ICYP ($[^{125}I]$ iodocyanopindolol) binding competition studies in membranes from Q227L did not show normal high affinity Iso binding, indicating that αsQ227L is in a GTP-bound conformation even in the absence of exogenous activators. These results indicate that codons 49 and 227 in αs are involved in guanine nucleotide binding and hydrolysis, and in regulation of the activating conformational change in G protein α chains.

PROTEIN KINASE C ACTIVITY IS REQUIRED FOR PTH DEPENDENT CONTROL OF Na-PHOSPHATE COTRANSPORT.
Heini Murer*and Gary Quamme. Institute of Physiology, University of Zurich, Zurich, Switzerland and Dept. Medicine, University Hospital, University of British Columbia, Canada.

Parathyroid hormone (PTH) inhibits Na-phosphate (Pi) cotransport in opossum kidney (OK) cells through two secondary messenger systems, cAMP and 1,2-diacylglycerol (DAG) acting on protein kinase A and protein kinase C, respectively. Two means were used to determine the requirement of protein kinase C in PTH regulation of Na-Pi cotransport. First, protein kinase C was down regulated with prolonged exposure to high phorbol ester, TPA (200 nM, 18 hr). Down regulated OK cells transport Pi at control levels (8.3 ± 0.1 nmole·mg^{-1} prot·5min^{-1}), respond normally by upregulating transport (12.6 ± 0.1 nmole·mg^{-1} prot·5min^{-1}) when grown on low Pi media and generate cAMP on exposure to PTH or forskolin. However, down regulation of protein kinase C activity resulted in the cells being refractory to a TPA rechallenge (200 nM), PTH (10^{-11} M or 10^{-8} M) and cAMP (8-bromo cAMP, 10^{-4} M). Second, the protein kinase C inhibitor, staurosporine (10 uM), was used to inhibit activity. As with down regulation the staurosporine-treated cells adapted normally to ambient Pi, however, Na-Pi cotransport was not altered by exposure to TPA (200 nM), PTH (10^{-8} M) or cAMP (10^{-4} M). These studies indicate that protein kinase C activity is required in the control of Na-Pi cotransport. They further suggest that protein kinase C and/or its substrates are required for inhibition of Na-Pi uptake through protein kinase A pathway.

TUMOR NECROSIS FACTOR (TNF) STIMULATES MESANGIAL CELL (MC) PROLIFERATION INDEPENDENT OF POLYPHOSPHOINOSITIDE TURNOVER OR PHOSPHATIDIC ACID FORMATION. Y. Nakazato*, M. Kester*, P. Mene'*, J.R. Sedor, Case Western Reserve University and University Hospitals, Cleveland, OH.

We have demonstrated that interleukin-1 (IL-1) stimulates both formation of 1,2-diacylglycerol (DAG) independent of polyphosphoinositide turnover and phosphatidic acid (PA) production in MC and have suggested that these transmembrane signals mediate IL-1-stimulated cellular responses. Since TNF has biological activities similar to IL-1, we have investigated its effects on proliferation, phospholipid-derived messengers and PGE_2 release in rat MC. Human recombinant TNF stimulated MC proliferation as measured by [^{3}H] thymidine incorporation at concentrations of 1-100 ng/ml. Like IL-1, TNF (100 ng/ml) did not stimulate total inositol phosphate (IP_n) formation over 15 min in MC labeled with [^{3}H]myoinositol whereas arginine vasopressin (AVP) (10^{-7}M) increased formation of IPs (35.7±2.4, 39.8±1.7, 192.5±51.8 cpm/ug protein in control, TNF- and AVP-stimulated MC, respectively). TNF also did not change either cytosolic free calcium in fura 2-loaded MC or intracellular pH in BCECF-loaded MC. TNF, however, did stimulate PGE_2 synthesis after incubations of at least 1 hour. In contrast to IL-1, TNF did not induce either DAG formation in [^{3}H]arachidonate-labeled MC or PA production in [^{32}P]orthophosphate-labeled MC. Platelet-activating factor and AVP stimulated DAG and PA formation respectively in the same experiments. We conclude TNF stimulates MC proliferation independent of phospholipase C activation or PA formation and apparently has certain signal transduction pathways distinct from IL-1.

ANION TRANSPORT INHIBITORS, DIDS/SITS, ATTENUATE GROWTH AND CALCIUM TRANSIENT STIMULATED BY FETAL CALF SERUM OF CULTURED RAT MESANGIAL CELLS VIA ENHANCED PROSTAGLANDIN PRODUCTION. T Okuda*, S Kremer*, K Skorecki, and K Kurokawa. IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo, and Toronto Gen Hosp, Univ Toronto Sch Med, Toronto, Ontario.

We reported recently that fetal calf serum (FCS) evokes Ca-transient, increases Cl conductance, and stimulates growth of cultured mesangial cells and suggested that stimulation of Ca-activated Cl conductance may be important in FCS-stimulated mesangial cell growth (Kid Int 33: 168, 1988). In addition, we showed Cl may affect mesangial cell function by modulating prostaglandin (PG) metabolism (Kid Int 33: 163, 1988). To further examine the possible role of anion transport in mesangial cell growth, we examined in the present study the effects of anion transport inhibitors on mesangial cell growth and Ca transient in response to 10% FCS and on PG metabolism. DIDS (10 nM-100 μM) and SITS (10 μM-1 mM) attenuated FCS-stimulated thymidine (THY) uptake in a dose-dependent manner. In addition, pretreatment with DIDS attenuated FCS-evoked Ca-transient. Attenuation by 1 μM DIDS of FCS-stimulated THY uptake was blocked by indomethacin, suggesting that the effects of DIDS may be via enhanced PG production. Indeed, DIDS increased PGE2 and PGF2α production and there was an inverse correlation between FCS-stimulated THY uptake and PG production. PGE2 also attenuated FCS-evoked Ca-transient. These data suggest that anion transport inhibitors attenuate FCS evoked mesangial cell growth by modulating Ca-transient possibly through modulating prostaglandin metabolism.

RENAL TUBULAR EPITHELIAL CELLS RELEASE FACTORS WHICH MODULATE FIBROBLAST FUNCTION: IMPLICATIONS FOR PATHOGENESIS OF INTERSTITIAL NEPHRITIS. J.T. Norman, K.S. Kleinman*, A. Bacay* and L.G. Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

The pathogenesis of most forms of tubulointerstitial nephritis is obscure. A common signal could be a primary perturbation of epithelial cell function which induces changes in interstitial cells. Interaction between the two cell types was examined in an in vitro model in which primary cultures of rabbit papillary collecting duct (PCD) or proximal tubule (PT) epithelial cells on porous filters were suspended above 3T3 fibroblasts. Both PCD and PT stimulated [^{3}H]thymidine incorporation in quiescent 3T3 fibroblasts. PCD were more potent than PT, possibly due to differences in the confluency of the cultures; sub-confluent PT cells had greater potentiating effect than confluent PT. Both epithelial cell types potentiated the effect of individual growth factors (IGF-I, TGFβ, basic FGF (10^{-9}M)) on fibroblast proliferation. The effect of the epithelial cells on fibroblast growth could be mimicked by addition of IGF-I (10^{-9}M) with individual growth factors. By autoradiography, ^{125}I-IGF-I binds to all regions of the rabbit kidney. In addition, PCD released IGF-I (0.25pg/cell/24 hours). Conclusions: (1) Tubular epithelial cells release factors which stimulate fibroblast proliferation; (2) the effect is enhanced by sub-confluent epithelial cells; (3) PCD release IGF-I, a potential mediator of the fibroblast response; (4) release of tubular cell factors may be important in the pathogenesis of interstitial nephritis.

ADENOSINE (ADO) STIMULATES INOSITOL PHOSPHATES (IPs), CALCIUM EFFLUX AND cAMP IN CULTURED RAT MESANGIAL CELLS (CRMC). A. Olivera*, A. Lopez-Rivas*, S. Lamas*, D. Rodriguez-Puyol*, J.M Lopez-Novoa*. (Intr. by p. Vinay). Fundacion Jimenez Diaz-CSIC.

ADO produces in the kidney a persistent decrease of GFR. We have previously shown that ADO 10^{-4}M contracts rat isolated glomeruli (Lopez Novoa et al. Eur J Pharmacol 134: 365-367, 1987) as well as CRMC. Now we have studied the intracellular mechanisms of action of ADO 10^{-4} M in CRMC. First, we studied cAMP levels in CRMC incubated with ADO for 5 min at 37°C. ADO stimulated cAMP levels both in presence of Isobuthyl methylxantine (IBMX) (Control: 75.5 ± 19.7; ADO: 175.6 ± 45.3 pmol/mg, $p<0.01$) and in absence of IBMX (C: 11.1 ± 1.6; ADO: 26.3 ± 5.5 pmol/mg, $p<0.01$). Second, we studied the effect of ADO on formation of IPs. After 30 s of incubation at 37°C, ADO increased the sum of ^{3}H-IPs separated by ion exchange chromatography, (C: 3,684 ± 68; ADO: 4,500 ± 407 dpm/mg, $p<0.05$), and slighly augmented ^{3}H-IP_3 formation (C: 324 ± 72; ADO: 516 ± 114 dpm/mg, $p < 0.1$) . Third, we assesed the effects of ADO on calcium mobilization. ADO stimulated the efflux of $^{45}Ca^{2+}$ from $^{45}Ca^{2+}$-loaded CRMC. It is concluded that in CRMC, ADO triggers phosphatidylinositide turnover, produces calcium mobilization and induces an increment of intracellular cAMP levels. However, the real implication of each of these pathways on ADO-induced CRMC contraction remains to be cleared out.

INHIBITION OF PTH-STIMULATED ADENYLATE CYCLASE IN PROXIMAL TUBULE CELLS: EVIDENCE FOR AMPLIFICATION OF CYCLIC AMP. J. J. Onorato, J. P. Middleton, V. W. Dennis. Duke Univ. Med. Ctr.,Durham, N.C.

PTH inhibition of sodium-dependent phosphate uptake is attributed to cyclic AMP but other messenger systems have been implicated. We analyzed the cyclic AMP pathway and phosphate uptake in opossum kidney cells in the presence and absence of 2,5-dideoxyadenosine, an adenylate cyclase inhibitor. PTH increased cyclic AMP and displaced 8-azido-32P-cyclic AMP from a 48 kDa protein band that co-migrated with Type 1 regulatory subunit of cyclic AMP-dependent protein kinase on SDS-PAGE. Pretreatment with 1 mM DDA decreased PTH-induced cyclic AMP by 95% and decreased the displacement of 8-azido-32P-cyclic AMP in a dose-dependent manner. In spite of these reductions, DDA had no effect on the actions of PTH to increase the cyclic AMP-dependent protein kinase activity ratio (PKA) or inhibit phosphate uptake (nmol/mg protein/3 min):

	Control	PTH	PTH + DDA
PKA:	0.30±0.04	0.38±0.04*	0.48±0.08*
Uptake:	5.4±0.7	4.0±0.3*	3.8±0.6*

(*, $p<0.05$ versus control). These data show that DDA markedly reduces cyclic AMP production but that even 95% inhibition is insufficient to prevent activation of protein kinase and inhibition of phosphate uptake. We conclude that extensive amplification occurs in the PTH-activated cyclic AMP pathway.

CALCIUM MEDIATED HORMONAL ACTIVATION OF POTASSIUM CHANNELS IN MADIN DARBY CANINE KIDNEY CELLS. M. Paulmichl, F. Friedrich, E. Wil and F. Lang (intr. by G. Giebisch). Univ. of Innsbruck, Dept. of Physiology, Austria.

Cultured Madin Darby Canine Kidney (MDCK) cells have been utilized to analyze the cellular effects of epinephrine (EN), acetylcholine (ACH), bradykinin (BK), serotonin (SER) and extracellular adenosine-triphosphate (ATP). To this end, experiments have been performed using conventional microelectrodes for continuous recording of cell membrane potential and identification of specific ion conductances, patch clamp techniques for analysis of single ion channels and quin2 fluorescence for determination of intracellular calcium concentration. Each, EN (α), ACH (muscarinic), BK (B1), SER (5-HT1), and ATP (P2), elicits a rapid, fully reversible hyperpolarization of the cell membrane by activation of inwardly rectifying potassium channels (single channel conductance 66 ± 3 pS). The same channels are activated by increase of intracellular calcium activity to 1 µmol/l. The hormones lead indeed to a profound enhancement of intracellular calcium concentration. The hyperpolarizing effects of EN, ACH and ATP are sustained, the effects of SER and BK transient. In the nominal absence of extracellular calcium the effect of either hormone is only transient. Pretreatment of the cells with pertussis toxin does not effect the effects of ATP and BK, but abolishes the effects of SER and ACH, and renders the effect of EN transient. In conclusion, several hormones activate potassium channels in MDCK cells by enhancement of intracellular calcium activity, utilizing distinct mechanisms of cellular transmission.

PATHWAY OF CELL ALKALINIZATION BY PTH IN PROXIMAL CONVOLUTED TUBULAR (PCT) CELLS IN SITU. E.Pastoriza*, R.Harrington*, and M.Graber. VAMC, Northport, and SUNY at Stony Brook, N.Y.

We have previously reported that PTH causes a decrease in luminal acidification in PCT and cell alkalinization, an effect which suggests an inhibition of basolateral base exit. The cell alkalinization effect is not mimicked by cAMP. We have also shown that PTH increases cell Ca suggesting a stimulation of phosphatidylinositol (PI) metabolism. To further examine the role of this pathway the effect of protein kinase C (PKC) stimulation by the phorbol ester PMA or the diacylglycerol OAG was studied in vivo in TPTX rats. Cell pH was measured in cells loaded with BCECF using the pH-sensitive 490/460 fluorescence ratio. PMA (5×10^{-7}M) and OAG (2.5×10^{-4}M) alkalinized the cell when applied to the basolateral area, Δ ratio=0.21±0.03 and 0.28±0.03. No effect on cell pH was seen when they were applied luminally. The effect of PTH on cell pH was also studied when the PTH-induced rise in cell Ca was prevented by loading cells with the calcium chelator BAPTA. As assessed by FURA 2 fluorescence, BAPTA totally obliterated the effect of PTH to increae cell Ca. Cell alkalinization by PTH was blunted in the presence of BAPTA, Δratio= 0.10±0.01 vs 0.24±0.03 in the absence of BAPTA, $p< 0.01$.

We conclude that stimulation of PI metabolism mediates the cell alkalinization effect of PTH. The cell signal for this action involves both stimulation of basolateral PKC and a rise in cell Ca. These two signal pathways may alkalinize the cell by inhibiting basolateral base exit.

REGULATION OF TISSUE-TYPE PLASMINOGEN ACTIVATOR (tPA) AND ITS TYPE 1 INHIBITOR (PAI1) IN CULTURED HUMAN MESANGIAL CELLS (MC). MN Peraldi, LM Villamediana, R Lacave, F Delarue, E Rondeau, JD Sraer. INSERM U 64, Paris, France (Intr. by Badr).

We previously demonstrated that MC released PAI1 and at a lesser extent tPA. The intracellular mechanisms regulating the synthesis of PAI1 and tPA by these cells have been now studied by ELISA, zymography and reverse fibrin autography. Phorbol myristate acetate (PMA, 16 nM), an activator of protein kinase C (PKC) increased the synthesis of PAI1 (5 fold) and tPA (3 fold) after 24 hours incubation. This effect was time and dose dependent. These syntheses were inhibited by the potent inhibitor of PKC, H7 (25 µM) : 58% for PAI1 and 50% for tPA. Inhibition (41 to 76%) was also observed with cycloheximide (0.36 µM) and actinomycin D (10^{-7} M) suggesting de novo protein synthesis. 8 bromocyclic AMP (8 b cAMP, 10^{-6} M), but not cGMP analogs inhibited the PMA-induced synthesis of PAI1 and tPA in a dose dependent manner (72 and 64% respectively) without affecting the basal secretion of PAI1 and tPA. By Northern blot analysis using cDNA probes, PMA was found to increase PAI1 and tPA mRNA whereas 8 b cAMP blocked this effect. We suggest that hormones or mediators which stimulate PKC activity could increase PAI1 and tPA synthesis in MC whereas those activating adenylate cyclase and protein kinase A have opposite effects.

VASOPRESSIN STIMULATION OF PLC AND PLA_2 IS COUPLED THROUGH TWO DISTINCT GTP-BINDING PROTEINS IN RAT GLOMERULAR MESANGIAL CELLS.

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We have designed experiments to determine if the vasopressin induced arachidonic (AA) release and increases in cytosolic Ca^{2+} are coupled to distinct guanine nucleotide binding proteins in rat glomerular mesangial cells. Mesangial cells were grown in culture as previously described. Experiments were designed to examine the Ca^{2+} signalling response to vasopressin and its modulation by pertussis toxin and phorbol myristate acetate (PMA) in intact cells in suspension. VP caused a dose-dependent increase in Ca^{2+}. PMA inhibited this response in dose-dependent manner. Pertussis toxin did not inhibit the calcium response, suggesting the presence of a pertussis toxin insensitive G-protein coupled to the activation of PLC. The effect of PMA 10^{-7} M was reversed by H7 but not by HA 1004 confirming the effect of PMA was mediated through protein kinase C. PMA 10^{-7} in contrast increased (AA) release from a basal level of 35.87 ± 8.4 ng/well to 69.37 ± 5 ng/well and this effect was inhibited by H7. GTPγS stimulated AA release in saponin permeabilize mesangial cells in the presence of VP 10^{-7}M from 49.8 ± 11.7 to 71.87 ± 10.67 ng/well. Pertussis toxin and GDPβS inhibited this stimulation, suggesting that the stimulation of AA release by VP was mediated through a GTP binding protein which was pertussis toxin sensitive. We conclude that VP stimulates Ca^{2+} mobilization and AA release through two distinct GTP binding proteins in mesangial cells.

CYCLOSPORIN A (CSA) INCREASES PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 (PAI1) SYNTHESIS BY HUMAN MESANGIAL CELLS IN CULTURE (MC).

E Rondeau, MN Peraldi, LM Villamediana, P Ruedin, JD Sraer. INSERM U 64, Paris, France (Intr. by C. Lechêne).

CSA is known to promote thrombotic microangiopathy. To determine a possible effect of CSA on MC, we studied the fibrinolytic components released by these cells known to produce mainly PAI1 and tissue type plasminogen activator (tPA), by ELISA, fibrin reverse autography and Northern blot analysis. PAI1 (ng/10^3 cells) was found to increase in the presence of CSA in a time (6-24 hours) and dose (0.10-10 µM) dependent manner. This effect was present both in the culture medium and in the cell extract. It was apparent at 0.10 µM with maximal effect at 5 µM.

		basal	+CSA(0.10 µM)
Supernatants	12 h	8.4±0.9	23.7±3.2*
Cell lysates	12 h	4.6±0.2	8.5±0.6*

* $p < 0.001$ vs basal value.

By contrast, CSA did not affect the release of tPA (basal : 26.9±3.9, CSA : 30.4±4.8 mU/10^3 cells/6 hours). CSA did not modify cell counts and cell viability. The effect of CSA was unaffected by protein kinase C inhibitor H7 (25 µM) but was reduced by 45% and 27% in the presence of cycloheximide (0.36 µM) and actinomycin D (10^{-7} M) respectively. In conclusion, CSA increases selectively PAI1 through de novo protein synthesis. This may contribute to the inefficient glomerular fibrinolysis occasionnally seen during CSA treatment.

MANNOSE-6-PHOSPHATE POTENTIATES IGF II-STIMULATED INOSITOL TRISPHOSPHATE PRODUCTION IN PROXIMAL TUBULAR BASOLATERAL MEMBRANES.

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We have shown that IGF II stimulates phospholipase C in the basolateral membrane of the canine renal proximal tubular cell (PNAS USA, 85:4037, 1988). The IGF II receptor and the cation-independent mannose-6-phosphate receptor are the same protein. To determine whether mannose-6-phosphate (M6P) affects IGF II-stimulation of phospholipase C activity we measured binding of ^{125}I-IGF II to isolated basolateral membranes and IGF II-stimulated production of inositol-trisphosphate (Ins-P3). ^{125}I-IGF II bound to its 260,000 M_r receptor. The specific binding capacity for IGF II was enhanced 26% by 5 mM M6P. Neither mannose-1-phosphate (M1P), nor mannose (MAN) enhanced binding. In the absence of IGF II, neither MAN, M1P or M6P affected Ins-P3 production. However, production of Ins-P3 measured in the presence of 10^{-9} M rat IGF II (rIGF II) was potentiated approximately 2.5 fold by inclusion of 5 mM M6P in incubations. No similar potentiation was effected by MAN, M1P, glucose-6-phosphate or fructose-1-phosphate. Enhancement of IGF II-stimulated Ins-P3 production by M6P was greater than would be expected by virtue of its action to increase binding of IGF II to its receptor alone. Our findings suggest that M6P potentiates stimulation of phospholipase C by IGF II in the basolateral membrane of the renal proximal tubular cell. Such potentiation could reflect a role for the M6P moiety of membrane or extracellular proteins as a modulator of IGF II "signal" transmission in vivo.

PARALLEL ALTERATIONS IN NA^+/GLUCOSE TRANSPORT AND PROTEIN KINASE C (PKC) LOCALIZATION INDUCED BY TPA OR HYPOXIA IN LLC-PK_1 CELLS: EVIDENCE FOR OXYGEN INDUCED CELLULAR DIFFERENTIATION.

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We reported previously that in contrast to standard still cultures adequately oxygenated rocked cultures of LLC-PK_1 cells exhibit a variety of differentiated functions of proximal tubular epithelium including enhanced Na^+-dependent alpha-methyl glucoside (α-MeG) transport activity.

Incubation of subconfluent rocked cultures with a tumor promoter TPA (10^{-7}M) produced significant inhibition of α-MeG uptake which became apparant within one hr of treatment (control 9.32 ±0.51, TPA (1 hr) 5.78 ±0.48, and TPA (8 hr) 2.99 ±0.06 nmol/mg/60 min, respectively). Inhibitory response of TPA on α-MeG uptake was unaltered by cycloheximide, suggesting a protein synthesis-independent inhibition of the transport activity by TPA in these cells.

PKC activity was found predominantly localized in membrane rich fraction of subconfluent cultures of still LLC-PK_1 cells. Rocked culture, which exhibit enhanced α-Meg uptake, show a shift in the distribution of PKC to the cytosolic pool. Treatment of these cultures with 10^{-7}M TPA for one hr resulted in complete translocation of cytosolic PKC with concomitant decrease in α-MeG uptake to the level of activities usually observed in hypoxic cultures of still cells.

Since PKC localization in cytosol is a feature associated with differentiated cells, these studies suggest that improved oxygenation by rocking induces cellular differentiation in LLC-PK_1 cells. Furthermore, PKC appears to play major role in the regulation of Na^+/glucose transport in LLC-PK_1 cells.

PURINERGIC RECEPTOR REGULATION OF CYTOSOLIC Ca^{2+} IN CULTURED OPOSSIM KIDNEY (OK) CELLS. H. Sakamoto, H. Kumagai, C.R. Filburn, and B. Sacktor. Lab Biol Chem, NIA, NIH, Baltimore, MD 21224 (Intro. by S. Guggino)

Cytosolic Ca^{2+} in renal tubular cells is regulated by IP_3 generated by phospholipase C catalyzed breakdown of phosphatidyl inositol-4, 5-bisphosphate in response to various hormones. To assess whether purinergic regulation of this mechanism also occurs, the effects of purine nucleotides and derivatives on $[Ca^{2+}]i$ and IP_3 generation in OK cells, which have proximal tubule characteristics, were measured. $[Ca^{2+}]i$ was monitored in cells loaded with the fluorescent Ca^{2+} indicator Indo-1 and IP_3 in cells prelabeled with $^3[H]$-inositol. ATP and ADP, but not AMP or adenosine, transiently increased $[Ca^{2+}]i$. ADP was more potent than ATP, with $ED_{50}=3x10^{-7}$ M; ATP was more effective (2.6 fold increase), but failed to saturate at 10^{-3} M, at which it increased $^3[H]$-IP_3 2.7 fold at 30 sec. Cells treated with 10^{-4} ATP gave blunted responses to subsequently added ATP or ADP; cells treated with ADP gave a small response to a second addition of ADP, but a large response to ATP. These effects persisted in the presence of EGTA or after washing the pretreated cells. Simultaneous addition of ATP and ADP elicited a nearly additive response. Thus, OK cells appear to have 2 distinct P-2 purinergic receptors linked to phospholipase C, and involved in regulation of $[Ca^{2+}]i$.

PAF INCREASES CYTOSOLIC FREE CALCIUM CONCENTRATIONS, $[Ca_i^{2+}]$, FROM BOTH EXTRA- AND INTRACELLULAR SOURCES IN $LLCPK_1$ CELLS. J. Schlosser*, A. Aboolian*, and E.P. Nord. Division of Nephrology, HSC, SUNY at Stony Brook, NY.

Platelet activating factor (PAF) is an important mediator of the inflammatory response. Since the renal cortex may be involved in certain inflammatory states, the mechanism of action of PAF was examined in cells of proximal tubule origin ($LLCPK_1$) by monitoring changes in $[Ca_i^{2+}]$ with the Ca^{2+}-fluorescent probe, FURA-2. In a Ca^{2+}-replete medium, acute exposure of cells to PAF (10^{-6}M) resulted in a small transient increment in $[Ca_i^{2+}]$, followed by a second, sustained Ca^{2+} increment. Deletion of Ca^{2+} from the bathing solution decreased the initial Ca^{2+} transient and completely obliterated the second sustained Ca^{2+} increment. In a Ca^{2+}-replete medium brief exposure of cells to verapamil (10^{-5}M, 5 min) or the protein kinase C (PKC)-activating phorbol ester, phorbol-12,13-myristate (PMA, 10^{-6}M, 2 min) blunted the second sustained Ca^{2+} increment by 78% and 85% respectively. Neither compound influenced the initial transient. Lower PAF concentrations evoked only the initial Ca^{2+} transient; rechallenge with PAF within 1 min evoked the second sustained Ca^{2+} increment. Conclusions: 1. In $LLCPK_1$ cells, PAF mobilizes Ca^{2+} from both intra- and extracellular sources. 2. The initial rapid Ca^{2+} transient represents mobilization of Ca^{2+} from internal stores. The second sustained increment is due to Ca^{2+} entry from the external medium. 3. The external Ca^{2+} entry pathway is inhibited by PMA, consistent with a PKC-regulated entry step.

IDENTIFICATION OF GTP-BINDING PROTEINS IN RAT GLOMERULAR MESANGIAL CELLS. D.Schlondorff, J.A.Satriano*, S.DeCandido*. Albert Einstein College of Medicine, Bronx, New York

Previous experiments by us and others showed involvement of GTP-binding protein in hormonal stimulation of phospholipase C in MC. This process was inhibitable by pertussis toxin pretreatment (PT). Recently a number of G alpha -i proteins have been isolated. We now attempted to identify the specific G alpha subunit involved in phospholipase C activation in M.C..

PT catalyzed ADP-ribosylation of two proteins of molecular weights 40 and 41KDa. Immunoblots with antibodies directed against different alpha -i subunits of G proteins (gift of Dr. A. Spiegel) showed both 40 and 41 kDa bands reacting with antibody AS/7 which reacts with G alpha -i proteins in general. The major, 40 KDa protein also reacted with LE/3 antibody directed against G alpha i-2. This was further confirmed by Northern blot which showed a major 2.3 Kb mRNA hybridizing with a cDNA probe for G alpha i-2 and a lesser amount of a 3.5 Kb mRNA hybridizing with a cDNA probe for G alpha i-3. Thus G alpha i-2 corresponds to the 40 KDa and G alpha i-3 to the 41 kDa substrate for PT ribosylation.

The results show that MC contain two types of G alpha -i subunits. The major one, G alpha i-2, has been linked to PT-inhibitable phospholipase C activation in leukocytes, and by analogy may fulfill the same role in MC. The use of immuno- and Northern blots should help to further clarify the role of the different G proteins in MC.

THE NUCLEAR GLUCOCORTICOID RECEPTOR (GR) OF TRANSPORTING EPITHELIAL CELLS IS ISORECEPTOR IB. G. Schulman, C. P. Bastl and G. Litwack, Temple Univ. Health Sci. Ctr. Phila., PA.

Previous studies suggested the activated GR of kidney and colon cytosol (IB) is a smaller less negatively charged molecule than that found in nonepithelial cells (GRII). The GR formed with exposure of intact epithelial cells to glucocorticoids (G) has never been characterized. Proximal colon cells were exposed to ^3H-Triamcinolone, $1x10^{-7}$M and the cytoplasmic, soluble nuclear (tris extractable) and chromatin bound (KCl-extractable) GR isolated and characterized. At 0°C only the unactivated receptor was found in cytoplasm and total specific nuclear binding was 19±47 fmol/mg DNA. At 30°C specific cytoplasmic binding was 289±33 fmol/mg protein. Both unactivated and activated GR were present in cytosol and nuclear binding markedly increased. At 20 minutes incubation the soluble specific nuclear GR content was 386±120 and the specific chromatin bound, 340±66 fmol/mg DNA. Nonspecific binding was 12% of total binding. Only one GR form was found in nuclei. It eluted in the prewash on DEAE cellulose and DEAE Sephadex anion exchange chromatography and had a Stokes radius determined by gel filtration of 34±0.7 A, identical to the activated form in cytosol. GRII, which elutes in the salt gradient on anion exchange and has a Stokes radius of 70A, was never found. Thus, the activated nuclear GR complex in intact cells, where G stimulate Na absorption is an isoreceptor. This suggests receptor form may determine the hormone's physiologic expression in different cell types.

AGONIST INDUCED CYTOSOLIC CALCIUM RESPONSES IN SINGLE VASCULAR SMOOTH MUSCLE CELLS. Ullrich S. Schwertschlag and Vincent W. Dennis,* Duke Univ. Medical Center, Durham, North Carolina.

Baseline and peptide hormone induced calcium (Ca_i) was measured in single cultured vascular smooth muscle cells (sVSMC) using FURA II microspectrofluorimetry. Baseline Ca_i was 107±14 nM, superfusion with 1 to 100 nM ANG II resulted in oscillating responses in Ca_i values not significantly different from control at 1 nM and 10 nM, but differed from control at 100 nM ANG II ($p<0.05$). At 1 uM ANG the Ca_i response was biphasic: Within 1-2 measurement cycles (4-8 sec) Ca_i increased to 245±39 nM ($p<0.05$), returned to 152±18 nM ($p<0.05$) within 60 sec., rose again to 264±27 nM ($p<0.01$) and returned to baseline within 120.8±21 sec. Arginine vasopressin (AVP) also caused oscillating responses at 1-100 nM and a monophasic Ca_i response at 1 uM: 311±66 ($p<0.01$) within 4-8 sec., which returned to baseline within 45±11 sec. These results differ from findings with VSMC populations where a agonist dose response curve could be established and the duration of the Ca_i response was between 5-15 min. We conclude that single cells differ from cell suspensions in their response to agonist stimulation, perhaps because of cell-to-cell communications.

RAPID FORMATION OF INOSITOL PENTAKISPHOSPHATE IN LLC-PK$_1$ CELLS. James A. Shayman, Bruce A. Davidson*, Dorothy Wu*, and Paul R. Knight*, University of Michigan, Ann Arbor, MI.

We have recently reported a precursor-product association between inositol 1, 4, 5 trisphosphate and inositol pentakisphosphate (IP5) in permeabilized renal epithelial cells and have defined its probable pathway. Because ion-pair chromatography permits the rapid resolution of IP5 and IP6 under isocratic conditions at low ionic strength, it has been possible to measure these products accurately without the attendant quenching associated with anion exchange chromatography. We sought to determine whether IP5 formation occurs in intact cells by use of this methodology.

LLC-PK$_1$ cells were labeled to equilibrium with myo [2-3H]inositol in inositol-free media with dialyzed fetal calf serum. Cells were stimulated by exposure to arginine vasopressin and extracted by acid precipitation and desalting over a C18 Sep-pak preequilibrated with tetrabutyl ammonium and eluted with 100% acetonitrile. Eluents were dried, resuspended in mobile phase (CH3CN 25%, KH2PO4 0.05M, TBA 0.01M, pH 3.25), and resolved by reverse phase chromatography. Under basal conditions, IP5 labeling was signifcantly greater than IP2, IP3, or IP4. Following stimulation (2x10-7M AVP) all inositol phosphates rose significantly with IP5 increasing to greater than 8 times its basal level. IP5 formation was enhanced by preincubation with 10mM LiCl. Moreover, IP5 formation was time and concentration dependent (ED50= 10-9M).

In conclusion, IP5 is the major product of hormone-stimulated phosphatidyl inositol bisphosphate hydrolysis in LLC-PK$_1$ cells. Based on our previous observations, inositol 1, 4, 5 trisphosphate is the likely precursor for this product. IP5 is therefore an important candidate for a second messenger in these cells.

ALTERATION OF A VIRAL PROMOTER BY RECOMBINATION IN HUMAN EMBRYONIC KIDNEY (293) CELLS SUGGESTS FLEXIBILITY IN THE INTERACTION OF TRANSCRIPTION FACTORS. R. Segal*, A.J. Berk* (intr. by L.G. Fine). Division of Nephrology, UCLA School of Medicine, and Department of Microbiology, UCLA, CA.

The 293 cell line was used to construct mutant adenoviruses with altered promoter regions in order to determine the requirements for effective interaction between transcription factors and the DNA template. This intracellular recombination technique allows mutant viruses with altered promoter regions to be isolated and purified. To determine whether insertion of multiple GC-boxes (Sp1 transcription factor DNA binding sites) can overcome the normal spacing requirements for interaction of transcription factors within the viral E1b promoter, we infected HeLa cells with mutant viruses containing two GC- boxes inserted 30 basepairs (bp) upstream from the TATA-box (normal separation: 8 bp). Transcription was quantitated by S1 assay of E1b mRNA. Insertion of two GC-boxes was found to overcome the 6 fold decrease in transcription achieved by a 30 bp separation of one GC-box from TATA, provided that the two GC-boxes are on the same side of the DNA helix as TATA. This finding supports a model of transcription factor interaction which involves flexibility of the transcription factors themselves and/or of the DNA template, e.g., looping to bring bound transcription factors into proximity. Conclusion: These insights into the mechanism by which transcription factors regulate transcription may provide a means for modifying the transcription of genes in vivo.

ROLE OF CALCIUM CHANNELS IN HUMAN MESANGIAL CELL (MC) PROLIFERATION. P. Shultz and L. Raij. Dept. of Medicine, VAMC and U. of Minnesota, Minneapolis, MN.

The stimulation of human MC proliferation by both platelet-derived growth factor (PDGF) and thrombin (T) is accompanied by changes in cytosolic calcium (Ca) levels. Both mobilization of intracellular Ca stores and influx of extracellular Ca appear to contribute to these increments in cytosolic Ca.

In order to determine whether activation of calcium channels is critical for the mitogenic effect of PDGF and T, we tested the effect of the calcium channel blockers, diltiazem (Dilt, 10^{-6}M) and nifedipine (Nif, 10^{-5}M) on PDGF and T-induced 3H-thymidine incorporation into DNA of MC. Quiescent MC in serum-free (SF) media were incubated with either SF control, PDGF(5 ng/ml) alone, PDGF + calcium channel blocker, T (5 U/ml) alone or T + calcium channel blocker for 28 hrs., with 3H-thymidine added for the last 4 hrs. of the incubation. The cpm/well incorporated into TCA-insoluble cell fractions was : SF = 613±9, PDGF = 1726±72, and PDGF + Dilt = 1295±61*. In separate experiments, SF = 882±119, T = 3749±234, and T + Dilt = 2385±90* (mean ± S.E., * $p<0.05$ vs mitogen alone). Dilt alone had no significant effect on 3H-thymidine incorporation. In cell counting experiments, PDGF alone caused a 140% increase in cell no. after 24 hrs., which was completely prevented by addition of Dilt (10^{-6}M). No decrease in cell no. was detected in wells exposed to Dilt alone, as compared to SF media. In separate experiments, Nif also inhibited both PDGF and T-induced increases in 3H-thymidine incorporation. In contrast, the Ca channel agonist, BAY K 8644 (BAY, 10^{-6}M) stimulated a 400% increase in 3H-thymidine incorporation over SF wells ($p<0.05$).

We suggest that influx of extracellular Ca via Ca channels in MC is an important signal in the mitogenic effect of PDGF and T, and perhaps other mitogens as well. Calcium channel blockers, such as Dilt and Nif, may be beneficial in attenuating the MC proliferation which frequently accompanies glomerular injury.

ENDOTHELIN STIMULATES MITOGENESIS IN QUIESCENT RAT MESANGIAL CELLS. Michael S. Simonson*, R. Guy McDermott*, Nnenna Njoku*, John R. Sedor, and Michael J. Dunn. Depts. of Medicine and Physiology and Biophysics, Case Western Reserve University, Cleveland Ohio.

We tested whether endothelin, a recently described peptide vasoconstrictor, would stimulate mitogenesis in cultured rat mesangial cells (MC). When added to quiescent (G_0) MC in DME/F12 with 0.5% fetal bovine serum (FBS), endothelin activated reentry into G_1 with progression to S phase 12-16 h later. [^{3}H]Thymidine uptake in response to endothelin was dose-dependent: threshold, 0.1 nM; half-maximal, 0.9 nM; and maximal stimulation at 10 nM. Endothelin at 10 nM increased [^{3}H]thymidine uptake from control values of 560 ± 101 cpm/well (n=4) to 2856 ± 79 (n=5). The potency of endothelin was comparable to 2.5% FBS, which in parallel experiments increased [^{3}H]thymidine uptake to 3175 ± 75 cpm/well (n=5). Endothelin-induced mitogenesis was also confirmed at 48 h by cell counts (0 time, 48 x10^3 cells/well; 48 h,control=50, 1 uM endothelin=75). In contrast to MC, endothelin failed to stimulate [^{3}H]thymidine uptake in Swiss 3T3 fibroblasts, showing that the endothelin response is specific for cell type. Endothelin did not stimulate MC mitogenesis in DME/F12 alone but acted synergistically with either 0.5% FBS or 0.1 ug/ml insulin, suggesting that endothelin acts as a comitogen. Endothelin (0.1 uM) also stimulated transcription of the proto-oncogene, *c-fos*, which peaked 30 min after addition and returned to control levels at 3 h. We conclude, therefore, that endothelin is not only vasoconstrictor but also mitogenic and MC proliferate in response to low concentrations of this endothelial-derived peptide.

G-PROTEIN MAY BE INVOLVED IN VOLUME REGULATION OF PROXIMAL CONVOLUTED TUBULE CELLS. M Suzuki*, Y Kawaguchi*, T Miyahara* (Intr by K Kurokawa). 2nd Dept Int Med, Jikei Univ Sch Med, Tokyo.

Animal cells swell in hypotonic media, but their volume is subsequently returns to normal by a net loss of KCl via Ca-dependent channels. It is thought that a rise in cell Ca concentration ([Ca]i) is an initial event in this volume regulation process. To understand cell volume regulation of proximal tubule cells, we measured [Ca]i using fura-2 in cultured single proximal tubule cells from rabbit kidney. We found a rapid rise in [Ca]i when cells were exposed from normotonic (300 mOsM) to hypotonic solution (250 mOsM) without changing Na concentration. The rise in [Ca]i was not observed in Ca-free medium and exposure to high K medium did not elicit a rise in [Ca]i. These data suggest that the rise in [Ca]i was due to Ca influx and not via voltage-dependent Ca influx nor decreased Ca efflux via Na/Ca exchanger. Pretreatment of tubule cells with pertussis toxin (1 µg/ml) for 2 hrs which irreversibly ADP-ribosylates guanine nucleotide regulatory protein (G-protein) abolished a rise in [Ca]i by hypotonic medium. The behavior is similar to recent observations by others of parathyroid-induced rise in [Ca]i in these cells. Furthermore, pretreatment with colchicine and cytokalasin B also blocked the rise in [Ca]i. These data suggest that the mechanism of volume regulation of proximal tubule cells in response to hopotonic milieu involves G-protein-operated influx of extracellular Ca leading to a rise in [Ca]i and that cytoskeletal microfilaments may play a role in this G-protein operated Ca influx.

SUSTAINED ELEVATION OF HEXOSE MONOPHOSPHATE (HMP) SHUNT IN EXPONENTIALLY GROWING RENAL AND NON-RENAL CELL LINES: SIGNAL TRANSDUCTION MECHANISMS AND GROWTH FACTOR EFFECTS. RC Stanton, E Zimmerman*, and LC Cantley*. Renal Division, Brigham and Women's Hospital, Harvard Medical School, and Tufts University; Boston, MA.

We have reported that the HMP shunt is activated within 1 min. following addition of epidermal growth factor (EGF) in renal primary cell cultures. To explore the mechanisms and importance of this activation, we examined two renal cell lines LLC-PK, of epithelial origin, and NRK, of fibroblast origin; and two non-renal cell lines Balb/c 3T3, of fibroblast origin, and A431, of epidermoid carcinoma origin. The first two HMP shunt enzymes, glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase(6PG) were studied by monitoring the conversion of $NADP^+$ to NADPH. In growing cells the activity of G6PD, the regulatory enzyme, was 130-200% of 6PG activity; quiescent cells G6PD activity was 80-110% of 6PG. In quiescent cells, fetal calf serum stimulated G6PD activity 59% ± 10.5 in LLC-PK and 60% ± 11 in NRK in 1 min. Platelet-derived growth factor stimulated G6PD 39% ± 11 in NRK. Ionomycin, a calcium ionophore, stimulated G6PD 61% ± 8.5 in NRK. Phorbol myristate acetate, a protein kinase C stimulator, had no effect. 6PG activity was not statistically altered in any experiment. We conclude; 1) Growing cell lines from different species and embryologic origin have an activated HMP shunt; 2) Growth quiescence corresponds with decreased HMP shunt activity; 3) Growth factors rapidly activate the HMP shunt in cell lines; 4) Calcium, but not protein kinase C appears to be an intracellular signal for NRK cells.

PARATHYROID HORMONE REGULATION OF PROTEIN KINASES A AND C IN OPOSSUM KIDNEY (OK) CELLS. Teiichi Tamura, Bertram Sacktor, and Charles R. Filburn. GRC, NIA, NIH, Baltimore, MD 21224 (Intro. by Gary M. Kiebzak)

PTH is known to stimulate both cyclic AMP production and cytosolic Ca^{2+} in OK cells, with the latter response linked to receptor-mediated stimulation of phospholipase C. Thus both protein kinase A (PK-A) and protein kinase C (PK-C), via diacylglycerol, may be regulated by PTH in these cells. Activation of protein kinase A, measured as an activity ratio (-cyclic AMP/+cyclic AMP) of cell extracts, and translocation of protein kinase C, measured as the ratio (membrane activity/membrane +soluble activity), were measured after exposure to rat 1-34 PTH. PTH activation of PK-A was maximal (0.07 to 0.55) at 15 sec, but quickly fell to a much lower, but elevated level (0.21) at 5 min. Half-maximal stimulation occurred at ~1x10^{-8} M, but in the presence of isobutylmethylxanthine at ~1x10^{-9} M. PK-C translocation was also transiently stimulated by PTH, with a maximum 2-fold increase at 10 sec and a return to a low basal level (0.06) at 1 min. Translocation of PK-C required higher levels of 1-34 PTH than activation of PK-A, with no effect at <10^{-8} M. Phorbol esters produced marked translocation of PK-C (>0.90), but also slightly activated PK-A. 3-34 PTH had no effect on PK-C. These data indicate that, while 1-34 PTH can activate PK-C, the effect is much smaller and requires higher levels of PTH compared to PK-A.

EFFECT OF INSULIN-LIKE GROWTH FACTOR (IGF-1) ON GLOMERULAR MESANGIAL (MS) CELL LIPIDS. Dean A. Troyer, and Oscar F. Gonzalez* Univ. of Texas Health Science Center, Dept. of Pathology, San Antonio, Texas.

Our previous studies have shown that insulin increases the content of diacylglycerol in MS cells. The high doses required (7 X 10^{-6} M) suggested that this might be mediated through the IGF-1 receptor. Arnqvist et al (Am J Phys 254:C411-C416, 1987) have shown that IGF-1 binding to MS cells was 200-fold greater than that for insulin, and that IGF-1 is a mitogen for MS cells. We therefore examined the effects of IGF-1 on MS cell lipids. Confluent cells were labelled for 12 hours with ^{3}H-arachidonic acid and then treated with IGF-1 (9 X 10^{-9} M) for 20 mins. Lipids were then extracted and neutral lipids separated by TLC and spots were scraped and counted with the following results:

	DG*	FA*	TG*
IGF-1 (n=4)	4152±528	1897±173	2145±211
Control	2333±103	1553±184	2062±242

*DG--Diglyceride;FA--Fatty Acid;TG--Triglyeride

Thus, IGF-1 may exert effects upon MS cells via an increase of diacylglycerol.

This work was supported by USPHS Grant DK 34234 and American Heart Association Grant 86-1051.

PLATELET ACTIVATING FACTOR (PAF) INDUCES ACTIVATION OF c-myc mRNA EXPRESSION IN MDCK CELLS. M. Vander Molen* and E.P. Nord., Division of Nephrology, HSC, SUNY at Stony Brook, NY.

Formation and release of platelet activating factor (PAF) in the kidney either from inflammatory cells, or from renal tissue per se, notably cells of glomerular and medullary origin, may play a role in renal inflammatory states. Since in other cell systems PAF has been shown to activate the phosphoinositol (PI) transduction pathway, and PI activation has been associated with regulation of gene expression, the role of PAF in activating proto-oncogene expression in cells of renal distal tubule origin was examined. Accordingly, confluent monolayers of cells derived from renal distal tubule (MDCK) were exposed to PAF for different time periods, and expression of c-myc mRNA determined on Northern blots. Exposure of cells to PAF (10^{-6}M) resulted in an early (15 min) increase in c-myc mRNA expression which plateaued at 60-120 min. In parallel control experiments baseline expression of c-myc mRNA remained unaltered at all time points examined. Conclusion: PAF induces activation of proto-oncogene expression in MDCK cells. Whether proto-oncogene expression is linked to the inflammatory response in this cell type remains to be elucidated.

SPARE ANGIOTENSIN II (AII) RECEPTORS IN CULTURED VASCULAR SMOOTH MUSCLE CELLS (VSMC). ME Ullian, and SL Linas. University of Colorado School of Medicine, Denver, Colorado.

Binding of AII to its membrane receptor in VSMC results in activation of phospholipase C and formation of inositol trisphosphate (IP_3). To examine the relationship between the IP_3 response to AII and cell surface receptor number (Bmax), we studied the phenomenon of homologous desensitization, i.e. the reduced biochemical response to AII upon repeated hormone exposure. Compared to buffer AII caused concentration-dependent increases in IP_3, with a 34% increase after a 30 sec exposure to 100 nM$_8$AII at 21°C. The AII receptor antagonist Sar^1, Leu^8-AII (SL-AII) did not effect an IP_3 response. Preincubation with AII resulted in time- and concentration-dependent reductions in Bmax; e.g. after 30 min exposure to 100 nM AII, Bmax was reduced by 30%. Reductions in Bmax were associated with desensitization. After 30 min exposure to AII the IP_3 response to 100 nM AII for 30 sec was reduced by 30%. Preincubation with SL-AII also resulted in time- and concentration-dependent reductions in Bmax, which were similar in magnitude to those produced by AII. Despite reductions in Bmax after SL-AII, desentization did not occur, as the IP_3 response to AII was not reduced.

Conclusions: Since AII-induced IP_3 responsivity was preserved despite a 30% reduction in Bmax by SL-AII, 1) at least 30% of AII surface receptors are spare receptors; and 2) homologous desensitization is not mediated by reductions in surface receptor number.

THROMBIN (THR) STIMULATES TISSUE-TYPE PLASMINOGEN ACTIVATOR (tPA) AND PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 (PAI1) SYNTHESIS BY HUMAN MESANGIAL CELLS IN CULTURE (MC). LM Villamediana, MN Peraldi, E Rondeau, F Delarue, JD Sraer. INSERM U 64, Paris, France (Intr. by C. Lechène).

In MC, the synthesis of PAI1 and tPA has been shown to increase when PKC is activated by phorbol esters. Because THR is a well known activator of PKC in platelets and endothelium, we tested its effect on PAI1 and tPA synthesis in MC. PAI1 and tPA antigens were measured by ELISA and their activities analyzed by zymography and reverse fibrin autography. Human α-THR (0.05-2.5 U/ml) increased PAI1 and tPA biosynthesis in a time and dose dependent manner. This effect was inhibited by 0.06-1 U/ml hirudin, an α-THR antagonist (61% for tPA, 32% for PAI1), and by H7 (more than 90% inhibition, 25 μM), an inhibitor of PKC.

	tPA (U/ml)	PAI1 (ng/ml)
control	1.78±0.71(4)	456±111(8)
THR	3.40±0.43(4)*	1,211±333(8)**
THR + H7	1.89±0.62(4)	463±111(8)

* $p < 0.05$, ** $p < 0.001$.

The stimulatory effect of THR was also inhibited (40-60%) by 8 bromo cAMP (5 10^{-4} M), an activator of PKA, and by α amanitine (70%, 5.4 10^{-6} M), an inhibitor of mRNA synthesis. By Northern blot analysis we found that activation of PKC resulted in an increased expression of both PAI1 and tPA mRNA. We conclude that in MC, THR locally accumulated during intravascular coagulation, stimulates PAI1 and tPA synthesis through PKC activation and de novo mRNA synthesis.

IMMUNOLOGICAL DETECTION OF TGF-β. David Deh-Ling Woo* (intr. by Ira Kurtz). Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

Transforming growth factor-beta (TGF-β) plays important regulatory roles in the growth and differentiation of many diverse cell types. Kidneys have been used as a source of TGF-β. The occurance of TGF-β in kidneys suggests that TGF-β may play key roles in the normal and pathological growth regulation of kidney cells. Four peptides reflecting potential antigenic domains of human TGF-β were synthesized. Their sequences are: 1) ALDINYCFSSTEKN; 2) ALYNGHNPGASSAP; 3) YVGRKPKVEQLSNM; 4) EQLSNMIVRSCKCS. These peptides were then conjugated to keyhole-lympet hemocyanin at a molar ratio of 50:1 and used as immunogens in rabbits. High titer anti-peptide antibodies (>1:50,000) were obtained for each of the peptides tested. Each of the anti-peptide antibody is specific for its corresponding antigenic peptide and does not cross-react with irrevelant peptides. All of the antibodies also specifically recognized purified native TGF-β in a direct ELISA assay. The limit of TGF-β detection of this assy is 0.1 ng TGF-β per microtiter (0.32 cm^2) test well. Kinetic studies of peptide antibody interactions indicated that peptides 1 and 3 contained more than one epitope. This phenomenon enabled these specific peptides to enhance antigen detection by 10-50 fold. We have also immobilized the immunizing peptides on FRACTOGEL-TSK chromatographic support to affinity purify the specific anti-peptide antibodies from total IgG fractions purified from immune sera using protein-G chromatography. These peptide affinity purified antibodies also recognized TGF-β in western analysis.

GOLGI APPARATUS ORGANIZATION IN MADIN-DARBY CANINE KIDNEY CELLS DURING THE FORMATION OF AN EPITHELIAL MONOLAYER.

Robert Bacallao*, Eric Karsenti, Michel Bornens§, Kai Simons
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Changes in the morphology of the Golgi apparatus (GA) in Madin-Darby canine kidney (MDCK) cells were studied during the formation of a polarized epithelium. The fluorescent ceramide analog N-6[7-nitro-2,3-benzoxadiazol-4-yl] aminocaproyl sphigosine (C6-NBD-ceramide) and an "anti-Golgi" monoclonal antibody (32D4) was used to examine the organization of the GA by confocal beam scanning laser microscopy.

MDCK cells plated at 50,000 cells/cm^2 on Costar tissue culture treated, polycarbonate filters, were labelled 6 hours after plating and each successive day until a polarized epithelial monolayer formed. Transepithelial resistance was measured to assess formation of epithelial polarity.

We found that isolated cells have a contracted, eccentrically located perinuclear GA. The Golgi staining is equatorial to the nucleus with some components extending basally. This same pattern is observed in cells on the border of cell nests formed 2-3 days later. Cells within the cell nest have a GA spreading around the nucleus. The GA forms "finger like" projections with the long axis oriented vertically. When a polarized epithelium is formed, Golgi staining reveals "finger like" projections which are suspended above thenucleus and just below the level of the tight junctions. The changes in Golgi morphology are coordinated with changes in centrosome location and microtubule organization.

The results show that during the establishment of a polarized epithelium there is a coordinated rearrangement in the organization of the GA.

TNFα INCREASES THE LEVEL OF GM-CSF mRNA IN HUMAN FIBROBLASTS VIA Na^+/H^+ ANTIPORT STIMULATION. K. Yamato*, Z.R. El-Hajjaoui*, E.J. Cragoe Jr.*, H.P. Koeffler*, and I. Kurtz. Division of Hematology/Oncology and the Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

We have previously demonstrated that tumor necrosis factor α (TNFα) increases the level of colony stimulating factor (GM-CSF) mRNA in human fibroblasts (WI-38) by increasing the rate of transcription three-fold, and by increasing the stability of the message more than two-fold. The purpose of the present study was to determine the signal pathway(s) mediating the increase in GMCSF mRNA. TNFα (25 ng/ml) increased intracellular pH (pH_i) measured with BCECF from 7.16 ± 0.06 to 7.28 ± 0.05 ($p<0.001$) in WI-38 cells bathed in HEPES (5 mM). The increase in pH_i was completely blocked by the Na^+/H^+ antiport inhibitor 5-(N-methyl-N-isobutyl)amiloride (MIA, 5 μM). In cells bathed in 25 mM HCO_3^-, pH 7.4, TNFα failed to increase pH_i, yet GM-CSF mRNA increased in response to TNFα. MIA (5 μM) prevented the increase in GM-CSF mRNA induced by TNFα in cells bathed in 25 mM HCO_3^-. Monensin (100 μM) increased the level of GM-CSF mRNA without changing pH_i in cells bathed in 25 mM HCO_3^-, pH 7.4. In addition, ouabain (1 μM) increased the level of GM-CSF mRNA in cells bathed in 25 mM HCO_3^-. Conclusions: 1) the activation of the Na^+/H^+ antiporter is required for the TNFα-induced accumulation of GM-CSF mRNA in human fibroblasts 2) the stimulation of the Na^+/H^+ antiporter by TNFα increases pH_i in cells bathed in HEPES (5 mM) but not in 25 mM HCO_3^- 3) changes in intracellular Na^+ rather than pH_i may mediate TNFα-induced accumulation of GM-CSF mRNA.

PROTON TRANSPORT IN ENDOSOMES OBTAINED FROM HUMAN RENAL CORTEX. K.M. Dawson*, R.W. Gurich, and T.D. DuBose, Jr. U. Tex. Med. Br., Galveston, Texas.

We have described proton transport properties of endosomes obtained from rabbit renal cortex (AJP 251:F702, 1986). The purpose of the present study was to define proton transport properties of endosomes obtained from human renal cortex of kidney rejected for transplantation. Horseradish peroxidase (HRP) was injected into the renal artery during perfusion on a Water's pump. Five minutes after HRP injection, the cortex was removed and homogenized immediately. Endosomal vesicles were prepared by differential and sucrose gradient centrifugation. Proton transport was measured by the acridine orange method. HRP and H^+ATPase activity co-migrated in the lightest area of the gradient (modal density=1.14 gm/cc). There was no measurable maltase, succinate dehydrogenase or N-acetyl-glucosaminidase activity at this density indicating there was no contamination by brush border membranes, mitochondria or lysosomes. Like other H^+ATPases, the human endosomal H^+ATPase was found to require halides for optimal proton pumping and was inhibited by N-ethyl maleimide. Addition of sodium, after steady-state pH gradient formation, collapsed the pH gradient. Voltage clamp experiments ($K^+_{in}=K^+_{out}$ plus valinomycin) showed this to be an electroneutral process. The endosomal Na^+/H^+ exchanger was amiloride-insensitive similar to that observed in the rabbit. Conclusions: 1) the human kidney endocytoses HRP into a cellular compartment distinct from other organelles. 2) human kidney endosomal H^+ATPase is functionally similar to other H^+ATPases, 3) endosomes from human kidney also contain an electroneutral Na^+/H^+ exchanger.

THE RATE AND pH PROPERTIES OF THE AGGREPHORE (AG) CYCLING SYSTEM. N.Franki,* G.Ding,* and R.M. Hays. Department of Medicine, Albert Einstein College of Medicine, Bronx, N.Y.

In toad bladder, AG's deliver water channels to the luminal membrane, and, as vasopressin (VP) stimulation continues, cycle back to the cytoplasm. How long a given AG remains fused, and whether the AG's are part of the acid endosome system is not known. We determined turnover time in toad bladders where luminal solutions (1/10 Ringer's) contained horseradish peroxidase (HRP). Bladders were fixed 2,5, and 10 minutes after VP stimulation, and the frequency (per 100 sq. microns) of HRP-labeled AG's, fusion events, small and large endosomes was determined by EM. In separate studies, endosome and AG pH was determined by the DAMP method (Anderson PNAS,1984). We found:

	2 min.	5 min.	10 min.
Fusion events	0.7±0.1	2.7±0.5	5.3±1.4
HRP labeled aggreph.	0	0.4±0.2	1.3±0.8
HRP labeled sm. endos.	3.3±2.2	4.5±0.7	6.4±2.2
HRP labeled lg. endos.	0	2.2±1.1	6.5±1.1

HRP recovery by small endosomes begins promptly, and by 2 min is approximately 50% of its 10 min value. Transfer of HRP to large endosomes proceeds after a 2 min delay. AG fusion begins by 2 min; return to the cytoplasm begins later, and proceeds at a low rate, with approximately 25% returning per 5 min. DAMP studies showed that small endosomes and AG's are not acidic, but large endosomes are. Thus, AG cycling is slow compared to the endosome system, and AG's do not have the acidifying properties of large endosomes. A mechanism separate from the general endocytic system may control AG cycling.

PROTEIN 4.1, A VESICLE-ASSOCIATED CYTOSKELETAL PHOSPHOPROTEIN, IS PRESENT IN TOAD BLADDER GRANULAR CELLS. L.M. Guay-Woodford,* O. Platt,* and H.W. Harris Jr. Div. of Nephrology and Hematology-Oncology, The Children's Hospital Boston, MA.

Protein 4.1 is a family of cytoskeletal phosphoproteins that bundle microtubules and actin filaments and associate with intracellular vesicles. In nerve terminals, changes in protein 4.1 phosphorylation modulate its binding to synaptic vesicles, thereby regulating vesicle movement and neurotransmitter release. We are investigating whether a 4.1-like protein plays a similar role in the ADH-stimulated water permeability response where aggrephore vesicles fuse with the granular cell apical membrane. Western blots of toad bladder granular cell proteins, probed with a specific anti-human erythrocyte 4.1 antibody, demonstrate a single 65kD immunoreactive band. Cell fractionation studies reveal that this protein 4.1 is present in both cytoplasmic and membrane fractions. Immunofluorescent localization of protein 4.1 shows that granular cells are diffusely fluorescent. Analogous to other tissue protein 4.1's, toad bladder protein 4.1 is basic with an isoelectric point of 7.5 when assayed by two dimensional immunoblot analysis. A protein that co-migrates with this amphibian protein 4.1 is phosphorylated in ADH-stimulated toad bladders. We conclude that protein 4.1 is present in granular cells of the toad urinary bladder. Amphibian protein 4.1 is structurally similar to previously described protein 4.1 species and may mediate aggrephore movement in the ADH-stimulated water permeability response.

HEPARAN SULFATE (HS) INHIBITS RAT MESANGIAL CELL (MC) PROLIFERATION IN CULTURE. G.C. Groggel, G. Marinides*, P. Hovingh*, A. Linker*. University of Utah School of Medicine and VA Medical Center, Salt Lake City, UT.

Heparin has been reported to have an antiproliferative effect on smooth muscle cells. The effect of HS, a component of the GBM, on MC proliferation was investigated by ^{3}H thymidine incorporation over 48 hours in 10% Nu-Serum. Subcultures of P3 to P5 rat MC were used near confluence in microtiter plates. The effect of adding either HS of different sizes and degrees of sulfation or chondroitin sulfate (CS) on MC proliferation was compared to MC in Nu-Serum alone. HS caused greater suppression than CS (Table; % suppression of Nu-Serum alone).

Dose(µg/ml)	HS	CS	p value
1000	85±1%	5±7%	<0.001
500	76±3%	6±5%	<0.001
250	73±3%	19±4%	<0.001
100	51±3%	8±5%	<0.001
50	40±3%	15±7%	<0.05
25	38±4%	4±12	<0.05

The less-sulfated HS fraction (1.0M) caused greater suppression than higher-sulfated fraction (1.3M), 90±1% vs 71±2%, p<0.001. Reduction in size of HS with heparitinase increased the degree of suppression, 71±1% vs 84±0.8% p<0.001, while heparitinase alone had no effect. Suppression by HS was completely reversible, 48 h of HS 49±4% vs 0±6%, 24 h of HS and 24 h of Nu-Serum, p<0.001.

These results demonstrate that HS has an antiproliferative effect on MC in culture, which is inversely related to the degree of sulfation. Heparan sulfate may be an endogenous regulator of MC growth.

COLCHICINE-INDUCED REDISTRIBUTION OF AN APICAL MEMBRANE PROTEIN (gp 330) IN KIDNEY PROXIMAL TUBULE. E.J. Gutmann, J. L. Niles, R. T. McCluskey and D. Brown. Depts. of Pathology and Medical Services (Renal Unit), Mass. General Hospital, Boston, M A.

Microtubules may be involved in the generation and maintenance of cell polarity. Therefore, we examined the effect of colchicine on the cellular distribution of an endogenous glycoprotein, gp330, which is normally inserted into the apical plasma membrane of proximal tubule epithelial cells. Six hours after colchicine injection (0.5mg/100g) into rats, kidneys were perfusion-fixed and examined by indirect immunofluorescence and immunoelectron microscopy, using antibodies against gp330. In control rats, gp330 was localized in the brush border, and in apical invaginations and vesicles. In contrast, in rats treated with colchicine, gp330-containing vesicles were dispersed throughout the cell cytoplasm. Many vesicles were packed into basolateral infoldings, close to the basolateral plasma membrane, but quantification of gold particle labeling revealed no significant insertion of gp 330 into the basolateral membrane. When rabbit anti-gp 330 antiserum was injected intravenously into colchicine-treated rats, immune complexes were found in the glomerular basement membrane but no basolateral rabbit IgG or immune complexes were detected in peritubular basement membranes. We conclude that microtubules are involved in the accumulation of gp 330-containing vesicles at the apical pole of the cell, but that additional factors control fusion with the plasma membrane.

3-D ORGANIZATION OF THE MEMBRANE-CYTOSKELETAL INTERFACE IN TOAD BLADDER EPITHELIAL CELLS. J. H. Hartwig* and D. Brown. Hematology-Oncology and Renal Units, Massachussets General Hospital, Boston, MA 02114

Dynamic interactions between the plasma membrane, cytoplasmic vesicles and the cytoskeleton are critical to epithelial cell function. Using the toad bladder as a model transporting epithelium, we have used short treatments with the nonionic detergent triton X-100 to expose filaments and vesicles in the apical cytoplasm of granular and mitochondria-rich cells, while retaining patches of overlying, intact apical plasma membrane. In freeze-dried, rotary shadowed specimens, myosin S1-labeled actin filaments in granular cells formed an anastomosing mesh directly beneath folds of plasma membrane that constitute ridges on the cell surface. Actin filaments also assembled into a cortical network along the cell margins. A dense network of intermediate filaments was present deeper in the cytoplasm. Points of insertion of actin into the underside of the plasma membrane were identified, and in many cases corresponded directly with anchoring regions of filamentous glycocalyx on the external surface of the cell. In addition, actin filament ends made direct contacts with the surface of vesicles in the apical cytoplasm suggesting a role in their movement. Different types of vesicles were identified by their distinct membrane features. In mitochondria-rich cells, the apical membrane appeared more resistant to detergent, and striking linear arrays of elongated particles were exposed. These particles had a characteristic subunit structure not visible in conventional replicas, and they may correspond to the transmembrane domain of proton pumps that we have previously described in these cells. The cytoplasmic surface of vesicles in granular cells also contained particle arrays differing in structure from those present on the plasma membrane of mitochondria-rich cells.

ENDOCYTOSIS AND PTH ACTION IN OK CELLS. Stephen A. Kempson and Heini Murer*. Physiology Inst., University of Zurich, Zurich, Switzerland.

Fluid-phase endocytosis in OK cell monolayers was determined from cell uptake of horseradish peroxidase (HRP). HRP uptake was decreased by low temp. and metabolic inhibitors, indicating that uptake was an endocytic process. This was confirmed by electron microscopy of sections stained for HRP activity. Reaction product was confined within endocytic vesicles and there was none on the cell surface. Increasing the osmolality (OS) of the incubation medium from 280 to 500 mOsm decreased HRP uptake by 75%. PTH action on Na^+/phosphate (Pi) cotransport was studied by incubating cells for 4 h with 10^{-8} M PTH at either 280 or 500 mOsm. At 280 mOsm PTH caused 67 $\pm$ 1% inhibition of Na^+/Pi cotransport compared to 39 $\pm$ 2% ($p < 0.05$) inhibition at 500 mOsm. Since high OS alone caused partial (34%) inhibition of Na^+/Pi uptake, the cells were given an additional 90 min recovery at 280 mOsm (PTH absent) following incubation at high OS. The normal OS group was treated similarly. This step allowed complete recovery of Na^+/Pi uptake, and also endocytosis, from the effect of high OS. With this protocol, PTH at 280 mOsm caused 56 $\pm$ 7% inhibition of Na^+/Pi uptake compared to only 25 $\pm$ 4% ($p < 0.05$) inhibition at 500 mOsm. High OS did not change basal intracellular cyclic AMP or the increase (70-fold) due to incubation with PTH. We conclude that inhibition of endocytosis by high OS was accompanied by a diminished effect of PTH on Na^+/Pi cotransport. PTH action may be mediated, in part, by endocytic internalization of Pi transport proteins present in the plasma membrane of the cell.

ORGANIZATION OF THE MICROTUBULES IN MADIN-DARBY CANINE KIDNEY CELLS DURING THE GENESIS OF EPITHELIAL CELL POLARITY

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The role of microtubules in the sorting of proteins to their respective membrane domains remains controversial. As part of our initial studies in this area we have studied the organization of microtubules in Madin-Darby canine kidney (MDCK) cells. We used monoclonal antibodies which recognize beta-tubulin and centrioles, to map the location and organization of these subcellular components. The immunofluorescence staining was studied by confocal laser scanning beam microscopy.

MDCK cells plated at 50,000 cells/cm^2 on Costar tissue culture treated, polycarbonate filters were fixed and stained at various times after plating. The transepithelial resistance (TER) was measured to determine the development of functional polarity. From 6 hours to 2 days after plating the microtubules are found in two distinct morphological patterns. There is an area of increased microtubule staining in a perinuclear region which co-localizes with centriole staining. This pattern is consistent with classical descriptions of a microtubule organizing center (MTOC). The microtubules spread out adjacent to the cell membrane. A second group of cells have no clearly defined MTOC. Again the microtubules are spread along the periphery of the cell.

By 2-3 days after plating the cells are 10-12 µm high. The centrosomes are split and occupy positions on opposite sides of the nucleus. The microtubules form a submembranous support network and a webbed, dome-like cap is formed apically. 4-5 days after plating the cells are 16-20 µm high and the monolayer has a high TER. The microtubules rearrange to form a dense apical web. The centrosomes are found in this region. Just basal to the web the microtubules unite, forming bundles which run helically down the cell next to the cell membrane. At the basal internal surface of the cell, the bundles break up to form a fine network at the basal membrane. These changes correlate with changes in the morphology of the Golgi apparatus. The results suggest that establishment of a polarized epithelium is coordinated with rearrangement of microtubules and centrosomes.

TRANSCELLULAR WATER FLOW DIRECTLY MODULATES WATER CHANNEL EXOCYTOSIS AND ENDOCYTOSIS IN KIDNEY COLLECTING TUBULE. Michio Kuwahara* and A.S. Verkman. CVRI, UCSF, San Francisco, CA.

The regulation of osmotic water permeability (P_f) by vasopressin (VP) in kidney collecting tubule involves a cycle of exocytosis and endocytosis where subcellular vesicles containing water channels are inserted into and removed from the apical membrane. To examine effects of transcellular water flow on vesicle movement, the time course of P_f was measured in the isolated perfused rabbit cortical collecting tubule in response to addition and removal of VP (250 µU/ml) in the presence of a bath-to-lumen (B-L), lumen-to-bath (L-B) and no osmotic gradient. P_f was measured continuously with 1s time resolution using a new quantitative microscopy technique. With VP addition, P_f increased from 12 to 262×10^{-4}cm/s (37°C) in 10min. At 1min, P_f was ~70×10^{-4}cm/s with all three gradient conditions. At later times, P_f increased fastest for L-B and slowest for B-L gradient; at 5min, P_f was 250×10^{-4}cm/s (L-B) and 140×10^{-4}cm/s (B-L). With VP removal, P_f decreased fastest for B-L gradient and slowest for L-B gradient; at 30min, P_f was 65×10^{-4}cm/s (B-L) and 183×10^{-4}cm/s (L-B). Endocytosis was also studied by apical uptake of rhodamine-dextran; in paired studies where the same tubule was used for + and - gradients, B-L and L-B gradients gave 168% and 82% of uptake measured with no gradient. These data provide evidence that transcellular volume flow modulates the vasopressin-dependent cycling of vesicles containing water channels, suggesting a novel driving mechanism to aid or oppose the targeted, hormonally directed movement of subcellular membranes.

CORTICOSTERONE 6β-HYDROXYLASE IN A6 EPITHELIA IS A STEROID INDUCIBLE MICROSOMAL P450. C. Watlington, V. Phillips, E. Schuetz, J. Schuetz, P. Guzelian and W. Grogan. Depts. of Med. Path. and Biochem., Med. Coll. of VA/VCU, Richmond, VA.

Stimulation of transepithelial Na^+ transport by corticosterone (B) in A6 cells is mediated by 6β-OH-B (synthesized from B in the effector cells) which activates nuclear receptors (Type IV) distinct from Type I and II. The enzyme system responsible for conversion of B to 6β-OH-B (6β-OHase) is a potential modulator of B induced active Na^+ transport stimulation. The purpose was to determine whether the glucocorticoid inducible liver cytochrome P450p found in rat (P450p), rabbit (LM3c) and human (HLp) (mammalian liver testosterone 6β-OHase) or a member of the same gene family catalyzes the 6β-hydroxylation of B in A6 cells. Assay for 6β-OHase (conversion of [^{3}H]B to [^{3}H]6β-OH-B) revealed catalytic activity only in the microsomal fraction which was induced specifically by glucocorticoids [B, 6β-OH-B, cortisol and dexamethasone (DEX)] and phenobarbital, and inhibited by highly specific antibodies to rat and rabbit liver P450p. Microsomes from A6 cells, X. laevis, human and rat kidney were immunoreactive with antibodies specific for rat P450p and human Hlp on Western blot analysis. Addition of B and DEX to the A6 culture medium increased the amounts of P450p immunoreactive protein. Northern blot analysis demonstrated hybridization of the cDNA for the human liver P450p to mRNA from A6 cells and human kidney under low stringency conditions, suggesting a related form of P450p. We conclude that a P450 protein immunochemically related to rat and human liver P450p catalyzes B 6β-hydroxylation in A6 cells.

ABNORMAL VISUAL EVOKED POTENTIALS IN HEMODIALYSIS PATIENTS. Altmann P*, Dhanesha U*, Hamon C*, Cunningham J*, Blair J*, Marsh F*, (intr. by Slatopolsky E). The London Hospital, London and The University of Aston, Birmingham, UK.

Dialysis patients with severe aluminum (Al) accumulation may develop encephalopathy. We have described abnormalities of tetrahydrobiopterin metabolism, which appear to be Al induced, in hemodialysis (HD) patients without encephalopathy. Similar abnormalities have been reported in Alzheimer's disease, in which there is a specific alteration in visual evoked potentials (VEP): the pattern (P) stimulated VEP remains normal, but the flash (F) stimulated VEP becomes delayed as the dementia increases, so that the flash-pattern VEP interval (F-P interval) increases. We have now studied the VEPs of 16 HD patients with no overt cerebral impairment and of 22 control subjects.

Patients' and controls' PVEPs were not significantly different, but FVEP was delayed in patients (135.4±2.2 ms) compared with controls (127.7±2.2 ms, p=0.02), and F-P interval was greater in patients (32.75±3.65 ms) than controls (19.41±2.36 ms, p=0.003). There was no relation between F and PVEPs in patients (r=0.15,p=0.6), or between these and patients' age (53.4±2.4 yr) or duration of dialysis (71.8±12 months). In controls F and PVEPs were weakly related (r=0.4,p=0.05) and both were related to age (50.8±3.3 yr; FVEP: r=0.72,p=0.0002; PVEP: r=0.45,p=0.03). Patients' pre-dialysis serum Al concentration was 63.1±9.4 µg/l, hemoglobin 9.4±0.5 g/dl, urea 23±1.3 mmol/l and creatinine 1137±43 µmol/l. None of these were related to the F-P interval. In 10 of the patients the F-P interval was related to the most important determinants of Al accumulation in our patients: cumulative oral Al intake from phosphate binders (r=0.7,p=0.02) and daily urine volume (r=-0.7,p=0.02).

Our results demonstrate a VEP defect in HD patients which appears to be related to accumulation of Al. The defect is similar to that found in Alzheimer's disease, in which Al has also been suggested as a causal factor

INCREASED ABSOLUTE REABSORPTION OF SODIUM AND WATER IN THE DISTAL TUBULE FOLLOWING AN ORAL PROTEIN LOAD(OPL) IN HEALTHY SUBJECTS. P.Anastasio, S.Coppola,G.Coscarella,G.DeSimone,D.R.Giordano,P. Strazzullo,R.Iacone,G.Capodicasa,G.Capasso,N.G. DeSanto,Università Federico II Napoli(Introd.by S.G.Massry)

The explanation of the renal hemodynamic response OPL is presently based on a primary metabolic stimulation of the proximal tubular function. Changes in the distal transport function have not been demonstrated at present time .The scope of this work is to investigate the distal tubule function following OPL by evaluating Lithium Clearance(CLi),Sodium Clearance(CNa),Potassium Clearance(CK),GFR,RPF,and by calculating 1.the absolute reabsorption of Na(CLi- CNa) and water(CLi- V)in the distal nephron,2.the excretion in urine of Na and water expressed as a fraction of the amount delivered from the proximal tubules(CNa/CLi and V/CLi).A group of 8 adult subjects underwent a total of seven 30 minute clearance studies 2 before and 5 after an OPL (30,60,90,120,180 min).The OPL consisted of 2 g /kg of protein as cooked red meat.Following OPL GFR increased fron 116 ± 3.35 to 142.8 ml/min xl.73 sq.m($p<0.0005$) and RPF from 491 ± 39.7 to 599 ± 42.8 ml/min x 1.73 sq.m($p<0.0005$).CLi - CNa and well as Cli - V increased significantly($p< 0.01$),while Cli/CNa and CLi/CK did not change.V/CLi was significantly reduced from 0.33 to 0.17($p< 0.0005$).The data indicate that in healthy subjects an increase in the reabsorbtion of water and sodium occurs following an OPL,but in final urine the sodium excretion expressed as fraction of the amount delivered from the proximal tubule is not affected while that of water is reduced.

LACK OF RENAL RESPONSE TO PROTEIN INTAKE (PI) IN LUPUS NEPHRITIS (LN) IS ASSOCIATED WITH "CHRONICITY" LESIONS IN RENAL BIOPSY. T.Bochicchio*, L.Tipan*, C.Calleja*, H.Pérez-Grovas* and J.Herrera-Acosta. Department of Nephrology, Instituto Nacional de Cardiología "Ignacio Chávez", Mexico City.

Glomerular and tubulointerstitial sclerosis and tubular atrophy (chronicity index) in LN are considered indicative of progressive loss of renal function. Decreasing renal mass induces glomerular hemodynamic adaptations (hyperfiltration:HF) that may contribute to renal damage. To test if progressive LN is associated with HF, the response to PI was evaluated in LN patients and correlated with renal biopsy (RB) findings.

Fifteen normotensive pts with LN were studied: serum creatinine (SCr), 24 hr protein excretion (U Prot) and the response of GFR (I-131-Iothalamate) and RPF (125-I-Hippuran) to 1.5 g/Kg protein intake were measured. RB was obtained in all pts and was classified according to WHO and the activity (AIx) and chronicity (CIx) indexes proposed by Austin et al. were determined. All pts were receiving 5 -20 mgs/D of prednisone. Pts were divided in two groups according to the response of GFR to PI. Results are expressed as mean ± SEM.

G	n	Age yrs	SCr mg/dl	U Prot g/24h	GFR—ml/min—B	GFR—ml/min—PI	RPF—ml/min—B	RPF—ml/min—PI	Renal Biopsy WHO	Renal Biopsy AIx	Renal Biopsy CIx
G-I	7	32 ±4	0.86 ±.09	2.5 ±2.2	67.0 ±8.2	98.0* ±12.0	239 ±29	335* ±40	IV-V	3.3 ±0.9	1.4 ±0.3
G-II	8	25 ±4	1.00 ±.04	2.6 ±.9	87.8 ±8.9	88.3 ±9.6	356¤ ±33	340 ±27	II-IV	5.9 ±1.5	3.6¤ ±0.5

* = $p < 0.05$ vs B, ¤ = $p < 0.05$ vs G-I

Supression of renal response to PI in patients with higher chronicity index in RB suggests that progression of LN is associated with HF and may thus contribute to renal damage. The response to PI may be a useful in evaluating LN.

SPECIFICITY & SENSITIVITY OF DIAGNOSTIC ASSAYS IN ACUTE PANCREATITIS (AP) IN END STAGE RENAL DISEASE (ESRD) PATIENTS (pts). G. Braden, J. Rosenbaum,* A. McGuire*, Baystate Med. Ctr., Springfield, MA, Tufts School of Med., Boston, MA.

Since serum amylase (SA) & lipase (SL) activity may be elevated in 50% of ESRD pts without AP, we reassessed whether these tests could be useful in diagnosing AP utilizing a new colipase method for SL which increases the sensitivity of SL in diagnosing AP in non-ESRD pts. We measured SA & SL in normal controls, 40 stable CAPD and hemodialysis pts, 12 CAPD pts with peritonitis (PER), 35 ESRD pts admitted with abdominal disorders (ABD) other than AP, and 8 ESRD pts with AP confirmed by laparotomy, autopsy, or CT scan. Results are expressed as the mean maximum SA & SL:

	HEMO Stable	CAPD Stable	CAPD PER	ESRD ABD	ESRD AP
SA	140	109	102	106	646 *
SL	277	278	348	267	2557 *

P < 0.01 AP compared to all groups for that assay. Peritoneal fluid A & L were markedly elevated in all 5 CAPD pts with AP compared to stable CAPD or PER pts ($p < .01$). We calculated the specificity & sensitivity of SA & SL in diagnosing AP using various cutoff levels above normal (NL) for SA & SL:

	SPECIFICITY	SENSITIVITY
SA > 2 x NL	92%	100%
SA > 4 x NL	100%	50%
SL > 3 x NL	93%	100%
SL > 5 x NL	100%	88%

Although SL is more sensitive than SA in diagnosing AP in ESRD pts, we found that either SA or SL was elevated above the 100% specificity level in all 8 AP pts. We conclude that SA & SL are specific & sensitive assays for AP in ESRD pts.

STABLE RENAL FUNCTION AND BENIGN COURSE IN AZOTEMIC DIABETICS TREATED WITH ERYTHROPOIETIN (EPO) FOR ONE YEAR. Clinton D. Brown*, Eli A. Friedman,. Department of Medicine State University of New York Health Science Center at Brooklyn, Brooklyn, N.Y.

Perturbations in blood rheology may contribute to the genesis and progression of microvascular complications in diabetes mellitus. To assess this risk, we conducted a one-year pilot trial of human recombinant EPO in five anemic azotemic diabetics (two insulin-dependent and three noninsulin-dependent subjects) who had diabetic nephropathy and concurrent extrarenal vasculopathy. Viscosity was measured at the high rate of shear (230 Sec^{-1}) at 37°C and reported in centipoise (cp). Plasma viscosity was measured by the same technique using 2 ml. of freshly separated plasma at 37°C at a shear rate of (230 Sec^{-1}). Normal values for our laboratory as measured in 25 adult men and 25 adult women are: hematocrit of 40.1±1.31%, whole blood viscosity at 230 Sec^{-1} of 4.30±0.37 cp, and plasma viscosity at 230 Sec^{-1} rate of shear is 1.61±0.11 cp.

All patients reported (subjective) improvement in effort tolerance and sense of well being. Mean hematocrit increased significantly ($p < 0.001$) from a baseline mean of 29.4% (range 26 to 33%) to a mean of 39.2% (range 36 to 44%). Whole blood viscosity also rose significantly ($p < 0.001$) from a pre-EPO treatment level of 3.75 ± 0.11 Sec^{-1} to 4.33 ± 0.11 Sec^{-1}. Plasma viscosity, however, was unchanged from a baseline of 1.61 ± 0.11 Sec^{-1} by EPO treatment. Mean serum creatinine concentration was 3.7 ± 0.27 mg/dl at the end of one year of EPO-treatment, a value unchanged statistically from its level of 3.5 ± 0.28 mg/dl at the start of the study. None of the subjects suffered a cardiovascular or cerebrovascular event or had deterioration in vision. Hospitalization was necessitated in one patient for three days due to an enalapril-induced reversible rise in serum creatinine. None of the patients sustained a cerebrovascular or cardiovascular *incident* during the year of study.

From this preliminary experience, we infer that in EPO-treated azotemic diabetics, the rise in whole blood viscosity that accompanies the increase in red cell mass is tolerable over the term of one-year and may not hasten progression of macrovasculopathy.

PROGRESSIVE RENAL DISEASE: ROLE OF RACE AND ANTIHYPERTENSIVE THERAPY P.C. Brazy, Durham VAMC and Univ. of Wisconsin, Madison, WI.

Patients with diastolic hypertension (dBP) have a more rapid rate of decline in renal function than normotensive patients (Clin.Res. 36:45A, 1988). This relationship may be due to the presence of hypertension or to other factors related to hypertension which may be affected by race or specific drug therapy. To address this question we reviewed the rate of decline in renal function (slope 1/creatinine vs time), dBP and drug therapy in 112 Black and 88 White patients from the Durham VAMC who had demonstrated at least a 20% decrement in 1/creatinine (1/Cr). The group's slope of 1/Cr averaged -0.0082 ± .0004 dl/mg month and diastolic BP averaged 89.9 ± 0.1 mm Hg. Antihypertensive medications were taken by 75% of patients. In White patients increasing levels of dBP were associated with a more negative 1/Cr slope by linear regression analysis ($P<0.025$). Similar data in Black patients had no correlation. In individual Black patients therapy with minoxidil or hydralazine improved the 1/Cr slope by +0.006 ± .003 and +0.007 ± .003 dl/mg month, respectively ($P<0.025$). Calcium channel blockers had a similar beneficial effect (+0.005 ± .002 dl/mg month; $P<0.025$) in White patients. These data indicate that White patients have an inverse association between dBP and rate of decline in renal function. In Black patients, however, other factors like renal vasoconstriction may determine the course of renal disease.

CORRECTION OF THE ANEMIA IN HEMODIALYSIS (HD) PATIENTS (PTS) WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo): HEMODYNAMIC CHANGES AND RISKS FOR HYPERTENSION. FS Buckner*, JW Eschbach, NR Haley*, RR Davidson, and JW Adamson*. Univ. of Washington, Seattle, WA.

Increased blood pressure (BP) has been associated with a rising hematocrit (hct) induced by rHuEpo in HD pts. Forty of our 64 rHuEpo-treated pts had an increase in mean arterial pressure >10 mmHg associated with a rising hct. BP was controlled with new or extra BP medication, and some pts eventually required less antihypertensives. Those pts with hct <22 at outset were at greater risk for becoming hypertensive ($p < 0.01$). Factors not associated: years on HD, age, sex, or presence of native kidneys. Non-invasive hemodynamic measurements on 7 normotensive pts not requiring BP medication were done at hct 22.6 ± 1.5 and when the hct was 36 ± 1.3 and showed: diastolic BP 61 ± 6 --> 75 ± 7 mm Hg ($p=0.06$); cardiac index 5.8 ± 0.6 --> 4.8 ± 0.6 L/min/m^2 ($p=0.02$; normal = 3.0 ± 0.5); total peripheral resistance index 1154 ± 138 --> 1553 ± 93 dyne-sec/m^5 ($p=0.025$; normal 2000 ± 300). Correction of anemia in HD pts may result in rising BP. These findings in normotensive pts suggest that the increased BP seen in HD pts on rHuEpo therapy is due to a reversal in compensatory vasodilation without complete normalization in elevated cardiac output.

IDIOPATHIC IgA NEPHROPATHY (IgAN) IS AMONG THE MOST FREQUENT CAUSES OF END STAGE RENAL FAILURE (ESRF). G. Cam,* P. Simon,* KS Ang,* MP Ramée, (intr. by G.S. Hill), Hôpital La Beauchée, St Brieuc, France.

The incidence of IgAN in a population of 400,000 was evaluated between January 1976 and December 1987. Renal biopsy was indicated in 781 patient who were born and lived in the studied area. Primary glomerular disease (PGD) was diagnosed in 401 pts (51.3%) correpsonding to a 12-year prevalence of 1/1000. The results were compared for three consecutive periods: A(1976-79), B(1980-83), C(1984-87) for which the number of pts with PGD was respectively 138, 148, and 115, and incidence was 86, 92 and 72 per 10^6 adults (> 15 yrs of age). IgAN was the most common PGD (137 pts, 34.2%) and its incidence remained stable for the three periods: 26(A), 31 (B) and 28(C) per 10^6. Sex-ratio M/F was 2.5 (A), 2.6 (B) and 1.2 (C) and mean age at the time of renal biopsy was 33 ± 15 (A), 37 ± 16 (B) and 41 ± 15 (C) ($p < 0.01$ with A). The disease began in subjects under 35 yrs of age in 62% of the cases, with a peak between 16 and 25 yrs. Seventy-three pts (63%) were symptom-free and the condition was diagnosed when they underwent routine screenings for renal disease. Among 278 pts who started dialysis during the period under review, 85 pts (30.5%) had PGD, 81% of whom had undergone renal biopsy. IgAN was the first cause of ESRF (11.5%, incidence rate 6.4% per 10^6) followed by diabetes (10.5%). Theoretical prevalence of ESRF was calculated as 21.2% for PGD and 22.4% for IgAN. Thus, IgAN is not only the most common form of glomerulonephritis but is also among the most frequent causes of ESRF.

SCREENING FOR ACQUIRED CYSTIC KIDNEY DISEASE: COMPARISON OF CT AND ULTRASOUND. E.P. Cohen*, A.J. Taylor*, S.J. Erickson*, W.D. Foley* and W.F. Piering. Depts. of Med. & Radiol. Medical College of Wisconsin, Milwaukee, WI

Acquired cystic kidney disease (ACKD), cyst formation in a failing non-cystic kidney, is of growing concern in dialysis and transplant patients. Malignant and non-malignant complications may occur, which while infrequent, may be lethal. The known increasing prevalence of ACKD with time on dialysis, and the report of increasing kidney volume after three years on dialysis led us to prospectively compare CT scan (CT) and ultrasound (US) in patients on dialysis three years or more. Forty-one patients (22 hemodialysis, 19 peritoneal) on dialysis 3 years or more (mean 4.5 years) were evaluated by contrast enhanced CT scan and US. For each of 79 kidneys, ACKD was graded first independently then by consensus among three radiologists. Five or more cysts in a kidney was defined as ACKD.

	US+		US-	US
CT+	14		33	sensitivity 30%
CT-	0		32	specificity 100%
	A	B	C	Conspicuity of the kidney was also graded
U.S.	45	25	9	with grade A as best, grade B as inter-
C.T.	79	0	0	mediate and grade C as ill-defined.

Three solid masses were found by both US and CT (mean diameter = 3.0 cm) and were malignant. Seven other masses were indeterminate by either CT or US. Correlation of CT and US images indicated 4 hyper-dense cysts, 1 simple cyst, 1 focal hyperrophic area and 1 mass that was still indeterminate. Thus, US and CT were equally sensitive in detecting masses 3 cm or larger. In 17% (7 of 41) of cases, both modalities were needed for further precision. Though three-fold cheaper and highly specific, low US sensitivity and low image conspicuity renders this test less optimal than CT, even for screening purposes. In conclusion, both the low sensitivity and image quality of US indicate that CT alone is the preferred technique to detect ACKD in patients having been on dialysis for 3 years or longer.

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) IN PATIENTS WITH END-STAGE RENAL DISEASE (ESRD): A LONG TERM FOLLOW-UP. Jhoong Cheigh, Kurt Stenzel, Luis Tapia, John Sullivan, Robert Riggio, Albert Rubin. The New York Hospital/Cornell Medical Center, New York.

Little information exists on the natural course of patients with SLE who develop ESRD and require long-term maintenance dialysis treatment or kidney transplantation. We studied the clinical course of 51 patients with SLE who developed ESRD and required dialysis for >3 months between 1970 and 1987. There were 44 females and 7 males, ages ranged 10-65 years (mean ± S.E.=27.9 ± 0.2) when they began dialysis and they were followed for 5-193 months (83.4 ± 1.1). At the time dialysis was initiated, 20 patients (39.2%) had clinically active SLE. Renal disease progressed in the majority of patients without clinically active SLE. 14 patients died, 8 from infectious and 6 from cardiovascular diseases. Cumulative patient survival was 94.1, 79.3 and 72.9% at 1, 5 and 10 years respectively. 18 patients received a total of 21 kidney transplants (14 living related; 7 cadaver) and their graft survival was 59.4 at 5 years and 44.5% at 10 years. 20% of patients surviving 5-10 years had at least one abnormal serological test for SLE, but none expressed clinical activity of SLE. We conclude: 1) Lupus nephritis often progresses to ESRD in the absence of clinically active SLE, 2) Most long-term surviving patients remain clinically inactive for SLE, 3) Patient survival on dialysis and renal allograft survival of these patients are comparable to that in the non-SLE population, and 4) This study suggests that the prognosis of SLE patients could be further improved by giving less immuno-suppression in the peri-and post-ESRD period.

THE ERRORS IN ESTIMATING DIETARY INTAKE IN CHRONIC RENAL FAILURE. G.A. Coles*, J.H. Meadows*, K. Tomlinson* (Intro. by R.B. Sterzel). KRUF Institute of Renal Disease, Royal Infirmary, Cardiff, Wales, UK.

Protein intake of uraemic patients is usually assessed from urine urea nitrogen losses (UUN). Recently it has been suggested that analysis of a spot nocturnal sample is sufficient. We compared a weighed dietary record with 24 hr and spot sample UUN estimates of N intake in 45 patients with chronic renal failure.

Urea/creatinine ratios in spot and 24 hr urines correlated well, r=0.91, but the degree of agreement was poor for individual subjects. When expressed as daily UUN excretion the spot samples gave a higher value than direct 24 hr measurement, mean difference +1.6 g, 95% limits +5.7 to -2.5. For 12 subjects taking a low protein diet the dietary record gave a N intake of +0.6 g, 95% limits +2.4 to -1.2, compared to estimates from 24 hr UUN + non urea N (NUN) and urine protein N loss. N intake as assessed from spot sample UUN + NUN and urine protein loss gave a value higher than the dietary record, +1.1 g, 95% limits +7.2 to -4.9. The agreement between dietary records and urine estimations of N intake was worse for subjects not on a protein restricted diet due to the day to day variability of food consumed.

We conclude that estimation of protein intake from a spot urine sample is too inaccurate for clinical use. A 24 hr urine estimate of N intake is reasonably accurate for subjects receiving protein restriction but not for those on a normal diet.

PLASMA AMINOACID(AA) CONCENTRATION IS INCREASED AT THE TIME OF THE MAXIMAL HEMODYNAMIC RESPONSE(MHR) AFTER A MEAT MEAL. S.Coppola, G.Spagnuolo,P.Anastasio,L.Bellini,D.R.Giordano,R.Alfieri,G.Coscarella, G.DeSimone,A.Lombardi,N.G.DeSanto, Università Federico II,Naples,introd. by S.G.Massry)

Plasma AA concentration and various hormones are usually taken into account along with the physical factors in order to explain the hemodynamic response to MM.This explanation is based on experiments where AA were infused intravenously and /or on old knowledge on postprandial AA.The aim of this work is to evaluate contemporarely the time of MHR plasma AA Concentration and insulin..Two experimental adult groups were studied:Group 1 of 7 control subjects and Group 2 of 7 patients with a mean plasma creatinine concentration of 2.7 md/dl,evaluating GFR(inulin),RPF(PAH),AA and Insulin by performing 7 clearance study periods(2 before MM each lasting 30 min and 5 after MM at 30,60,90,120 and 180 min.AA were meaured(Thr,Val,Leu,Ile,Phe,Met, Lys,Tau,Asp,Asn,Ser,Glu,Gln,Pro,Gly,Ala,Cys,His, Arg,Trp,Tyr,Orn and Phosphoserine(Phs) at baseline and at the time of MHR.AA increased significantly ($0.05<p<0.01$) at exception of Orn,Tyr,Glu and Phs in group 1 and of Tau,Ser,Glu,Cys,Arg in Group 2. Essential AA,Glycogenic AA,Chetogenic AA(Leu,) Non Essential AA,Total AA,Branched chain AA increased significantly($0.05<p<0.0005$ in both groups as increased insulin($p<0.01$).At the time of MHR GFR and RPF increased over baseline values($0.01 <p<0.0005$ without changes in Filtration Fraction. The data provide the first demonstration that GFR,RPF,Plasma AA and Insulin are increased contemporarely following MM.

RENAL ARTERY DISEASE IN CHRONIC ADVANCED RENAL FAILURE (CARF). Jose Roberto Coelho da Rocha, Tania B. Rios, Celso Fianco and Ronaldo C. Borges, Serviço de Nefrologia do Hospital da Beneficência Portuguesa do Rio de Janeiro, Brazil.

Renal artery disease (RAD) is a cause of acceleration of chronic renal failure. In the past, surgical approach was deemed risky, but percutaneous angioplasty (PAN) brought new hopes for such problem.

Based on existing informations, we developped a criteria for suspicion of RAD in patients with CARF: hypertension of diabetes for many years, evidence of other vascular diseases (coronary, carotid or aortoiliac), rapid renal failure, renal asymetry or irregular renal silhouette on X-ray. Applying this criteria, 14 patients with CARF (Ccr 10ml/min), age 46 to 87 (mean 64.1), underwent angiography, which confirmed the presence of DAR in all: 9 with unilateral stenosis, 3 with bilateral, 1 with unilateral thrombosis and 1 with aortic thrombosis, left renal artery thrombosis and right stenosis.

In 3 patients with unilateral stenosis PAN was not done, and nephrectomy was done in 1 with unilateral thrombosis. PAN was tried in 10 patients, but achieved only in 8. Improvement of renal function was seen in only 1 patient, which was able to stay off dialysis for a few monts. One patient developped a brachial A-V fystula and another thrombosed the renal artery.

This study suggests that, in CARF, renal PAN does not improve GFR.

PTERIN METABOLISM IN HEMODIALYSIS PATIENTS. Cunningham J*, Altmann P*, Hamon C*, Marsh F*, Blair J*, (intr. by Slatopolsky E). The London Hospital, London and The University of Aston, Birmingham, UK.

Brain dihydropteridine reductase (DHPR) deficiency, causing impaired metabolism of the pterin, tetrahydrobiopterin, causes mental dysfunction and may be identified by reduced erythrocyte DHPR activity, raised serum biopterin but normal neopterin concentrations. We have previously found raised concentrations of serum biopterin derivatives in hemodialysis (HD) patients without overt cerebral impairment, in whom there was inhibition of erythrocyte DHPR activity by low levels of aluminum (Al) accumulation. There was no relationship between serum biopterin derivatives and DHPR activity, possibly due to impaired excretion of pterins in renal failure and to their formation by activated leucocytes.

To study this further we measured serum neopterin and biopterin concentrations in 17 HD patients and 7 controls: neopterin (66.2±7.4 vs 2.8±0.4 µg/l, p=0.0001) and biopterin (13.6±1.3 vs 2.4±0.4 µg/l, p=0.0001) were raised but neither was related to serum creatinine. The concentrations were also expressed in µg/mg creatinine to correct for impaired excretion in renal failure. Erythrocyte DHPR activity was not related to either uncorrected or corrected neopterin (r=-0.5,p=0.2), but was significantly related to the corrected biopterin (r=-0.99,p=0.0001). Bone Al was related to corrected biopterin only (r=0.68,p=0.03), although serum Al and oral Al intake were not related to biopterin or neopterin.

The results suggest that factors in addition to renal excretion, such as immune activation or Al accumulation, may influence pterin metabolism. The lack of correlation between neopterin and DHPR implies that the neopterin/biopterin ratio in HD patients is not a valid indicator of DHPR activity. Biopterin, which predictably would rise due to reduced salvaging of quinonoid dihydrobiopterin by DHPR, was not only raised but, when corrected for creatinine, related closely to DHPR activity. Thus, accumulation of biopterin in HD patients is due to inhibition of DHPR activity and, to a lesser extent, reduced excretion.

DURATION AND AMOUNT OF ABUSE IS NOT PREDICTIVE FOR THE DEVELOPMENT OF ANALGESIC NEPHROPATHY (AN). Monique M. Elseviers*, Marc E. De Broe* (Intr. by G.A. Porter). Univ. of Antwerp, Dept. of Nephrology-Hypertension, Antwerp, Belgium.

In 1983, the prevalence of AN still was 18% in the Belgian dialysis population. Within the framework of a large investigation of AN, we studied the effect of duration and amount of abuse on the development of this disease. No difference in intake was observed between a group of abusers without any document renal damage, a group of patients of the outpatient clinic and a group of dialysis patients both with documented AN.

	N	Duration*	Amount**
Abusers	181	21.7	34.4
Outpatients clinic	104	23.6	43.5
Dialysis patients	33	21.0	42.2

Within the group of patients of the outpatient clinic with documented AN, the duration and amount of abuse is not related to the degree of renal failure (expressed as calculated creatinine clearance (CrCl; resp. r=.07 and r=-.02).

	N	Duration*	Amount**
CrCl > 25 ml/min	36	22.4	47.0
10-25 ml/min	31	22.1	40.5
< 10 ml/min	13	18.6	27.8

These observations suggest that unidentified predisposing factors play an important role in the development of AN.

* duration of abuse in mean years
** amount of abuse in mean of total units/1000

CORRECTION OF ANEMIA IN PROGRESSIVE RENAL FAILURE WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo). JW Eschbach, MR Kelly, NR Haley*, RI Abels*+, and JW Adamson*. Univ. of Washington, Seattle, WA, and Ortho Pharmaceutical Co.+, Raritan, NJ.

As renal failure progresses, anemia develops and patients (pts) may become debilitated even before they require hemodialysis (HD). To test the effectiveness of rHuEpo in such pts, and to determine the effect of correction of anemia on the rate of progression of renal failure, we treated 9 pts with rHuEpo (doses: 50 or 150 U/kg 3X/wk IV). The mean (± SD) hematocrit (hct) rose from 27.2 ± 3.2 to 39.4 ± 1.8 in 8 wks and was maintained with 25 to 100 U/kg given 3X/wk subcutaneously. Pts treated with 150 U/kg responded identically to HD pts receiving the same dose: a hct rise of 2 points/wk. Mean serum creatinine rose from 7.3 ± 2.1 to 8.1 ± 1.8 mg/dl as the hct increased, a rate of decline in renal function not accelerated from pretreatment levels. Hypertension, present in all pts before rHuEpo treatment, increased in 3 pts as the hct rose. Quality of life improved in all. Ability to work was maintained even after renal function decreased and HD was initiated. Conclusion: rHuEpo is as effective in anemic predialysis pts as it is in HD pts. Although the numbers of pts are small, there was no evidence for acceleration of the progression of renal failure with correction of the hct.

THE EFFECT OF BLOOD PRESSURE REDUCTION AND DIETARY RESTRICTION OF PROTEIN ON THE PROGRESSION OF DIABETIC NEPHROPATHY. G. V. Evanoff, C.S. Thompson, and E. J. Weinman. University of Texas Medical School, Houston, Texas.

To examine the effects of control of blood pressure and restriction of the dietary intake of protein on renal function in diabetic patients, the rate of decline in renal function (RD = $creatinine^{-1} \cdot 10^{-2} \cdot months^{-1}$) was examined in 14 patients with diabetic nephropathy and hypertension. For an average period of 10.8 months during which the blood pressure was not adequately treated (172/98) and the patients ingested an unrestricted protein diet, RD averaged -1.79 ± .26. With control of blood pressure (156/90) for a period of 29.2 months RD was significantly decreased to -0.97 ± .42. The patients were placed on a protein restricted diet of 40 gms/day for 11.6 months while blood pressure control was maintained. Restriction of the dietary intake of protein significantly decreased RD further to -0.34 ± .45.

These findings indicate that reductions in the systemic arterial blood pressure and in the dietary intake of protein slow the progression of diabetic nephropathy. The results also suggest that blood pressure and protein intake effect the progression of diabetic nephropathy by different mechanisms.

EFFECT OF PARATHYROID HORMONE (PTH) ON IMMUNOGLOBULIN (Ig) PRODUCTION IN NORMALS AND IN CHRONIC RENAL FAILURE (CRF) PATIENTS. Zbigniew Gaciong*, Jadwiga M. Alexiewicz*, Thomas O. Pitts, Mariana Linker-Israeli*, Ira Schulman* and Shaul G. Massry. Div. of Nephrol., Univ. of So. Calif., Los Angeles, CA.

B-cell proliferation is inhibited in uremia and this is due to excess PTH. This defect may affect Ig production. We examined production of IgG, IgM and IgA by B-cells from normals and CRF patients under stimulation of Staphylococcus aureus Cowan I (SAC) and Pokeweed mitogen after 8 days culture. IgG, IgM and IgA production by B-cells from CRF patients were lower ($p<0.02$) than normals. Both 1-34 PTH and 1-84 PTH inhibited production of all Igs by cells from normals in a dose-dependent manner. PTH also inhibited Igs production in CRF patients but less ($p<0.01$) than in normals. Forskolin which causes a rise in cAMP levels, calcium ionophore (0.25uM), and TPA, an activator of protein kinase C, mimicked the effects of PTH. Kinetic studies showed that for PTH to exert its action, it must be present on day 1 of the culture. The results show that PTH inhibits Ig production by B-cells most likely due to PTH-receptor interaction resulting in cAMP production, ionophoric property of the hormone and an effect on protein kinase C. Since the latter stimulates B-cell proliferation but inhibits Ig production, one may suggest that this effect of PTH may be at least in part due to an action on B-cell differentiation. This new action of PTH may underlie the defects in humoral immunity in CRF.

LOW TESTOSTERONE (T) LEVELS INCREASE IN MALE HEMODIALYSIS PATIENTS (HDP) TREATED WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo). NR Haley*, AM Matsumoto*, JW Eschbach, JW Adamson*. Univ. of Washington, Seattle, WA.

Sexual interest and potency are impaired in many HDP. After correction of anemia with rHuEpo, many male HDP report improved sexual performance. We measured serum T and FSH levels in 12 male HDP prior to rHuEpo therapy and after the hematocrit (hct) had increased and stabilized for 4 to 12 months. Six HDP had baseline T and FSH levels within the normal range (T = 3-10 ng/ml; FSH = 30-230 ng/ml). The other six had low T levels and elevated FSH levels. With rHuEpo therapy, T levels normalized as the hct increased.

	pre-rHuEpo	post-rHuEpo	p
Hct	20.4 ± 3.2	33.0 ± 1.9	<0.001
T	2.1 ± 0.6	3.1 ± 1.0	<0.01
FSH	314 ± 181	498 ± 218	=0.02

The ages of the HDP were 22-59 in the normal T group and 23-74 in the group with low T. T and FSH levels were unchanged from baseline 2 wks after rHuEpo therapy was started, but before the hct increased. Conclusion: T levels increase as a result of a rise in hct and not as a direct effect of rHuEpo, and this increase may account for the improved sexual potency observed in these HDP.

INTERNATIONAL HUMAN REMNANT KIDNEY REGISTRY: FIRST REPORT. J.T. Harrington, J.F. Donohoe*, G.R. Sant*, M.H. Foster*. Newton-Wellesley Hospital, Newton, MA; Beaumont/Mater Hospitals, Dublin; Tufts-New England Medical Center, Boston, MA.

Surgical ablation of 5/6 renal mass in rats leads to focal sclerosis in the remnant kidney and progressive renal failure. Such a phenomenon has not been observed in dogs and rabbits. Data in humans are limited to patients(pts) with unilateral renal agenesis or uninephrectomy. To obtain data in humans with a remnant kidney, we surveyed > 800 urologists and nephrologists in the U.S. and abroad. Clinical data were requested on all pts with the following characteristics: 1) Surgical resection resulting in presence of only part of one kidney; 2) Follow-up of $\geq$ 5 yrs postop. 13 pts were identified, one of whom initially had a solitary kidney: 12 pts had renal cancer and 1 had tuberculosis. All 6 pts followed 10 or more yrs postop have stable serum creatinine (S.Cr.); 2 of these are 25 and 30 yrs postop. At 5 to 7 yrs postop the other 7 pts have S.Cr $\leq$ 3.0 mg/dl; only one of these pts has had a rise in S.Cr. Seven of the 13 pts. are known to be hypertensive.

Data on 2 additional pts were obtained. One pt with one functioning and 1 atrophic kidney required partial nephrectomy for cancer in the functioning kidney. S.Cr is stable at -3.0 mg/dl 6 yrs postop. The second pt had both uninephrectomy for cancer and ligation of a 50% A-V malformation of the 2nd kidney; this pt., treated with postop irradiation, developed focal sclerosis and ESRD 11 yrs postop.

Conclusion: Survival of at least 25 to 30 years with a stable serum creatinine is possible in humans with only a remnant kidney.

PSYCHOLOGICAL WELL-BEING OF TRANSPLANT VS. NONTRANSPLANT CATEGORIES OF END-STAGE RENAL DISEASE PATIENTS IN THE MICHIGAN STUDY.
M. Julius,* J.D. Kneisley,* V.M. Hawthorne,* P. Carpentier-Alting,* R.A. Wolfe,* and F.K. Port. Univ. of Michigan, Depts. of Epidemiology and Internal Medicine, Ann Arbor, Michigan.

Scores on a number of psychological measures (e.g. affect, satisfaction with life) were assessed in personal interviews and compared across treatments for the Michigan ESRD study population, n=929. Patients were stratified by their transplantation status: (1) dialysis patients who wanted to remain on dialysis, (2) dialysis patients who were on the transplant waiting list, (3) dialysis patients prefering transplants but not on the list, (4) patients with a functioning transplant, and (5) dialysis patients with a failed transplant.

Using analysis of variance and covariance, adjusting for sex, race, age, education, marital status, primary cause, and duration of ESRD, and mean duration of each treatment modality, indicated presence of highly significant differences between these five categories of treatment on psychological well-being.

Consistently across all measures the highest psychological well-being was reported by patients with functioning transplants and the lowest by patients who wanted, but were not on the transplant waiting list (Scheffe multiple comparison test). The second best scores on well-being were between hemodialysis patients from categories one and two. Whereas the findings for most categories are in the predicted direction, the low ranking of category three appears to relate to a discrepancy of reality and expectations.

DEVELOPMENT OF HYPERTENSION (HT) AND UREMIA (U) IN CHILDHOOD PYELONEPHRITIS (PN) - A 30-YEAR FOLLOW-UP (FU).
S Jacobson*, O Eklöf*, LE Lins*, B Tidgren*, J Winberg. (Intr by CM Kjellstrand). Depts of: Med, Peds, Radiol, Phys. Karolinska Hosp, Stockholm, Sweden.

Development of HT and U following acute PN is controversial. We followed 30 children who had nonobstructive acute PN (at age 6; range 1-13 years) for >27 years. Glomerular filtration rate (GFR), renal plasma flow (RPF), systolic (SBP) and diastolic (DBP) blood pressure, plasma renin (PRA), angiotensin II (AII), fractional clearance of Na (FeNa) and K (FeK), S-B2 microglobulin, U-B2 and U-Albumin (U-A) was determined in 27 of the patients (mean age 33 years, range 22-41) and in 13 healthy controls (mean age 34 years, range 22-41)

Results at follow-up: Three patients (10%) had developed end-stage renal disease and 7 patients (23%) had HT (>140/90 mmHg). Seven patients (23%) had undergone nephrectomy or resection. The table shows results from the remaining 20 patients.

	GFR	RPF	SBP	DBP	PRA	AII	FeNa	FeK	S-B2	U-B2	U-B2
pat.	91	483	122	82	1.5	40	1.3	20	1.8	0.6	5
cont.	108	587	116	72	0.9	21	1.3	27	1.5	0.4	4
t-test	**	**	ns	**	*	ns	ns	*	ns	ns	ns

*p<0.05 **p<0.01

Regression analyses: PRA vs DBP (r=0.5, p<0.05),PRA vs FeNa (ns), PRA vs FeK (r= -0.4, p=0.053), PRA vs AII (ns), SBP vs FeNa (r=0.54, <0.01), SBP vs FeK (ns), DBP vs FeNa (r=0.51, p<0.01), DBP vs FeK (ns), GFR vs DBP (ns), RPF vs DBP (r=-0.4, p<0.05).

Conclusions: Children with acute PN are at high risk of developing renal failure and hypertension and should be closely followed. Hypertension in this group of patients is renin dependent which should be considered in the treatment of these patients.

SEVERE HYPERCALCIURIA AS A RESULT OF COMBINED 1,25-DIHYDROXYVITAMIN D3 (D3) AND GROWTH HORMONE (GH) IN UREMIC RATS. G Kainer, M Nakano, F Boyle, JW Foreman, JCM Chan. Medical College Virginia, Richmond, VA.

Chronic uremia in children is associated with growth failure; GH treatment has been tried to improve growth. As D3 is commonly used to treat renal osteodystrophy, we investigated the effects of combined GH and D3 therapy on renal handling of divalent ions in growing uremic rats.

23 days old male Sprague-Dawley rats were fed a diet containing 8% protein, and 0.6% calcium and phosphorous. At 26 days one stage 75% nephrectomy or sham surgery was performed, and rats assigned to five groups: Sham control (SC), Uremic control (UC) Uremic-D3 treated (UD3), Uremic-GH treated (UGH), and Uremic-D3-GH treated (UD3GH). GH (0.5 mg/day) was injected S.C., D3 (20 ng/kg/day) was gavaged, rats not receiving drugs received vehicles only. After 40 days on treatment, fasting (water ad lib) 24 hour urines and bloods were collected. Results are Mean $\pm$ S.E.M.

	SC n=6	UC n=22	UD3 n=20	UGH n=20	UD3GH n=20
Scr	0.52$\pm$.11	1.07$\pm$.05	1.21$\pm$.05	0.94$\pm$.06	1.13$\pm$.09
Ccr	0.47$\pm$.11	0.16$\pm$.01	0.14$\pm$.01	0.19$\pm$.01	0.16$\pm$.01
Ca^{2+}	1.45$\pm$.02	1.48$\pm$.02	1.48$\pm$.02	1.46$\pm$.02	1.47$\pm$.03
FECa	0.19$\pm$.04	0.77$\pm$.08	2.04$\pm$.23	1.04$\pm$.22	4.50$\pm$.82*

* p<0.001 (ANOVA) UD3GH vs. all other groups
Scr = serum creatinine, Ca^{2+} = ionized calcium
CCr = creatinine clearance(ml/min/100g body weight)
FECa = fractional excretion of calcium.

These data show marked hypercalciuria in uremic rats treated with both GH and D3, and raise concerns regarding the use of GH in uremic children.

THE EFFECT OF RECOMBINANT HUMAN ERYTHROPOIETIN (EPO) UPON QUALITY OF LIFE (QL) AND FUNCTIONAL CAPACITY (FC) OF ANEMIC PATIENTS ON CHRONIC HEMODIALYSIS. Canadian Erythropoietin Study Group, Robarts Research Institute, London, Ontario (presented by Paul A. Keown)

Eight Canadian centres conducted a randomized, double-blind trial in 118 hemodialysis patients with a hemoglobin (H) less than 90 gm/l to determine whether correction of the anemia with EPO improved their QL and FC. Patients were randomized to 3 groups: I placebo, II EPO to achieve a H of 95-110 gm/l, III EPO to achieve a H of 115-130 gm/l. Neither the patients, dialysis or research nurses knew the treatment allocation. QL and FC measures were assessed prior to randomization and 4 months later. Baseline characteristics of the 3 groups were similar. Mean H at 4 months was 73.3 gm/l (Group I), 103.7 gm/l (Group II), and 116.3 gm/l (Group III). There was no difference in QL or FC between Groups II and III. When all patients on EPO (Groups II and III) were compared to placebo, there were clinically and statistically important improvements in the Sickness Impact Profile ($p=.021$), physical and fatigue dimensions of a kidney disease specific questionnaire ($p < .002$), six minute walk test ($p=.02$), and stress test ($p=.001$). There were no changes in echocardiographically measured left ventricular end systolic and end diastolic diameters or fractional shortening in any groups. No significant differences in mean blood pressure or serum potassium were found between groups. SUMMARY: In a randomized, double-blind placebo controlled trial, EPO appears to statistically and clinically improve the QL and FC of anemic hemodialysis patients.

MAJOR RESULTS OF THE FEASIBILITY STUDY OF THE MODIFICATION OF DIET IN RENAL DISEASE (MDRD) STUDY. MDRD Study Group (prepared by S.Klahr, A.S.Levey, A.M.Sandberg*, G.W.Williams*), National Institutes of Health, Bethesda, MD.

The MDRD Study is a multicenter, randomized, controlled trial to determine acceptance, safety, and efficacy of low protein and phosphorus diets in patients with progressive renal disease. The purpose of the feasibility study was to test procedures and recruitment strategies and to estimate sample size for the full-scale trial. It was not the purpose of the feasibility study to compare rates of progression among diet groups.

A limited number of patients aged 18-75 years, with current ^{125}I-iothalamate clearance (C_I) from 7.5-56 ml/min/1.73m^2 and previous progressive rise in serum creatinine, were randomly assigned to one of four diets (g/kg/d protein): M (1-1.4); L (.575); K (.28 and keto acids); J (.28 and amino acids). Compliance was estimated from dietary protein intake, calculated from urinary urea excretion. Nutritional status was monitored by anthroponetry and serum protein concentration. Progression of renal disease was calculated as the rate of decline of C_I.

Ninety-six patients were randomized and followed for a mean duration of 11.6 months. Compliance with low protein diets was less than expected; nonetheless, mean estimated protein intake differed among diet groups. Weight declined slightly in low protein diet groups, but serum protein concentration was stable. Decline in C_I was linear; rate of decline was faster in patients with higher mean blood pressure (BP). Based on variability of C_I slopes, design for the full-scale trial is 800 patients randomized to 3 diet groups and 2 BP goals and followed for 2-4 years.

LYMPHOKINES IN RENAL DISEASE (RD): UREMIA (U), HEMODIALYSIS (HD) AND CAPD. Paul L. Kimmel, Terence Phillips*, Juan P. Bosch. George Washington Univ. Med. Ctr. Dept. of Medicine, Washington, D.C.

In order to determine the role of Interleukin 1 and 2 (IL 1 and IL 2) in patients with RD 38 pts were studied and compared with 12 normal subjects.

Methods: Blood sampling schedule:

	Pre	Post
Normal subjects	(n=12): Time 0	4 hrs later
Pts on HD	(n=22): Beginning	End Rx
Pts on CAPD	(n= 8): Beginning	End exchange
Uremic pts	(n= 8): In AM	

IL1 and 2 determinations: Monocytes were separated by density gradient centrifugation and incubated. The cells were then stimulated, incubated and pulsed with H3 Thymidine. For IL 1, CRL 1424 cells and for IL 2, CTTL 2 cells were used. (mean: * $p < .05$ by paired t test, ** $p < .01$ by ANOVA)

	Normal		HD		CAPD		Uremia
	Pre	Post	Pre	Post	Pre	Post	Pre
IL 1	16432	16347	11496	17392*	11179	15994*	10924
IL 2	6233	6293	10210	16090*	9029	11981*	11455
IL2/IL1	.35	.35	**.94	.91	**.82	.77	**1.09

The IL system is abnormal in pts with RD. While absolute IL 1 and 2 levels are not discriminatory, the IL2/IL1 ratio was significantly elevated in pts with RD, suggesting a greater IL2 response per level of IL1. No differences were shown between uremics and pts on maintenance therapy. Thus, neither HD nor CAPD restores the IL2/IL1 ratio to normal. Both treatments resulted in increased IL1 and IL2 production. In U, HD and CAPD there is an increased IL2 production capacity. The abnormal and hyperreactive IL system observed in uremia responds to HD and CAPD with increased synthesis of IL1 and 2.

KINETICS OF ALLOISOLEUCINE [AIL] AFTER INGESTION OF KETOACIDS [KA] IN NORMAL AND CHRONIC RENAL FAILURE [CRF] ADULTS. JD Kopple, JT Dichiro*, S McKay*, SG Adler and SA Laidlaw*. Harbor-UCLA Medical Center, Torrance, California 90509.

With the increasing use of KA analogues of essential amino acids in renal failure, it has become important to monitor compliance to the KA prescription. Evidence indicates that plasma AIL rises in patients given ketoisoleucine, but there are few data regarding the consistency, rapidity of rise, or duration of elevation in plasma AIL. We studied this in 7 normal and 3 CRF (SCr 3.2±1.3SEM mg/dl) adults fed the KA mixture EE. All received a KA load, 1.4 g/10 Kg DBW, providing 0.138 g/10Kg of ketoisoleucine. Four normals and 1 CRF patient also were studied for 6-10 days while they ate 2.1-2.8 g/10 kg/day KA with meals. All subjects lived in a CRC, were faculty or co-authors. AIL was measured by the method of Ponto and Anderson. Since there were no differences in plasma and urine AIL between normal and CRF subjects, data were combined. After the KA load, plasma AIL increased from 3±1 to 20±3 μM by 30 min in the 2 groups combined. Peak levels of 43±3 μM were reached by 180 min, and values began to decline by 240 min. At 300 min, plasma AIL was 36±3 μM. During the 6-10 day study, fasting a.m. plasma AIL was 36±1 μM. After stopping KA, plasma AIL fell below a.m. fasting levels by 4 hours and reached baseline range 36 hours after the last dose. Urine AIL also rose in most individuals but was more variable. The data suggest that plasma AIL is a sensitive indicator of the intake of KA mixtures containing ketoisoleucine. Plasma AIL may therefore be used to monitor compliance to KA diets.

ANEMIA OF CHRONIC RENAL FAILURE: A COMPARISON OF PATIENTS ON AMBULATORY PERITONEAL DIALYSIS (PD) VS HEMODIALYSIS (HD). Stephen M. Korbet, Roger A. Rodby*, Edmund J. Lewis. Rush Medical College, Chicago, Ill.

The hematologic status of ESRD patients with hematocrits ≤ 30% on PD was compared to a similar group of HD patients. No patient received androgens or had an obvious secondary cause for anemia (ie. hemolysis, Fe deficiency, GI blood loss or aluminum intoxication). Twenty one PD patients (M/F 8/13) and sixteen HD patients (M/F 6/10) were evaluated. No significant difference in age (48.0±14.1 vs 46.6±14.4 (SD) years) existed between PD vs HD patients. A significantly higher ($P < 0.005$) hemoglobin (Hgb) (8.4±1.3 vs 7.3±0.9 gm) and hematocrit (Hct) (25.2±3.8 vs 21.7±2.8%) concentration was observed in PD vs HD patients. A Hgb concentration ≥ 9.0 gm was observed in 43% of PD patients as compared to 6% of HD patients. The Hct was ≥ 27% in 33% of PD patients but no HD patients had a Hct of ≥ 27%. Furthermore, 38% of HD patients had required transfusions (0.6 units/month/pt) vs 24% of PD patients (0.2 units/month/pt) in the preceding 6 months. Serum creatinine (13.1±4 vs 15.8±5.6 mg%) and BUN (60.4±19 vs 85.3±22.3 mg%) concentration was significantly lower ($P < 0.05$) in PD vs HD patients, but no significant difference in weight was demonstrated between the two groups (72.9±19.6 vs 78.0±26 kg). Our observations reveal significantly less bone marrow depression in the PD group. This results in fewer transfusions required by PD patients. The improved hematologic status of PD patients may reflect more efficient dialysis.

RENAL RESERVE (RR) IS INTACT IN CHRONIC RENAL FAILURE (CRF) AND IS INDEPENDENT OF ANGIOTENSIN II (A II) G.G. Krishna, G. Deuter, S.C. Kapoor, Temple University, Philadelphia, PA.

The increased GFR from acute protein loading (APL), the "renal reserve (RR)," is said to be mediated by A II and thought to diminish markedly with advancing CRF. We examined the RR in 8 normal (N) subjects and 12 patients with CRF. APL (1g/kg BW protein as beef steak) was given twice to each subject, pretreated with placebo (P) or enalapril (E) (10mg in N or 5mg in CRF) in a double blind randomized fashion.

	NORMAL				CRF			
	PLACEBO		ENALAPRIL		PLACEBO		ENALAPRIL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
C_{In}	90	118*	99	120*	31	40*	30	37*
	(8)	(6)	(6)	(8)	(6)	(9)	(6)	(8)
C_{PAH}	540	600*	518	653*	207	235*	203	255*
	(59)	(44)	(82)	(76)	(47)	(49)	(35)	(42)

*$p<0.05$ pre-APL vs post-APL; C_{In} and C_{PAH} indicate inulin and PAH clearances mL/min/1.73 m^2. Data are given as mean ± (SEM).

The mean percentage increase in GFR following APL in N (35±9 %) and CRF patients (36±12%) was equal and unaltered by E. RR was intact and similar in early CRF (GFR > 40 mL/min/1.73 m^2, n=5) (RR=29%) and advanced CRF (GFR < 40 mL/min/1.73 m^2, n=7) (RR=39%). In all groups APL reduced renal vascular resistance, while filtration fraction rose in all P groups and fell in all E groups. Plasma renin activity was unchanged by APL in all P groups while it increased in all E groups. We conclude that RR is intact in CRF. A II inhibition does not alter RR in normal or diseased kidneys.

RAPID CARBAMOYLATION OF TYROSINE AND PHENYLALANINE MEASURED BY RP HPLC IN VITRO AND EX VIVO IN CHRONIC RENAL DISEASE L M Kraus* and A P Kraus, Jr.* (intr. by F E Hatch) Depts. of Biochemistry and Medicine, University of Tennessee, Memphis, TN 38163

In chronic renal disease the elevated urea results in the formation of urea-derived cyanate which is available for carbamoylation of amino acids. Both tyrosine (Tyr) and phenylalanine (Phe) may be measured by RP HPLC at 215 nm without derivitization. Therefore, their modification by urea-derived cyanate to carbamoylated forms namely, N-C-Tyr, O-C-Tyr, N-O-C-Tyr and N-C-Phe in vivo has been measured. Without cyanate, concentration of Tyr or Phe does not change when measured at intervals from 5 minutes to 4 hrs. in vitro. However, in plasma from patients with chronic renal disease, Tyr and the three forms of carbamoyl Tyr are present and change upon ex vivo incubation at 37° without the addition of exogenous cyanate. The Tyr or Phe precursor cross-over curve with both N-carbamoyl forms, crosses at 5 to 8 minutes in vitro with the t1/2 appearing to be 5 minutes. This indicates that, in patients with chronic renal disease, cyanate available for in vivo carbamoylation of amino acids is in a dynamic state and is almost immediately removed through reaction with free amino groups.

DESCRIPTION AND PERFORMANCE CHARACTERISTICS OF A KIDNEY DISEASE SPECIFIC QUESTIONNAIRE (KDQ) IN A CLINICAL TRIAL OF RECOMBINANT HUMAN ERYTHROPOIETIN (EPO). Canadian Erythropoietin Study Group, Robarts Research Institute, London, Ontario (presented by A. Laupacis)

A KDQ for use in clinical trials was developed by asking 50 hemodialysis patients which aspects of their quality of life (QL) were affected by their disease and how important these were. The items with the highest frequency-importance product were incorporated into a 26 item questionnaire with 5 dimensions (physical function, fatigue, depression, relationships with others, frustration). The physical function dimension was patient specific. The KDQ was administered to 118 hemodialysis patients in a double-blind, placebo controlled trial of EPO, along with other QL measures (Sickness Impact Profile (SIP), time-trade off, stress test (ST), 6 minute walk (6MW). In placebo patients the test-retest reliability coefficient was .83-.98. There was a highly statistically significant improvement in the physical and fatigue KDQ dimensions in EPO treated patients compared to placebo, which was as great or greater than the other QL measures used. The t-statistics were KDQ fatigue 3.67, ST 3.53, KDQ physical 3.34, 6MW 2.39, SIP -2.35.

SUMMARY: The KDQ is a measure of QL that is reliable, responsive to change, and potentially useful in other therapeutic trials in hemodialysis patients. The actual questionnaire as well as its performance characteristics in the EPO trial will be demonstrated.

POOR CORRELATION OF RATES OF CHANGE OF CREATININE CLEARANCE, RECIPROCAL SERUM CREATININE AND GFR. Modification of Diet in Renal Disease (MDRD) Study Group (prepared by A.S.Levey, J.J.Gassman*, P.M.Hall, W.G.Walker), National Institutes of Health, Bethesda, MD.

The MDRD Study is a multicenter, randomized, controlled trial to determine acceptance, safety, and efficacy of low protein and phosphorus diets in patients with progressive renal disease. One of the aims of the feasibility phase was to compare methods for measuring progression.

Ninety-six patients aged 18-75 years, with previously declining reciprocal serum creatinine ($1/P_{Cr}$) and current ^{125}I iothalamate clearance (C_I) from 7.5-56 ml/min/1.73m^2, were randomly assigned four study diets. During follow-up (mean duration 11.6 months) C_I, creatinine clearance (C_{Cr}) and $1/P_{Cr}$ were measured every three months; rates of change (slopes) of C_{Cr} and $1/P_{Cr}$ were compared to C_I slopes. Values for Spearman (rank) correlation coefficients (r) and p are below:

initial C_I (ml/min/1.73m^2)	C_{Cr} slopes r	p	$1/P_{Cr}$ slopes r	p
25-56	.54	<.01	.36	NS
7.5-24	.42	<.01	.60	<.01

Mean change in $1/P_{Cr}$ slope after diet assignment was +0.0031 dl/mg/mo (slopes became less steep, p<0.05). Change predicted simply from regression to the mean is +0.0015 dl/mg/mo.

We conclude: 1) Correlations of C_{Cr} and $1/P_{Cr}$ slopes with C_I slopes are significant but weak. Factors other than changes in GFR, such as changes in creatinine secretion and metabolism, may affect C_{Cr} and $1/P_{Cr}$ slopes after dietary intervention. 2) Changes in $1/P_{Cr}$ slope after dietary intervention may be due, in part, to regression to the mean.

RENAL DISEASE AND ESRD DUE TO MESANGIALPROLIFERATIVE GLOMERULONEPHRITIS (mesGN) IN NAVAJO INDIANS: D Megill,*W Hoy, S Smith,*M Hughson*Phoenix AZ, Albuquerque NM and El Paso TX.

We have reported that mesGN of unknown etiology comprises a high proportion of Southwestern Indian renal biopsies. This study defines the pathology and clinical features of mesGN and its contribution to ESRD in the Navajo. The Navajos are the largest American Indian tribe, with a population of 200,000, and age-adjusted ESRD incidence 4 times that of US Whites.

Of 87 sequential Navajo renal biopsies, 55 or 63% showed mesGN. Additional changes were sclerosis in 66% capillary loop involvement in 66% and crescents in 42%. IF showed mesangial IgA, usually with IgM, in 88% of biopsies, IgM without IgA in 10%, and C3 in 95%. Mesangial electron dense deposits were found in 90% of those with preterminal biopsies. Mean age at biopsy was 33 years, and numbers of males and females were equal. At time of biopsy, 66% of subjects had renal insufficiency and >2 gm proteinuria, and 50% of those biopsied ≥5 years ago have reached ESRD.

Age-adjusted Navajo ESRD rates due to GN are 1.8 times those of the US Whites, and rates of Navajo ESRD due to GN and suspected GN together are double this. Of biopsied GN-ESRD Navajos, 63% have mesGN. Navajo GN-ESRD subjects are much younger at the start of treatment than the US GN-ESRD population (37 vs 53 years, p<.001). Their annual mortality rate with ESRD treatment is only 6.9%. GN and suspected GN comprised 38% of Navajo ESRD incidence and 58% of the ESRD prevalence in 1985, with Type 2 diabetes comprising 50% of incidence and 40% of prevalence.

Thus mesGN is extraordinarily prevalent and unusually aggressive in the Navajo, and is contributing a large portion of the ESRD load. Its associations, causes and possible treatment warrant further studies.

ANGIOTENSIN CONVERTING ENZYME INHIBITOR TREATMENT REDUCES PROTEINURIA IN CHILDREN WITH CHRONIC GLOMERULOPATHY. T. Linné*. Department of Pediatrics, Karolinska Institute, St. Goran's Hospital, Stockholm, Sweden.

Nine patients (age 16.6±3.8 years, range 9.6-21.0) with different forms of chronic glomerulopathy and constant proteinuria were put on angiotensin converting enzyme inhibitor (ACEI) treatment (captopril or enalapril). Before the start of treatment, the GFR (C_{In}) was decreased in five of the patients (range 10.5-79 ml/min/1.73m^2). The effects on urinary protein excretion, blood pressure and renal function were evaluated.

The 24-hour excretion of protein was invariably lower 1-4 months after the initiation of ACEI treatment (mean 0.5 g, range 0-1.2 g) than before (mean 1.8 g, range 0.1-7.3 g). After 12 months, 5 of 6 patients still had decreased protein excretion. The mean arterial pressure decreased in 6 of 9 patients (mean 99.1 vs. 89.3 mm Hg). This was also seen in some patients with normal blood pressure. The serum creatinine did not change.

Thus, the ACEI treatment invariably reduced the proteinuria. In most cases the effect was longstanding. Blood pressure also decreased in normotensive patients. Whether these effects will improve the long-term prognosis, however, has yet to be proven.

LONG-TERM TOLERANCE OF CYCLOSPORINE A (CsA) TREATMENT IN NEPHROSIS.

Alain Meyrier* and the Collaborative Group of the Société de Néphrologie (Intr. by G.S. Hill), Dept. of Nephrology, Hôpital Avicenne, Bobigny, France.

Efficacy of CsA in nephrosis (N) due to minimal change (MCL) or to FSG has been established. Most cases being CsA dependent, long-term tolerance remains to be assessed. Among 56 pts. with N, 18 have now been treated for 12 to 26 mo. Tolerance was judged by comparison of pre-CsA and final serum creatinine (Cr) and in 14, of initial and final histology. Initial Cr was 103±47 umol/L (49-210). Initial biopsy disclosed FSG in 5 and MCL in 9: 4 FSG and 3 MCL already had interstitial and/or vascular lesions. At final evaluation Cr was 89±32 (50-185). On biopsy 3 cases of MCL had developed FSG. Two were among those with pre-CsA interstitial or vascular lesions. In the 8 FSG, repeat biopsy showed interstitial and/or vascular lesions now present in 7, but typical lesions of CsA toxicity were found in only 2. Interestingly, in 2 cases final lesions were less than pre-CsA. Among the remaining 6 MCL, minor vascular and interstitial lesions were found in 2. Conclusions: 1) Development of vascular and/or interstitial lesions is mostly apparent in FSG and may be due to progression of disease more than to CsA toxicity. 2) Maintaining remission of N with CsA does not entail unreasonable renal hazards. 3) Nonetheless renal biopsy at 1 yr is advisable before deciding to continue CsA, whatever the apparent stability of renal function.

SERUM THYMOSIN β_4 (TB_4) LEVELS IN PATIENTS WITH CHRONIC RENAL FAILURE (CRF) S.D. Migdal, M.G. Mutchnick*, S.K. Mahajan, and D.K. Abu-Hamdan, and F.D. McDonald. Dept. of Medicine, Wayne State Univ. School of Medicine, Detroit, MI, and VA Med. Ctr., Allen Park, MI

TB_4 is a well characterized polypeptide originally derived from thymus gland and which has been shown to exert a suppressive effect on T cell responses and to induce macrophage inhibition. Since patients with CRF display a variety of immunologic abnormalities, we used an ELISA to measure TB_4 in the sera from 19 hemodialyzed patients (HD), 19 CAPD patients, and 11 nondialyzed CRF patients (NDCRF) [Serum creatinine range 2.8-10 mg/dl; mean 5.4 $\pm$ 2.8 SD]. Results were compared to normal controls. No correlation was found for either age or sex. The serum levels of TB_4 were significantly elevated compared to controls in: HD patients [181 $\pm$ 99 ng/ml VS 37 $\pm$ 16 ng/ml, ($p < 0.001$)], CAPD patients [115 $\pm$ 55 VS 34 $\pm$ 16, ($p < 0.01$)], and NDCRF patients [63 $\pm$ 49 VS 34 $\pm$ 16 ($p < 0.001$)]. Data from 5 HD patients pre- and post HD demonstrated no effect on TB_4 levels by dialysis. The NDCRF group had significantly lower TB_4 levels than either the HD ($p < 0.001$) or CAPD ($p < 0.01$) groups. Furthermore, HD patients had higher TB_4 levels than the CAPD group ($p < 0.001$). These data demonstrate that patients with CRF have increased serum TB_4, and that these levels are not altered by HD. This finding suggests a possible role for TB_4 in the immunologic abnormalities obvserved in patients with CRF.

RISK FACTORS FOR PROGRESSION AND RENAL SURVIVAL IN PATIENTS (pts) WITH CHRONIC RENAL FAILURE (CRF). L.Oldrizzi*,C.Rugiu*,G.Maschio - Div.of Nephrology University Hospital, Verona, Italy.

A series of 390 pts with CRF on long-term low-protein diet (0.6 gm/kg) was studied.CRF was due to interstitial nephritis (IN)in 112 pts,glomerulonephritis(GN) in 94,hypertensive nephrosclerosis(HN)in 44,PKD in 44,unknown etiology(UE)in 96.The initial SCr was 2.25±1.11 mg/dl.The compliance with the diet was good in all pts during the follow-up(54±28 months).Renal survival probability at 84 months(RS84)(renal death:SCr > 10 mg/dl) was 72% in overall population. RS84 were 79% in IN pts, 75% in UE,70% in HN,65% in GN,55% in PKD(Log-rank test 12.8, p 0.012).Progressive CRF(monthly increase in SCr > 0.02 mg/dl) was observed in 166 pts(43%),224 pts(57%)had no change in renal function.Four out of 31 clinico-biochemical variables were indicators of an increased risk for progressive CRF:initial SCr,proteinuria(Upr),systolic(SBP)and diastolic(DBP)blood pressure.RS84 adjusted by SCr were 86% (SCr< 2 mg/dl),61% (SCr 2-3),41% (SCr 3-4),32% (SCr > 4)(Log-rank 51.3,p 0.000).RS84 adjusted by Upr were 84% (Upr < 0.5 gm/24 hr),70% (Upr 0.5-1.5), 47% (Upr 1.5-3.0),40% (Upr > 3.0)(Log-rank 20.1,p 0.001).RS84 adjusted by SBP were 78% (SBP < 145 mmHg),63% (SBP > 145)(Log-rank 4.81,p 0.024).RS84 adjusted by DBP were 90% (DBP < 85 mm Hg),64% (DBP > 85)(Log-rank 9.7,p 0.002).The Cox multivariate analysis showed that 3 features were independent risk factors for renal death:initial SCr(Relative Risk-RR-4.09 if > 2 mg/dl) Upr (RR 2.63 if > 0.5 gm/24 hr),DBP (RR 2.44 if > 85 mmHg). Underlying renal disease,late dietetic and pharmacologic treatment,poor control of BP,proteinuria > 0.5 gm/24 hr seem to be important prognostic indicators in CRF.

URINARY PROCOAGULANT AND FIBRINOLYTIC ACTIVITY IN PATIENTS WITH PRIMARY IgA NEPHROPATHY (IgAN). RELATIONSHIP WITH THE SEVERITY OF DISEASE. L.F. Morrone, G. Pallotta, P. Montemurro, R. Triggiani, M. Colucci, N. Semeraro, F.P. Schena (intr. by S.N. Emancipator), Cattedra di Terapia Medica and Istituto di Patologia Generale, University of Bari, Italy.

Fibrin has been suggested to play an important role in the progression of IgAN. The deposition of fibrin in the kidney may result from pathological activation of blood coagulation and/or impaired removal by the fibrinolytic system. In this study we evaluated the urinary procoagulant (PCA) and fibrinolytic activity (FA) in 22 patients with IgAN and in 9 matched controls. PCA was measured in urine samples by a clotting assay and FA by a fibrin plate method. The calculated results for 9-h urine excretion of PCA (arbitrary units x 10^{-3}) and FA (urokinase (UK) units x 10^{-3}) are shown in the Table.

	Contr.(n=9)	Class I-II(n=13)	Class III-IV(n=9)
PCA	0.13 ± 0.1	0.21 ± 0.2	1.1 ± 0.8
FA	5.3 ± 2.3	6.4 ± 4.5	2.1 ± 2.0

PCA was significantly increased ($p<0.01$) and FA significantly decreased ($p<0.01$) in pooled class III-IV patients, as compared to class I-II patients. No correlation was found between these two parameters and proteinuria. The bulk of FA (<90%) was of UK-type (by immunological criteria and electrophoretic mobility). These data suggest that in IgAN the balance of PCA and FA in the kidney is shifted toward promotion of fibrin deposition. This could play a role in the progression of the disease.

RECOMBINANT-HUMAN ERYTHROPOIETIN (EPO) IN CHRONIC RENAL FAILURE (CRF): NO ADVERSE EFFECT ON RENAL HEMODYNAMICS OR PROGRESSION OF DISEASE. JA Opsahl, CE Halstenson*, KM Rachael*, PA Abraham, Henn Cty Med Ctr and Univ of MN, Minneapolis, MN.

Correction of anemia with EPO may worsen hypertension (HTN) and accelerate renal injury in experimental CRF. We studied the acute and chronic renal effects of therapy (Rx) with EPO in 8 pts (5m:3f, 4 diabetics, age 46±13 yrs) with CRF. Initially, pts were randomized to either placebo (P, n=4) or various doses of EPO (n=4). Hematocrit (Hct), serum creatinine (Cr), and para-aminohippurate (RPF) and inulin (GFR) clearances were assessed at baseline (B) and after 9±2 and 8±3 weeks of P or EPO, respectively.

Mean (SD); $^{*}p < 0.05$ EPO vs B; $^{+}p < 0.05$ EPO vs P

	Hct(%)	Cr(mg/dL)	RPF(mL/min)	GFR(mL/min)
B	28(2)	3.8(0.4)	79(21)	27(9)
P	29(3)	4.0(0.8)	71(42)	19(7)
B	32(3)	5.2(2)	73(88)	16(13)
EPO	37(2)*+	5.7(2)	91(76)	18(11)

Subsequently, all 8 pts were treated chronically with various doses of EPO. After 33±18 wks of EPO Rx, HCT increased from 31±5 to 39±3 %. Normotension (final BP 135±22/74±9) was maintained although HTN Rx was increased in 6 pts. In all pts, multiple linear regression analysis was performed to assess the effect of EPO Rx on the slope of 1/Cr vs time. In 4 pts the slope significantly decreased and in 4 no significant change in slope was observed.

Thus, treatment of anemia with EPO in pts with CRF and well controlled HTN does not appear to adversely effect renal hemodynamics or the rate of progression of renal disease.

THE EPIDEMIOLOGIC FEATURES OF TREATED END-STAGE RENAL DISEASE IN THE POPULATION OF A LARGE PRE-PAID HEALTH PLAN. Juan D. Ordonez, Robert A. Hiatt(*), Charles Quesamberry. Oakland, California.

We examined the incidence of treated End Stage Renal Disease (ESRD) among the 2 million members of the Kaiser Permanente Medical Care Program (KPMCP) of Northern California from 1973 through 1985. The collection of data on treated ESRD patients in this large, well defined, pre-paid health plan population has been complete since the inception of Medicare coverage of ESRD treatment in 1973. The age-adjusted incidence rates rose from 36.8 to 81.7 per million during that period, and showed no signs of stabilization. Rates were higher in men than in women, and in adult and older age groups than in younger ones. Glomerulonephritis, diabetic nephropathy and hypertension were the most frequent diagnoses, with rates of 15.4, 11.2 and 8.6 per million respectively. Significant ($P<.0001$) upward trends in the rates of diabetic nephropathy and hypertension were noted during this period. The continued increase in incidence of treated ESRD may be due to several factors, including a liberalization of the criteria for use of hemodialysis and changes in the natural history of some diseases leading to kidney failure.

LATENT DISTAL TUBULAR ACIDOSIS: A BEDSIDE CLUE TO THE DIAGNOSIS OF SYSTEMIC DISEASE IN THE COURSE OF CHRONIC RENAL FAILURE.
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Distal acidification is preserved in chronic renal failure (CRF). Inappropriately high urinary pH in the face of metabolic acidosis can be a clue to overlooked systemic disease. We studied renal acidification by a short NH_4 loading test in 86 pts with CRF. Group 1 comprised 64 pts with no evidence of systemic disease. GFR was 43 ± 24 ml/mn. Group 2 included 22 pts with systemic disease (autoimmune disease, with sicca syndrome (SS) in 10 and without SS in 11, "Randall" in 1). GRF was 59 ± 32 ml/mn. The degree of acidosis was the same in the 2 groups (bicarbonate 22 ± 4 mmol/L in Group 1, 20.5 ± 6.3 in Group 2). There was no intergroup difference in % of acid load excreted within 8 hrs of urinary NH_4 excretion. Titrable acidity was slightly lower in Group 2. The most clearcut abnormality was the inability in Group 2 to lower urinary pH, lowest value 5.85 ± 0.60 vs. 5.17 ± 0.43 in Group 1 ($P < 0.001$), provided serum bicarbonate was < 22 mmol/L. The same discrimination was found when urinary pH was simply determined in a morning fasting urine sample collected at bedside under mineral oil. This led us to suspect systemic disease in 4.

Conclusion: Fasting urinary pH > 5.6 in a pt with CRF whose total CO2 is < 22 mmol/L is an excellent clue to undertake diagnostic workup of systemic disease.

EFFECTS OF LONG-TERM CAPTOPRIL IN PATIENTS WITH MODERATE TO SEVERE RENAL FAILURE. Min Sun Park*, Kyung Soo Kim*, Seung Duk Hwang*, Hi Bahl Lee. Dept. of Internal Medicine, Soon Chun Hyang Univ. Hosp., Seoul, Korea.

The effects of the angiotensin converting enzyme inhibitor captopril(C) on BP, renal function and proteinuria were evaluated in 18 hypertensive patients(pts) with moderate to severe renal failure. The pts were followed for 6 to 15 months(mos) after C. Serum creatinine(Scr) and potassium(Sk), glomerular filtration rate by ^{99m}Tc-DTPA(GFR) and 24-hour urinary excretion of protein (UPE) were obtained before and every 3 mo after C.

In 7 pts (Group I: mean age 50 yrs) Scr rose from 5.1±1.3 to 10.3±1.7mg% and GFR fell from 24.7± 10.3 to 19.5±10.6 ml/min and dialysis was begun in 9.6 mo (6-15 mo) after C while in the remaining 11 pts (Group II: mean age 45 yrs) Scr (4.3±1.6 vs. 4.6±1.7) and GFR (32.4±13.4 vs. 35.5±15.6) remained stable over a period of 10.9 mo (6-12 mo). Control BP, Scr and GFR were not different between the 2 groups but Sk (5.4±0.94 vs. 4.4±0.75 mEq/L, P=0.025) and UPE(4.78±4.59 vs. 1.53±1.20 g/gcr, P=0.037) were signifcantly higher in Group I. BP control after C was similar in 2 groups. In Group I, Sk and UPE did not change after C. In Group II, Sk rose significantly after C (4.4±0.75 vs. 4.9±0.66, P=0.01) but remained within normal range and UPE fell significantly after C (1.53±1.20 vs. 0.60±0.36, P=0.017).

In conclusion, C appears to exert renal protective effects in the majority of pts with moderate to severe renal failure but may have deleterious effects in some pts with heavy proteinuria and elevated Sk.

CHRONIC AMBULATORY PERITONEAL DIALYSIS (CAPD) AS AN ALTERNATIVE TO HEMODIALYSIS FOR PATIENTS WITH END-STAGE RENAL DISEASE (ESRD) SECONDARY TO MULTIPLE MYELOMA (MM). Roger A. Rodby*, Stephen M. Korbet, Edmund J. Lewis. Rush Medical College, Chicago, Il.

Although the prognosis of patients with multiple myeloma is considered to be worsened when accompanied by irreversible renal failure, some groups have reported similar one year survival rates (50%) for hemodialyzed multiple myeloma patients compared to their non-uremic counterparts. Long term survival on hemodialysis is not uncommon. We report our experience with 3 patients with ESRD secondary to multiple myeloma who were maintained on CAPD from 1986-1988. The mean age at diagnosis was 60 years (50-65). All had Stage 3 disease. They consisted of one kappa light chain MM, one IgD kappa MM, and one IgG kappa MM. All had irreversible renal failure at diagnosis of multiple myeloma. All received intermittent chemotherapy with melphalan and prednisone. Two patients survived 16 and 25 months on CAPD before death. One patient is alive after 16 months on CAPD. There were 12 episodes (E) of peritonitis; one pt: 6E/25 mo, one pt: 5E/16 mo, one pt: 1E/16 mo. (mean 2.5E/pt/yr). Seven episodes were secondary to gram positive organisms and 5 were secondary to gram negative organisms. Six episodes were treated as in- patients and six were treated as out-patients. Although both deaths were secondary to sepsis neither were associated with peritonitis. CAPD appears to be a viable alternative to hemodialysis in the management of multiple myeloma patients with end-stage renal disease.

COMPARATIVE INCIDENCE RATES OF END STAGE RENAL DISEASE (ESRD) TREATMENT BY STATE. SJ Rosansky, TL Huntsberger, KL Jackson, PW Eggers, WJBD Veterans' Hospital and the Univ of South Carolina, Dept. of Medicine, Computer Science, & Public Health, Columbia, SC, and HCFA Baltimore, Maryland.

State specific direct adjusted incidence rates (DAIR) of ESRD treatment for sex and race subgroups as well as indirect adjusted rates by major primary etiology (IAR) of ESRD were calculated, utilizing data from 75,494 patients reported to Medicare. Utilizing a multiple regression analysis, state demographic and environmental factors were used to predict DAIR. DAIR rates for 1980-1983 varied from 45/million in North Dakota to 96/million in Washington D.C. For males and females, rates varied from 70-122 and 54-84 respectively. Regional patterns of DAIR were demonstrated. For blacks, DAIR were low (125-164 per million) in Southcentral states compared to Florida, Georgia, and New Jersey (rates 207-242 per million). For whites DAIR were relatively low in the Northwestern U.S. (all < 54 per million) compared to S.W. U.S. (all > 78 per million). Of 19 factors utilized in the regression model to predict DAIR, only the percent of a state's population residing in metropolitan areas ($p<.0001$, $r^2=.37$) and the gallons of gasoline consumed per capita ($p<.004$, $r^2=.06$) were predictive of DAIR. Examination of IAR by primary etiology as predictors of DAIR found diabetic nephropathy as the best predictor ($r^2=.63$). The reasons for the marked variation of diabetic nephropathy rates across the U.S. and the factors in gasoline consumption and metropolitan living that contribute to ESRD need to be explored.

RECOVERY OF RENAL FUNCTION IN PATIENTS WITH ESRD BY PRIMARY DIAGNOSIS. M.A. Sekkarie, F.K. Port, R.A. Wolfe and C.W. Ferguson. Univ of Michigan Ann Arbor, MI.

Even though the term ESRD denotes irreversibility, we observed recovery of renal function in cases reported as ESRD to the Michigan Kidney Registry. From 1976 through 1985, 7,860 patients entered chronic dialysis while residing in the State of Michigan. Of those who remained on dialysis for a minimum period of 30 days and excluding all cases of presumed ATN, 227 (3.0%) recovered renal function, i.e. alive and off dialysis for at least 30 days. Among 12 diagnostic categories examined the following were associated with significantly higher percentages of recovery than expected (P 0.005): Vasculitis and secondary GN (15.5%), RPGN (11.2%), SLE (9.8%) and myeloma (9.8%). Lower percentages of recovery than expected (P 0.005) were observed for diabetes (1.3%) and congenital cystic diseases (0.2%). Recovery usually occurred early but late recovery by treatment modality is of particular interest. We conclude that recovery of renal function from presumed ESRD is not rare in certain diagnoses. Therefore, in dialysis patients with such diagnoses, (1) caution should be used in the declaration of irreversibility and (2) renal function should be reassessed.

COMPARATIVE EFFECTS OF ANTIHYPERTENSIVES ON RENAL INJURY. ME Rosenberg and TH Hostetter, University of Minnesota, Minneapolis, MN.

Control of hypertension improves the course of chronic renal disease. To determine whether a differential effect of antihypertensives on renal injury exists, we examined the effects of two drugs in patients with renal disease. After a 21 day baseline period, 9 adult patients were randomly assigned in a cross-over study to two 28 day periods during which either a converting enzyme inhibitor (enalapril) or an α_1-antagonist (prazosin) was added to their existing antihypertensive regimen. At the start (Day 0) and end (Day 28) of each period 24 h urine collections followed by renal clearance studies were performed. Results (mean ± SEM; *p<.05 Day 0 vs Day 28;†p<.05 CEI vs α_1-antagonist):

	BP (mmHg)	GFR (ml/min/1.73m^2)	FRACTIONAL CLEARANCE ($\times 10^{-2}$) Albumin	IgG
CEI:				
Day 0	108 ± 3	29 ± 5	38.3 ± 12.4	13.0 ± 4.8
Day 28	94 ± 4*†	26 ± 5*	18.8 ± 5.3*†	5.7 ± 1.7*†
α_1-ANTAGONIST:				
Day 0	108 ± 3	29 ± 4	38.0 ± 10.8	11.6 ± 3.7
Day 28	98 ± 4*	28 ± 5	29.9 ± 8.4*	9.7 ± 3.2

Thus, despite a comparable degree of BP reduction, CEI resulted in greater improvement of glomerular permselectivity when measured during the clearance period. The 24 h excretion of albumin and IgG, however, decreased similarly with both drugs. Albumin excretion decreased from 2659 ± 812 to 1887 ± 617 during CEI and from 2492 ± 643 to 1722 ± 499 during α_1-antagonism (both $p<.05$), while IgG excretion went from 233 ± 95 to 194 ± 85 during CEI, and from 208 ± 68 to 173 ± 71 during α_1-antagonism (both $p<.05$). PRA was higher with CEI (5.6 ± 1.9 vs 1.3 ± 0.3; $p<0.05$). We conclude that reduction of BP reduces renal injury with perhaps a differential effect favoring CEI over α_1-antagonism.

APPLICATIONS AND LIMITATIONS OF RECIPROCAL SERUM CREATININE VS TIME PLOTS. B.V.Shah, A.S.Levey. New England Medical Center (NEMC), Boston, MA.

The slope of reciprocal serum creatinine vs time (1/cr vs t) has been used to assess the rate of progression of chronic renal disease, predict the interval until initiation of dialysis and judge the response to therapeutic interventions.

In order to determine the applicability of this practice in predicting the interval until dialysis, we reviewed all 70 adult patients initiating dialysis for ESRD at NEMC during a 3 year interval. Only 28 fulfilled selection criteria used by Mitch et al (Lancet 1976); only 21 (30%) had a linear decline in 1/cr vs t ($r^2 \geq 0.70$).

In order to determine the limitations of using a single slope of 1/cr vs t, we applied a method of linear regression analysis described by Jones and Molitoris (Anal Biochem 1984) to search for spontaneous changes in slope of 56 patients in 3 published studies in addition to the 21 patients from NEMC (mean follow-up interval 59 months). Spontaneous changes (breakpoints) were identified in all 77 patients. The change in slope was significant in 25 (32%); the second slope was less steep in 49 (60%); median value for serum creatinine at the time of the breakpoint was 5.4 mg/dl; mean change in slope (absolute value) was 0.0097 (adults) and 0.2751 (children) dl/mg/no; mean error in prediction of the interval until dialysis was ±20% of the follow-up interval.

We conclude 1) 1/cr vs t plots are applicable in only 30% of patients initiating dialysis; 2) in patients with a linear decline, spontaneous breakpoints are very frequent; 3) breakpoints limit the usefulness of this method for predicting the interval until dialysis and for judging the response to therapeutic interventions.

ONE-YEAR, ALTERNATE DAY DOSAGE, CORTICOSTEROID Rx REDUCES RATE OF FURTHER RELAPSES IN ADULT MINIMAL CHANGE NEPHROSIS

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Minimal change (MCL) nephrosis is usually steroid (CS)-sensitive but often follows a multi-relapsing course. Relapses might be due to a rebound effect after too short initial Rx or too abrupt termination of Rx. To test this hypothesis we compared 2 groups of adult MCL. Group A (14 F + 14 M, aged 32±17, range 13-69) was treated 2-4 months with 1 mg/kg/d prednisone. Group B (13 F + 13 M, aged 34±15, range 15-68) was treated with prednisone, 2 mg/kg every other day for 3 months, then dosage was progressively tapered to zero over the ensuing 9 mo. There were 3 CS-resistant pts in each group. In the other pts, initial remission rate was similar in the 2 groups. Conversely, there was a significant difference in subsequent rate of relapses (R): Group A, 1 R in 9 pts, 2 R in 7 pts, 3 R in 2 pts and multi-R in 10 pts; Group B, 1 R in 19 pts, 2 R in 2 pts, 3 R in 0 and multi-R in 5 pts. Finally, there were 8 indications for immunosuppressive Rx in Group A vs. 5 in Group B. Tolerance of Rx in Group B was excellent and in retrospect cumulative dosage of CS had been less in Group B than in Group A. In conclusion: long initial alternate day CS Rx followed by slow tapering is effective in obtaining protracted remission in adult MCL nephrosis, and long-term tolerance of such therapeutic mode is superior to the conventional schedule.

ATHEROGENESIS IN UREMIC C57BL/6J MICE

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This study was undertaken to determine if mice with chronic renal failure develop atherosclerosis. Uremia was induced in female C57BL/6J mice by the sequential electrocoagulation of the right renal cortex and left nephrectomy. After 6 weeks, during which time they were fed normal mouse chow, the mice were sacrificed and their aortas were examined for atherosclerotic lesions. At the beginning and end of the experiment, serum total- and HDL-cholesterol and triglyceride levels were measured and a lipoprotein electrophoresis profile was established. The results were compared with those from unoperated, normal-fed C57BL/6J mice and unoperated animals fed a diet containing 15% saturated fat and 2.5% cholesterol.

The aortas of uremic and atherogenic diet-fed mice were found to contain fatty lesions, which were approximately equal in size and number. These mice also exhibited a similar increase in serum total cholesterol level compared with unoperated, normal-fed animals. However, while uremic mice exhibited increases in HDL-cholesterol and triglyceride levels, the diet-fed mice showed significant decreases in these parameters. Furthermore, the predominant serum lipoprotein in uremic mice was found to be a slow-migrating HDL, whereas in diet-fed mice it was VLDL.

The results demonstrate that uremic C57BL/6J mice would provide a good model for the study of accelerated atherosclerosis in chronic renal failure.

GFR DETERMINATION IN CHRONIC RENAL FAILURE (CRF) BY 3 RADIONUCLIDE MARKERS AND INULIN (IN). Coefficient of Variation (CV) of the Methods.

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GFR was measured in 16 stable CRF patients (pts) on 2 study days one month apart to determine the reliability and reproducbility of different methods compared to each other. Simultaneous markers of Ytterbium (YTTB), Iothalamate (125-I) and Technitium (TECH) were compared to IN before and after a cold iothalamate (C-IO) infusion. Four collection periods in the morning (AM) and 4 in the afternoon (PM) were separated by 100 mg C-IO. Data are presented as the mean GFR, mean between period (BePe) CV(%) and mean natural logarithm (ln) CV, with the standard deviation (SD) for all markers.

Marker	GFR ± SD	BePeCV ± SD	lnCV±SD
YTTB	23.2±11.9	21.1±17.0	2.73±0.82
125-I	23.0±12.0	20.7±16.3	2.69±0.86
TECH	21.6±11.0	21.3±17.1	2.72±0.89
IN	20.5±11.9	22.6±18.1	2.78±0.89

YTTB and 125-I give larger values than TECH and IN, with YTTB always giving significantly larger values than IN. C-IO between AM and PM does not significantly change GFR values. Mean BePeCV by time and day is the same for all 4 markers and is variable. The between day CV is less than the BePeCV, ranging 11-16 %. for all 4 markers. Therefore, the mean GFR between days in the same pt is less variable than the BePe measurement on a given day.

"ENDOGENOUS FOX GLOVE" IN PATIENTS WITH RENAL FAILURE - INFLUENCE OF UREMIA AND HYPERTENSION.

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Since first discovered disturbing analysis of digoxin plasma levels in uremia, endogenous digitalis like substances (EDLS) have been found to exert biological activity on the Na-K-ATPase system and have been associated with hypertension. Various chemical identities have been suggested.

We studied 25 predialysis or dialysis patients, 11 with hypertension. A RIA method measuring percent inhibition of digoxin tracer binding in plasma was used. Fractionation according to water or lipid solubility was performed using SEP-Pak and acetonitril CH_3CN elution.

The uremic patients had higher levels than normal subjects of EDLS ($p<0.05$). These were present in mainly water soluble fractions (20% CH_3CN uremics 33.9% ± 11.6 vs normals 24.7% ± 7.6 $p<0.05$, 40% CH_3CN uremics 30.6% ± 4.7 vs normals 19.5% ± 4.3 $p<0.05$). One of these fractions in 40% CH_3CN was increased only in uremic patients with hypertension (41.3% ± 8.4 vs normals 27% ± 6.9 $p<0.001$, uremics without hypertension 31.9% ± 8.9 vs normals $p>0.05$).

Conclusions: EDLS are present in excess in uremic patients as mainly water soluble substances. Both renal failure and hypertension are associated with the increased plasma levels, but hypertension alone with one fraction eluted in 40% CH_3CN.

Do EDLS represent nature's attempt to defend against cardiac failure induced by uremia and by hypertension?

SIGNIFICANTLY HIGH QUANTITY AND LARGE SIZE OF SERUM IgA RHEUMATOID FACTOR IN PATIENTS WITH ACTIVE PHASE IgA NEPHROPATHY. Masatomo Yashiro,* Eri Muso, Munehiro Matsushima,* Kenji Sawanishi,* Haruyoshi Yoshida, and Chuichi Kawai.* Univ. of Kyoto, Fac. of Med., Dept. of Art. Kid., 3rd Div. of Int. Med. & Pathol., Kyoto, Japan.

To investigate the pathogenic role of IgA type rheumatoid factors(IgARF) in IgA nephropathy, serum IgARF was analysed and compared with the microscopic findings of renal biopsies. Serum levels of IgA and IgARF were examined in 39 patients of IgA nephropathy and 21 normal controls with ELISA plates coated with anti-human IgA antibody and IgG-Fc,respectively. Serum levels of IgARF were significantly higher in IgA nephropathy patients ($P<0.01$). In comparison with the light microscopic findings, the patients with active glomerular lesions including severe mesangial proliferation and crescent formation showed significantly higher levels of IgARF ($P<0.02$) which decreased with the improvement of renal damage. To investigate the size characteristics, the serial sera from one severely active case who was treated successfully to remission, were fractionated using high performance liquid chromatography (HPLC) in the buffer conditions of pH 7.0 and 3.5. In both conditions, the ratio of large size (>500kd) IgA and IgARF to monomeric forms were significantly higher in active phase than those in inactive phase, with more apparent tendency of IgARF. The acid fractionation of active phase serum showed remarkable shift of IgARF from large size to monomeric size. It could be concluded that the high amounts of large size IgARF in sera correlate with the active glomerular lesion forming immune complexes in IgA nephropathy.

THE HEMODYNAMIC AND COAGULATION RESPONSES TO dDAVP ARE HOMOGENOUS IN A LARGE GROUP OF MALE PATIENTS WITH CONGENITAL NEPHROGENIC DIABETES INSIPIDUS (CNDI): EVIDENCES FOR A SINGLE PATHOPHYSIOLOGICAL MECHANISM. D.G. Bichet, M. Razi*, D.J. Hirsch[1], S. Ligier*, M.F. Arthus*, M. Lonergan*. Nephrology and Research Center, Sacre-Coeur Hosp., Montreal and [1] Dalhousie University, Halifax, Canada.

Moses et al. (JCEM 1988; 66:1259) recently suggested on a single observation that two distinct pathophysiological mechanisms could be involved in patients (pts) with CNDI, an X-linked disorder: they studied a female polyuric pt resistant to the antidiuretic action but increasing normally her coagulation factors to the V_2 agonist, 1-desamino[8-D-arginine]vasopressin (dDAVP). We studied 14 male pts with CNDI and 11 female obligatory carriers of the gene for nephrogenic diabetes insipidus. Seven male pts have been reported earlier (Bichet, NEJM 1988; 318:881-7). These pts were from 8 families: 5 were French-Canadians, 1 Irish, 1 Iranian and 3 pts were from the Nova-Scotia-Hopewell pedigree (Bode, NEJM 1969; 280:750-4). None of the male pts had any hemodynamic response to dDAVP (no decrease in blood pressure or increase in pulse) and coagulation factors remained stable (Factor VIIIc, von Willebrand Factor, tissue plasminogen activator). Female obligatory carriers had low to normal hemodynamic and coagulant responses most probably due to random X chromosome inactivation ("lyonization"). We conclude that the V2-mediated hemodynamic and coagulant effects of dDAVP are always defective in male pts with CNDI and that female obligatory carriers could demonstrate normal responses.

URINARY EXCRETION OF LYSOSOMAL HYDROLASES (LH) BY RENAL GRAFT IN FABRY'S DISEASE (FD). S. Adler, R. Berty* and R. Glew*. Montefiore Hosp. and U. Pitt., Depts. Med. and Biochem., Pittsburgh, PA

FD, an inherited deficiency of alpha-galactosidase (AG), causes renal failure. Although renal grafts synthesize AG, most of it is excreted in the urine. Our earlier study showed that AG excretion was altered by acute acid-base changes in FD and normal controls. To determine the effect of chronic acid-base changes on LH excretion a 44 y.o. patient with FD and a renal graft ingested NH..4..Cl 0.06 g/kg daily for 7 days. Fractional excretion of AG (enzyme activity/creatinine) and 2 other hydrolases, N-acetyl-beta-glucosaminidase (NAG) and beta-glucuronidase (BG) were measured. Studies were performed before acid (PRE), after 6 days of acid (ACID) and on the sixth day post acid (POST).

RESULTS

	PRE	ACID	POST
Serum HCO3(mEq/l)	25	17	25
Urine NH3(ug/mgCr)	369	684	179
Urine AG(U/mgCr)	36.5	20.9	29.1
Urine NAG(U/mgCr)	304	245	348
Urine BG(U/mgCr)	92.0	60.6	84.5

Serum AG activity, however, did not change significantly. The data show that: (1) Urinary excretion of LH is altered by chronic acid-base changes. (2) Despite reduced AG excretion serum AG activity does not rise. (3) Renal tubular excretion and presumably processing of all 3 lysosomal enzymes is affected similarly by chronic A-B changes. These alterations in urinary LH show the danger of using LH excretion as a diagnostic marker for renal disease in the absence of knowledge of acid-base conditions.

ASSESSMENT OF A LINKED DNA MARKER FOR PRESYMPTOMATIC DIAGNOSIS OF ADULT POLYCYSTIC KIDNEY DISEASE IN NORTH AMERICA. J.E. Brissenden*, J.M. Roscoe and M. Silverman. Clin. Molec. Biol. Unit, Toronto General Hosp., and Div. of Nephrology, Wellesley Hosp., Toronto, Ont.

The present report represents an expansion of our early experience (Kidney Int. 33:184,1988) with the use of 3'HVR (Nature 317:542,1986) as a diagnostic probe of adult polycystic kidney disease (APCKD). We now report our results from 46 members of 12 unrelated families. Buffy coat DNA was extracted by standard methods and PvuII digests of the DNA were separated in 1% agarose gels, transferred to nylon membranes, hybridized with a ^{32}P-labelled, 4Kb HinfI fragment of the 3'HVR region in the plasmid pSP64 and autoradiographed. Polymorphic DNA fragments of 1-8kb correlated with the clinical picture of APCKD. The recomination frequency between APCKD and 3'HVR was 14% (2/14) in our completed families, higher than that reported for European families (4%). The two cross-overs were contributed by one family which when investigated further showed two additional crossovers. This family is being followed further to determine if they may represent an alternate genetic type of APCKD. This could be similar to the second genetic locus for APCKD (PKDf2) which has been suggested for two families of Italian descent (Rameo et al, Lancet 8601 ii:8, 1988).

NORFLOXACIN IN POLYCYSTIC KIDNEY DISEASE (PKD) PATIENTS. M. Carr*, L. W. Elzinga, A. Rashad*, W.M. Bennett. Depts. of Medicine & Microbiol., Oregon Hlth. Sci. Univ., Portland, Oregon.

Most antibiotics achieve subtherapeutic levels within cyst fluid, accounting for the frequent failure to eradicate cyst infection in PKD patients. Ciprofloxacin, due to its nonpolar lipid-soluble nature, is a notable exception (Elzinga et al, Antimicrob Agents Chemother 32:1988). Accordingly, Norfloxacin (Nor), a fluoroquinolone with similar properties, was given to 4 PKD patients (400 mg bid x 1 wk) and fluid from 56 cysts was aspirated for antibiotic determination. Cysts were identified as "non-gradient" (NG), "gradient" (G) or indeterminate (I) by [Na^+]. Mean ± SE Nor are:

	Total* (n=46)	NG* (n=22)	G† (n=15)	I (n=9)
Nor(μg/ml)	17.8±6	1.2±.1	38.0±14	3.2±.7
Nor cyst\serum	24.4	1.2	59.5	5.0

* Excludes 10 NG cysts with levels < 0.5 μg/ml.
† p<.01 vs NG.

Nor efficacy, judged by inhibitory/bactericidal activity (BA) of the cyst fluid vs E. coli and P. mirabilis, was usually excellent. Of particular note, fluid from 10 NG cysts failed to achieve detectable levels and BA (usually associated with low serum levels). An additional 83 cysts from 5 patients were sampled after Nor x 2d. With few exceptions, Nor levels and BA were undetectable. Conclusion: Nor preferentially accumulates in G cysts were it achieves high BA. Cyst entry is slow, and uniformly low (often subtherapeutic) levels (≤ serum) occur in NG cysts. Clinically, high Nor serum levels should be maintained to assure adequate levels in NG cysts.

GLOMERULAR HYPERFILTRATION (GH): AN EARLY MANIFESTATION OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD). A. Chapman,* A. Johnson,* W. Kaehny, R. Schrier, P. Gabow. Univ of Colo Hlth Sci Ctr, Denver, CO.

GH, defined as creatinine clearance (Ccr) >150 ml/min/1.73 m^2, occurs in diabetes mellitus, but has not been reported in other progressive renal diseases. Therefore, we assessed GH by 2 consecutive 24 hr Ccr in 213 ADPKD subjects and 155 family members without renal cysts (NADPKD). ADPKD subjects with GH clustered in the lower age range. 25% (3/12) of ADPKD subjects <18 yrs and 2.5% (5/201) >18 yrs manifested GH (p<0.007). Within the ADPKD group with GH the degree of GH decreased with age (p<0.041). Adult ADPKD subjects with GH had a smaller mean renal volume than adult ADPKD subjects without GH (355 ± 69 vs 705 ± 46 cm^3, p<0.05), suggesting less renal cystic involvement. The only ADPKD subject with GH studied serially had a fall in Ccr from 168 to 96 ml/min/1.73 m^2 from age 24 to 25 years. In the NADPKD group 11.8% (2/17) <18 yrs and 5.8% (8/138) >18 yrs manifested GH (p=NS). 6 of 10 NADPKD subjects with GH belong to a PKD family with the PKD-2 gene (not linked to 3'HVR on chromosome 16) with an apparent familial age of onset of ADPKD later than other ADPKD subjects (39 vs 32 years, p<0.009). 2 of 2 NADPKD subjects in whom gene linkage was informative had a 95% chance of carrying the PKD-1 gene. Therefore, we suspect that NADPKD subjects with GH carry a PKD gene but have not yet manifested renal cysts. Thus, GH appears to be an early manifestation of ADPKD, but not necessarily a bad prognostic sign.

ALPORT'S SYNDROME IS X-LINKED. Cyril Chantler, Frances Flinter* and Martin Bobrow*. Paed. Res. Unit, Guy's Hosp., London, England.

The clinical course and genetics of hereditary nephritis have been controversial for 30 years. We identified 41 clinically homogeneous families containing 192 individuals affected with the same disease characteristics as Alport's original family. Every family studied had evidence of at least three out of four of the following:

1. **Positive family history of haematuria with or without chronic renal failure**
2. **Electron microscopic evidence of extensive thickening and splitting of the glomerular basement membrane**
3. **Diagnostic eye signs (lenticonus or macular flecks)**
4. **High-tone, sensorineural deafness**

The progression of the disease was fairly uniform and predictable in males; but females showed more variation and although 15% developed CRF, the majority retained normal renal function throughout a normal life span. This sex difference is typical of X-linked inheritance. All female obligate gene carriers had haematuria.

Analysis of the pedigrees was entirely compatible with X-linked inheritance and there was no male-to-male gene transmission.

A formal gene linkage study showed linkage of the Alport gene to probe S21 (DXS 17) with a maximum LOD score of 4.72 at $\theta = 0.06$, confirming that 'classic' Alport's Syndrome is an X-linked disease.

DETECTION OF A FAMILY WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE LOOSELY LINKED TO DNA MARKERS FROM 16p. D.B. Dawson,* V.E. Torres, J.W. Charboneau, and S.N. Thibodeau,* Depts. Lab. Med. and Pathol., Intern. Med., and Radiology, Mayo Clinic, Rochester, MN.

Autosomal dominant polycystic kidney disease (ADPKD) has been shown to be linked to the α-globin gene cluster on 16p by Reeders et al. More recently, the DNA probe 24-1 (Breuning et al.) has been shown to flank the ADPKD locus. In this study, linkage analysis has been performed on 10 families segregating for ADPKD in an effort to further define the recombination frequencies between these 2 markers and the ADPKD locus. Furthermore, we have examined the linkage relationship of these to 2 additional loci defined by the DNA probes EKMDA2 and CMM65, which have also been mapped to this region of chromosome 16. The clinical diagnosis of ADPKD was based on the studies of Bear et al., while the markers used included DH7 and α-3'HVR (Weatherall), 24-1 (Breuning), EKMDA2 (White), and CMM65 (White). DNA was isolated from peripheral blood and the linkage analysis was performed using the program LINKAGE (Lathrop and Lalouel) with age dependent penetrant classes to allow for the possibility of false negative diagnoses which is increased in family members <30 years of age. Two point linkage analysis for the linkage groups ADPKD - α3'HVR, and ADPKD - 24-1 demonstrate loose linkage for one of the families examined, suggesting the presence of genetic heterogeneity. The possibility of heterogeneity for the disease locus which causes ADPKD is consistent with a recent report on an Italian family (Lancet July 2, 1988, 8).

ACUTE RENAL FAILURE DUE TO RENAL INFARCTIONS IN A PATIENT WITH NEUROFIBROMATOSIS. D.A.DiPrete* and J.G. Abuelo. RI Hospital and Brown Univ. Program in Medicine, Providence, Rhode Island.

VonRecklenhausen's neurofibromatosis is a hamartomatous disorder of neural crest tissue that can involve the skin, the eye, and musculoskeletal, vascular, and nervous systems. Renal involvement includes: coarctation of the abdominal aorta, renal artery stenosis (usually proximal), saccular aneurysm formation, and intraparenchymal vessel abnormalities leading to chronic stenosis and ischemic scarring. These lesions may cause renovascular hypertension; however, renal infarction and renal insufficiency have not been reported.

In May 1988 a 35 year old white female with neurofibromatosis and renovascular hypertension was admitted for acute left flank pain. She had a serum creatinine of 1.4 mg/dl, LDH 916 and the following angiographic abnormalities: hypoplastic right kidney and right renal artery, mid-pole infarct of the left kidney with two abruptly occluded interlobular arteries. The CAT scan suggested areas of new and old infarction in the left kidney. A left renal infarction was diagnosed. Approximately one month later, the patient presented again with acute onset left flank pain, creatinine 2.2 mg/dl and an LDH of 1328. Angiography at that time demonstrated a new, complete occlusion of the left upper pole renal artery secondary to thrombus with resultant infarction of that kidney segment. An attempted reperfusion with urokinase was unsuccessful. Echocardiogram showed no myxoma, vegetations or thrombus.

Comment: Radiographic evaluation defined two separate episodes of left renal infarction in a 35 year old female with neurofibromatosis and renovascular hypertension. Mild renal insufficiency resulted.

LONG-TERM OUTCOME OF RENAL TRANSPLANTATION (TX) AND FREQUENCY OF CYSTS IN THE RENAL ALLOGRAFT (GX) IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) AND CONTROLS. P.M. Fitzpatrick,* V.E. Torres, K.P. Offord,* H. Zincke,* J.W. Charboneau.* Mayo Clinic, Rochester, MN.

To determine the long-term outcome of renal TX for ADPKD, we reviewed the records of all pts with ADPKD transplanted between 1964 and 1987 (n=54) and of 107 pts without ADPKD or diabetes mellitus matched for age, sex, date of TX, and source of the GX (C). No differences in pt survival (ADPKD: 71, 59 and 47%; C: 71, 53 and 47% at 5, 10 and 15 years) and pt survival with functioning first GX (ADPKD: 59, 52 and 42%; C: 55, 44 and 33%) were observed. Causes of death were similar. One ADPKD pt had a ruptured intracranial aneurysm (1/318 ADPKD pt-years). No major morbid events related to native kidneys were observed in 13 ADPKD pts transplanted without bilateral nephrectomy (36 pt-years). To determine whether cysts recur in the GX, CTs with I.V. contrast and 1 cm cuts through the GX were obtained in 20 pts with good renal function (s.cr. 1.2±0.1) 10-22 yrs (14±1, x±SEM) after TX (10 ADPKD pts, 10 pts without ADPKD matched for age, sex, and date of TX). Five ADPKD pts (16 cysts) and 3 C pts (5 cysts) had cysts (NS). The source of the GX and the age of donors and recipients had no detectable influence on the number of cysts. Nonsignificant trends were noted in correlations of the number of cysts with the time from TX in C pts (p=0.06) and the male sex of the recipients (p=0.07). In summary: 1) the outcome of renal TX for ADPKD pts is not different from that for age- and sex-matched C pts, 2) no evidence for recurrent cystic disease in the GX was found but additional observations and longer follow-ups are needed.

ASSOCIATION OF HLA-DR4 WITH THE RISK OF OCCURRENCE OF CHRONIC HYPERTENSION AFTER HYPERTENSIVE PREGNANCIES. R. Fauchet,* P. SIMON,* G. Semana,* KS. Ang,* G. Cam,* I. Blanchard,* B. Cloup.* (Intr. by G.S. Hill), Hôpital La Beauchée, St. Brieuc and University of Rennes Immunology Research Group, Rennes, France.

Hypertensive pregnancy in 96 caucasian women including 50 with pre-eclampsia and 46 with gestational hypertension was analyzed in relation to HLA class II phenotypes. The risk of recurrence of disese and of occurrence of chronic hypertension in 71 patients who had later pregnancies and who were followed from 3 to 24 years (mean: 8.5 yrs) was studied. HLA-DR antigen distribution was determined by a search on B lymphocytes for the 10 antigens of locus DR. The normal population included 38 control couples with normotensive pregnancies and 200 healthy controls recruited from a local donor population. There was a higher occurrence of HLA-DR4 in women with pre-eclampsia (38%) and gestational hypertension (54.3%) than in female control subjects (5.3%, $p < 0.001$). When HLA-DR4 was considered in later pregnancies, there was evidence for an HLA-DR4-associated recurrence in women with gestational hypertension (62.5%). HLA-DR4 was also strongly associated with the risk of chronic hypertension after hypertensive pregnancies (73%, $p < 0.0001$, RR=39). The number of couples sharing DR4 antigens was signficantly higher in gestational hypertension couples (1/38, 2.6% $p < 0.01$) or pre-eclampsia couples (4/44, 9% $p < 0.05$). These data argue in favor of a non-fortuitous association of HLA-DR4 with hypertensive pregnancy and suggest that an immune response gene may be relevant to its pathogenesis and to the risk of future chronic hypertension.

INHIBITION OF URINARY CALCIUM OXALATE MINERALIZATION IN PRIMARY HYPEROXALURIA (H) DETECTED BY CALCIUM ION ELECTRODE. D.S. Fraley, F.J. Bruns, and S. Adler. Dept. Med., Montefiore Hosp. and Univ. Pittsburgh, Pittsburgh, PA

Renal stone formation is a serious complication of H. Using a calcium ion (Ca++) electrode method in which the electrode measures the rate of fall in Ca++ after addition of oxalate (OX) to a Ca++ containing urine, we previously showed a more rapid removal of Ca++ in fasting urine from idiopathic CaOx stone formers (ISF) compared to normals (N). Slopes of the Ca++ fall derived from urines standardized against saline controls are expressed as a calcium solubility index (CSI). The CSI of 10 N urines (1.60±0.09) differed from 10 ISF urines (0.69±0.17) ($p < .001$). The CSI, the urinary citrate (C) and urinary phosphate (P) concentration per mg creat. were measured in 12 adult members of an H family, 4 of whom have hyperoxaluria. The mean value for these 12 subjects and the N and ISF groups are shown:

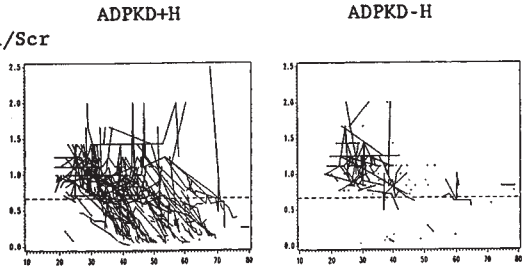
	Number	CSI	C/creat	P/creat
N	10	1.60	2.15	.40
ISF	10	.69**	1.82	.61
H	12	2.03*	2.93	.75+

**$p < .001$, +$p < .01$, *$p < .05$ compared to N

Interestingly, CSI and citrate excretion were greatest in the H members with hyperoxaluria. Thus, the data show that in H factors promoting solubility are enhanced rather than diminished. These results indicate that stone formation in H is due solely to the hyperoxaluria and that reduction in oxalate excretion or concentration should decrease stone formation.

HYPERTENSION: A DETERMINANT OF RENAL FUNCTION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD). P. Gabow, A. Johnson,* R. Jones,* D. Lezotte,* W. Kaehny, R. Schrier. Univ of Colo Hlth Sci Cntr, Denver, CO.

The effect of hypertension (H) on renal function was assessed in 293 ADPKD subjects with H (ADPKD+H) and 103 ADPKD subjects without H (ADPKD-H). Hypertension was defined as a history of H or a blood pressure >150/90 mmHg. Renal function, measured by 1/serum creatinine (1/Scr), is plotted against age.



Longitudinal data analysis was used to model a mean line of 1/Scr for each group. The slopes of the mean lines were similar, but the ADPKD+H group had significantly lower renal function for a given age than the ADPKD-H group ($p<0.001$). Thus, H alters the natural history of ADPKD with a significant detrimental effect on renal function.

STEROID SENSITIVE NEPHROTIC SYNDROME (SSNS) OF CHILDHOOD: EVIDENCE FOR GENETIC SUSCEPTIBILITY. Clara C. Lagueruela,* Thomas L. Buettner,* Alan M. Robson, John M. Kissane, and Barbara R. Cole. Washington Univ., Depts. of Pediatrics and Pathology, St. Louis, MO

Single alleles of the major histocompatibility complex (MHC), such as HLA-B8, B44, DR3, DR7, BfF and C4A*QO, have been found more frequently in SSNS patients than in the normal population, suggesting an inherited susceptibility to this disease. Extended haplotypes, the association of Class I, II, and III alleles of the MHC which segregate nonrandomly, may be helpful in pinpointing candidate susceptibility genes. To elucidate a possible genetic basis for SSNS in childhood, we analyzed the associated Class I, II and III alleles in 32 SSNS patients and their families. Standard methods were used for HLA typing and complotyping. Control groups were family non-disease-associated haplotypes, and the Caucasian population reported by the VIII Int'l. Histocompatibility Workshop.

Genotypic analysis revealed 346 haplotypes. Fifty percent of these SSNS patients had one or both of only 2 specific extended haplotypes. The first (HLA-B8, DR3,SC01) occurred in 11 of 64 haplotypes, 17% compared to only 3.8% in the control population ($P=0.008$). The second (HLA-B44, DR7, FC31) was observed in 10 of 64 haplotypes, 16% compared to 3.8% of the controls ($P=0.016$). Five patients had both extended haplotypes. Clinical data analysis showed that the affected individuals with one or both of the extended haplotypes had more frequent relapses than those without them ($P<.05$).

Thus we have described, for the first time, two genetic markers for SSNS. Future study may elucidate the pathogenetic relationship between the MHC and the disease.

THE ALPORT LOCUS ASSOCIATED WITH A DEFECT IN TYPE IV COLLAGEN NC1 DOMAIN MAPS TO REGION Xq21.3-q22. C.E. Kashtan, P. Szabo*, S. Rich*, A.F. Michael, B. de Martinville*. U. of Minn. Medical School, Mpls., MN, & New York Hospital-Cornell Medical Ctr., NYC, N.Y.

Pedigree analyses have shown X-linked dominant inheritance in most kindreds with Alport syndrome. Results of epidermal basement membrane (EBM) studies using an Alport-specific antibody that identifies an epitope in the NC1 domain of type IV collagen support this mode of inheritance (J Clin Invest 78:1035-1044, 1986). Affected males in most Alport kindreds completely lack this epitope, while it is partially absent in affected females. Atkin et al (A J Hum Genet 42:249-255, 1988) previously assigned the Alport locus to a region distal to the DXS3 locus defined by probe p19-2, located in the region Xq21.3-q22. We performed linkage analysis in 2 kindreds in which EBM of affected males lacked reactivity with the Alport-specific antibody. In family A with juvenile onset of end-stage renal disease (ESRD) 11 members of 3 generations were studied, including 2 affected males and 2 affected females. In family B (adult-onset ESRD) 14 members of 3 generations were studied, including 4 affected males and 4 affected females. Affected males were defined by persistent hematuria and affected females by having hematuria or an affected child. DNA purified from peripheral blood or lymphoblastoid cell lines was cleaved with the restriction endonucleases *Taq I*, *Pst I* and *Msp I*, and analyzed with the probes pDP34 (DXYS1), pXG3 (DXS96), pXG7 (DXS95), pXG12 (DXS94) and pXG17 (DXS91). pDP34, pXG3 and pXG17 were noninformative. For the informative probes pairwise analysis was performed with the ILINK program. pXG7, assigned to the region Xq21, was linked (theta = 0) to the Alport locus, although not with statistical significance (lod score = 1.8). pXG-12, assigned to the region Xq21.3-q22 (Hum Genet 77:172-174, 1987), was tightly linked (theta = 0) to the Alport locus, with statistical significance (lod score = 3.3). The gene order and distances between the loci are being investigated. These data support assignment of the Alport locus to region Xq21.3-q22 in families exhibiting a defect in the NC1 domain of type IV collagen.

THE CONCEPT OF POLYCYSTIC KIDNEY DISEASE AS A THRESHOLD TRAIT A.T.J. McDonald, D.E.C. Cole, S.R. Blecher, S. Digout, and J.F.S. Crocker. IWK Children's Hospital and Dept of Pediatrics, Dalhousie University, Halifax, NS, and School of Human Biology, University of Guelph, Guelph, ONT, CANADA.

Administration of hydrocortisone acetate (250 mg/kg) to newborn mice (n > 2100) caused PKD of varying proportions in each of 18 different inbred strains; none of the injected controls (n > 500) were affected. All kidneys were histologically examined and scored for degree of cyst formation using a semi-continuous (0 to 4+) grading scheme. Results suggested a threshold trait. For each strain, estimates of the mean (m) and standard deviation (s) for liability to PKD were determined by maximum likelihood methods. Subsequent analysis demonstrated significant goodness of fit of the observed data to normal distributions in the 9 strains tested and yielded the following results: 1) isolation and quantitation of a significant extraneous environmental effect--i.e., a change in threshold with a change in the source of the drug; 2) quantitative estimates of thresholds (in relation to liability means) that ranged from 0.93 ± 0.34 (95% CI) for B10.M (n=46) to -0.71 ± 0.12 for C57Bl/6J (n=297); and 3) separation of 8 of the 9 strains into 2 statistically homogeneous groups of different susceptibility and distribution of liability. This indicates a new method of analysis of the variable penetrance and expressivity of human PKD.

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE IN CHILDREN. PS Parfrey, J. Morgan, M.Singh, B.Cramer, J.Bear, . Memorial University of Newfoundland, St. John's, Canada.

To determine the prevalence of renal disease among young subjects at risk of autosomal dominant polycystic kidney disease (ADPKD), we studied 122 members of 17 families aged < 20 years. Among persons aged < 10 years, 40 were at 50% risk of inheriting ADPKD, of whom 9 (23%) had renal cysts on ultrasound. 18 were not at risk (cousins of persons at risk, whose potentially transmitting parent were aged > 30 years, and did not have renal cysts). In these three groups, blood pressure (BP) was similar for persons aged < 10 years. Among persons aged 10-19 years 45 were at 50% risk, of whom 12 (27%) had cysts. 19 were not at risk. Blood pressure was significantly higher in those with cysts (127 ± 18 / 80 ± 9 mm Hg SD) than in those at risk but without cysts (111 ± 13 / 71 ± 10) or those not at risk (116 ± 13 / 72 ± 9). 33% of those with cysts had BP > 2 SD above the general population mean for their age and sex, compared to 6% of those at risk without cysts and 0 of those not at risk. There were no differences in renal function or age among the three groups. Mean serum creatinine in those aged 10-19 who had cysts was 69 ± 23 umol/l (0.78 ± 0.26 mg/dl). We conclude that about half of persons with the ADPKD gene have renal cysts before the age of 20 years; in such persons BP starts to rise in the second decade, well before deterioration in renal function is apparent.

ATTITUDES ON GENE LINKAGE ANALYSIS (GLA) FOR PRENATAL TESTING OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD). E. Sujansky,* S. Kreutzer,* A. Johnson,* D. Lezotte,* R. Schrier, and P. Gabow. University of Colorado Health Sciences Center, Denver, CO.

ADPKD is a hereditary disorder for which there is now a possible prenatal diagnosis with GLA. There is little information on attitudes regarding its use. Therefore, we gave questionnaires to 141 affected (AF) and 137 at risk (AR) subjects from 107 families. The AR and AF groups were similar in sex (50% males vs 43% males, NS), mean age (39 ± 1 vs 42 ± 1 yrs, NS), and ethnicity (86% white vs 85% white, NS). 97% of AR would use GLA to define their own gene status and most AR and AF would use GLA to establish a diagnosis in their offspring (92% vs 89%, NS). Fewer would utilize it for fetal testing (FT) (52% AR vs 64% AF, $p<0.04$). GLA use for FT was not influenced by age, religion, education, income or severity of disease. Only 11% of AR and 7% of AF would terminate a pregnancy for ADPKD. 36% of AR and 31% of AF were uncertain about termination of pregnancy for ADPKD. However, 25% of AR and 25% of AF would terminate a pregnancy for a serious medical disorder in the fetus. No subject would terminate for ADPKD but not for a serious medical problem. Thus, although most AF and AR subjects would utilize GLA to define gene status in an offspring or a fetus, few perceived ADPKD as a reason to terminate a pregnancy. Therefore, it appears that FT with GLA will not significantly reduce the frequency of ADPKD.

COAGULATION FACTORS AND PLASMA RENIN ACTIVITY (PRA) ARE STIMULATED BY ADRENALINE BUT NOT BY dDAVP IN PATIENTS WITH CONGENITAL NEPHROGENIC DIABETES INSIPIDUS (CNDI). M. Razi*, M. Lonergan*, M.F. Arthus*, and D.G. Bichet. Nephrology and Research Center, Sacré-Coeur Hosp., Univ. of Montreal, Montreal, Canada.

We recently showed that male patients with CNDI did not decrease their blood pressure nor increase their PRA and coagulation factors in response to the antidiuretic V_2 specific agonist 1-desamino[8-D-arginine]vasopressin (dDAVP) (D. Bichet, N. Engl. J. Med., 318: 881-887, 1988). In normal subjects, Factor VIIIc (FVIIIc), von Willebrand factor (vWF) and tissue plasminogen activator (t-PA) are stimulated by dDAVP and by adrenaline. The purpose of the present study was to demonstrate the specificity of the absence of responses to dDAVP in CNDI patients. We infused adrenaline (5 ug/kg body weight during 15 to 25 minutes) in 3 brothers (age 29 to 31) with CNDI. FVIIIc, vWF and t-PA increased from 75 to 100% ($p < .01$, Dunnett) and PRA increased by 130% ($p < .05$, Dunnett). None of these parameters changed when the same subjects received dDAVP (0.3 ug/kg body weight intravenously in 20 minutes). Since the hemodynamic and vasodilatory responses to dDAVP have been observed in anephric dogs (Liard, Clin. Sci., 74: 293, 1988), these results demonstrate the specificity of the extrarenal V_2 receptor defect in patients with CNDI. The stimulation of intact V_2-like receptors are responsible for the vasodilating and coagulant effects of dDAVP. These V_2 receptors or the V_2-receptor-transduction process, or both, are defective in CNDI pts.

INDOMETHACIN IN THE TREATMENT OF LITHIUM-INDUCED NEPHROGENIC DIABETES INSIPIDUS (NDI). Henry M. Allen,* R.L. Jackson*, M.D. Winchester*, L.V. Dack*, M. Allon. Dept. of Med., Univ. of Okla HSC and VAMC, OKC, OK

Prostaglandins (PG) have been implicated as a factor in the polyuria of lithium-induced NDI. We report the successful use of indomethacin, a PG synthetase inhibitor, in reducing the urine output in a patient with severe lithium-induced NDI. Following long-term lithium carbonate therapy, the patient was hospitalized because of marked polyuria and dehydration (24 hour urine volume, 6.9L, plasma osmolality 347 mOsm/kg). NDI was confirmed by inappropriately dilute urine (189 mOsm/kg) following water deprivation, and lack of improvement with aqueous vasopressin. A single dose of indomethacin, 50 mg orally, resulted in a 75% reduction of urine volume and doubling of U_{osm} to 408 mOsm/Kg. The effect was sustained for at least 5 hours, and independent of change in GFR. Serial 24 hour urine collections were obtained during 3 days on indomethacin 50 mg tid, followed by 3 days off the drug, followed by 3 days on the drug. Urine outputs were: 3.4, 6.8, and 3.1L/day and U_{osm} were 121, 98, and 134 mOsm/kg, respectively, during these experimental periods. Following 3 months of chronic indomethacin therapy, urine volume was 2.9 L/day, and U_{osm} was 174 mOsm/kg. Indomethacin was held for 3 days and repeat values were 4.6 L/day and 114 mOsm/kg, respectively. GFR (C_{Cr}) was stable. These observations suggest that PG may play an important role in the vasopressin-resistance of lithium-induced NDI, and that PG inhibition may alleviate the polyuria.

GLOMERULAR ATRIAL NATRIURETIC FACTOR (ANF) RECEPTORS IN PRIMARY GLOMERULOPATHIES: STUDIES ON HUMAN RENAL BIOPSIES. A.C. Bacay*, C.R. Mantyh*, A.H. Cohen, P.W. Mantyh* and L.G. Fine. Departments of Medicine and Pathology, UCLA School of Medicine, Los Angeles, CA.

To determine whether differences in glomerular ANF binding exist in different forms of primary renal disease, we used quantitative autoradiography of ^{125}I-human ANF to determine the characteristics of ANF binding sites in the normal human kidney. Specific ANF binding was highest in the glomeruli but lower levels of specific binding were localized to the inner medulla and the interlobular arteries. K_d for glomeruli, inner medulla and interlobular arteries were approximately identical at 4.0×10^{-11}M. Competitive binding inhibition studies with unlabeled human ANF demonstrated highly specific binding shared by the glomerulus, inner medulla and interlobular arteries; the apparent half-maximal inhibition concentrations (K_i) being 9.2 , 8.0 and 8.2×10^{-10}M, respectively. Needle biopsy specimens of minimal change disease (MCD), membranous nephropathy (MN) and focal glomerulosclerosis (FGS) showed no differences in ANF binding between the different groups. Conclusion: This study demonstrates the feasibility of studying receptor physiology on biopsy specimens of the human kidney. The absence of significant differences in glomerular ANF binding in the MN, MCD and FGS, is consistent with recent studies which have failed to delineate important pathophysiologic differences in renal function and volume homeostasis in these disease states.

EFFECTS OF ANGIOTENSIN OR THROMBOXANE RECEPTOR ANTAGONISM ON GFR IN RATS WITH CONGENITAL HYDRONEPHROSIS. Frank G. Boineau, R.C. Vari, L. Ziegler*, P.J. Kadowitz*, and J.E. Lewy. Tulane University School of Medicine, New Orleans, Louisiana.

Traditional urinary clearance measurements can underestimate GFR in hydronephrotic kidneys due to residual pelvic fluid dilution of the clearance substance in the urine. To obviate this problem GFR was measured by a constant infusion-technique not requiring collection of urine. From an inbred colony of Wistar rats in which over 80% of males have unilateral right hydronephrosis four groups of rats were studied. At 10 weeks of age the left normal kidney of all rats was removed and 4 weeks later GFR was measured in GR1 (n=6), rats with a normal kidney; GR2 (n=12), rats with a congenital hydronephrotic kidney; GR3 (n=7) and GR4 (n=5) rats with a hydronephrotic kidney that were systemically infused with [SAR^1; Val^5,Ala^8] AngII, an angiotensin II receptor antagonist, or SQ 29548, a thromboxane receptor antagonist, respectively.

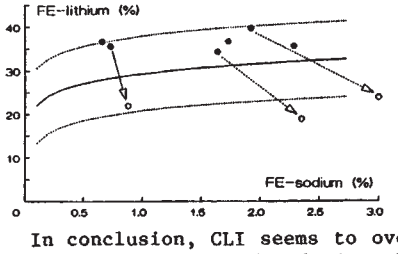
Results:

GFR (ml/min)	GR1	GR2	GR3	GR4
	1.96±.12	1.48±.10*	2.12±.15	2.31±.19

The GFR of GR2 was significantly (*$P \leq .001$) less than GR1 control rats. The GFR of GR3 and GR4 rats were both significantly greater than GR2 rats and were not different ($P \geq .05$) from the GFR in the control rats of GR1. These data confirm a significant reduction in GFR associated with congenital hydronephrosis and provides further evidence that this reduction in renal function is mediated by angiotensin and thromboxane mechanisms.

PARADOXICAL HIGH LITHIUM CLEARANCE (CLI) IN BARTTER'S SYNDROME (BS). W.H.Boer*, R.J.Hene*, H.A. Koomans*, E.J.Dorhout Mees. Dept. of Nephrology, Univ. of Utrecht, The Netherlands.

Presumably, the abnormalities in renal Na handling in BS are a primary defect in Na reabsorption in the diluting segment and a secondary fall in Na delivery to this site. Thus, one would expect CLI, advanced as an index of Na output from the proximal tubules, to be low in BS. We studied CLI on 6 occasions in BS, 3 times also during chronic converting enzyme inhibition (CEI), which reversed hypokalemia (hypo-K) (pK: 2.4 -> 3.9 mmol/l). We simultaneously measured the maximum urine flow during water diuresis (Vmax), an index of Na and H_2O delivery to the diluting segment. Vmax/GFR was reduced (11.3±4.0%; normal value: 15.3±1.4%); no change occurred during CEI. In contrast, CLI/GFR was in the high normal range, falling below normal during CEI:



● = control
o = CEI (enalapril)

(dotted lines: 95% confidence limits in 204 controls)

In conclusion, CLI seems to overestimate distal Na delivery in BS. Reduced Li reabsorption in Henle s loop (hypo-K induced rise in prostaglandin synthesis ?) could be the cause, since CEI (not affecting CLI in normal subjects), corrected hypo-K and strongly enhanced tubular Li reabsorption. Consequently, CLI may not correctly reflect Na and H_2O delivery from the proximal tubules in man.

SUSTAINED REMISSION OF MEMBRANOUS GLOMERULONEPHRITIS (MGN) WITH CYCLOPHOSPHAMIDE (CY) AND PREDNISONE (P) TREATMENT (Rx). F.J. Bruns, S. Adler, D.S. Fraley, and D.P. Segel. Dept. Med., Montefiore Hosp., and Univ. Pitt., Pittsburgh, PA

To evaluate effects of Rx, 9 consecutive MGN patients with at least 6 months documented progressive renal functional decline were given CY, 100 mg qd for 12 months. Eight also received P, 1.5 mg/kg qod initially, then tapered. Initial plasma creatinine ranged from 2.1 to 4.2 mg/dl and decreased in every patient by 6 mo post Rx. Each patient served as his own control. Five patients were followed 1 year and 3 patients 2 years after discontinuation of Rx. Two years after Rx 1 patient again had renal functional decline so Rx has been restarted. Mean values for the 9 patients are:

	at Rx	6 mo	12 mo	24 mo	36 mo
Number patients	9	9	5	5	3
Pcreat mg/dl	2.8	1.9**	1.8*	1.8*	1.8
Palbumin g/dl	2.5	3.4**	3.7**	4.0*	3.9*
Pcholesterol mg/dl	462	375	268	262*	268*
Uprotein g/24 hr	13.6	7.0**	2.3*	3.0*	3.6*

*$p < .05$, **$p < .01$ versus at Rx value

In each patient the final Pcreat was less than the initial value. Edema disappeared in the 8 patients with edema pre-Rx. Repeat renal biopsy done in 3 of the 5 followed for over one year showed persistent changes of MGN by light and EM. Thus, despite histologic persistence of MGN, Rx stabilizes renal function and markedly improves the course of MGN. These results show that CY plus P can induce prolonged remissions in MGN even when renal function is already impaired.

RESULTS OF A CONTROLLED TRIAL OF ALTERNATE DAY PREDNISONE (P) IN MEMBRANOUS NEPHROPATHY (IMGN).
D.C. Cattran, E. Cole, C. Cardella, R. Charron, J. Roscoe, T. Delmore, S. Ritchie and the Toronto Glomerulonephritis Study Group, Dept. of Medicine. University of Toronto, Canada.

Either 6 months of alternative day P 45mg/m^2 or no specific therapy were given to 158 patients with IMGN. Mean follow up 48 ± 3.2 months. There were 77 controls (C) and 81 treated (T) patients. At presentation their age, duration of disease, creatinine clearance (CrCl), serum albumin, hypertension % and pathological stage were similar. The % of females in the control group was greater (p=0.03). Creatinine difference (p=0.05) and proteinuria (p=0.01) were less in the control patients. The rate of deterioration in renal function as measured by the slope of the CrCl (mls/sec/yr) was not different in the C versus T group at 6 months (-0.05 vs -0.04 p=0.7, β=.24) or at last follow up (-0.02 vs -0.07 p=0.2, 95% CI -0.03 to 0.13, β=.01). Similarly, complete remission of proteinuria (<0.3gms/day) was not different at 6 and 12 months or at last follow up. Multivariate analysis which adjusted for entrance sex, proteinuria and creatinine failed to provide an explanation for the lack of steroid effect. Five failed to complete 6 months of therapy, 3 withdrew, 1 myocardial infarction, 1 mood variation. Minor adverse effects were seen in 22% and included cushingoid features, bloating, acne, mood swings and nausea.

Actuarial survival was the same in both groups at 5 years (93%) and 8 years (90%). Eleven patients developed renal failure or died during the study, 5 in C and 6 in T group.

We conclude that 6 months of ADS in patients with IMGN confers neither a short nor long term benefit on preservation of CrCl or on severity of proteinuria.

RED BLOOD CELL (RBC) CHARGE IN MINIMAL CHANGE NEPHROPATHY (MCN). H.T. Cohen, A.K. Singh, B.S. Kasinath, E.J. Lewis. Rush Medical College, Chicago, IL.

The functional and morphological lesions of the glomerulus in MCN are believed to be the result of decreased glomerular epithelial cell surface anionic charge density. Others have proposed that a circulating factor capable of reducing cell surface charge either by neutralization or by reducing synthesis of charged moieties may be responsible for the lesion. These investigators reported a reduction in RBC charge in MCN patients based on an indirect technique of Alcian blue binding. In this study, we have retested the hypothesis by directly measuring RBC surface charge using (1) cell electrophoretic mobility (EPM) in microns/sec/ volt/cm and (2) sialic acid content by slug lectin binding assay. RBC were sampled from MCN patients (n=8), patients with other glomerular lesions and nephrotic syndromes (NS) (n=14) and healthy controls (C) (n=17).

GROUP	EPM	SIALIC ACID
MCN	2.66*	4.23 units
NS	2.62	4.00
C	2.51	4.09

*p is < 0.01 compared to control

These data show that in MCN, RBC charge is not decreased but may be slightly higher than normal. Our results fail to support the hypothesis that in MCN there is a circulating factor which is capable of diminishing cell surface charge as a generalized systemic phenomenon.

HYPOURICEMIA (HU) IN AIDS. A.J. Cusano*, H.L. Thies F.P. Siegal*and J.K. Maesaka. Dept. of Med., Long Island Jewish Med Ctr, New Hyde Park, NY.

HU, serum uric acid ≤ 3.0 mg/dl, was frequently observed while evaluating patients with HIV infection. A review of 96 consecutive patients with AIDS or ARC seen at our institution between 1985 and 1988 revealed that 21 patients (22%) had HU. Patients with HU had an increased incidence of opportunistic infections (OI) (2.24 ± 0.21 vs 1.43 ± 0.09 OI in non-HU patients; $p < 0.0002$). HU patients also had a higher mortality than their cohorts without HU($p<0.02$). Of the 21 HU patients 16 (76%) died during a mean follow-up period of 17.3 ± 2.6 months vs the non-HU group in which 29 out of 75 (39%) died during 20.6 ± 1.6 months of follow-up ($p<0.01$). No correlation was found between HU and any specific type of OI, malignancy, drugs, or SIADH. Fractional excretion of uric acid (FEua; normal < 10%) was assessed in a randomly selected subset of 23 patients. Elevated FEua (range 11.9% to 61.5%) was found in 19, 12 of whom had HU. Twenty-four hour total UA excretion was assessed in 10 patients and was normal in 7 and above normal in 3. Six patients with HU also had orthostatic hypotension. Central venous pressure (CVP) measurements in these 6 were low (0-1cm H20) and 4 of them also had elevated renin levels suggesting significant volume depletion. Clearance studies revealed elevated FEua in each patient (range 12.2% to 26.9%).

The high incidence of HU is unexpected as is its association with increased mortality. HU was associated with elevated FEua even as volume depletion occurred when decreased FEua is expected. This data suggests abnormal renal tubular handling of UA in patients with HIV infection.

ASSOCIATION OF SEGMENTAL CRESCENTIC GLOMERULONEPHRITIS (SCGN) WITH AUTOANTIBODIES DIRECTED AGAINST DIFFERENT LYSOZOMAL ENZYMES. J.W. Cohen Tervaert*, R. Goldschmeding*, J.D. Elema* G.K. van der Hem*, C.G.M. Kallenberg* (Intr. by L.W. Statius van Eps) Univ. Hosp. Groningen and CLB, Amsterdam, The Netherlands.

To test the hypothesis that SCGN is part of the spectrum of systemic vasculitides we evaluated the clinical and serological data of 28 consecutive patients with SCGN biopsied between June 1984 and March 1988. Patients with anti-GBM-nephritis, SLE-nephritis and Henoch-Schonlein purpura were excluded. Autoantibodies were tested with a "sandwich" ELISA in which mouse monoclonal antibodies directed against the anti-neutrophil cytoplasmic antibodies (ANCA)-antigen, myeloperoxidase (MPO) and elastase were used as catching antibodies. 9 patients had biopsy proven WG, 13 patients were clinically suspected of WG, 3 patients had idiopathic SCGN, and 3 patients had SCGN secondary to an infectious origin. Antibodies to lysozomal enzymes were found in 25 out of 28 patients (table).

	Biopsy proven M.Wegener	Suspected M.Wegener	Idiop. SCGN	Infectious SCGN
ANCA	9/9	11/13	1/3	0/3
MPO	0/9	2/13	2/3	0/3
elastase	0/9	0/13	0/3	0/3

In sera from patients with other renal lesions (n = 41) and from normal donors these autoantibodies could not be detected. In conclusion, SCGN of non-infectious origin is associated with either ANCA or anti-myeloperoxidase antibodies. These antibodies may be an early marker of systemic vasculitis. Our data suggest that SCGN is part of the spectrum of systemic vasculitides.

IGG CIRCULATING IMMUNE COMPLEXES (CIC) AND THE PROGRESSION OF IGA NEPHROPATHY. M.K. Dasgupta,* L.D. Jewell,* K. Solez. Departments of Medicine & Pathology, University of Alberta, Edmonton, Alberta, Canada.

Although raised serum IgA and IgA-containing CIC are common in IgA disease, their relationship to renal functional deterioration has not been established. Since the original descriptions of IgA disease emphasized codeposition of IgG, we have investigated the role of IgG CIC in disease progression. IgG- and IgA-containing CIC were analyzed by Raji-cell radioimmunoassay in 126 blood samples prospectively collected over a three year period from 24 biopsy-proven adult IgA disease patients. The 8 patients who developed IgG CIC (persistent in 4) had a significantly higher histologic activity score on biopsy reflecting severity of glomerular lesions and IgG deposition by immunofluorescence ($4.5\pm.4$ versus $2.7\pm.4$ on a $0\text{-}8\pm$ scale, $p<.01$). Three of the 8 patients in the IgG CIC group had crescents on biopsy, a feature not found in any of the other patients. These 8 patients with IgG CIC also had significantly higher levels of serum creatinine than patients with no IgG CIC (195 ± 40 versus 116 ± 14 umol/L, $p<.05$). There was a significant correlation between the level of IgG CIC and serum creatinine ($r=0.62$, $p<.001$) but no similar relationship was found for IgA CIC ($r=0.23$, NS). Serum IgA levels were correlated with IgA CIC ($r=.66$, $p<.001$) but serum IgG levels did not correlate with IgG CIC ($r=0.09$, NS). We conclude that although IgA CIC predominate in IgA disease patients, IgG CIC are more closely related to deterioration in renal function and histologic progression.

APPARENT RENAL RESPONSE TO MELPHALAN AND PREDNISONE (MP) IN LIGHT CHAIN DEPOSITION DISEASE (LCDD). R.L.Heilman, Keith Holley, Ken Offord,* J. Velosa, R. Kyle.* Mayo Clinic and Foundation, Rochester, Minnesota.

We reviewed our renal biopsy records and selected all 19 cases that met our criteria for LCDD. Patients had to have monoclonal deposition of LC in renal tissue (15 kappa, 4 lambda) and evidence of monoclonal LC production in serum, urine or by bone marrow histochemical studies. Mean age was 60 yrs (range 37 to 77). Hematologic diagnoses were multiple myeloma in 6 Waldronstroms macroglobulinemia in one and LCDD in 12. Three patients had no demonstrable monoclonal protein on repeated studies of both serum and urine. 11 had a classic mesangial nodular lesion and 3 had membranoproliferative glomerulonephritis. Follow up (F/U) was available in 18.

	6 months (m)	1 year	3 years
Patient survival	100%	91%	76%
Renal survival	82%	66%	40%

Six presented with initial serum creatinine (Scr) > 10.0 mg/dL. Mean F/U was 26 m. One patient regained some function and another died. The remainder progressed to ESRD. Response to cycled therapy with MP in the remaining patients is illustrated in the following table.

Initial Scr	Number	Mean F/U m	Renal function worse	stable	Death
4.1-10.0	5	13	4	1	1
1.8-4.0	7	28	1*	6(2 improved)	0

(*Received Prednisone only)

We conclude that in patients with LCDD presenting with Scr < 4.0 mg/dL, therapy with MP may stabilize or improve renal function.

MEAN CORPUSCULAR VOLUME (MCV) OF GLOMERULAR (GN) HEMATURIA (HMT). P. Goldwasser*, A. Antignani*, N. Mittman, F. DiPillo*, M. M. Avram. The Long Island College Hospital, Brooklyn, New York

Red cells (RBC) of GN origin have been shown to have low MCV values on sizing in isotonic hematology diluent (IsoD). RBC may lose volume on deforming in passage across the GBM. Distal tubule (DT) hypotonicity (HT) may also contribute to RBC injury. The influence of urinary (U) composition on MCV has not been studied. In 6 cases of GN HMT we measured U Na, K, Cl, Na+K, Na+K-Cl, osmolality, pH, MCV, and peripheral (P) MCV. Mean U-MCV was 55±6.0[SD]fl. U-MCV (normalized to P-MCV) correlated only with pH ($r=-.87$ $p<.06$). In one case we studied the effect of furosemide diuresis (FD). FD increased MCV from 54 (pre-FD) to 93fl (peak-FD). The increase correlated strongly with the changing urinary composition and weakly with flow rate. On incubation in IsoD the enlarged RBC lost most but not all the increase, returning to microcytic range (<70fl) with a $t_{1/2}$ of 3 min. Since FD partly corrects the low MCV, we propose that GN RBC lose volume in a FD-insensitive step (GBM passage) and a FD-sensitive step (HT injury in DT). One might wonder how RBC, in the absence of FD, survive such extreme HT without total hemolysis. We speculate that RBC are protected from total HT hemolysis by a viscous coat of Tamm-Horsfall protein which allows loss of solute and water, but not hemoglobin, under HT stress. FD both abolishes HT injury and reversibly enlarges GN RBC. The reversible effect may be due to RBC buffering of H and Cl in the acidic DT, buffer-poor due to FD. A similar mechanism could explain the correlation between MCV and pH in the absence of FD.

RENAL LYSOZYME (LY) HANDLING IS UNALTERED BY ALBUMINURIA IN RATS RECEIVING LOW DOSE AMINONUCLEOSIDE (AMNS). Mark T. Houser. Univ. of Nebraska Med. Ctr., Depts. of Pediatrics & Pharmacol., Omaha, Nebraska.

The renal handling of low molecular weight proteins such as Ly, are known to be excellent markers of proximal tubular injury or dysfunction. However, it remains unclear whether lysozyme excretion is also increased in the face of altered glomerular permeability, which might lead to a competitive inhibition of Ly reabsorption by filtered large molecular weight proteins such as albumin (alb). To examine this issue, albuminuria was induced in Sprague-Dawley rats by injecting a single dose (5mg/100g) of AMNS (IP). Albuminuria peaked in the experimental group on Day 10 and resolved by Day 28. Day 10 data is presented:

	CCr (µl/min)	Proteinuria (mg/Day)	CAlb/CCr ($\times10^5$)	CLy/CCr ($\times10^5$)	CLy/CAlb
Control	467±20	6.8±0.9	3.0±0.3	4.5±0.5	1.8±0.4
AMNS	438±21	82.0±18.7*	38.2 ±14.2*	7.8±1.7	0.3±0.1*

(mean ± SEM; n=10 (Control) or 8 (AMNS); * p <0.005)

Although CAlb/CCr and total protein excretion increased more than tenfold, this was not associated with any significant ($p=0.10$) increase in CLy/CCr. Furthermore, no correlation was found between CLy/CCr and CAlb/CCr in either normal animals ($r= 0.18$, $p=0.20$) or in those receiving AMNS ($r=0.17$, $p=0.20$). These data suggest that in states associates with moderate albuminuria, renal tubular Ly handling is unaltered. However, it is conceivable that more severe albuminuria might impair renal tubular Ly reabsorption.

SULFONAMIDE INDUCED ACUTE INTERSTITIAL NEPHRITIS (AIN): ROLE OF MULTIPLE DRUGS AND CELL-MEDIATED IMMUNITY. K.S. Kant, M.A. Weiss, Depts. of Med. and Pathology, Univ. of Cincinnati Medical Ctr., Cincinnati, Ohio.

That sulfonamide (S) agents cause AIN is well known. Pathogenesis, believed to involve hypersensitivity mechanisms, is not understood. The role of multiple agents and of cell mediated immunity is unknown. We hereby report 4 patients who had been taking one S agent for months/years and who developed AIN within days after taking another such agent. The index patient, B.C., took hydrochlorthiazide (HCTZ) for years with no adverse effects. Three days after starting glyburide (GLY), she developed AIN. Renal function improved when HCTZ, not GLY, was withdrawn. Monoclonal Abs showed a largely T4 positive interstitial infiltrate. Peripheral blood mononuclear cells from this patient, 5 normal controls and 5 individuals taking HCTZ without problems were cultured in the presence of phytohemaglutinin (PHA) and varying concentrations of HCTZ and GLY and ^{3}H-thymidine incorporation measured at 96 hr. Results are expressed as a stimulation index = cpm with PHA + drug/cpm with PHA:

Agent	PHA +HCTZ (1 ug)	PHA +GLY (1 ug)	PHA +HCTZ (10 ug)	PHA +HCTZ+GLY (1 ug)
Pt. B.C.	2.89	2.50	2.93	2.34
Normals	1.09±0.48	1.10±0.42	1.06±0.38	0.99±0.46
Controls	0.87±0.19	1.33±0.45	0.69±0.43	0.99±0.49

We conclude that sulfonamide agents produce AIN through mechanisms that involve cell mediated immunity and that multiple drugs are more likely to precipitate AIN than single agents.

EFFECT OF LONG TERM NORMALIZATION OF COMPLEMENT (CH50) ON THE COURSE OF LUPUS NEPHRITIS (LN). R.Laitman,* D.Glicklich, L.Sablay, A.Grayzel,* N.Bank. Montefiore Medical Center, Bronx, N.Y.

39 pts with LN were studied prospectively to determine whether continuous control of CH50 by adjustment of immunosuppression influences long term renal and pt outcome. All pts had one renal biopsy (bx), 24 had a 2nd bx (mean 16.7glomeruli), with interbx interval of 41±6 mo. Prednisone(1-2 mg/kg/d) and Imuran(1-2mg/kg/d)were given up to 6 mo to normalize CH50. Thereafter,therapy waa adjusted to actively maintain nl CH50 as feasible. Over follow-up of 117±10 mo, 17pts (Group 1A) remained CH50-controlled, 14 had chronic low CH50, mean 34±4 mo(Group 2),8 initially normalized CH50 but later developed persistently low CH50 as therapy was not appropriately adjusted. There was no initial difference in creatinine (creat), proteinuria or histology between Groups 1(A+B)and 2.

	Group 1A	Group 2	Group 1B
Initial creat	0.9±.06	0.9±.08	0.8±.09
Final creat	1.2±.25	2.7±.51*	3.7±.52*
ESRD	1 (5.9%)	5 (36%)*	5 (62.5%)*
Nephrotic	3 (17.6%)	9 (75%)*	8 (100%)*
Chronic HTN	6 (35.3%)	10(71%)*	8 (100%)*

*p<.05 Group 1A vs Group 1B or Group 2

Two observers unaware of pt data separately evaluated all bx for chronicity index (CI). Serial bx showed that the 14 pts with normal CH50 had less progression of CI than 10 pts with chronic low CH50(1.2±.3 vs 3.9±.9, p<.01).Mortality (0 vs 44% p<.02) and liklihood of doubling creat (31 vs 89%, p<.01) were less in the CH50 controlled pts. We conclude that therapy adjusted to continuously control CH50 results in improved renal/pt outcome.

INTRAVENOUS PULSE CYTOXAN (IVPC) TREATMENT OF RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS (RPGN). B. Kiss*; V. D'Agati; G. Williams; C.L. Kunis; G.B. Appel; Depts. of Med. & Path. Columbia U. NY, NY.

Pts with RPGN have a poor prognosis and no therapy has proven universally effective and safe. To test the safety and efficacy of IVPC in RPGN we treated 7 Pts (1M/6F, age 23 to 82 yrs) with 6 monthly or bimonthly doses of IVPC (0.5-1g/m2) plus rapidly tapering high dose corticosteroids. Diagnoses included microscopic periarteritis (n=2), idiopathic RPGN (n=3), crescentic IgA nephropathy (n=1) and crescentic immunotactoid glomerulonephritis (n=1). At biopsy (Bx) all pts had a rapid decline in renal function, with active urinary sediment, and 2 required dialysis. Lab data at Bx, 6 mos, one yr, and 18 mos include:

	Pcr (mg/dl)				24 Hr Prot.(g/d)			
	N	>10	4-10	2-4	<2	N	>3	1-3	<1
Bx	7	2	4	1	0	7	3	2	2
6mo	7	0	0	5	2	7	0	5	2
1yr	6	0	1	4	1	5	0	1	4
18mo	5	0	1	3	1	4	1	0	3

Inital Bx showed mean activity index (AI) 13.1/21 and mean chronicity index (CI) 4.5/12. 5 repeat Bxs at 9-33 mos showed markedly decreased AI (X 2.1) and increased CI (X 8.2). Side effects of therapy were minimal with no severe infections, leukopenia, alopecia, or hemorrhagic cystitis. We conclude that IVPC plus rapidly tapered high dose steroids are potentially effective initial therapy for pts with various forms of RPGN. Despite the favorable initial clinical response and striking reduction in histologic activity at repeat Bx, the high CI with evolution to glomerulosclerosis suggests a guarded long term prognosis.

DETERMINANTS AND MONITORING OF GFR IN DIABETIC GLOMERULAR INJURY (DGI). NR Loon, E Morelli,* BD Myers. Div. Nephrol Stanford Univ. CA.

The GFR is said to decline progressively in DGI and to be the best measure of progression rate in this entity. To examine this proposition we studied 56 proteinuric diabetics: DGI was divided according to proteinuria into mild (subnephrotic, n=20) and severe (nephrotic, n=36) categories. Clearances (C) of inulin (GFR), PAH (RPF), and plasma oncotic pressure (π) were determined. Fractional clearances of dextran (r_s=26-42Å) were used to estimate hydraulic pressure gradient (ΔP) and ultrafiltration coefficient (K_f): * p<0.05.

	GFR	RPF	π	ΔP	K_f
Subnephrotic	69±8	459±42	22.8±0.5	35	8.7±1.0
Nephrotic	34±8*	344±33*	20.8±0.7*	40*	2.5±0.4*

Inefficient emptying of a neuropathic bladder resulted in intra- and interstudy coefficients of variation for C_{in} of 28±2% and 11±3% respectively, vs 11±1% and 5±2% in healthy controls. Whereas C of iothalamate and inulin were identical, C of creatinine overestimated C_{in} in inverse proportion to GFR, reflecting tubular creatinine hypersecretion. Thus prolonged (24hr) C_{creat} cannot be used to compensate for bladder dysfunction when estimating GFR.

We conclude that (1) GFR falls in DGI because of declining K_f; (2) GFR underestimates extent of DGI because of an offsetting increase in ultrafiltration pressure and (3) the adequacy of GFR as a measure of progression rate in DGI is suboptional because of autonomic bladder dysfunction. We submit that GFR is too insensitive to monitor outcome of new therapies for DGI in single center studies.

AUTOANTIBODIES TO HUMAN GP330 IN SERA OF PATIENTS WITH IDIOPATHIC MEMBRANOUS GLOMERULARNEPHROPATHY. Sudesh P. Makker and John J. Kanalas. Univ. of Texas Health Science Ctr., Dept. of Pediatrics/Nephrology, San Antonio, TX.

Autoantibodies to human gp330 were searched in the sera of patients with idiopathic membranous glomerularnephropathy (MGN) by enzyme-linked immunosorbent assay (ELISA) and immunoprecipitation assays. Human gp330 was prepared by a method similar to that used for the preparation of rat gp330. Ten patients with MGN, 18 normals and 6 patients with systemic lupus erythematosus (SLE) were studied. Significant levels of autoantibodies to gp330 were detected in sera of patients with MGN when compared to normals ($P < 0.005$) and to SLE patients ($P < 0.02$). No significant difference was observed between normals and SLE patients ($P > 0.1$). The autoantibodies were characterized by immunoprecipitation, sodium dodecyl sulfate polyacrylamide gel electrophoresis and autoradiography to be reactive against gp330 and another glycopeptide, gp450. These results suggest that MGN in humans may be an autoimmune disease and that its pathogenesis may be similar to the active Heymann nephritis of rats.

THE ACUTE EFFECTS OF CAPTOPRIL (CAP) ON PROTEINURIA IN NEPHROTIC PATIENTS: DEPENDENCE ON HEMODYNAMIC EFFECTS. C. Pasque*, M. Rodriguez, M. Allon. Section of Nephrology, Univ. of Oklahoma HSC and VAMC, Okla. City, Okla.

A study was designed to evaluate the relative contributions of changes in glomerular filtration rate (GFR), renal plasma flow (RPF), and filtration fraction (FF) to the antiproteinuric effect of captopril. Protein excretion and renal hemodynamics were measured in 7 nondiabetic nephrotic patients (pts) (24 hr urine protein 5-25 gm; GFR 19-118 ml/min) during water diuresis, at baseline and for 8 consecutive 30-minute clearance periods following CAP (25 mg). The changes in parameters at 2 and 4 hours after CAP are summarized. (*$P<0.05$ vs baseline value).

Parameter (% baseline)	2 Hours	4 Hours
Protein excretion	80±5*	76±9*
GFR (C_{inulin})	89±5	94±7
RPF (C_{PAH})	104±7	103±10
FF	87±2*	91±3*

CAP produced a significant decrease in protein excretion. There was a nonsignificant increase in RPF, and a nonsignificant decrease in GFR, resulting in a significant decrease in FF. Moreover, linear regression analysis revealed a strong correlation between the change in protein excretion and the variation in GFR for each individual pt. ($p<0.001$), as well as for the group ($R=0.85$, $p<0.001$). For any clearance period in an individual pt., if FF decreased without an associated decrease in GFR, there was no reduction in protein excretion. In conclusion, in nephrotic pts, CAP acutely decreases protein excretion by a mechanism that is dependent on both a decrease in FF as well as in GFR.

EFFECT OF CONVERTING ENZYME INHIBITOR (CEI) ON DIABETIC GLOMERULAR INJURY (DGI). Ester Morelli,* Nicholas. R. Loon, and Bryan D. Myers. Div. of Nephrology, Stanford Univ., CA.

We studied 15 nonazotemic patients with IDDM and DGI. Each was treated with the CEI, Enalapril (5 to 20 mg/day), for 90 days Fractional clearances (θ) of dextrans (radius 20-60 Å), albumin and IgG were determined before and after. Clearances of Iothalamate (GFR), 72+9 vs 71+8 ml/min, and PAH, 536+39 vs 555+48 ml/min, remained unchanged at 0 and 90 days. Urinary protein excretion (UV) declined from 2.4+0.8 to 1.8+0.7 g/24h ($p=0.08$). Other findings (Mean+SEM, * = $p<0.05$)

	MAP (mmHg)	PRORENIN	RENIN (ng/ml/h)	θalb ($x10^{-5}$)	θIgG
Pre	98±2	244±78	9±1	145±61	60±29
Post	90±2*	362±105*	29±6*	105±47	42±22*

Lower MAP and high renin levels confirm inhibition of converting enzyme. The θ of dextrans (radii 28-60 Å) declined significantly and uniformly. A theoretical analysis with a heteroporous membrane model (Am J Physio 249:F374-389, 1985) revealed a decline ($p<0.05$) of restrictive pore radius from 56 to 54 Å after CEI. The fraction of filtrate volume permeating large non-restrictive pores declined from 19+3 to $14+3x10^{-3}$ ($p<0.05$). Following a 30 day wash out, 8 patients received clonidine (1-2μg/day) for 90 additional days. UV, θ albumin and θ IgG on day 90 were significantly higher than corresponding values on CEI. We conclude that Enalapril exerts an antiproteinuric effect in early DGI by shifting glomerular pores towards smaller size, thereby enhancing barrier size-selectivity.

IV PULSE CYTOXAN (IVPC) THERAPY OF SEVERE LUPUS NEPHRITIS (LN) A. Pernis*; V. D'Agati; D. Estes*; C.L. Pirani; G.B. Appel; Depts of Med and Path, Columbia U, NY,NY

20 Pts with severe LN (x age 32 yrs; 15 female) treated with monthly IVPC and rapid steroid taper have been followed more than 1 yr (12-42 mos). 18 Pts received 5-6 monthly doses of 0.5-1g/m^2 IVPC; one Pt received 9 doses and one died after 4 doses. At initial biopsy (Bx), 70% had extrarenal lupus activity, 65% were hypertensive, 90% had active urinary sediment. Initial Bxs (19/20) showed: 13 DPLN, 5 FPLN, 1 Memb LN. Mean Activity Index (AI) was 10/24 , mean Chronicity Index (CI) was 3/12. Data at Bx, 6 mo, 12 mo, and 18 mo are:

	N	Abnl Pcr	Prot >1g/d	Prot >3g/d
Bx	20	11/20	16/20	11/20
6mo	19	7/19	11/19	4/19
12mo	19	6/19	9/19	2/19
18mo	13	5/13	7/13	2/13

15/19 pts were serologically inactive at 6 mo and at 1 year. Repeat Bxs at 12-18 mo showed reduced AI (x=5) and increased CI (x=5) in 10/14. Third Bxs in 3 showed further decreased AI and increased CI. 4 Pts were treatment failures: 2 persistent severe renal failure; 2 persistent histologic activity. Side effects during the first year included major infections (3), H. zoster (2), significant alopecia (2). We conclude: IVPC produced a favorable early response in severe LN with correction of serology, reduction of proteinuria and stabilization or improvement of Pcr in most Pts. Some Pts failed to respond to this short course of IVPC and most developed increased chronicity on repeat renal Bx suggesting the need for either further maintenance therapy with IVPC or additional treatment modalities.

CHANGING PERSPECTIVES IN CHILDREN WITH POSTSTREPTOCOCCAL ACUTE GLOMERULONEPHRITIS (PSAGN). SHANE ROY, III. Dept. of Pediatrics,Univ. of TN., Memphis, Memphis, TN.

Prompted by an observed resurgence of acute rheumatic fever in children in our area over the last several years our experience with PSAGN since 1980 was reviewed. Between 1961 and 1970 from 25 to 37 patients per year ($\bar{x}$=31±6.3) from Memphis with PSAGN were admitted to our hospital. Since 1980 we have evaluated 72 children, 50 male and 22 female, 41 black and 31 white whose mean age was 8.2±1.5 years at onset of PSAGN. Thirty children lived in Memphis and 42 were referred from rural communities in a 4 state area. Fifty-one patients (70.8%) were school age and 21 (29.2%) were between 2 and 6 years of age. PSAGN was preceeded by sore throat in 46 patients (63.9%), by pyoderma in 24 patients (33.3%) and in 2 patients (2.8%) the etiology was undetectable. Edema (55/72), cola colored urine (40/72), hypertension (44/72), elevated ASO titer (43/54) and/or streptozyme titer (69/72), RBC casts (60/72) and decreased C'3 levels (56/72) characterized these patients at onset of PSAGN. Renal biopsies were necessary for definitive diagnosis in 21 patients whose laboratory data and/or clinical course were not completely consistent with classical PSAGN. Hospital stay ranged from 0 to 24 days ($\bar{x}$=6.4±4.1). In contrast with past years when 70% of our cases were pyoderma related and 40% were admitted between July and Sept., in the current study 64% of patients were both sore throat related and admitted between October and March. In comparison to the decade ending in 1970 these data show:1) a marked decline in prevalence of PSAGN in our hospital (p=<0.0005), 2) a predominance of pharyngeal rather than skin infection preceeding PSAGN, 3) a change in seasonal presentation of patients with PSAGN and 4) a marked decline in urban origin and an increase in rural origin of PSAGN cases referred to our hospital.

GLYCOGEN STORAGE DISEASE TYPE I (GSD-I) NEPHROPATHY: AMELIORATION WITH THERAPY. Jon I. Scheinman, Y. T. Chen*, Rosiland A. Coleman* and Robert H. Wilkinson*. Duke University Med Center Depts. of Pediatrics and Radiology, Durham, NC

We identified a chronic nephropathy of GSD-I with focal glomerulosclerosis (N Engl J Med 318:7-11, 1988). Early renal dysfunction is high creatinine clearance (ClCr) and proteinuria. Thereafter, ClCr decreases and progresses to renal failure. Further systematic studies were needed: ClCr may accurately reflect GFR (by ^{99}Tc-DTPA): Three proteinuric patients had simultaneous ClCr and GFR :75 vs. 73, 116 vs. 120, 222 vs. 252. Extrapolated ClCr (from height/Cr) in two patients without proteinuria were less secure: 136 vs. 148, 141 vs. 105. Reversal of GSD-I nephropathy was explored by cornstarch feeding 2g/kg/6 hrs, over 2 weeks. Two patients showed decreased ClCr (138 to 122, 100 to 74) and decreased proteinuria (2.0 to 1.0g, 6.1 to 2.6g). We investigated renal tubular dysfunction in GSD-1: aminoaciduria, phosphate reabsorption (TRP), concentration, and Beta$_2$ micro-globulin (B_2MG). Of 5 patients with proteinuria, 4 had elevated B_2MG (0.9 + 0.37 mg/l, range 0.5-1.4, n1<0.37 mg/l); concentration was decreased (<7 30mOsm/Kg) in two; TRP and aminoaciduria were normal. After cornstarch treatment decreased proteinuria, B_2MG decreased from 0.8 to 0.3 mg/l in one patient examined. Isolated tubular dysfunction was found in one of five GSD-I patients without proteinuria or hyperfiltration: Aminoaciduria, phosphaturia (TRP<75%), hypophosphatemia (3.1-3.4mg/dl) and increased B_2MG (4.3mg/l). With treatment, B2MG decreased to 0.5mg/l, TRP increased to >85%. Mechanisms of GSD-I nephropathy hyperfiltration with proteinuria, and related tubular dysfunction remain obscure.

SPECTRUM OF RENAL INVOLVEMENT IN FAMILIAL MEDITERRANEAN FEVER (FMF). Riyad Said, Yousef Hamzeh*, Musleh Tarawneh*, and Mohammed Al-Khateeb*.

Twelve patients with FMF were evaluated for evidence of renal involvement. Eight were males and their ages ranged between 10-40 years. The duration of their illness ranged between 3-15 years. Fever and recurrent abdominal pains were present in all, in addition to arthralgias in eight and pleurtic chest pain in five patients. Proteinuria was present in all and it ranged between 750mg - 12gm per day, and in four patients it was 1gm or less. Hematuria was present in five patients. Oligonauria was seen in two patients in association with acute renal failure(ARF). Serum creatinine ranged between 0.6-10mg/dl,and in three patients it was more than 6mg/dl. Dialysis support was needed in the two patients with ARF, and one patient recovered function, while the second patient continued to need dialysis support and finally he received a kidney transplant. Kidney biopsies were done in all,and the findings were as follows: Amyloidosis in six patients, Mesangial Proliferative glomerulonephritis in four and rapid progressive glomerulonephritis in two. Immunofluorescence microscopy was positive for IgM in three patients two of them have no Amyloidosis, and for IgA in two patients without Amyloidosis.

In conclusion, renal involvement in FMF includes in addition to Amyloidosis, mesangial proliferative glomerulonephritis (IgM, and IgA) and rapid progressive glomerulonephritis.

RENAL AND ELECTROLYTE DISORDERS IN 50 PATIENTS WITH AIDS IN DALLAS. Frank D Seney, Jr, Dennis K Burns*, Fred G Silva, Bruce Baker*. University of Texas Southwestern Medical Center, Dallas, TX.

We reviewed the first 50 AIDS patients to undergo autopsy at UT Southwestern (12/83-8/87). Patients were predominantly homosexual males; 32% reported IV drug use. Renal failure and proteinuria were detected in 24% and 12%. Severe hyponatremia (hypoNa), hyperkalemia (hyperK) and hypocalcemia (hypoCa) were present in 40%, 16% and 44%. HypoNa and hyperK were usually transient. HypoCa was often more severe than expected on the basis of hypoalbuminemia alone.

Formalin-fixed renal tissue from the same 50 patients was prepared for and examined by light microscopy. The following lesions were observed:

Renal tubular casts	66%
Cortical and/or medullary nephrocalcinosis (Ncalc)	44%
Focal interstitial nephritis	38%
Prominent mononuclear cells in congested vasa rectae	34%
Renal invasion by CMV, M. avium intracellulare, C. neoformans, Histoplasma capsulatum	26%
Hyaline droplets, proximal tubule	12%
Global glomerulosclerosis	8%
Glomerular capillary aneurysms associated with cryptococci	6%
Focal/segmental glomerulosclerosis	2%

Ncalc did not correlate with administration of amphotericin but did correlate with hypoCa. We conclude that extraglomerular lesions and electrolyte disorders including hypoNa, hyperK and hypoCa are common in patients dying of AIDS.

EVIDENCE FOR AN HIV-RELATED NEPHROPATHY A CLINICO-PATHOLOGICAL STUDY. Anita Soni,* Arun Agarwal,* Praveen Chander and Gerhard Treser. New York Medical College, Dept. of Medicine and Dept. of Pathology, Valhalla, New York.

The existence of an HIV related nephropathy as a distinct disease entity is controversial. We observed a high incidence of renal disease in our AIDS patients. Of 182 patients, 59 patients (32.4%) were found to have heavy proteinuria (greater than 2 grams/24 hrs.). Of these, 24 patients had slow progression of renal insufficiency and 2 patients had rapid deterioration to end stage renal disease. There was a notable absence of hypertension in these cases. The incidence of proteinuria was similar in blacks and hispanics, however 22.8% of blacks had renal insufficiency as compared to 6.9% of hispanics. There was no difference in the incidence of heavy proteinuria between intravenous drug abusers (32.3%) and non-abusers (33.3%). Renal morphology when examined showed characteristic changes, including cytomembranous structures and virus-like particles. These changes were similar in patients with heavy or non-heavy proteinuria, though they were less severe in the latter. We conclude that a HIV related nephropathy exists and the presence of cytomembranous structures and virus-like particles in the renal tissue raises the possibility of a viral etiology for this disorder.

TUBULAR DYSFUNCTION IN PROLIFERATIVE GLOMERULONEPHRITIS IN SLE. EJ ter Borg*, PE de Jong*, S Meyer*, CGM Kallenberg* (Intr. by LW Statius van Eps). Univ. Hosp. Groningen, The Netherlands. Though glomerular pathology predominates, tubulo-interstitial changes probably also play a role in lowering GFR in active lupus nephritis. We prospectively studied tubular function during exacerbations of proliferative glomerulonephritis in SLE. Data are available prior to and during exacerbation (n=7) or during and after exacerbation (remission)(n=9). Complement C3 and C4 (mg/100ml), GFR, ERPF (ml/min), FF (GFR/ERPF x 100), fractional excretion (FE) of beta-2-microglobulin (beta-2m, %x100), FE of DMSA (%), proteinuria (prot, g/day) and urinary LDH (ur.LDH, U/mmol kreat) were measured. Therapy consisted of prednisolone and cyclofosfamide in all cases.

	prior to	during	during	after
	n = 7		n = 9	
C3	72±15	38±15 *	42±14	82±26 *
C4	14±4	10±3 *	11±5	16±6 *
GFR	95±22	47±23 *	60±34	88±41 *
ERPF	539±160	394±188 *	462±191	416±133 *
FF	18.7±3.1	11.6±4.2 *	12.9±4.2	20.6±3.6 *
FE B-2m	0.15±0.16	0.32±0.10 *	0.33±0.11	0.12±0.15 *
FE DMSA	11.1±1.5	18.7±6.8 *	17.3±2.6	11.9±2.5 *
prot	1.8±2.4	5.3±3.3 *	3.5±2.1	1.6±1.8 *
ur.LDH	7.0±5.7	10.5±10.9	4.9±3.4	2.5±0.8

*:p<0.05 vs value in the period before.

Since FE B-2m and FE DMSA can be used as a marker of tubular function, the rise in these parameters during exacerbation, and the fall to control values during remission indicate that tubular dysfunction frequently is present during proliferative glomerulonephritis in SLE.

RANDOMIZED TRIAL OF CYCLOSPORINE AND LOW DOSE PREDNISONE (P&CS) VS CONVENTIONAL PREDNISONE (P) THERAPY IN RECENT ONSET (<1 YR) NEPHROTIC SYNDROME (NS). A. Tejani, M. Suthanthiran*, D. Rajpoot*, R. Gonzalez*, A. Pomrantz* SUNY-Health Science Ctr. at Brooklyn and Rogosin Institute, New York.

We have previously shown (Kid Int:33:729, 1988) that cyclosporine (CS) can induce a remission in relapsing NS but remission is not sustained. In this study we evaluated the effect of P&CS against P in children with recent onset NS. 28 patients were randomized to receive (Group A) cyclosporine 7mg/kg daily for 8 weeks and daily 20mg/m^2 p for 1st 4 weeks and then 10mg/m^2 p alternate day 2nd 4 weeks OR (Group B) 60mg/m^2 of p for 4 weeks followed by 40mg/m^2 alternate day for 4 weeks. Patients in Group A had Con A, PhA stimulation and IL2 production measured.

Results:	Group A(14)	Group B(14)
Histology - Minimal change	7	7
F.S.G.S.	4	4
IgA	0	1
IgM	2	1
Membranous	1	1
Mean age at onset in years	4.8(2-16)	5.1(2-13)
Remission	13/14	8/14 p<.05
Duration remission	3.6 mo	4.8 mo NS

Patients with low mitogen stimulation and low IL2 were either resistant to P&CS or tended to relapse soon after discontinuation of therapy, whereas patients who were high responders had a sustained remission.

Conclusion: (1) Combined P&CS is more effective in producing a remission but remission is not sustained by short term combined therapy. (2) Immunological studies using a variety of markers may identify patients who will respond to P&CS with sustained remission.

FOCAL PROLIFERATIVE GLOMERULONEPHRITIS ASSOCIATED WITH ANKYLOSING SPONDYLITIS. Melvin Yudis, Robert Sirota, Harold Stein., Dept. of Medicine., Abington Memorial Hospital., Abington, PA

The renal findings seen in ankylosing spondylitis previously reported have included IgA nephropathy, membranous nephropathy and amyloidosis. We have recently seen a patient with ankylosing spondylitis present with a nephritic illness who had a focal segmental proliferative glomerulonephritis. Although she had received non-steroidal anti-inflammatory drugs, it was felt unlikely that these drugs caused the glomerular abnormalities.

Our patient is a 40 year old female who had ankylosing spondylitis and peripheral arthralgias who had received non-steroidal anti-inflammatory drugs in the past. She developed microhematuria and proteinuria. Mild azotemia developed with a creatinine of 2mg%. Renal biopsy revealed focal segmental proliferative lesions. Immunoflorescence showed plus three mesangial deposits of complement C3 and plus two IgM. Electron microscopy revealed scattered subendothelial and subepithelial dense deposits.

The presense of focal segmental proliferative glomerulonephritis with immune deposits suggests aberrant immunity in a patient with ankylosing spondylitis. Although IgA nephropathy has been described in patients with seronegative spondyloarthropathy, we found no evidence of IgA deposits. This patient had previously taken non-steroidal anti-inflammatory drugs but the above described biopsy appearance was not felt to be compatible with these drugs. One should consider the possibility that focal glomerulonephritis may represent an extraskeletal manifestation of ankylosing spondylitis.

SALUTARY EFFECTS OF MODEST FLUID REPLACEMENT IN THE TREATMENT OF DIABETIC KETOACIDOSIS (DKA). HJ Adrogue, J Barrero*, and G Eknoyan. VA Med. Ctr., and Baylor Coll. of Med., Houston, TX.

Volume depletion is a major derangement that triggers the release of counterregulatory hormones and substantially contributes to the morbidity and mortality of DKA. The optimal rate of fluid administration to properly correct this volume deficit remains to be determined. The present study prospectively evaluated two regimens of therapy that differed exclusively in the rate of infusion, in patients admitted for severe DKA (TCO_2 <14 mmol/l). Protocol I (n=12): 0.9% NaCl iv 1000 ml/hr for 4 hrs and 500 ml/hr for the next 4 hrs; and II (n=11): 500 ml/hr for 4 hrs and 250 ml/hr for the next 4 hrs. After 8 hrs oral intake was allowed and rate of iv fluids reduced accordingly. Initial mean plasma values were 7.10 and 7.13 for pH, 17 and 21 mmHg for $PaCO_2$, 5.2 and 6.6 mmol/l for HCO_3^- and 559 and 682 mg/dl for glucose in groups I and II, respectively. The increment in $[HCO_3^-]_p$ from admission levels estimated at 2, 4, 8, 16 and 24 hrs after admission was larger with protocol II at all times, reaching statistical significance at 4 hrs (3.7±1.3 vs 0.7±0.6) and 24 hrs (13.2±1.3 vs 8.4±1.9). The slower recovery with high infusion rates is most likely accounted for by relatively greater acute expansion with a bicarbonate-free solution (initial component at 4 hrs); and a sustained loss of bicarbonate precursors in the urine due to continued volume expansion (late component at 24 hrs).

These data support cautious restraint in the volume of fluid replacement since prompt recovery and a significant reduction in overall cost of medical therapy are achieved. This might also decrease the risk of pulmonary and cerebral edema in DKA.

SPURIOUS ASSESSMENT OF SYSTEMIC ACID-BASE STATUS BY ARTERIAL SAMPLING IN CIRCULATORY FAILURE: THE CASE FOR CENTRAL VENOUS BLOOD GASES. HJ Adrogue, MN Rashad*, AB Gorin*, J Yacoub*, and NE Madias. VA Med. Ctr., Baylor Coll. of Med., Houston, TX and Tufts - New England Med. Ctr., Boston, MA

The acid-base status of arterial (A), central venous (CV) and mixed venous (MV) blood was examined during graded degrees of circulatory compromise in 105 patients and 63 dogs (pharmacologic vasodilation; increased intrathoracic pressure; hemorrhage; ventricular fibrillation; or pericardial tamponade). Humans and dogs with normal cardiac index had similar A-V ΔpH of ↓0.03 and ΔPCO_2 ↓-6 mmHg but severe circulatory failure enlarged these indices by fourfold. Cardiac arrest further extended A-V gradients to ΔpH of ↓0.35 and ΔPCO_2 of ↓-50 mmHg in humans, who had extreme V acidemia despite near-normal A pH. Widening of A-V indices was due to A hypocapnia/eucapnia and V hypercapnia. Whole-body CO_2 retention was evident by decreased end-tidal PCO_2. Thus, the prevailing respiratory acidosis eluded detection fully on A sampling. Bicarbonate administration aggravated CO_2 retention but was not prerequisite to V hypercapnia. Development of respiratory arrest prior to or concomitantly with circulatory arrest blunted markedly the A-V ΔpH and ΔPCO_2. CV blood sampling provided comparable information to that of MV blood.

We conclude that whereas normal A blood gases do not rule out major abnormalities in systemic acid-base status, normal V blood gases attest to the adequacy of both tissue perfusion and pulmonary CO_2 excretion. The data suggest strongly the introduction of CV blood sampling for monitoring of the systemic acid-base status in the critically ill.

INFLUENCE OF INSULIN SECRETION, ACID BASE STATUS AND CATECHOLAMINE ACTION ON THE IMPAIRED RESPONSE TO A K LOAD IN HEMODIALYSIS PATIENTS. Miriam Alvo* and Patricia Krsulovic* (intr by Andres Valdivieso) Department of Medicine and Dept of Physiol and Biophys, University of Chile, Santiago Chile.

Hemodialysis patients exhibit an impaired extrarenal tolerance to a K load. To clarify the factors involved in this deteriorated response, we have studied several parameters in a group of patients in chronic hemodialysis (HD) compared to a control group: a) The insulin plasmatic levels in response to an exogenous K (0.5 mEq/kg) and carbohydrate (0.5 g/kg) oral load. b) The correlation of the the acid base status of the patients with the maximal plasma K increase. c) The response of plasma K to an epinephrine infusion (0.05 µg/kg/min during 80 min).

In response to the K and carbohydrate load, plasma K levels increase in 1.07 ± 0.1 mEq/l in HD patients vs 0.39 ± 0.005 meq/l in controls ($p < 0.001$), only 58% of the K load was translocated to the intracellular space compared to 81.6% in controls ($p < 0.001$).

Basal insulin levels were higher in patients than in controls (21.3 ± 52 µU/ml vs 9 ± 48 µU /ml ($p < 0.001$) and increased in both groups in response to the oral load. Although HD patients were acidotic mean total CO2 15.8 ± 1.2 mEq/l (range 13 to 19.5 mEq/l) no correlation was found between the degree of acidosis and the maximal K increase. In response to epinephrine infusion plasma K levels decreased significantly in HD patients and controls.

We conclude that the impaired response to a K load in hemodialysis patients is not explained by diminished insulin levels, decreased tisular response to catecholamines or altered acid-base status.

SEVERE HYPERNATREMIA (SH) IN RATS: EFFECT OF TREATMENT ON BRAIN FUNCTION AND HISTOLOGY. J.C. Ayus, R. Krothapalli, J. Spark, * and M. Frergber. Baylor College of Medicine, Houston Texas.

SH carries significant morbidity and mortality in humans (Ann. Int. Med., 107: 309, 1987). The present study in rats was done as there is no information regarding appropriate treatment for SH.

Group I (acute SH, n=10): Following i.p. administration of hypertonic saline (H/T) and 24h water restriction, serum sodium (SNa) increased from 143 mEq/L to 171 mEq/L. The rats were then allowed to drink water ad lib. SNa decreased to 147 mEq/L by the end of 24h correction. At the time of SH, the rats were irritable, hyperactive and were jumping all over the cage. All rats became neurologically normal by the end of 24h correction and remained normal during observation. All brains were histologically normal at the end of one month.

Group II (chronic SH, n=10): Following i.p. administration of H/T, these rats were water restricted (ad lib food) for 10 days. SNa increased from 142 mEq/L to 174 mEq/L by the end of 10 days. The rats were then allowed to drink water ad lib. SNa decreased to 149 mEq/L by the end of 24h of correction. All rats lost weight. At the time of SH, they were less hyperactive than in Group I, but showed similar neurological dysfunction. All rats became neurologically normal by the end of 24h of correction and remained normal during observation. All brains were histologically normal at the end of 1 month.

We conclude that clinical neurological dysfunction seen in both acute and chronic SH in rats is totally reversible with correction to mildly hypernatremic levels in the first 24h without any evidence of histological brain lesions.

ENDOGENOUS VIP: A PARACRINE REGULATOR OF ALKALI EXCRETION. William A. Brodsky, Cristina Matons*, John H. Durham*. Dept. of Physiol. and Biophys., Mt. Sinai School of Medicine, N.Y., N.Y. 10029.

In short circuited bladders from alkalotic or euhydric turtles, serosally added, porcine derived vasoactive intestinal peptide (p VIP) induces an active, electrogenic, cyclic AMP mediated excretion of alkali (Durham et al, AJP,1987). These data, together with the known effects of VIP on electrolyte secretion in non-urinary epithelia (Said, 1984), suggest that: there are VIP receptors on the serosal surface of alkali excreting cells, and that endogenous reptilian (r) VIP is stored in sub-mucosal neuronal cells of the turtle bladder. Using a standard radio immuno assay system containing ^{125}I-labelled, porcine VIP and a specific anti-porcine VIP antibody, it was found that lyophilized acid extracts of turtle bladder and small intestine (as well as unlabelled p-VIP) inhibit the binding of labelled p-VIP to antibody; and that with increasingly diluted aliquots, the apparent immunoreactivity of turtle tissue extract increases relative to that of equally diluted aliquots of p-VIP. Therefore, an endogenous r-VIP indeed exists in the turtle bladder and intestine. Moreover the calculated concentration of r-VIP in these epithelia from euhydric turtles is greater than that in epithelia from alkalotic turtles. On the basis of this and more recent evidence, it is now suspected but not as yet proven that neural-stimulating agonists, such as norepinephrine act as VIP releasing factors during regulation of alkali excretion.

HYPOKALEMIA ASSOCIATED WITH DOBUTAMINE ADMINISTRATION. Michael D. Clayman, Christine Lawless* and Irvin Goldenberg*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, Hines VA Hospital, Hines, IL, and Minneapolis Heart Institute, Minneapolis, MN.

The purpose of this study was to evaluate the possible contribution of dobutamine therapy to the development of hypokalemia and hypomagnesemia, factors which could contribute to the modest arrhythmogenic potential of this drug. Seven NYHA Class III and IV CHF patients were treated with increasing doses of dobutamine and changes in serum potassium and magnesium were determined at each dosage level. No arrhythmias developed during this study. Data are as follows:

Mean Values	Dobutamine Dose (μg/kg/min) 2.5	5.0	10.0
↓K+ from baseline ($\frac{mmol}{L}$)	-0.09	-0.17	-0.60*
↓Mg++ from baseline ($\frac{mmol}{L}$)	-0.02	-0.03	-0.02
Duration on dose (min)	26	23	33
Cumulative duration on dobutamine (min)	26	49	82

*p <.005
All other K+ and Mg++ values not significantly changed from baseline.

All seven patients experienced a decrease in serum K+ at the highest dobutamine dose. The present data support the concept that dobutamine may exert a hypokalemic action, but do not distinguish between dose and duration of therapy effects.

THE ROLE OF CALCITRIOL (CTR) IN PROLONGED HYPOCALCEMIA DUE TO TUMOR LYSIS SYNDROME (TLS). Mark Camp*, R.W. Dunlay*, M. Allon, P. Fanti, H.H. Malluche, F. Llach. Depts. of Med., Univ. of Okla. HSC and VAMC, Okla. City, OK, and Univ. of Kentucky, Lexington, KY.

The mechanism of sustained hypocalcemia in TLS is not well understood. We studied a patient who developed severe TLS, complicated by acute oliguric renal failure, following chemotherapy for Burkitt's lymphoma. Hypocalcemia persisted for 10 days, even after normalization of plasma phosphorus. The hypocalcemia was resistant to intravenous calcium infusion, despite appropriately elevated parathyroid hormone (PTH) levels. Plasma CTR levels were subnormal throughout the period of hypocalcemia. Exogenous CTR replacement was started on Day 11. Serial measurements of plasma calcium (Ca; mg/dl) phosphorus (Phos; mg/dl) PTH (normal, 10-55 pg/ml), and CTR (normal, 25-60 pg/ml) were obtained until recovery of normocalcemia.

	Hospital Day 2	3	5	6	10	11	12	14
Ca	10.1	7.1	5.3	4.4	6.0	7.4	8.5	9.1
CTR		33	24	8.0	7.0	10.4	13.1	49
PTH		13	254	224	183	227	88	61
Phos	2.7	20.5	30.5	34.4	20.2	6.3	3.7	2.4

Hypocalcemia persisted as long as CTR levels were low, and resolved when CTR had normalized. These observations suggest that CTR deficiency plays a major role in the sustained hypocalcemia in TLS. Exogenous CTR replacement may hasten the recovery from hypocalcemia in TLS.

HYPONATREMIA (HN) IN AIDS AND ARC. A.J. Cusano*, H.L. Thies, F.P. Siegal*, J.K. Maesaka. Dept. of Med. Long Island Jewish Med. Ctr., New Hyde Park, NY.

We studied 96 pts with AIDS or ARC to determine the prevalence, pathogenesis and significance of HN. Pathogenesis was based on extracellular volume (ECV), renal function and blood sugar. Hypovolemia (HV) was defined by historical and clinical evidence, including CVP, renin and aldosterone, and by correction with saline. Normovolemia (NV) was defined by absence of HV, edema, or ascites.

Prevalence of HN was 31.3%. In 63.3% HN first developed as an outpt. Age, risk factors, duration of AIDS and weight loss did not differ between groups. The HN group had a higher mean number of opportunistic illnesses. The mortality was higher in the pts with HN (70% vs 36.4%, $p<0.005$) over a comparable followup (17 ± 2 vs 21 ± 2 mos). Pathogenesis was HV in 21 (88%) and NV is 9 (12%). Seventeen of the 21 with HV had no evident source of fluid loss. All responded to saline, 8 had CVP 0-1 cm H..2..0 and 6 had high renin and aldosterone. Two of the 17 had hyperkalemia, low ACTH stimulation test, and high urine sodium concentration. The remaining 15 had unexpectedly high urine sodium concentration (mean = 49.9 ± 9.1 mEq/L), normal serum creatinine (all), low (6) or normal (9) serum potassium, normal ACTH stimulation test, and normal renal histology (4) at autopsy.

HN is common in AIDS and occurs in pts with a poor prognosis. The association of HV and high urine sodium concentration in pts with no evident fluid losses suggests that a defect in renal salt conservation may contribute to the HN.

PATHOPHYSIOLOGIC MECHANISMS OF HYPONATREMIA IN CRITICALLY ILL PATIENTS. Maria V. DeVita*, Alan Konecky*, Leonida L. Rasenas*, Mark H. Gardenswartz, Paul M. Zabetakis, Gilbert W. Gleim, Michael F. Michelis, Section of Nephrology, Lenox Hill Hospital, New York, New York.

To evaluate the incidence and causes of hyponatremia (low SNa) in intensive care unit (ICU) patients, a retrospective and prospective study was done. Low SNa was defined as a serum sodium (SNa) equal to or less than 134 mEq/L. Retrospective evaluation revealed that 24 of 98 (24.5%) patients exhibited low SNa. Prospectively 29 of 98 (29.6%) patients displayed low SNa. Complete physiologic data were obtained in 12 patients with persistent low SNa. In 7 patients urine osmolality (Uosm) was higher than simultaneous serum osmolality (Sosm). Measured antidiuretic hormone (ADH) levels were elevated (> 0.5 pg/ml)and urine sodium (UNa) concentration was greater than 30 mEq/L suggesting lack of sodium conservation. The data suggest that at least 24.1% of ICU patients with persistent low SNa may exhibit inappropriate vasopressin secretion (SIADH). Mean data for patients with SIADH were:

Age	SNa	Sosm	Uosm	UNa
66±4.0	131±.88	272±.96	484±42	63±14

Other data revealed that two patients with low SNa had Sosm at normal or increased levels, and,in three patients,Uosm was equal to or less than Sosm and ADH levels were suppressed (< 0.5 pg/ml).62.5% of SIADH patients were on ventilators. The data suggest that hyponatremia is common in ICU patients, that SIADH may be seen to occur in 24% of low SNa patients and that hypotonic fluid should be administered to seriously ill patients with care.

EVALUATION OF THE RENAL CONTRIBUTION TO K BALANCE DURING HYPOKALEMIA. J.H. Ethier, H. Sonnenberg, J. Lemann and M. Halperin. Dept. of Medicine and Physiology, Univ. of Toronto and Medical College of Wisconsin, Toronto and Milwaukee.

To detect an abnormal value it is imperative to know the expected response to a provocative stimulus. The focus of this study was to determine the renal response to hypokalemia in the presence or absence of aldosterone. The parameter we chose to use was a non-invasive in vivo index of K secretion in the cortical collecting duct, the transtubular [K] gradient (TTKG) (Min Elect Met 12:234, 1986). Patients were drawn from both primary studies and previous reports. Group 1 had hypokalemia due to low K intake (n=7); 4 of these patients received DOCA after 1 week of K depletion. Group 2 had hypokalemia due to hyperaldosteronism, exogenous or endogenous (n=15); 10 patients received a water load after 1 week of DOCA administration.

		PLASMA K	TTKG
Group 1	No DOCA	3.3±0.2	0.8±0.2
(low K diet)	DOCA	3.0±0.2	1.8±0.6
Group 2	No H_2O	3.2±0.2	7.6±2.1
(hyperaldo)	H_2O	3.1±0.2	0.9±0.2

(Mean±SD)

The TTKG differences between the groups were independent of the cumulative K depletion. Conclusion: The expected TTKG during hypokalemia with low mineralocorticoids is close to 1. The TTKG response to mineralocorticoids during hypokalemia is markedly diminished if the K depletion is not the result of hyperaldosteronism or if hypoosmolar urine is excreted.

HYPERKALEMIA IN ACUTE GLOMERULONEPHRITIS: A MANIFESTATION OF TRANSIENT HYPORENINEMIC HYPOALDOSTERONISM. B.R. Don* and M. Schambelan. Div. of Nephroendocrinology, San Francisco Gen. Hosp., Univ. of Calif., San Francisco, CA.

Hyperkalemia occurs frequently in patients with mild to moderate chronic renal insufficiency (CRI), usually as a consequence of hyporeninemic hypoaldosteronism (HH). Hyperkalemia has been reported infrequently as a transient phenomenon in patients with acute glomerulonephritis (AGN) but the pathogenic mechanism has not been investigated systematically. We studied the mechanism of hyperkalemia (K^+ 6.0-6.7 mEq/L) in 4 men with post-infectious AGN (hypertension, edema, proteinuria, RBC casts, elevated ASO, and moderate azotemia) confirmed by biopsy in 3/3.

Pt	CrCl ml/min	UprotV g/24h	K^+p meq/l	PRA ng/ml/h	UaldoV µg/24h
1	65	3.1	5.2	0.2	2.0
2	39	7.5	5.7	0.7	1.4
3	35	14.5	6.4	<0.1	*
4	24	6.1	5.6	0.2	2.0

* Plasma aldosterone 6.6 ng/dl

Hyporeninemia and hypoaldosteronism was evident in all. Hyperkalemia resolved rapidly (1-2 wks) without treatment in Pts 1 and 2 as AGN resolved, diuresis ensued and PRA and aldosterone levels returned toward normal. Hyperkalemia persisted despite furosemide-induced natriuresis in Pts. 3 and 4, but was ameliorated with fludrocortisone. Hyperkalemia and azotemia resolved in Pt 3 within 1 month. Hyperkalemia also resolved in Pt 4 but progressive CRI developed. Thus, hyperkalemic pts with AGN have HH that is usually transient in nature and resolves with improvement in renal function and the onset of diuresis.

ROLE OF CORTICAL COLLECTING DUCT (CCD) IN THE PATHOGENESIS OF METABOLIC ALKALOSIS IN BARTTER'S SYNDROME (BS). D. Ben-Ezer Gradus, J. Rapoport, C. Chaimovitz (Intr. by R.B. Ettenger). Dept. of Pediatrics & Nephrology, Soroka Med. Ctr., Ben-Gurion Univ., Beer-Sheva, Israel.

The alkalosis of BS has been explained by a distal tubular H^+ loss obligated by a defect in Henle's loop. However, studies of BS have not shown net acid loss. The mechanism of alkalosis in a patient with BS was studied. We used amiloride which blocks distal Na channels in the CCD, thereby inhibiting H^+ secretion at this site. A 5 year old girl with BS was studied. Net acid excretion (NAE) on 3 consecutive days was negative, thus the alkalosis could not be explained by her NAE. Blood samples and hourly urine collection were obtained 2 hours before and 6 hours after 10mg of amiloride.

Urinary values (uEq/min.)

	Na	K	HCO_3	ΔpCO_2
Before amiloride	34	33	23	25
After amiloride	178	20	93	15

High urinary minus blood pCO_2 (ΔpCO_2) indicates H^+ secretion by the distal nephron in BS and the decrease in ΔpCO_2 after amiloride suggests that the CCD is the site of H^+ loss. The alkalosis in BS may result as follows: A transport defect in Henle's loop leads to increased delivery of NaCl to the CCD causing an increase in NAE and generating alkalosis. Subsequently the large filtered load of HCO_3 floods the CCD with this anion which is titrated by H^+. The high ΔpCO_2 is the evidence for this hidden H^+ loss which could only be unmasked following administration of amiloride. However, this H^+ loss is occult because of titration with HCO_3 and thus cannot be measured as NAE.

BRAIN EDEMA (BE) AND PLASMA VASOPRESSIN DEPENDENCE ON GLUCOSE AND INSULIN DURING DIABETIC KETOACIDOSIS Johannes Hensen*, William H. Hoffman*, Allan H. Sklar, Taher El Gammal*, Curt M. Steinhart*, Jacques A. Durr.* Univ. of Colorado, Dept. of Nephrol., Denver, Co, and Med. College of Georgia, Dept. of Pediatr., Med., and Radiol., Augusta, Georgia.

Plasma electrolytes, glucose and AVP were determined serially in 7 pediatric patients prior to and during treatment of diabetic keto(acido)sis. BE was assessed by cranial CT scans. Admission plasma HCO_3 and glucose were 10 ± 3 mEq/l and 537 ± 62 mg/dl (±sem) respectively. Plasma AVP correlated weakly with [2PNa] ~ ([Posm - BUN/2.8 - Glu/18]), ($r = .52$, $p = .03$) and unlike in normals, correlated better when glucose was considered as an impermeant solute [2PNa + Glu/18], ($r = 0.81$, $p < .01$). AVP correlated best with the tonicity form [2Na + σ(Glu/18)] ($r = .965$, $p < .001$) of "effective" osmolality, where σ represents the reflexion coefficient for glucose ($0<\sigma<1$). The empiric formula $\sigma = [(26 - HCO_3)/26]$, with plasma HCO_3 as an index of cell permeability to glucose, accounts for the shifting value of σ during treatment.

Six patients had asymptomatic BE which, paradoxically, preceded therapy. AVP decreased and correlated with plasma tonicity irrespective of the BE which worsened transiently in half of the patients. The dissociation between BE and AVP osmoregulation support an insulin sensitive osmoreceptor located outside the blood brain barrier which becomes glucose responsive during uncontrolled diabetes. These osmoreceptor cells probably do not accumulate "idiogenic osmoles" in response to hyperglycemia since the threshold tonicity for AVP secretion was unaltered. Finally, current concepts on the pathogenesis of BE in diabetes need revision since BE precedes any form of therapy.

URETERAL OBSTRUCTION DECREASES ATP DEPENDENT H PUMP ACTIVITY OF RABBIT RENAL OUTER MEDULLA. H. Kanemitsu,* S. Sasaki, T. Akiba,* K. Tomita, and F. Marumo. Dept. of Internal Medicine, Tokyo Medical and Dental Univ. Tokyo, Japan

Obstructive uropathy is accompanied by distal renal tubular acidosis. H pump in outer medullary collecting duct plays a dominant role in distal acidification. It is not well known whether this pump activity is reduced in obstructive uropathy. To examine this possibility, ATP dependent H pump rich membrane vesicles were obtained from outer medulla of rabbit kidneys. Unilateral ureteral obstruction was performed 24hr prior to the studies. H pump activities were determined by intravesicular H accumulation measured by acridine orange quenching. ATP dependent H pump was almost completely inhibited by N-ethylmaleimide(0.5mM) and dicyclohexylcarbodiimide(0.2mM), but not inhibited by oligomycin(10μg/ml), vanadate(0.1mM), and removal of ambient K. In 9 rabbits, H pump activities were significantly reduced in obstructed kidneys (4.88±0.93 vs. 6.28±0.94 AFU/min/mg protein, $p < 0.05$). A possibility that this difference was due to altered permeability of the vesicles to H/OH was denied by the observation that collapsing rates of pH gradient imposed inside the vesicles were not significantly different in both kidneys (the rates of obstructed kidneys were 116.6±21.3% of intacts, n=6).

In conclusion, 1)In rabbit renal outer medulla, there is ATP dependent H pump which is not mitochondrial origin, and does not require K. 2)Decreased activity of this pump may contributes to distal acidification defect in obstructed kidneys.

IMPORTANCE OF FUEL COMPETITIONS AND ENERGY TURNOVER RATE IN KETOACIDOSIS. K Kamel*, S Cheema-Dhadli*, ALA Fields and ML Halperin. Department of Medicine, University of Toronto and Alberta, Toronto and Edmonton, Canada.

The purpose of this study was draw attention to the importance of fuel competitions and energy turnover rate on the degree of ketoacidosis. In chronic fasted man, the rate of ketoacid (BHB) production is 1500 mmol/day; this exceeds the normal buffer capacity (1000 mmol). Thus even a 10% reduction in the rate of BHB oxidation could lead to severe ketoacidosis within a few days. The first objective was to displace BHB oxidation by promoting carbohydrate as a fuel with dichloroacetate (DCA). Obese rats were fasted 6 days; on days 3-6, half received 450 umol DCA/kg ip bid and controls were given equimolar NaCl. The plasma BHB was 4.3 mmol/l in the DCA group whereas it was 1.5 mmol/l in the control group, ($p < .05$). The second protocol was designed to decrease BHB oxidation by lowering cerebral oxygen consumption. An alternate ketogenic pathway was also used, metabolism of 1, 3-butanediol (BD) by liver alcohol dehydrogenase. Fasted rats given BD were then divided into 2 groups awake or anaesthetized. Despite the fact that BD levels fell to a lesser extent in anaesthetized rats (awake 28.4 mmol/l, anaesthetized 33.2 mmol/l, $p < .05$), the plasma [BHB] rose to a greater extent in anaesthetized vs awake rats (+ 1.5 mmol/l, $p < .05$). Conclusion: The degree of ketosis can be influenced significantly and promptly by factors which modulate the rate of BHB oxidation.

RENAL SALT WASTING IN AIDS. J.K. Maesaka, A.J. Cusano,* F.P. Siegal*and H.L. Thies. Dept. of Med. Long Island Jewish Med. Ctr., New Hyde Park, NY.

We performed sodium (Na) balance studies to define renal salt wasting in AIDS. One of several patients with AIDS who had orthostatic hypotension (OH), normal serum creatinine, normal urinalysis, low central venous pressure (CVP), normal autonomic and adrenal function tests and high plasma renin activity (PRA) and aldosterone (AL) levels was placed on a 15-20 mEq Na diet for 5 days prior to study. The CVP was 0 cm H..2..0 when saline infusion was started at a rate of 300 ml/hr for 13 hrs until the CVP increased to 3-4 cm. The infusion rate was adjusted to maintain CVP constant at 3-4 cm for 36 hrs then stopped and the patient placed on a low Na diet.

The initial infusion resulted in a positive Na balance of 520 mEq, weight gain of 7.5 lbs, correction of OH and decrease in PRA and AL to normal levels. Over the ensuing 36 hrs the patient required an average of 978 mEq Na/day to maintain CVP and weight constant without developing hypertension or edema. Creatinine clearance (Ccr) increased from a baseline of 55 to 90 ml/min, urine Na from 60 to 134 mEq/L and fractional excretion (FE) of Na from 0.29 to 6.31% as Na input matched Na output. On a low Na intake, there was a negative Na balance of 557 mEq in 33 hrs as the CVP and weight decreased to baseline values, OH recurred, PRA and AL levels increased, Ccr decreased to 45 ml/min urine Na was 138 mEq/L and FE Na 0.50%.

This Na balance study demonstrates a significant renal salt wasting syndrome in AIDS that may have profound clinical effects.

A NEW METHOD FOR ESTIMATING G-I ABSORPTION OF ALKALI. Man S. Oh and Hugh J. Carroll. State University of New York, Health Science Center at Brooklyn, Department of Medicine, Brooklyn, N.Y.

Alkali absorption from the G-I tract has been estimated as the difference between non-combustible cations and anions in the food minus that in the feces. On a normal diet, G-I absorption of alkali is substantial, but is difficult to quantify; current techniques are cumbersome, requiring prolonged stool collection, open to errors in collection and measurement. A new method for measuring net G-I absorption of alkali is presented here, which is easy and accurate and is based on electrolytes in urine instead of stool and food. Net G-I alkali absorbed is estimated as: urinary (Na+K+Ca+Mg)-urinary (Cl+1.8 P). This method assumes that, if serum bicarbonate is stable and bone and cellular buffering negligible, the difference between non-combustible cations and anions in urine should reflect the difference between these ions as they are absorbed from the G-I tract. A comparison was made of net G-I absorption of alkali by both the conventional technique and the new method in steady state conditions of acid-base metabolism in humans. The correlation between the 2 sets of results was highly significant ($r=0.99$ $p < 0.001$). Only during acute acid or alkali loading or immediately after acid loading did the two methods produce discrepant results. Analysis of G-I alkali absorption using urinary electrolytes can provide a useful tool for assessment of the role of the G-I tract in acid-base balance.

RADIOACTIVE IODINE INDUCED HYPOPARATHYROIDISM A RARE CAUSE OF HYPOCALCEMIA (HYPOCA) IN ADVANCED CHRONIC RENAL FAILUR (CRF). R.M. SHAH, S.A. SADJADI, VAMC, WILKES-BARRE, PENNSYLVANIA.

In advanced CRF, hypo.Ca. is usually caused by hyperphosphatemia and decreased renal production of 1-25 $(OH)_2D_3$. A 68 year old man with Paget's disease of bone and CRF due to chronic interstitial nephritis developed thyrotoxicosis and was given Radioactive iodine. Sixteen months later, while in the hospital for CHF, he was found to have: Creatinine clearance 20ml/min, serum ionized calcium (ca) 1.8 mEq/l (Normal 2-2.75), total Ca 3.6 mEq/L (Normal 4.25-5.25), phosphorous 1.6 mmol/L (Normal 0.8-1.5), Alkaline phosphatase 582 I.U. (Normal 30-103), PTH 0.5 ng/ml (Normal <0.9 ng/ml), serum 1-25 $(OH)_2D_3$ 69.5 pg/ml (Normal 37-69), spot urine cyclic AMP 0.2 micromol/l (Normal 1-7), Bone Biopsy: increased osteoblastic and osteoclastic activity. Patient received Vitamin D and supplemental oral calcium but few weeks later died because of cardiac arrhythmia. This is a rare condition, and only six cases of it have been described in the past 35 years.

Conclusion: Radioactive Iodine induced hypoparathyroidism is a rare cause of hypo. Ca in advanced chronic renal failure. It should be differentiated from hypocalcemia of CRF and its attendant secondary hyperparathyroidism. It responds readily to vitamin D and calcium supplementation.

CISPLATIN-ASSOCIATED SALT WASTING TUBULOPATHY. S. Owen*, and F. Llach. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, Okla.

Nephrotoxicity with a dose-dependent acute renal failure and magnesium wasting is a well documented consequence of cisplatin therapy. However, salt losing tubulopathy has only rarely been recognized. The present study evaluates 3 patients in whom the use of cisplatin was associated with sodium wasting. These patients, ages 62, 47, and 28 years, had colonic adenocarcinoma, squamous cell carcinoma, and extragonadal nonseminomatous germ cell tumor, respectively. Cumulative cisplatin doses of 156-360 mg were administered over a period ranging from one day to 4 weeks. Each patient presented with severe orthostatic symptoms and clinical volume depletion concurrent with a spot urine sodium ranging from 117 to 206 mEq/L. Plasma cortisol levels ranged from 11.2 to 9.9 µg/dl at baseline and 24.5 to 34.4 mcg/dl after ACTH (.25 mg) stimulation. Baseline creatinine clearances ranged from 62 to 80 ml/min. Each patient initially required intravenous crystalloid ranging from 554 to 125 mEq of sodium (4-8 liters of normal saline) per 24 hours to remain free of orthostasis. Nuclear blood volume determination in patient #1 was 2.6 liters and increased to 4.1 at discharge. All patients were discharged asymptomatic and free of orthostasis 30-32 days after admission.

In summary, three patients treated with cisplatin developed renal salt wasting which subsided after appropriate therapy. It is likely that the proximal nephron may be the site of cisplatin tubular toxicity.

OSMOTIC DEMYELINATION SYNDROME (ODS): AN EXPERIMENTAL MODEL. Richard H. Sterns, Darbbie Thomas,* and Robert M. Herndon*. Univ. of Roch. Sch. of Med., Dept. of Med. & Neurol., Rochester, NY.

Delayed neurologic findings and demyelinating brain lesions may develop a few days after treatment of severe hyponatremia. We studied the pathogenesis of this clinical syndrome (known as ODS) in an experimental model. Rats were made hyponatremic (Na=98 mEq/L) over 3 days with DDAVP and electrolyte-free tube feedings. The serum Na was then rapidly increased to 116-130 mEq/L and was maintained at this level for 24-48 hours. The animals were mildly sluggish during the induction of hyponatremia and became more alert immediately after treatment. One to two days after correction, seizures, stupor and gait disturbances began to develop. The brains of animals with untreated hyponatremia were histologically normal. Within 30 minutes after the administration of 1 M NaCl or 6 hours after the withdrawal of DDAVP, disruption of myelin was seen. Electron microscopy showed that this early lesion represented unraveling of the myelin sheath from large axons. We conclude that in our experimental model, ODS is caused by a large rapid increase in the serum Na and cannot be ascribed to hyponatremia itself or to anoxia. Demyelination appears to develop because of osmotic shrinkage of the axon, which severs the connections from its surrounding myelin sheath. Subsequent pathologic events (infiltration of macrophages, etc.) initiated by this primary lesion take place over several days and may explain why the neurologic manifestations of the disorder are delayed.

NEUROPATHOLOGICAL SEQUELAE OF CONTROLLED CORRECTION OF CHRONIC HYPONATREMIA IN RATS. J. G. Verbalis* and A.J. Martinez* (intr. by A. Greenberg). Depts. of Medicine and Neuropathology, University of Pittsburgh, Pittsburgh, PA.

Numerous investigators have produced myelinolysis by correction of experimental hyponatremia, but such studies have generally used acutely hyponatremic animals with high mortality rates. We reexamined this question using methods that maintain chronic hyponatremia in healthy non-catabolic rats (Verbalis and Drutarosky, KI, Sept. 1988). After 21d of hyponatremia, rats were corrected only to normotonicity via either slow intravenous infusion of hypertonic saline (HS, n=22), or water diuresis by withdrawing DDAVP (WD, n=26). A control group did not undergo correction of hyponatremia (CTRL, n=20). Blood was collected every 2h to calculate both maximal and 24h rates of correction. Rats were sacrificed either when severe neurological deficits became apparent or after 5-7d.

	$p[Na^+]$ (mEq/L)	$\Delta p[Na^+]$ (mEq/L/h) MAX	24 h	Mortality	Demyelination
HS	112 ± 2	5.5 ± 0.2	1.1 ± 0.1	32%	90%
WD	111 ± 2	2.0 ± 0.2	1.2 ± 0.1	12%	21%
CTRL	111 ± 2	0	0	0%	0%

Consequently, correction via WD alone also can cause neuropathological changes in hyponatremic animals, though less frequently than HS probably due to slower maximal rates of correction despite equivalent 24h rates in both groups. These results are consistent with clinical findings suggesting that correction of hyponatremia at rates under 2 mEq/L/h is only infrequently associated with neurological morbidity. However, they raise the possibility that future use of AVP receptor antagonists causing rapid water diuresis in chronically hyponatremic patients may be accompanied by a small but clinically significant incidence of myelinolysis.

IMPORTANCE OF BROMIDE AS A CAUSE OF HYPERCHLOREMIA AND LOW ANION GAP. Thomas M. Zipp, Michael Sealfont and Glenn Rech. Mt. Sinai Medical Center, Cleveland, Ohio.

Whereas bromide is no longer used therapeutically, it is complexed with pyridostigmine as Mestinon (M). Average daily intakes of bromide in patients with myasthenia gravis on M are 2-3 meqs. per day. Spurious elevation of chloride from pyridostigmine bromide has been reported (Archives of Neurology Vol. 38:321, May 1981.) The extent of this problem in a tertiary care 500 bed community teaching hospital is described.

A computer generated 5 year retrospective search was made for myasthenia gravis. Of 22 different patient charts, 16 were suitable for review. Eleven of the 16 patients were on M upon admission. Ten of these 11 patients had an anion gap of less than 6. Five of the 16 patients were not on M and none of these had an anion gap less than 6. Similarly, chloride determinations were reviewed. The average serum chloride was 113 meqs./L in patients on M (95% confidence interval 110-117 meqs./L) and was 102 meqs./L in patients with myasthenia gravis not on M (95% confidence interval 96-108 meqs./L).

In conclusion, M is probably the commonest cause of a significant low anion gap, it should also be considered as a cause of hyperchloremia.

PSEUDOHYPOCHLOREMIA AND ANION GAP ELEVATION IN PATIENTS WITH RENAL FAILURE DUE TO ALLOPURINOL AND PURINE METABOLITE ACCUMULATION

Bruce Wall, Steven Rinner, Michael Emmett, Depts. Nephrology & Pathology, Baylor University Medical Center, 3500 Gaston, Dallas, Texas 75246.

The accumulation of allopurinol and purine metabolites in patients with renal failure and accelerated purine generation can produce a systematic underestimate of chloride concentration (Cl) when measured with certain ion specific electrode instruments (Ektachem , Eastman Kodak). We report 3 examples of this phenomenon and results of in vitro addition of purine metabolites and allopurinol to normal serum.

	DX	BUN	Na	HCO_3	Ektachem Cl	AG	Chloridometer Cl
1	AML	76	120	32	53	35	--
2	CGL	150	134	34	57	43	99
3	ALL	100	142	25	65	52	104

In Vitro Addition	mg%	Ektachem Cl (mEq/L)	Chloridometer Cl (mEq/L)
Control		104	104
Allopurinol	3.5	69	104
Allopurinol	7.0	48	104
Hypoxanthine	5.0	62	103
Hypoxanthine	10.0	48	105
Uric Acid	15.0	105	104
Uric Acid	30.0	106	103

The in vitro study shows that the negative interference is due to the accumulation of allopurinol, hypoxanthine or both.

This artifact suggests mixed metabolic acidosis alkalosis in this patient population.

QUANTITATION OF PROTEINURIA BY DIPSTICK AND PROTEIN:CREATININE INDEX (U P/CR) IN NEPHROTIC CHILDREN. Carolyn Abitbol, Gaston Zilleruelo, Michael Freundlich, Jose Strauss. Dept. of Pedi., Univ. of Miami Sch. of Med., Miami, Fl.

The rapid, accurate assessment of proteinuria in children with nephrotic syndrome (NS) remains difficult. This study examined the relative feasibility of the random urinary dipstick (Dip) and random and 24 hour U P/Cr (mg/mg) in quantitating daily proteinuria (TP). Sixty-four children (aged 1.5 to 16 years) with relapsing NS contributed 145, 24 hour urine collections and 150 random specimens analyzed by Ames® protein Dip, and/or quantitation of protein and creatinine. Degrees of TP were designated as physiologic (< 0.1 $g/m^2/d$), intermediate (> 0.1 < 1.0 $g/m^2/d$) and nephrotic (> 1.0 $g/m^2/d$). The log regression analyses of 24 hour U P/Cr (x) and random U P/Cr (x) to TP (y) were highly significant ($r > 0.95$, $p < 0.001$) and yielded the identical regression equation $y = x + 0.2$. The lower confidence limits of U p/cr from this regression designated physiologic and nephrotic proteinuria as 0.1 and 1.0 respectively. The relative validity of these tests to quantitate TP was assessed.

	Dip	24 hour U P/Cr	Random U P/Cr
Sensitivity (%)	68	93	100
Specificity (%)	63	59	100
Predictive + (%)	86	90	92
Predictive − (%)	42	72	100

The random U p/cr demonstrated a distinct advantage over 24 hour U P/Cr and random dipstick in quantitating TP. Calculation of TP from the linear regression is provided by the equation TP ($g/m^2/d$) = 0.6 (U P/Cr).

RACIAL DIFFERENCES IN RENAL INVOLVEMENT ASSOCIATED WITH HYPERTENSION IN NON-INSULIN DEPENDENT DIABETES MELLITUS (NIDDM). J. Anderson, P. Duggal*, J. Zachary and W. G. Walker. Johns Hopkins Univ. Sch. of Med., Baltimore, Maryland.

Microalbuminuria (μAlb) measured by RIA, GFR, glycosylated hemoglobin (GHb) and blood pressure (BP) in 56 black and 56 white hypertensive NIDDM after stopping BP Rx revealed identical mean GFRs in both groups: (W:80.3 ml/min ± 26 SD; B:80.3 ml/min ± 25 SD), but whites were older (W:60.8 yrs ± 8.3 SD; B:56.9 yrs ± 7.8 SD, p<.02). No differences in syst. BP (W:158 mmHg ± 14 SD; B:163 mmHg ± 22 SD), diabetes duration (W:11.2 yrs ± 7.7 SD; B:9.2 yrs ± 5.2 SD) or GHb (W:9.9% ± 3.1 SD; B:11.1% ± 3.6 SD) were seen but blacks exhibited greater μAlb (B:257 μg/ml ± 430 SD; W:118 μg/ml ± 266 SD; p<.02) and only the blacks showed μAlb negatively correlated with GFR (r=-.34; p<.01). A stronger negative correlation between GFR and BP was present in blacks (r=-.46; p<.001) than in whites (r=-.21; p=.1). Although diuretic usage was slightly increased in blacks (B:36/56; W:24/56; p<.05), only whites exhibited a decreased GFR associated with diuretic use (diur. 69.8 ml/min ± 4.3 SE; non-diur. 88.4 ml/min ± 4.9 SE; p<.008). The strong association between GFR and both BP and increased μAlb in blacks suggests that hypertension may be at least one factor accounting for the greater incidence of ESRD in blacks with NIDDM. The apparent racial difference seen with diuretic usage requires further study.

LY146032 RENAL EXCRETION IN NORMAL SUBJECTS AND PATIENTS WITH RENAL INSUFFICIENCY. George R. Aronoff, and Rebecca S. Sloan*. Depts. of Med. and Pharmacol. Univ. of Louisville and VAMC, Louisville, Ky.

LY146032 (LY) is the first member of a new class of antibiotics, the lipopeptides, bactericidal against gram positive bacteria. Because LY may be used like vancomycin to treat Staphylococcus infections in patients with ESRD, we tested the hypothesis that renal failure alters LY disposition. We gave 6 normal subjects (N), 4 renal insufficiency patients (CRI), and 2 hemodialysis patients (HD), 1 mg/Kg i.v. Plasma LY was measured 18 times after infusion. A compartment independent kinetic analysis was performed. The creatinine clearance (GFR), peak level (Cmax), half-life (T1/2), distribution volume (Vd), renal (Clr), nonrenal (Clnr), and plasma clearances (Clp) were:

	N	CRI	HD
GFR (ml/min)	133	21#	0#
Cmax (mg/L)	14.6	14.5	13.0
T1/2 (hr)	8.8	16.4#	22.5#
Clr (L/hr)	.335	.055#	0#
Clnr (L/hr)	.268	.215	.230
Clp (L/hr)	.603	.270#	.230#
Vd (L/Kg)	0.12	0.09	0.11

#significantly different than N

56% was recovered in the urine in N. We conclude that renal insufficiency slows LY elimination by decreasing LY renal excretion but does not affect nonrenal clearance or distribution. Dose reduction is needed to prevent drug accumulation in CRI and HD. Like vancomycin, LY has a long T1/2 in renal failure and therapeutic levels can be maintained in HD by giving the drug after dialysis.

MORPHOLOGY AND MEAN CORPUSCULAR VOLUME (MCV) OF NONGLOMERULAR (NG) HEMATURIA (HMT). A. Antignani*, P. Goldwasser*, N. Mittman, F.DiPillo*, M.M.Avram. The Long Island College Hospital, Brooklyn, NY

Red cells (RBC) of NG origin have been shown to have normal to increased MCV's when sized in isotonic hematology diluent (IsoD). Stomatocytes and ghost cells have been described in NG sediments, compatible with RBC overhydration, but occasionally crenated cells are described, suggesting loss of volume. To study the influence of urine composition on MCV, we measured urinary (U) MCV, Na, K, Cl, Na+K, Na+K-Cl, osmolality, pH and peripheral (P) MCV in 6 cases of NG HMT. Mean U-MCV, normalized to P-MCV, was 1.05±0.12[SD]fl. Normalized U-MCV correlated only with pH (r=0.97 p<.002). NG-RBC or P-RBC incubated in pH-adjusted aliquots of various urine supernatants or 1mM phosphate-buffered Ringer's lactate (PBRL) also showed this correlation. The acid-related volume increase reversed with a $t_{1/2}$ of approximately 3 minutes if the RBC were allowed to incubate in IsoD. In PBRL solutions of pH>7.7, P-RBC appeared crenated; their MCV's (in IsoD) were at or below P-MCV. Incubation of P-RBC in unbuffered saline solutions (0.5% to 6%) did not affect MCV, compatible with the fact that osmotically-driven uptake or loss of water alone should be reversed in seconds after placing the cells in IsoD. In contrast, based on the $t_{1/2}$ of its reversal, acid-related swelling is probably mediated by RBC update of ions (H and Cl) and, secondarily, water.

We conclude that pH is an important determinant of the size and morphology of NG RBC.

USE OF $1,25D_3$ IN PATIENTS WITH MODERATE RENAL FAILURE. L.R.I.Baker*,S.M.L.Abrams*,C.J.Roe*,M-C.Faugere*,P.Fanti,Y.Subayti*,H.H.Malluche Dept.of Neph.,St. Bartholomew's Hosp.,London, U.K.; Div.of Neph., Bone & Mineral Metabolism, Univ. of Kentucky Medical Center, Lexington, KY.

To evaluate the effects of $1,25D_3$ in patients (pts) with moderate renal failure, we conducted a randomized prospective double-blind study. Sixteen pts with a mean creatinine clearance of 39±4 ml/mn were enrolled. 13 pts completed the study. 7 pts received $1,25D_3$ (0.25-0.5. μg/day) for 12 mos., the others received placebo. Blood and urine samples were taken at baseline and every mo. after. Bone biopsies were performed before and at the end of the study. At baseline, no pts complained of bone pain and no X-ray changes were seen. Serum 1,25D levels were low (16.3±1.9 pg/ml) and PTH levels elevated in 7/13 pts (0.78±0.10 pmol/ml). At the start of the study, bone histology was abnormal in all pts. $1,25D_3$ treatment was associated with a fall in serum phosphorus (p<0.01) and alkaline phosphatase concentrations (p<0.01). Histologically, there was improvement or normalization of bone as evidenced by a decrease in lamellar osteoid (4.7±1.1 vs. 2.2±0.7 mm^3/cm^3), woven osteoid (1.95±0.70 vs. 0.76±0.60 mm^3/cm^3), number of osteoblasts (429±58 vs 127±17 cells/100 mm) and osteoclasts (42±7 vs. 24±7 cells/100 mm). No such changes were seen in the placebo group. Despite episodes of hypercalcemia in 5 pts, no significant adverse effect of $1,25D_3$ treatment upon renal function was detected. These data show that $1,25D_3$ can be used for the management of bone disease in moderate renal failure if meticulous monitoring of urinary and serum calcium is done.

EFFECTS OF THE COMBINED INTAKE OF CALCIUM CITRATE AND ALUMINUM HYDROXIDE IN NORMAL SUBJECTS: Asad A. Bakir, Sarosh Ahmed*, and George Dunea. Cook County Hospital and the University of Illinois, Division of Nephrology, Chicago, Illinois.

We previously showed that serum aluminum values (SAl) in uremic and normal subjects, and the 24-hour urinary aluminum (UAl) in the latter increase upon the combined intake of aluminum hydroxide [Al $(OH)_3$] and Shohl's solution (sodium citrate and citric acid). We have now conducted this further study to see if calcium citrate (CaCit), used by many as a phosphate binder, has the same effect as Shohl's solution. Six healthy subjects took 1900 mg CaCit thrice daily for four days, washed out for two weeks, then took 0.6 g $Al(OH)_3$ thrice daily for four days, washed out for two weeks, then took both agents in the same doses for four days. Atomic absorption spectroscopy was used to measure SAl (µg/L), UAl (µg), and the percent fractional Al clearance (FcAl).

	SAl	UAl	FcAl
Baseline	4±1	12±3 *	6±2 *
CaCit	4±1	28±5	12±2
Washout	4±1	14±4 ††	6±3
$Al(OH)_3$	10±5	124±33	57±40
Washout	4±1 *†	26±10 **	38±33
CaCit + $Al(OH)_3$	9±2	95±30	16±6

*p<.05, † p<.05 vs. CaCit, †† p<.01 (p<.05 vs. CaCit), **p=.05 (All numbers rounded, results expressed as mean ± SEM).

We conclude that, like Shohl's solution, CaCit alone increases UAl and FcAl over the basal state. However, SAl and the high UAl seen with $Al(OH)_3$ alone do not increase further when CaCit in the above dose is added, but an additive effect cannot be ruled out with larger doses or in azotemic patients.

STEROID THERAPY IN THE FEBRILE SLE PATIENT. WH Bay, LA Hebert, D Middendorf, FG Cosio, NS Nahman, Dept of Med, Ohio State Univ, Columbus, OH.

The present study was undertaken to test the following hypotheses 1) when fever is caused by active SLE, the fever can be expected to resolve rapidly (usually within 24 hr) on a moderate dose of prednisone (P) (~30 mg) or an equivalent steroid. 2) When fever in an SLE patient does not resolve on a moderate or greater dose of P, or if fever develops while the patient is receiving a moderate or greater dose of P, it is likely that the fever is due to a process other than SLE, usually infection. To test this hypothesis, we analyzed the cases of 17 consecutive febrile SLE patients in whom detailed clinical data were available so that a determination of the presence or absence of active SLE vs another cause for fever, could be made. We found that of the 17 patients, 9 had the fever of active SLE, 7 had the fever of infection, and 1 had the fever of severe acute cholecystitis. In the patients with fever due to active SLE, the fever resolved in all patients within 24 hr, and usually within 12 hr, following the first dose of P (mean initial dose 35 mg, range 20-60 mg). By contrast, in the other 8 patients, the fever did not resolve on P therapy (mean daily dose 187 mg/day, range 40-960 mg day). In the patients with the fever of SLE, the peak oral temperature 24 hr after starting P was 98.2°F (range 97.8 to 99.0). By contrast, in the patients with fever from other causes, the mean peak oral temperature while receiving moderate to high doses of P was 101.9° (range 100.4 to 103). Furthermore, a fatal outcome from infection was observed in the 2 cases in which there was prolonged use of high dose steroid therapy in the face of continuing fever. Conclusions: 1) Moderate doses of P cause the fever of SLE to resolve within 24 hr. 2) Fever that develops or fails to resolve in SLE patients receiving moderate to high doses of P is due to a process other than SLE, usually infection.

PURIFIED PLASMA FACTOR FROM SUBJECTS WITH MINIMAL CHANGE DISEASE (MCD) AFFECTS ANIONIC SITES OF THE RAT KIDNEY EX VIVO. Winston W. Bakker†, Wilma H.J. van Luijk†, Jan Koudstaal* and Juul F.W. Baller†. Univ. Hosp. Groningen, Dept. of Pathol. and Pediatrics, Groningen, The Netherlands.(Intr.by Statius v.Eps)Circulating factors are thought to play a role in the pathogenesis of increased glomerular permeability in Minimal Change Disease (MCD). We recently isolated a vasoactive plasma factor (approx. 100 kD) from plasma of patients with MCD and from healthy donors. Following incubation with kidney tissue in vitro, this factor was able to affect glomerular sialoglycoprotein. We now studied the effect of this factor upon anionic sites (as) in the GBM of the rat kidney after perfusion ex vivo. Either 5 ml 100 kD factor (1.8 mg protein/ml saline) or equal amounts of human albumin (HA) were perfused ex vivo in kidneys of anesthetized PVG rats (n=10). Using polyethyleneimine (PEI, 1.8 kD pH 7.4) as an anionic marker in vitro, kidney sections were examined following perfusion, by randomly photographing capillary loops (47000x) of 15 resp. 16 glomeruli from 100 kD factor versus HA perfused kidneys. Numbers of as were counted over 400-700 cm distance and expressed per 1000 nm. A significant decrease in the number of as was found exclusively in the LRI's of kidneys perfused with the 100 kD factor versus those perfused with HA (13.6 ± 1.7/1000 nm vs 19.2 ± 3.0/1000 nm $p \leq 0.005$, Mann Whitney). No significant differences were seen in the number of stained LRE as. (43.2 ± 4.8/1000 nm vs 39.0 ± 7.1/1000 nm). Since it has been reported that the 100 kD factor is activated in MCD in relapse, we suggest that promotion of proteinuria in MCD may be mediated through direct affection of the endothelial anionic sites in MCD.

VEGETABLE VS ANIMAL PROTEIN: TO MINIMIZE RENAL HYPERFILTRATION Giorgio Bazzato*, Agostino Fracasso and Paolo Toffoletto*. Nephrology Division Umberto I Hospital, Venice-Mestre, Italy.

It has been clearly demonstrated that protein load induces an increase of GFR, thus this test has been used to determine the renal functional reserve (RFR). Nevertheless the effect of qualitatively different proteins (animal Vs vegetable) on GRF is still little known. Elevel healthy subjects following a protein diet of 1.1 gm/kgBW, underwent the study. Creatinine and Inuline clearances as well as urea and Na urinary excreation were evaluated in basal condition and hourly for 4 hrs after acute load of 80-90 gm (1.5 gm/kgBW) of animal (red meat) and vegetable proteins (yellow soya). The results obtained showed an increase of GFR from 107.3±6.2 to 161.8±11 and 133.2±7 ml/min/m^2 respectively 3 hrs after the meat and soya load with a net increase of 33.7 and 20% ($P < 0.001$) from the basal values. The urea and sodium excreation were comparable into the two phases of the study.

Conclusions: qualitatively different protein load determines a different hyperfiltrative response. These data suggest the possibility of integrating low animal protein diet with vegetable ones in the dietary management of CRF patients on conservative therapy, thus preventing renal hyperfiltration and maintaining an adequate protein intake.

COMPARISON OF RED CELL VOLUME DISTRIBUTION BY COULTER COUNTER (CC) TO PHASE CONTRAST MICROSCOPY (PCM) IN THE DIFFERENTIATION OF GLOMERULAR (G) FROM NON-GLOMERULAR (NG) HEMATURIA. William M. Bennett, Steven Fairley*, Douglas Birch*, Kenneth Fairley*, Department of Nephrology, Royal Melbourne Hospital, Melbourne, Vic., Australia.

PCM is a sensitive technique to distinguish G from NG hematuria. Recently, Schichiri et al reported similar results using automated red cell sizing with a CC to eliminate observer variability (Lancet 1:908,1988). The sensitivity of these techniques was compared using midstream urine samples from 44 patients with > 10^5 rbc/ml. Bleeding was classified as NG by PCM if there was a uniform isomorphic cell population and by CC if there was a distribution peak of cells > 75 μm^3. G bleeding was diagnosed by dysmorphic red cells on PCM and a unimodal or bimodal red cell population < 75 μm^3 on CC. The phase microscopist was blinded to the clinical diagnosis or CC results. 27 patients had biopsy proven glomerulonephritis and 14 patients had a single NG bleeding source. 3 patients had both G and NG sources.

Correct diagnosis and (%):

	PCM	CC
G (n = 27)	26 (96)	24 (89)
NG (n = 14)	14 (100)	10 (71)

Only PCM was successful in identifying 2 simultaneous bleeding sources. Conclusion: Both techniques have excellent sensitivity for G hematuria however PCM microscopy is more accurate for NG sources. PCM and CC are simple, inexpensive ways to avoid unnecessary invasive procedures. PCM is recommended to confirm G obtained by CC to avoid overlooking small isomorphic cells which require urologic studies.

NEPHROCALCINOSIS IN VERY LOW BIRTH WEIGHT (VLBW) INFANTS: EFFECTS OF TOTAL PARENTERAL NUTRITION (TPN) ON URINARY OXALATE & CALCIUM EXCRETION. G. Braden, T. Campfield*, Baystate Med. Ctr., Springfield, MA, Tufts School of Med., Boston, MA.

Nephrocalcinosis is common in VLBW infants, but the mechanisms for this disorder are not defined. Since TPN given to VLBW infants contains the oxalate precursors glycine and ascorbic acid, we evaluated the effects of TPN on urinary oxalate (Uox) and calcium (UCa) excretion. We measured Uox and UCa in timed 8 h urine samples in 10 VLBW infants not receiving diuretics who were fed daily first with Dextrose 10% (D_{10}) and then TPN with protein levels of 0.5 gm & 1.5 gm/kg/d. Ascorbic acid was given in fixed amounts of 35 mg/kg/d. Urinary ascorbic acid was measured in samples in all groups by HPLC and was below a level that interferes with the oxalate assay. Results are expressed as mean ± SEM:

	D_{10}	0.5 gm	1.5 gm
Uox (ug/ml)	13±5	27±3 *	40±8 * +
Uox/U creat	.19±.09	.30±.03	.41±.05 * +
UCa (ug/ml)	42±9	86±17 *	115±20 *
UCa/U creat	.53±.14	.87±.23	1.15±.15 *
U[Ca Ox]x10^{-7}M2	1.8±0.4	7.7±2.2	12.8±4.7 *

* p<.05 compared to D10; + p<.05, .5 vs 1.5 gm.

Uox excretion increased significantly between the D_{10} and 1.5 gm groups (p < .001). Changes in U creat excretion did not account for these results.

We conclude that TPN given to VLBW infants is associated with significant increases in Uox & UCa concentration & excretion and increases in urinary [Ca Ox]. These findings may be involved in the pathogenesis of nephrocalcinosis in VLBW infants.

TRIMETHOPRIM (TMP) BLOCKS TUBULAR CREATININE (Cr) SECRETION BUT NOT % MAXIMAL RISE IN Cr CLEARANCE (C) AFTER PROTEIN INGESTION IN MAN. Alison Boyle*, A Chapman*, S Mulroney*, R McPherson*, S Bailey*, J Winchester. Georgetown Univ, Wash, DC, USA & Edinburgh Univ, Scotland.

TMP blocks tubular secretion of Cr, but it's effect on CCr after protein ingestion is unknown. After an overnight fast, we gave 200 mg oral TMP to 13 healthy subjects (11m, 2f: age 32 ± 8.7 yrs) 6 hr prior to hydration (800 ml water over 20 min, followed by 300 ml/hr) and ingestion of 1 g/kg (mostly red meat) protein. Serum Cr (SCr), CCr, Na, and K excretion at timed intervals, were compared in the TMP study (study 2) to prior studies without TMP (study 1) in same individuals.

	Baseline		120 min		240 min	
Study	1	2	1	2	1	2
SCr (mg/dl)	1.03 ± 0.15	1.14* ± 0.19	1.05 ± 0.16	1.19** ± 0.23	1.02 ± 0.16	1.21** ± 0.22
Na (nEq/min)	106.9 ± 66	91.2 ± 55	99.5 ± 74	100 ± 39	145 ± 107	130 ± 56
K (nEq/min)	65 ± 24.6	50.7 ± 28.2	44.5 ± 18.6	38.8 ± 14.2	56.3 ± 25.6	51.4 ± 18
			Study 1	Study 2		
Max CCr (ml/min)	102.7 ± 21.8	78& ± 14	138.8** ± 22.8	106.8** ± 27.7		
% Max rise CCr			36.9 ± 15%	37.4 ± 26.5% (p >0.05)		

* higher than study 1 (p <0.05), & lower than study 1 (p <0.05)
** higher than comparable baseline period (p <0.05)

These results indicate a 21% contribution of tubular Cr secretion to CCr in these subjects. Moreover, TMP is associated with an increase in SCr after ingestion of protein (and Cr in red meat), which was not seen without TMP (in this and other studies). Percent maximal CCr response to protein ingestion was not impaired by TMP. It is possible that use of TMP gives C more accuracy when measuring glomerular filtration rate after protein ingestion. Further studies are necessary to determine duration of effect of TMP.

IMPACT OF RACE ON RENAL DISEASE IN PATIENTS WITH AIDS. E. Scott Cantor*, Paul L. Kimmel and Juan P. Bosch. George Washington Univ. Med. Ctr., Dept. of Medicine, Washington, D.C.

Controversy exists regarding the racial distribution of renal disease associated with AIDS (AAN). One group reported an elevated incidence of AAN in a population of AIDS patients with a high percentage of blacks and IV drug abusers (IVDA). In another center, with few black patients or IVDA, the incidence of AAN in AIDS patients was low. To determine the racial distribution of AAN we retrospectively studied 330 patients (62% black, 37% white) with AIDS (defined according to CDC). 53 (16%) had concurrent renal disease . Acute Renal Failure (ARF) was defined as a rise of 1.0 mg/dl of serum creatinine (Baseline < 2.0 mg/dl). Chronic Renal Failure (CRF) was defined as a persistent elevation in plasma creatinine > 2.0 mg/dl. Tissue diagnosis was available in 30% of the ARF and in 40% of the CRF subjects. Results:

	Total # Subjects	Black	White	Gay	IV	Others
AAN	53	34	19	46	6	1
Acute RF	39	21(39%)	18 (34%)	34	5	0
Chronic RF	14	13 (25%)	1 (2 %)	12	1	1

In ARF mortality was 34%, 5% progressed to CRF and 61% recovered. In CRF mortality was 50%; 57% required dialysis. Of those on dialysis 50% had long term survival. In CRF all tissue material revealed focal glomerulosclerosis. In ARF and CRF there were no differences between black and white patients in age, sex, incidence of homosexuality or mortality. Chi square analysis demonstrated a significant difference in the percentage of CRF (but not ARF) in black and white patients (p<.009).We conclude CRF associated with AIDS occurs in subjects without a history of IVDA. Race is an important risk factor in the development of CRF in patients with AIDS.

MECHANISM OF RENAL DAMAGE FOLLOWING PYELONEPHRITIS (PN) IN YOUNG RATS. G Celsi*, L Hannerz*, S Olling*, and I Wikstad*. Dept of Pediatrics and Radiology, Karolinska Institute, Stockholm, and Dept of Pathology, Univ. of Gothenburg, Sweden.

Childhood PN is a common cause of reduced renal function and hypertension in young adults. Early diagnosis is essential but difficult. We report a model of experimental ascending PN that allows studies of the natural history and the pathophysiology of this disease. A solution containing E. Coli (10^7/ml) was injected intravesically with constant pressure and infusion rate in 20-day-old female rats. Several groups of rats were studied either 5 or 30 days after infection (n = 12-26 rats in each group). At 5 d, E. Coli was cultured in 100% of the kidneys. Renal areas with decreased ^{99m}Tc-DMSA uptake were found in 25/26 rats. Those areas always had histopathological changes typical of acute PN. Histopathological changes were generally absent in tissue where DMSA uptake had been normal. After 5 d the rats were left untreated (U) or treated with trimethoprim-sulfa (T) for 5 d. After 30 d, body weight was the same in U, T and control (C) rats. No histopathological lesions were detected in 10/12 U rats and in 11/13 T rats. Kidney area and weight and GFR were the same in T and C but significantly reduced in U. The number of filtering glomeruli was the same in U, T, and C. Cortical DNA content was significantly reduced in both T and U and was significantly lower in U than in T. Cortical protein/DNA ratio was the same in T and U and significantly higher than in C. Childhood PN inhibits cell growth. Conclusion: There is a compensatory increase in cell size which may blunt the gross signs of renal growth retardation in treated PN. DMSA-scan is valuable for early detection of PN.

PLASMAPHERESIS DECREASES THE PROGRESSION OF CHRONIC RENAL FAILURE. Paul G. Cohen and Larry Bennett, Atlanta, Georgia.

Most patients with chronic renal failure and high grade proteinuria inexorably progress to endstage renal failure. Attempts to prevent this progression usually fail. The present reports details the features of two patients with chronic renal failure and proteinuria who have been treated with monthly plasmapheresis with an IBM 29-97 blood cell separator for 4 and 8 years respectively.

Both patients experienced a marked decrease in proteinuria with only a minimal decrease in renal function.

	Patient #1		Patient #2		
	1980	1988	1983	1984	1988
Creat.	3.1	3.2	1.2	2.2	2.2
Creat.Cl.	44	39	78	41	39
Total Protein	3.7	0.3	14.4	14	3.1

These findings suggest that in addition to dietary and hypertension control, plasmapheresis may reduce the progression to endstage renal failure. Possible mechanisms include alterations of the thixotrophic properties of blood and lipid levels as well as immunological factors.

RENAL FAILURE ASSOCIATED WITH HEMOLYTIC UREMIC SYNDROME (HUS): A COMPLICATION OF BONE MARROW TRANSPLANTATION (BMT). M.Chandra, M.McVicar, M.Susin, I.Sahdev,* North Shore Univ. Hosp., Manhasset, N.Y. & Cornell Univ. Medical College New York, N.Y.

HUS with renal failure was observed in 2 of 25 patients who received BMT. Both patients received high dose Cytoxan, Ara-C and total body radiation prior to, & Methotrexate post BMT. Patient A (18 yr male) with leukemia in 1st remission, underwent a renal biopsy 5 mos post BMT because of microhematuria, WBC casts, normal protein excretion & serum creatinine (Scr) 1.7 mg%. His Hct & platelet (plt) count were stable at 35% & 130,000/µl. Glomeruli showed marked narrowing of capillary lumina from subendothelial expansion with deposition of fibrin & plasma like material, & new basement membrane formation. There was loss of endothelial cells & ballooning of glomeruli from expanded fibrillar mesangial matrix. Overt HUS emerged 1 mo after the biopsy with fragmented RBC, Hct 22%, plt 32,000/µl, BP 190/120mm Hg & Scr 3.3 mg%.

Patient B (20 yr male) received BMT for leukemia in 4th remission. HUS developed 6 wks after BMT (plt 10^3/µl, blood transfusion dependent, fragmented RBC, Scr 1.3 mg%, hematuria & moderate proteinuria). Chronic dialysis was initiated 7 mos after BMT. He died 1 mo later. At autopsy, light microscopy of kidneys revealed changes as in patient A.

Our findings indicate that BMT patients are at risk for development of renal failure from HUS. The HUS may be precipitated by capillary endothelial & mesangial cell damage from radiation and/or chemotherapy.

CONTINUOUS ARTERIOVENOUS HAEMOFILTRATION AS TREATMENT FOR ACUTE RENAL FAILURE COMPLICATING CEREBRAL MALARIA. *SL Cohen, *JC Kingswood, *P Street, *R Langford, *A Hall, *P Chiodini. Intr. by A. Watson. University College Hospital and Hospital for Tropical Diseases, London.

CAVH is a useful treatment for acute renal failure (ARF) particularly suited to intensive care units not requiring specially trained dialysis personnel. As malaria usually occurs in countries with limited trained renal personnel, we investigated the use of CAVH in treating malarial associated ARF.

2 Patients presented with cerebral malaria in London, one 10 days after returning from Malawi with hypovolaemic shock, oliguria and a toxic confusional state. Urea rose to 42mmol/l and creatinine to 561 µmol/l. She was treated with I.V. quinine for the falciparum malaria and CAVH for 3 days and made a complete recovery. A second patient presented with fever and an acute confusional state 14 days after returning from Zaire. He had falciparum malaria and pneumonia, toxic confusional state and two epileptic fits. B Urea rose to 50 mmol/l and creatinine to 337 µmol/l. He was treated with quinine, one exchange transfusion and CAVH for 4 days. He also made a complete recovery. CAVH controlled the biochemistry in our patients and facilitated management of the fluid balance. Cerebral oedema related to malaria may be exacerbated by dialysis disequilibrium making CAVH a safer form of treatment than dialysis. CAVH is cheaper and simpler than haemodialysis and a more suitable treatment for ARF in countries where malaria is endemic.

NITROPRUSSIDE "NEPHROPATHY": AN UNRECOGNIZED CAUSE OF ACUTE RENAL FAILURE (ARF). Louis Cotterell*, Lawrence W. Elzinga. Oregon Health Sciences University, Division of Nephrology, Portland, Oregon.

Nitroprusside (NP) is commonly used for the acute management of congestive heart failure (CHF). NP induced ARF has been reported in a single patient (Reid and Muther, Am J Neph, 1987). We describe 3 additional patients who developed reversible oliguric ARF during NP therapy for severe CHF. Three men (25-47 yrs) were hospitalized with CHF due to dilated cardiomyopathy. All had right heart catheterization for hemodynamic monitoring. All had constant dopamine (DP) infusions (2-4 μg/kg/min) with NP dose adjusted for optimal hemodynamics (0.082-4 μg/kg/min). Despite improvements in cardiac hemodynamics, acute oliguria unresponsive to fluid bolus and loop-diuretics occurred in the absence of hypotension. Urine output (UO) was inversely related to NP dose. Following discontinuation of NP a brisk diuresis immediately ensued in all patients. Representative data from one patient are shown.

	BP (mmHg)	PCWP (mmHg)	CI ($L/min/m^2$)	SVR ($dyn \cdot S/cm^5$)	UO (cc/hr)
Pre NP	58	28	1.84	1316	212
NP	51	16	3.31	599	<25
Post NP	52	26	1.93	1267	200

In conclusion, NP can induce oliguric ARF, unresponsive to diuretics, complicating the management of patients with severe CHF. A potential mechanism is the disproportionate vasodilation of non-renal vascular beds creating a "steal" syndrome.

IATROGENIC PROBLEMS IN NEPHROLOGY
M.Davidman, P.Olsson*, J.Kohen*, CM.Kjellstrand. Depts.Med: Hennepin Co Med Center, Minneapolis, Minnesota and Karolinska Hospital, Stockholm, Sweden.

We studied the incidence of nephrological iatrogenic disease (IAD) in a tertiary care hospital, by prospectively classifying patients seen by nephrology consultation (NC) into 10 presenting syndromes: Acute renal failure (ARF), chronic renal failure (CRF), nephritis, nephrosis, electrolyte fluid imbalance (el-fl), obstruction, hypertension, infection, asymptomatic urinary abnormalities and stone) and 7 etiologic groups (drug induced, decreased perfusion - cardiac, decreased perfusion - volume, post-operative, infection, immunological and other).

In 4 months there were 100 NC, 2.2% of all admissions to the hospital, 24% of NC were due to IAD. 64 of 100 NC improved, 23 were unchanged and 13 died. Most common syndrome was ARF (59 patients) and el-fl (14 patients). Of ARF patients 21 (36%) were iatrogenic. 17 were drug induced: 4 by amino-glycosides, 4 by NSAID, 3 by ACE-inhibitor, 2 by pentamidine (for pneumocystic carinae in AIDS-patients), 1 to cephalosporin, 1 to contrast agent and 2 to diuretics. 3 patients died, 2 secondary to NSAID intoxication, 1 to contrast. 18 patients had ARF secondary to dehydration, 14 came with this referred to the hospital, 4 developed it in the hospital. Of 14 patients with el-fl, 3 were secondary to the use of diuretics, all 3 patients survived after correction.

Thus, iatrogenic disease with a nephrologic presenting syndrome will develop in 0.5% of all patients admitted to a tertiary care hospital and it carries a 13% mortality. ARF is the most common presenting nephrological syndrome, and among ARF-NC, IAD is the most common etiology present in 36% of all ARF. It is most common secondary to antibiotics, NSAID, ACE-inhibitors, diuretics and dehydration.

IS THE SLEEP APNEA SYNDROME (SAS) ASSOCIATED WITH PROTEINURIA? **G. Cowell*, S. Hatseras*, O. Ruiz*, S. Lietz*, E. Önal* and J.A.L. Arruda, Dept. of Medicine, Univ of IL and WSVAMC, Chicago, IL.**

Recent reports have suggested an association between the SAS and proteinuria. Most of these reports are anecdotal yet convincingly describe a waxing and waning of proteinuria in proportion to the severity of the SAS. A retrospective study showed an increased prevalence of proteinuria in SAS which was not correlated with obesity. We prospectively examined the prevalence of proteinuria in all patients presenting to the sleep laboratory for evaluation of possible SAS. Patients with diabetes or prior renal disease were excluded. When an initial urine specimen had a protein:creatinine ratio greater than 0.2 a 24 hour urine collection was obtained and used to confirm the presence of proteinuria. A diagnosis of SAS was made when snoring and hypersomnolence were associated with sleep disordered breathing. Seventy patients have been studied of whom 14% had proteinuria. In patients with SAS proteinuria was found in 17% and 16% in the presence and absence of obesity, respectively (mean urine protein:creatinine ratio 0.47, range 0.21 - 1.7). Among those without SAS proteinuria was present in 10% (mean protein:creatinine ratio 1.67, range 0.16 - 4.05), a value not significantly different from that found in SAS patients.

	+SAS	Obese	Non-Obese
% with proteinuria	17%	17%	16%
	-SAS	Obese	Non-Obese
% with proteinuria	10%	5%	25%

These initial results fail to confirm an increased prevalence of proteinuria in SAS.

UNILATERAL RENAL PARENCHYMAL DISEASE WITH CONTRALATERAL RENAL ARTERY STENOSIS: U P C R A S SYNDROME. PE de Jong*, JH van Bockel*, D de Zeeuw* (Intr. by LW Statius van Eps). Univ. Hospitals Groningen and Leiden, The Netherlands.

Hypertension with a unilateral small kidney suggests either a vascular (renal artery stenosis) or a non-vascular (urological) cause. Generally, the hypertension can be attributed to the abnormal kidney and correction of the stenosis or nephrectomy of the shrunken kidney will result in normalization of the blood pressure. We describe 8 young female patients (median age: 34, range:18 to 42 yr) referred for hypertension (diastolic blood pressure range 110 to 130 mmHg) with a small kidney on intravenous urography, which was considered to be due to either agenesis or dysplasia and reflux. Although the diseased kidney was suspected to be responsible for the high blood pressure, this group of patients was unique in having a stenosis of the contralateral renal artery. This stenosis was diagnosed to be of the fibromuscular-dysplasia (FMD) type, predominantly localized in the distal part of the renal artery and frequently extending into the peripheral branches. Anatomical successful correction of the stenosis without surgery of the contralateral small kidney resulted in normalization of blood pressure. We argue that an urological abnormality of one kidney with a renal artery stenosis of the FMD type at the contralateral side is an up to now unrecognized combination. It may well represent a distinct clinical entity: Unilateral Parenchymal disease with Contralateral Renal Artery Stenosis - U P C R A S - syndrome.

TYROSINE-PEPTIDES AS A NOVEL TYROSINE SOURCE IN AMINO ACID THERAPY OF RENAL FAILURE W.Druml, E.Roth, K.Lenz, H.Lochs, P.Balcke. I^{er} Medical, I^{er}Surgical and I^{er}Gastroenterological University Clinic, University of Vienna, Austria

Tyrosine (TYR) is regarded a conditionally essential amino acid in renal failure, because its formation from phenylalanine is reduced. However TYR is not included in conventional amino acid solutions because of its low solubility. Alternatively, TYR-containing peptides and/ or N-acetyl-TYR (NAC-TYR) could serve as TYR source for parenteral supply.The utlisation of these substances has to be proven in renal failure, because the kidney is the predominante organ for peptide disposal.

In 5 patients on regular hemodialysis therapy and a urinary output of <200 ml/d elimination and hydrolysis of alanyl-TYR (ALA-TYR), glycyl-TYR (GLY-TYR) and NAC-TYR was investigated by a bolus injection protocol (dosage corresponding to 10 mg TYR/kg b.w.)(Values are given as µmol/l,Mean±SEM)

(*p<0.01,**p<0.001)	ALA-TYR	GLY-TYR	NAC-TYR
basal ALA/GLY	255±33	279±49	-
maximal ALA/GLY	1086±96*	650±80*	-
Peptide/NAC-TYR 5'	17±8	182±18*	366±18*
TYR basal	45±5	33±4	47±6
maximal TYR	805±87**	368±69**	64±9ns
Half life k_{1e} (min)	0.9±0.1	3.6±1.3*	61.2±22*
Clearance ml/min	6211±1325**	941±148**	111±37

Thus the peptides ALA-TYR and to a lesser degree GLY-TYR, but not NAC-TYR are readily hydrolyzed even in patients with absent renal function und present promising new subtrates for parenteral amino acid therapy in renal failure.

TRUE RENAL HYPERTROPHY OCCURS IN HUMAN PREGNANCY. Jonathan S. Fain* and Leon G. Fine. Departments of Pathology and Medicine, UCLA School of Medicine, Los Angeles, CA.

Chronic elevation of GFR is almost invariably associated with renal hypertrophy. In human pregnancy, GFR and renal plasma flow increased during the first trimester and remain elevated throughout pregnancy. In the pregnant rat the same elevation in GFR and plasma flow is observed but kidney dry weight does not increase (J.M. Davison et al., J Physiol 301: 129, 1980; Baylis et al., Kidney Int 28: 140, 1985). To determine whether true hypertrophy, as opposed to renal vascular engorgement, is the basis for renal enlargement in human pregnancy, we analyzed the autopsy records of deaths during pregnancy at UCLA from 1957-1978. Mean ± SEM two-kidney weight in normal females in the 20-40 age group is 250 ± 10 G. In the autopsy cases, kidney weights were: 2nd trimester (n=8): 350 ± 31 G and 3rd trimester (n=16): 416 ± 26 G. Conclusion: An increase in kidney weight accompanies the elevation of GFR and renal plasma flow of human pregnancy. Since renal weight is increased in the absence of renal perfusion (i.e. autopsy) this represents true hypertrophy

EVALUATION OF MICROALBUMINURIA DETECTION IN DIABETES MELLITUS USING MICROBUMINTEST(R). P. Duggal*, J. Hermann*, J. Schunk* and W.G. Walker, Johns Hopkins Univ. Sch. of Med., Baltimore, Maryland.

The sensitivity and specificity of a new qualitative test tablet, Microbumintest(R) to detect microalbuminuria in diabetics as a screening procedure was evaluated by comparison with quantitative radioimmunoassay (RIA) of urine albumin (Alb) in 344 urine samples (294 from diabetics and 50 from normal healthy subjects). Results were read as negative, one plus (+) or two plus (++), as per package insert and were impressively consistent at Alb levels >50 µgm/ml with 99% sensitivity. When (+) and (++) were read as positive; specificity was only 80%. With 40 µgm/ml as cutoff point the sensitivity was 95% and specificity decreased to 64%. If 30 µgm/ml of Alb is accepted as the level indicating pathological proteinuria the sensitivity and specificity dropped to 90% and 65% respectively. However when only the (++) result was considered as positive, two samples only out of 216 with Alb <30 µgm/ml were ranked positive (sensitivity of negative test >99%). The dual utility of the test is confirmed by this study. First it effectively and reliably lowers the detectable level of Alb to 50 µgm/ml (compared to ~200 µgm/ml available by Albustix(R)) and secondly it will be very useful for screening as a negative test, routinely employed at regular intervals in diabetics, at little cost, and thus requiring confirmation by quantitative test of only those graded as (+).

RHABDOMYOLYSIS (RM) AND ACUTE RENAL FAILURE (ARF) FOLLOWING COCAINE ABUSE. M. Faulkner*, P. Singhal, A. Peters, A. Santiago*, M. Grosser, S. Levine, D. Schlondorff, Long Island Jewish Medical Center and Albert Einstein College of Medicine, New York.

An increase in the use of cocaine by drug addicts has led to the recognition of renal toxicity associated with it. From July, 1986 through June 1988, Renal services of two city hospitals were consulted for evaluation of 310 patients with ARF. Five of these 310 pts (1.6%) developed RM and ARF following cocaine abuse: in two after cocaine smoking and in the others after I.V. injection. All were male and young.

As potential contributing factors, 3 pts developed excitation syndrome with intense muscular activity. A fourth pt sat in squatting position in a bath tub continuously for 10 hrs. During this period, he experienced repeated episodes of severe cramps of the lower limbs. The fifth pt developed an acute compartmental syndrome of the left leg and required emergency fasciotomy. Despite vigorous fluid therapy, two developed oliguria. The others were non-oliguric. Peak blood values (Mean±SEM) were as follows:

BUN	CREATININE	CPK	LDH
108±16	9.2±0.9	95,023±45,518	2008±1183

Only the two oliguric pts required dialysis (2 wks). All pts recovered renal function. We suggest that cocaine may induce RM leading to ARF. This may be brought about by the intense muscular activity, in combination with the cocaine-induced vasoconstriction which may contribute to both the muscle and renal injury, the latter by reducing urinary flow and pH, thus increasing tubular precipitation of myoglobin.

FAVORABLE OUTCOME OF RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS (RPGN) IN SYSTEMIC VASCULITIS WITH ANTI-NEUTROPHIL CYTOPLASMIC AUTOANTIBODIES (ANCA). R.O.B.Gans*, M.C.Kuizinga*, F.Huysmans*, P.G.G.Gerlag*, S.J.Hoorntje* (intr.by L.W.Statius van Eps). Free Univ., Dept. of Med., Amsterdam, The Netherlands.

In a retrospective study over a period of 13 years (1975-1988) we evaluated the renal outcome of 42 patients with systemic vasculitis. Thirty-nine had a positive ANCA test, considered highly specific for Wegener's granulomatosis. Another three were ANCA negative but fulfilled the clinical and histological criteria for this disease. Thirty-two (76%) presented with RPGN. Of these patients 3 died during the acute phase and 3 need chronic dialysis. Of the remaining 26 patients, 16 revealed a serum creatinine concentration > 350 µmol/l at the start of prednison and cyclophosphamide. In this group A 10 patients needed temporarily dialysis (A+) with a mean duration of 11,5 (range 1-120) days and 6 didn't (A-). Ten patients had a twofold increase in serum creatinine within 2-120 days. In this group B none needed dialysis. Mean values and ranges:

	A+	A-	B
1 creat. at presentation	820(563-1374)	441(369-980)	199(160-290)
2 creat. after acute phase	144(119-277)	126(103-290)	123(80-230)
3 creat. at last control	156(140-623)*	162(136-1094)*	170(82-630)*
4 months of follow up	44(4-89)	23(7-54)	16(3-103)

* p<0.01 (Wilcoxon) 2 vs 3.

Renal outcome after the acute phase did not differ significantly between the various groups. Although hemodialysis (sometimes for up to 120 days) was needed in the vast majority of the patients in group A this was not associated with a worse prognosis. In the long-term a slight decline in renal function was noted in all groups, but only one patient progressed to end-stage renal disease after 54 months. In this large retrospective study a high percentage of RPGN occurred; RPGN was associated with a better prognosis than previously assumed.

DEVELOPMENT AND USE OF DISTRIBUTED DATA ENTRY AND ELECTRONIC COMMUNICATION IN A MULTI-CENTER STUDY OF PROGRESSIVE RENAL DISEASE. JJ Gassman*, MJ Drabik*, JR Leatherman*, KJ Fatica*, JA McPherson* and the MDRD Study Group (introduced by LG Hunsicker) Cleveland Clinic, Cleveland, Ohio

The Modification of Diet in Renal Disease (MDRD) Study is a multi-center randomized clinical trial comparing effects of diets with varying levels of protein intake on progression of renal disease. Patients are enrolled at 15 clinical centers (CC), and data are stored and analyzed at a Data Coordinating Center (DCC) in Cleveland. Centrally trained staff at each CC use microcomputers to enter data into an intelligent key entry package which has been programmed to check data ranges and within form agreement. Most discrepancies are thus detected and corrected immediately at the CC. A customized system for electronic data transmission provides for speed and accuracy in moving data from the CC's to the DCC. The data from the CC's is loaded into a central database along with data from biochemistry and GFR laboratories and a Nutrition Coordinating Center. The database is programmed to generate reports using this central information. The reports are sent to CC's via electronic mail, providing rapid feedback of laboratory results. In addition, electronic mail is used as an efficient means of communication between staff members at the CC's and central units, and can provide documentation of all messages. The MDRD Study computer system was developed and pilot tested during the feasibility phase of the MDRD Study and has been enhanced for the full scale study. The system is instrumental for conducting the MDRD Study in a high quality manner.

PROTEINS C AND S ARE NOT LOW IN THE DIABETIC NEPHROPATHY (DN).

Garcia-Maldonado M*, Comp PC*, Kaufman CE. University of Oklahoma, Health Sciences Center, and VA Medical Center, Department of Medicine, Oklahoma City, OK.

Thrombosis a leading complication of the nephrotic syndrome (NS), is associated with low free levels of the natural anticoagulant Protein S. We assessed the Protein C Protein S (PCS) system in 16 DN patients (pts), to determine if similar abnormalities occur. PC and total and free (FPS) levels of PS were measured by standard immunoelectrophoretic techniques, and the results compared with eleven age-matched controls (C). The results are shown as mean ± SD: DN pts, age 48.9 ±15, creatinine clearance 40.8 ±28 ml/min, urinary protein excretion (UP) 3.9 ±3.9 gm/24 hours. PC in DN 113.7 ±14% vs 108.0 ±30% in C, p=NS. PS in DN 108.7 vs 84.3 ±8.7. FPS in DN 100.6 ±20 vs 104.1 ±10 in control plasma, p=NS. PC levels, but not PS, correlated directly with UP, r=0.64, p<.005. Also eleven DN pts with UP ≥3.5, had higher PS (117.0 ±30.7) than C , p<.05, but FPS (104.1 ±23.4) and PC (120.6 ±13.4) were not different, p=NS. Conclusions: DN pts show normal to high levels of the PCS system. These levels of PCS are unlikely to promote thrombosis in pts with DN, including those with NS.

RELATIVE CONTRIBUTION OF TYPE I AND TYPE II DIABETICS TO THE CHRONIC DIALYSIS POPULATION.

L. Gimenez, G. Briefel, J. Zachary and W.G. Walker, Johns Hopkins Univ. Sch. of Med., Baltimore, Maryland.

The incidence of diabetic nephropathy among new enrollees in the national ESRD program increased at an annual rate of 23.5% per year between 1979 and 1984 (HCFA report, 1985). Although ESRD incidence among Type I diabetics is higher than in Type II diabetics, since the incidence of both Type II diabetes and ESRD, are higher in older (>35 year old) than younger (<35 year old) patients, one would suspect that Type II diabetics outnumber Type I diabetics on dialysis, but data on this important question are extremely limited. We surveyed all the patients from our 2 major units who are currently on dialysis and found that of a total of 339 patients 89 (26%) had diabetes; 14 (4%) of these were Type I and 75 (22%) were Type II. Data from 1983 to present in one unit revealed 71% of 41 diabetics currently on dialysis presented after Jan. 1986. The yearly percentage of diabetics enrolled from the total number of new patients was 27% in 1986, 31% in 1987 and 37% up to 07-01-88. According to these data, a large majority of diabetic patients currently on dialysis are Type II, with a sustained and progressive increase each year. Thus, any preventive program which attempts to reduce the incidence of ESRD must include a major focus upon preventing or reducing diabetic nephropathy in Type II diabetics.

ENHANCED RELEASE OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN PATIENTS WITH BILATERAL URETERAL OBSTRUCTION. F.A. Gulmi,* S.Y. Chou, U.M.M. Mooppan* and H. Kim.*Department of Urology and Division of Nephrology & Hypertension, The Brookdale Hospital Medical Center, Brooklyn, New York.

Renal response to release of bilateral ureteral obstruction resembles that to intravenous administration of ANP. In the present prospective study we measured plasma ANP levels before and serially after relief of obstruction in 9 patients (age, 69±3 years) with bilateral ureteral obstruction and azotemia. Obstruction was documented by renal ultrasonography. Before relief of obstruction, BUN and serum creatinine (SCr) levels were 85±18 (mean ± SE) and 8.2±1.3 mg/dl, respectively, accompanied by metabolic acidosis (serum HCO3 16±2 meq/L) but not hyperkalemia. Plasma ANP (measured by radioimmunoassay) was 129±28, markedly elevated when compared with 46±7 pg/ml in 7 age-matched control subjects (P<0.01). After relief of obstruction marked diuresis and natriuresis ensued. The patients' fluid intake was kept below 80% of the urinary output. By the 5th day after relief of obstruction the diuresis and natriuresis abated; BUN and SCr had declined to 25±9 and 2.1±0.5 mg/dl, respectively; plasma ANP levels also declined progressively to 35±8 pg/ml. These findings were associated with a significant weight loss and a rise in plasma renin activity (from 1.57±0.68 to 5.27±1.82 ng/ml/hr, P<0.01). These results suggest that ANP release is augmented in patients with bilateral ureteral obstruction and azotemia, probably due to hypervolemia, and may contribute to postobstructive diuresis and natriuresis.

ACUTE RENAL FAILURE (ARF) SECONDARY TO ORAL CIPROFLOXACIN (C)
Robert Hootkins, Andrew Z. Fenves, Barry Brooks, Robert Farkas, Michael K. Stephens, Baylor University Medical Center, Dallas, Texas.

The fluoroquinolones represent a new class of antimicrobial agents with broad spectrum activity. There is a reported less than 1% incidence of renal toxicity.

We report 4 cases of ARF in patients without a previous history of renal insufficiency placed on C therapy. The average baseline creatinine (Cr) was 1.1 mg/dl and rose to 5.5 mg/dl during therapy. The length of therapy ranged from 4 days to 2 weeks. Prerenal causes of ARF and post-obstructive uropathy were excluded. Kidney size was normal-to-increased and a gallium scan in one case was positive, consistent with acute injury. In addition, a positive urine eosinophilia suggested an acute hypersensitivity reaction. In each case the ARF was non-oliguric, and was partially or completely reversible with the discontinuation of C.

The nephrotoxic potential of these drugs has been linked to the development of crystalluria, yet even with crystalluria, renal damage is not thought to occur (Christ et al, 1978). There have been only 2 previous cases of ARF due to oral C, 1 with biopsy-proven acute interstitial nephritis.

We conclude that oral C may lead to ARF and the potential of C for nephrotoxicity needs to be reevaluated.

ANTIPROTEINURIC EFFECT OF THE ACE-INHIBITOR (ACEi) LISINOPRIL (Lis). JE Heeg*, PE de Jong*, GK van der Hem*, D de Zeeuw* (Intr. by LW Statius van Eps). Univ. Hosp. Groningen, The Netherlands.

ACEi have been shown to lower proteinuria in renal disease. However, the response varies and only patients with hypertension, and/or (severe) renal function impairment, and frequently only low proteinuria, have been studied. Moreover, sodium intake was not standardized. To evaluate whether ACEi can be used as antiproteinuric treatment and what determines the response, we studied the effect of Lis in 12 patients with overt proteinuria (range 3.2-10.5 g/day), with normal or elevated blood pressure (diastolic BP 64-105 mmHg), and with varying GFR (34-127 ml/min). Sodium intake was set at 50 mmol/day, and all medication was withdrawn for at least 2 weeks. The study involved four 2-month periods: (1) control, (2) α-methyldopa (MD) to test the effect of BP-lowering, (3) Lis 5 mg/day, and (4) Lis 10 mg/day. Proteinuria did not change from control to MD (6.0±2.3 to 6.1±2.1 g/day), and fell by 26±20% (p<0.002) on 5 mg Lis, and by 50±17% (p<0.001) on 10 mg Lis to 3.1±1.4 g/day. BP decreased compared to control by 11±7%, 13±7% and 16±6% respectively. The fall in proteinuria was similar in patients with MAP > or <100 mmHg, and in patients with GFR > or <75 ml/min. Moreover, no correlation existed between fall in proteinuria and initial BP, GFR, or proteinuria. Interestingly, when the patients switched during Lis from low to high sodium intake (200 mmol/day), proteinuria rose to control value (5.9±3.0 g/day). We conclude that Lis reduces overt proteinuria irrespective of initial BP, GFR, and proteinuria. This effect is dose related, and appears dependent on adequate sodium restriction.

EFFECTS OF ERYTHROPOIETIN ON ANEMIA AND HEMO DYNAMICS IN CHRONIC RENAL FAILURES WITHOUT DIALYSIS TREATMENT. Kei Hori*, Harumitsu Kumagai*, Kaoru Onoyama*, Kunitoshi Iseki* and Masatoshi Fujishima*.(intr by Massry SG). Kyusyu Univ, 2nd Dept of Internal Med, Fukuoka, Japan.

Effects of recombinant erythropoietin(EPOCH, Chugai, Japan) on anemia, hemodynamics and volume states in chronic renal failure patients without hemodialysis(CRF) was compaired with those with hemodialysis(HD). Seven CRF, average Scr 5.3mg/dl 4 males 3 females, were treated with EPOCH(1,500U 3 times a week) for 4 weeks and 6 HD, average HD duration of 58 months, 2 males 4 females, with EPOCH(1,500-4,500 U, 3 times a week) for 6 weeks. Before and end of EPOCH treatment cardiac output (CO, M-mode echo) and blood volume(RISA) were examined. CO was expressed as cardiac index(CI) and total peripheral index(TPRI) was calculated. GFR(inulin) and RPF(PAH) were also examined in CRF. Hematocrit(Hct) elevated 6.5 and 12.4% in CRF and HD, respectively. MBP increased 15mmHg in CRF but not in HD. TPRI elevated significantly in both groups and CI decreased in HD and unchanged in CRF. An unchanged MBP associated with elevated TPRI might be compensated by the decreased CI in HD. BV increased in CRF and unchanged in HD. GFR and RPF were not changed but filtration fraction increased significantly in CRF. Improvement of anemia by EPOCH was equally obtained in both CRF and HD. Hemodynamic and volume regulation seem to be different between the patients with and without hemodialysis in this observation period. Blood pressure response to the changes of Hct and TPRI may be more blunted in HD than CRF.

DEATH (D) AND CHRONIC RENAL FAILURE (CRF) IN SEVERE ETHYLENE GLYCOL (EG) INTOXICATION. B.Hylander*, K.Karlsson*, H Persson*, CM.Kjellstrand. Deps.Med-Intox Ctr,Karolinska Hosp,Stockholm,Sweden.

EG is sporadic and prognosis unclear. We studied 63 EG cases in 1 yr, 36 were serious (>250 ml). In 17 cases we had complete records and follow up >6 months. We studied 100 clinical, and chemical factors for D and CRF.

6/36 patients (17%) died. 11/17 survived, 3 with CRF (creatinine 132, 134 and 213 μmol/l > 6 mths). All but 2 had ARF, 4 anuric, 9 oliguric.

Neither amount EG taken (481 ±217 ml) nor delay to adm, dialysis or to IV HCO_3 or alcohol related to outcome. More patients who died vs survived: were comatose (5/6 vs 3/11 p=0.086), had higher K: (6.1 vs 4.4 p=0.019) were more acidotic (pH 7.0 vs 7.2 p=0.02. BE -28.5 vs -19.6 p=0.04). Urine contained oxalate crystals in only 10 cases. At 24 hours, K was higher in those who died (4.8 vs 3.7 p=0.01), BE lower (-14 vs -3 p=0.02). At 48 hours, K was identical 3.7, BE had been over corrected (4 vs -1.6 p=0.07). Blood EG levels were lower in the dead group, both at 0, 24 and 48 hours. At 72 hours there was no difference in any parameters. BUN and creatinine did not differ at 0,.24, 48 and 72 hrs. Survivors received more HCO_3 day 1 and 2, less day 3. Same vol.IV ethyl alcohol was given day 1 (1.6 vs 1.3 l) but less day 2 (8 vs 0.6 l) and 3 (0.5 vs 0 l). All survivors were dialysed (9 HD, 2 PD), of the dead 2 had no dialysis, 1 PD and 3 HD. There was no difference in any parameters among the survivors who developed CRF and others. No survivors needed chronic dialysis or had organic brain lesion.

In patients with severe EG intoxication severe acidosis, hyper-K and coma at admission carry a dismal prognosis, other factors are unimportant. Immediate and large amounts of HCO_3, alcohol and hemodialysis should be given and sustained.The renal damage of EG is more similar to acute tubular necrosis than oxalate nephropathy and CRF unusual.

RENAL CHOLESTEROL EMBOLIZATION (RCE):OCCULT RENAL FAILURE WITHOUT EVIDENCE OF PERIPHERAL EMBOLIZATION: P.H. Juergensen*, F.O.Finkelstein, A.S. Kliger, H.B. Carey*, and K. Cooper. Yale Univ. Dept. of Medicine, Hospital of St. Raphael, New Haven, CT.

RCE as a cause of acute renal failure (ARF) usually occurs after vascular procedures in association with stigmata of peripheral embolization. The reported spectrum of associated abnormal lab findings includes thrombocytopenia (TCP), eosinophilia (↑EOS), elevated sedimentation rate (↑ESR) and hypocomplementemia (↓C3). Renal disease usually progresses inexorably to ESRD.

We report 9 patients (6 male, 3 female; age 60-81) who presented with ARF secondary to RCE, without coincident stigmata of peripheral embolization. RCE was confirmed by renal biopsy in all patients. Four patients had no antecedent arterial vascular procedure. Clinical data revealed the following:

	n	HTN	↑EOS	TCP	↑ESR	↓C3	ESRD
Vascular Procedure	5	5/5	3/5	1/3	3/3	0/3	3/5
No Procedure	4	4/4	2/4	0/3	2/2	0/2	3/4

We conclude that RCE as a cause of ARF needs to be considered in elderly patients with or without recent invasive vascular procedures, even in the absence of peripheral stigmata of embolization. Many, but not all patients progress to ESRD.

MANAGEMENT OF CONGENITAL HYDRONEPHROSIS. Kikuo Iitaka*, Takeo Ishidate* and Tadasu Sakai*(int.by Kouju Kamata). Kitasato Univ. School of Med., Dept. of Pediatrics, Sagamihara, Kanagawa, Japan

Thirteen children with congenital hydronephrosis(CHN) have been followed for the mean period of 2.5 years. Their lesions were detected by the fetal echography in 9(Gr.A) and also detected neonatally in 4(Gr.B). In Gr.A the lesions were bilateral(BL) in 5 and unilateral(UL) in 4 and were due to the obstruction(OB) at the ureteropelvic junction(UPJ) except for one baby with urethral OB who died but did not have the oligohydramnios. The nephrostomy(NS) tube was inserted in 2 with severe UL HN, but nephrectomy was performed at 5 months old in 1 and the NS has been successfully continued for 2 years in 1. The other 6 have been followed conservatively and spontaneous improvement of HN was observed in 5 and the lesion was unchanged in 1. In Gr.B the lesions were due to the BL vesicoureteral OB in 2 and UL UPJ OB in 2. The NS tube was inserted in 1 with severe UL HN due to the OB at UPJ, but it was removed after 3 months when the passage of the contrast media was confirmed at the UPJ. Spontaneous improvement was also observed in the other 3. Recently the increasing number of children with CHN have been detected during their intrauterine life by the fetal echography and also at their neonatal period. Severe CHN due to the OB of the urinary tract requires surgical treatments, but the spontaneous improvement of these lesions with conservative treatments or the NS was observed in 10 of 13 patients in this study. These observations should be considered in the management of the surgical correction of CHN due to the OB of the urinary tract.

THE FRACTIONAL EXCRETION OF UREA (FEUr) AS A GUIDE TO RENAL PERFUSION. Andre A. Kaplan, Orly F. Kohn*. Division of Nephrology, University of Connecticut Health Center, Farmington, CT.

Several urinary indices can be employed to determine the existence of a diminished renal perfusion (Miller et al. Ann Int Med 89:47-50, 1978). Unfortunately, many of these may be rendered useless by the preexistence of chronic renal failure or the use of diuretics. To determine the usefulness of the FEUr as a clinical guide to renal perfusion, we evaluated urinary indices in 7 patients with decreased renal perfusion. Diagnoses included congestive heart failure, hepatorenal syndrome, pre-eclampsia, renal artery stenosis and non-steroidal induced renal failure. 5 patients were taking furosemide at time of diagnosis, 4 patients had preexisting chronic renal failure. Mean urinary indices at time of renal dysfunction (pre) (n=7) and after return to baseline (post) (n=6) are listed:

	Urine Na#	FENa	FEUr	serum Ur/Cr	Urine Osm#	U/P ratio (Ur)	(Cr)
Pre	38	2.0	19	25	343	10	36
±sd	±25	±2.0	±8	±8	±154	±14	±52
Post	71*	2.2	46**	22	342	17	37
±sd	±37	±2.1	±17	±11	±87	±18	±31

=mEq/l, * = p<0.05, ** = p<0.002 (pre vs post)

The results reveal a significant increase in the FEUr despite chronic renal failure or the use of diuretics. Although the urinary Na concentrations were also significantly higher at return of renal function, in only one case was the original value compatible with a pre-renal state (<20 mEq/l).

We conclude that the FEUr can be a useful guide to the state of renal perfusion.

THE USE OF RECOMBINANT HUMAN ERYTHROPOIETIN IN THE CORRECTION OF ANEMIA IN PRE-DIALYSIS PATIENTS AND ITS EFFECTS ON RENAL FUNCTION: A DOUBLE BLIND PLACEBO CONTROLLED TRIAL. Kenneth S. Kleinman M.D., Suzanne U. Schweitzer R.PH., M.P.H., Sondra T. Perdue Dr. P.H., Kenneth H. Bleifer M.D., Robert I. Abels M.D. Van Nuys and Los Angeles CA and Raritan, NJ.

Fourteen patients with chronic renal insufficiency and not dialyzed (serum creatinine 3.0 to 11.0 mg/dl) with severe anemia (hematocrit less than 30%) were randomized to receive either recombinant human erythropoietin (rHuEPO) to reach a hematocrit of 38 to 40% or a placebo subcutaneously thrice weekly for 12 weeks. Anemia was significantly ameliorated in the treated patients. No acceleration in the progression of renal failure (change of 1/serum creatinine versus time) or change in serum potassium was noted for either the placebo or treated group. Six of seven treated patients had a significant fall in serum ferritin and percent transferrin saturation (plasma iron/total iron binding capacity). This resulted in functional iron deficiency and the requirement for iron supplementation. At the end of the study period, the average systolic and diastolic blood pressure did not differ significantly between these two groups of patients. Quality of life was improved in all rHuEPO treated patients, but not in the placebo group. This study demonstrates the safety and efficacy for the use of rHuEPO in the correction of anemia in pre-dialysis patients without adversely affecting renal function over a 12 week period. Improved patient well being, as a result of the correction of anemia, resulted in one patient refusing appropriate initiation of dialysis therapy.

PREDICTORS OF AMINOGLYCOSIDE NEPHROTOXICITY: IMPORTANCE OF DURATION OF THERAPY. D.J. Leehey, B.Braun*, L.Chung*, C.Gross*, J.R. Lentino*. Dept. of Medicine, Hines VA Hospital, Hines, IL.

As part of an ongoing randomized clinical trial designed to evaluate the cost-effectiveness of pharmacokinetic dosing of aminoglycosides, an interim analysis of data from 134 pts. was performed to identify predictors of toxicity. Nephrotoxicity was defined as a >30% decrease in creatinine clearance. Serum creatinine was monitored until 14 days post-therapy. By univariate analysis, the factors associated with toxicity were ICU stay (p=.001), liver disease (p=.001), duration of therapy (days) (p=.002), shock (p=.0025), furosemide (p=.007), clindamycin (p=.017), total dose of drug (mg) (p=.021), contrast media (p=.022), and vancomycin (p=.049). Using stepwise multivariate discriminant analysis, the most important variables predictive of toxicity were duration of therapy (p<.001), shock (p=.004), and liver disease (p=.007). Mean duration of therapy was 9.1 days in the non-toxic and 14.1 in the toxic group. Initial peak, initial trough, percent toxic troughs, bacteremia, renal insufficiency (SCr>1.5 mg/dL), and hypokalemia were not associated with or predictive of toxicity. Overall, 37 pts. (28%) became nephrotoxic. Interestingly, over half (19 pts) demonstrated evidence of toxicity only after aminoglycoside therapy had been discontinued for up to one week. We conclude that aminoglycoside toxicity remains a common clinical problem; moreover, its incidence is very dependent on duration of therapy and will be underestimated unless serum creatinine is monitored after cessation of treatment.

RENAL FUNCTION IN PROLONGED OBESITY. Sidney Kobrin,* Gary Levine,* and Rasib M. Raja. Albert Einstein Medical Center, Kraftsow Division of Nephrology, Philadelphia, Pennsylvania.

Rats subjected to 1 5/6 nephrectomy develop glomerular hyperfiltration (GH) in remaining nephrons, leading to focal segmental glomerulosclerosis and renal dysfunction (RD). GH occurs commonly in young obese humans. Focal segmental glomerulosclerosis occurs in 53% of obese pts with nephrotic syndrome, versus only 6% of non-obese controls (Arch Intern Med 146:1105). The prevalence of RD in obesity is uncertain. We evaluated renal function in 86 obese pts participating in a weight (W) reduction program. Blood pressure (BP), urinalysis, W, Body Mass Index (BMI), serum creatinine (Cr), Blood Urea Nitrogen (BUN), Albumin (A), Glucose (G) and Cholesterol (C) were measured initially and at 4 and 8 months.

Results (Means):

TIME	N	W kg	BMI UNITS	YO YRS	BP mmHg	Cr mg/dl	BUN mg/dl	A g/dl
Initial	86	129	47	31	137/87	0.9	13.2	4.0
8 Mons	54	121	45	31.7	135/86	0.9	13.0	4.1

YO = YEARS OF OBESITY, NORMAL BMI < 25

Mean age 40 years. Diabetes Mellitus (DM) was present in 16% of pts and HT in 40%. Mild proteinuria (30 mg/dl-100 mg/dl) occurred in 10% of pts, half these pts had microscopic hematuria (2-20 red cells/high power field). One pt, with DM and HT, had serum Cr > 1.5. No pts had nephrotic syndrome. Renal function did not change in 52 pts with a mean of 8 kg W loss, or 2 pts with W gain. These data suggest a low prevalence of RD in pts with prolonged obesity. GH may not lead to significant RD in obese humans in the absence of additional renal insults.

ORNIPRESSIN AS A TREATMENT OF HEPATO-RENAL SYNDROME (HRS): EFFECT ON VASOACTIVE HORMONES Kurt Lenz, Wilfred Druml, I[st] Medical University Clinic, Heide Hörtnagl, University of Vienna, Austria and Alexander L.Gerbes, Klinikum Großhadern, Munich, FRG

As we have demonstrated recently, low dose ornipressin (8-ornithine vasopressin (6 U/h) is an effective therapy of HRS inducing a marked diuresis, increasing sodium-excretion and creatinine clearance. To identify the causes of this effect we measured concentrations of atrial natriuretic peptide (ANP), catecholamines (A, NA) renin and aldosterone together with hemodynamics and renal function in 6 patients before and 120 min during ornipressin infusion:

Results:	before	120 min	unit	p<
ANP	28.4±4.8	71.2±16.6	ng/ml	0.001
Epinephrine	0.79±0.5	0.52±0.3	ng/ml	n.s.
Norepinephr.	1.74±0.3	0.85±0.2	ng/ml	0.001
Renin	13.5±3.9	5.9±2.1	ng/ml	0.001
Aldosterone	238±25	210±18	ng/ml	0.05
C-Creatinine	44.6±14	76.3±24	ml/min	0.001
Diuresis	1.0±0.2	2.7±0.7	ml/min	0.001
Sodium excret.	10.7±0.7	21.6±15	mmol/2h	0.01
mean art.press.	82±5	101±5	mmHg	0.001
cardiac index	5.6±0.6	4.1±0.4	l/min/m²	0.01
heart rate	101±5	78±5	beats/min	0.001
vasc.resistance	532±72	958±66	dyn.sec.cm^{-5}	.01

Ornipressin causes a pronounced increase of ANP despite elevated basal levels and decrease of norepinephrine and renin activity. Thus, the amelioration of renal function in HRS is mediated by a complex pattern of additive/ synergistic effects as normalisation of hemodynamics, increase of venous return, reduction of reflex activation of sympathetic tone and renin-angiotensin axis and increase in ANP.

ALBUMIN (Alb) EXCRETION RATE (ER) IN NORMAL MAN: DAILY AND DAY TO DAY VARIATION. Susie Q. Lew, Daniel Drell*, Juan P. Bosch. George Washington Univ. Med. Ctr., Dept. of Medicine, Washington, D.C.

Urinary alb is a sign of renal dysfunction. The aim of the present studies was to determine the daily and the day to day variation in urinary alb excretion in normal subjects . An ELISA method was employed using a rabbit anti-human alb antibody to immunoselect alb from dilute human urine specimens. Three 24 hr urine collections were obtained at baseline, 7 and 14 day intervals from 4 healthy volunteers with no evidence of renal dysfunction. Alb was measured in each voided and 24 hour specimen. Results: (mean values)

	Daily Variation (ug/min): Collections		
Subject#	Morning 8AM - 4PM	Afternoon 4PM-12AM	Evening 12AM-8AM
1	3.0	3.7	2.5
2	5.2	4.8	3.7
3	5.3	4.6	4.0
4	8.4	6.9	4.7

	Day to Day Variation (ug/min): Collections		
Subject#	1	2	3
1	5.1	2.5	1.2
2	5.3	5.4	2.2
3	7.5	3.1	3.6
4	4.6	3.7	5.5

Alb ER was always less in the evening collection. During the awake period ER was constant. There was a significant day to day variation within subjects. All values were < 7.5 ug/min. Because the subjects voided about 1000 ml per day, the concentration measured in the spot urine was not different from the ER. Thus, for screening purposes, a single spot sample during waking hours is sufficient to identify significant albuminuria without the need for timed collections.

ACUTE EFFECTS OF DOBUTAMINE (Db) AND LOW-DOSE DOPAMINE (Dp) ON RENAL HEMODYNAMIC FUNCTIONS (GFR AND RPF) IN SEVERE CONGESTIVE HEART FAILURE (CHF). R.C. PABICO, G.J. ROGAL,* B.A. MCKENNA,* J. RICHESON,* W.B. HOOD.* Univ. of Rochester Medical Center, Rochester, New York.

The treatment of severe CHF with low cardiac output, index (CO, CI) and elevated pulmonary venous pressure (PCWP) is made more difficult by the marked reduction in GFR and RPF. Profound Na^+ retention perpetuates the edema. Low-dose Dp (≤5µg/kg/min), with its renal vasodilatory effect, and, inotropic dose of Db (7-19 µg/kg/min) were given i.v. to 7 patients with cardiomyopathy and severe CHF (mean age: 60 yrs; 5 men, 2 women; no known renal disease; on no diuretics or digitalis; CI <2.5 L/min/m^2; PCWP>18 mm Hg). The study was done in the Medical Intensive Care Unit after signed consent was obtained (approved by Human Research Committee). CO was measured by thermodilution; PCWP by Swan-Ganz catheter; GFR and ERPF by clearances of inulin and PAH. Measurements were made at baseline, during Db alone, and during Db and Dp. CI rose from baseline 1.9±.17 to 2.7±.19 L/min/m^2 during Db with no further change during Db/Dp. PCWP decreased from 25±1.9 to 16±2.3 mm Hg on Db with no further change on Db/Dp.

	Baseline	Db	Db/Dp
GFR (ml/min/1.73m^2)	66±11	65±7	82±10
ERPF " "	220±39	226±20	360±42*
FE Na^+ (%)	.50±.04	.57±.05	*.77±.06

*p < 0.05 vs baseline

ERPF increases on Db/Dp; though it did not attain statistical significance, GFR rose on Db/Dp. Natriuresis and diuresis also occurred. Thus, inotropic dose of Db and low-dose Dp improved cardiac/renal hemodynamics in CHF.

SHIGA-LIKE TOXINS (SLT) IN ARGENTINE CHILDREN: ASSOCIATION WITH HEMOLYTIC UREMIC SYNDROME (HUS). E. L. Lopez, M. Diaz, F. Mendilaharzu, S. Grinstein, E. Rubeglio, M. Vazquez, M. Woloj, M. Turco, S. Devoto, B. E. Murray, L. K. Pickering, T. G. Cleary, (intr. by J. Lemire). Hospital de Ninos, Buenos Aires, Argentina, and Dept. of Pediatr., Univ. of Tex. Med. Sch., Houston, Tex.

Because of the high frequency of HUS in Argentina, we conducted a prospective study of HUS cases and healthy age-season matched children for evidence of SLT-infection. Fecal cytotoxin as detected in a ^{3}[H] Hela cell assay was found in 10/31 (32%) children with HUS and in 0/19 (0%) healthy children ($P<0.01$). Free fecal cytotoxin was detected more often in those HUS patients who had stool collected within 48 hours of diarrhea resolution ($P<0.02$). Presence of free fecal cytotoxin was not related to either presence or duration of anuria. 320 E. coli strains isolated from these patients were evaluated using DNA probes for SLT-I and SLT-II. Four HUS patients and no controls had E. coli that were DNA probe positive. E. coli 0157:H7 was detected in only 1 HUS patient and in no controls. 51% of HUS cases and 5% of healthy control children had serum neutralizing titers of >1:4 to shiga toxin ($P< 0.0005$). 75% of HUS patients on whom all specimens were available had evidence of recent SLT infection. These data show that in Argentina, HUS is commonly SLT-associated but that E. coli 0157:H7 is not the major toxin producing strain related to HUS in this setting. (Supported by the Thrasher Research Fund.)

PLASMAPHERESIS (PP) DOES NOT ENHANCE THE SUSCEPTIBILITY TO INFECTIONS IN IMMUNOSUPPRESSED PATIENTS WITH LUPUS NEPHRITIS. M. Pohl, T. Berl, S. Lan* and the Lupus Nephritis Collaborative Study Group (LNCS). Denver, CO, Cleveland, OH, Chicago IL

PP is employed in a variety of disorders characterized by rapidly progressive renal insufficiency. The possibility that PP enhances the infection susceptibility of concomitantly administered immunosuppressive agents has been suggested (JAMA 244: 2423, 1980). To assess the validity of this postulate the infectious complications of 86 patients who were entered in the LNCS were analyzed. Forty-six patients received only corticosteroids + cytoxan for 8 weeks followed by discontinuation of cytoxan and tapering of corticosteroids (control group, C) while 40 patients were identically treated but also received a variable number of PP in the first 8 weeks (PP group). In the first 9 weeks 35% of C and 24% of PP patients had infections (p=NS). The infection rates during the steroid taper phase (42% in C and 30% in PP) or during remission at which time patients were on 20 mg q.o.d. prednisone (35% in C and 41% in PP) were likewise not significantly different. Overall, 74% of C patients sustained 61 infectious events while 67% of PP patients sustained 48 infectious events (p=NS). Of 14 deaths, 7 were attributed to infections (4 in C and 3 in PP patients). Except for an increment in systemic viral infections in C patients, the pattern of infections was not different in the 2 groups. This study therefore clearly dispels the notion that PP imparts an additive risk for infection in immunosuppressed patients.

INTRAPERITONEAL REINFUSION OF ASCITIC FLUID AFTER EXTRACORPOREAL CONCENTRATION AS A TREATMENT FOR OLYGURIC PATIENTS WITH REFRACTORY ASCITES.
Claudio Ronco,Luciano Fecondini, Pierantonio Conz, Giuseppe La Greca (Intr. by J.P. Bosch). Dept. of Nephrology, St.Bortolo Hospital, Vicenza, Italy.

I7 patients with refractory ascites have been treated with new technique. Ascitic fluid is drained by gravity in a unit consisting of an hemofilter and a bag used as a transit reservoir, placed below the patient. When the bag is full the unit is rised to a height sufficient to let the fluid flow back though the filter into peritoneum. During this step ultrafiltration occurs and the concentrate is returned to the patient. The machine cycle is automatically repeated as many times as necessary to achieve the scheduled weight loss. I7 patients have been treated for a total of I.4 sessions/patient with reinfusion of the concentrate in the abdominal cavity. In all patients a significant reduction of the amount of ascitic fluid and of its rate of formation have been achieved. The tretment was well tolerated and no side effects were observed. After treatment the diuresis and Na excretion increased significantly in all patients (Urinary Na excretion from 45.2 mE/l ± I2.2 to 84.4 mE/l ± 22.2 ; Urine output from 844.I ml/24 h ± 4II.5 to I667.6 ml/24 h ± 585.2). The system is safe and reliable for the treatment of refractory ascites. The new equipment is easy to use, it is reliable and it does work without any pump thus avoiding possible complications.

CONTRAST NEPHROTOXICITY: A RANDOMIZED PROSPECTIVE TRIAL OF IONIC VERSUS NONIONIC RADIOGRAPHIC CONTRAST.
SJ Schwab, M Hlatky*, K Morris*, CJ Davidson*, T Bashore*. Duke University Medical Center, Durham NC

To examine the relative nephrotoxicity of ionic and nonionic radiographic contrast, we randomized 443 patients to receive either iopamidol or diatrizoate for cardiac catheterization. Patients were stratified into low risk (N=283) or high risk groups (N=160) prior to randomization based on the presence of the following factors: 1) diabetes mellitus, 2) CHF or, 3) baseline serum creatinine (BCr) >1.5 mg/dl. Serum and urinalysis were performed at baseline, 24 and 48 hours after contrast. The median maximal rise in BCr was 0.2 mg/dl in the 233 patients randomized to diatrizoate and 0.2 mg/dl in the 210 patients randomized to iopamidol (P=NS). BCr increased by >0.5 mg/dl or more in 10.2% of patients receiving diatrizoate and 8.6% of patients receiving iopamidol (P=NS). Among high risk patients, BCr increased by 0.5 mg/dl in 17% of the patients receiving diatrizoate vs. 15% of the patients randomized to iopamidol (P=NS). BCr >1.5 mg/dl was the only predictive risk factor associated with nephrotoxicity. No other urine or serum values, or historical factors correlated with nephrotoxicity. This randomized study shows no overall difference in the incidence of contrast nephrotoxicity between ionic versus nonionic agents.

INCREASED INCIDENCE OF HLA-B40 IN HEMOLYTIC UREMIC SYNDROME (HUS).
K.J.Sheth,J.B.Hunter*,H.E.Leichter, J.C.Gill*.Med Coll of Wisconsin & Blood Center of SE Wisconsin, Dept of Pediat,Milwaukee, Wisconsin.

Not all patients (pts) with verotoxin producing E.coli infections develop HUS suggesting that genetic predisposition may determine the development of HUS. To elucidate genetic susceptibility and to identify the possible association of HLA antigens with the severity of HUS, we evaluated HLA A,B,C,DR,DQ antigens in 22 children (age 1½-14 yr; 7M,15F) who previously had classical HUS [mild(6), moderate(9), severe(7)]. Distribution of HLA antigens were compared between the study and the random population. There was no significant difference in the distribution of HLA-A,C,DR or DQ antigens. Frequency of HLA-B antigens in HUS were:

		HLA - B Antigens			
	Pt #	B40	B13	B44	B7	Total
Random	(5000)	14%	6%	23%	23%	66%
HUS	(22)	45%(10)	14%(3)	18%(4)	23%(5)	95%
Mild	(6)	2	1	1	2	
Mod.	(9)	4	2	2	1	
Severe	(7)	4	0	1	2	

Relative Risk (RR) of developing HUS with the presence of HLA-B40 alone was 5.0($\chi^2$10.6,p<0.001; p corrected <0.05).This positive association can be maintained by extending the comparison to cross reactive HLA antigens. 21/22 (95%; RR=5) of HUS pts type for B40(60,61,41),13,44,7. With the exception of B7 all these antigens share a distinct amino acid sequence at positions 67-74 in the α_1 domain. Conclusions: The presence of a short sequence shared (public) epitope increased the RR of developing HUS 5 fold. The severity of HUS was not related to the presence of these antigens but may be determined by environmental factors.

LONG-TERM SEQUELAE OF CHILDHOOD HEMOLYTIC UREMIC SYNDROME (HUS), Richard L. Siegler, Mark K. Milligan†, Ted H. Burningham† Univ. of Utah Med. Center, Dept. of Peds., Salt Lake City, UT.

The acute phase of classical childhood HUS has been well described, but there are few reports of long-term sequelae and there are no sizable reports detailing the North American experience.

We therefore studied 45 patients from the intermountain region of Utah and Idaho (mean age 11.4 ± 3.4 yrs; range 5-21 yrs) who had experienced classical childhood HUS 5 or more years earlier (mean 8.9 ± 2.7 yrs). We found the following:

	Number	Percent
Hypertension (>95 percentile)	7	15.6
Neurological residual	1	2.2
Proteinuria	14	31
Hematuria	2	4.4
Pyuria	1	2.2
Granular casts	13	29
1 or more urinary abnormalities	21	47
Ccr <90 ml/min/1.73m^2	11	24
Ccr <80 ml/min/1.73m^2	6	13
Ccr <70 ml/min/1.73m^2	2	4.4
Ccr <60 ml/min/1.73m^2	0	0
Children with 1 more sequalae	26	58

While we found few serious or advanced sequalae, the high incidence of mild hypertension, proteinuria, cylindruria, and impaired GFR indicate a need for long-term monitoring of HUS patients.

RECOVERY OF RENAL FUNCTION FOLLOWING PROLONGED OLIGO-ANURIA: A THREE YEAR STUDY. R. F. Spurney*, R. E. Coleman*, S.J. Schwab. Duke University Medical Center. Durham, North Carolina.

Prolonged oligo-anuria following acute renal failure is associated with limited return of renal function. We evaluated 320 consecutive episodes of dialysis-dependent acute renal failure over 5 years. Forty-two of 159 survivors (mean age 55 years) were oligo-anuric for >3 weeks (4.6±0.2weeks). Ten of 42 were anuric >3 weeks (3.4±0.1 weeks). Mean duration of dialysis dependence was 6.5 weeks. Pre-ARF creatinine was known in 26 of 42 (1.1± 0.2 mg/dl). Twenty-seven patients recovered to a serum creatinine <1.5 mg/dl. Fourteen patients recovered with a creatinine >1.5 but <4.0 mg/dl. Two patients remained dialysis dependent. Hippuran radio-nuclide scans were performed in 20 patients. Prominent uptake of tracer occurred in 16; all recovered renal function. Faint uptake occurred in 4 patients, 2 of whom remained dialysis dependent. Risk factors for permanent renal impairment were duration of anuria, prior serum creatinine, and faint hippuran uptake on renal scan. We conclude that prolonged oligo-anuria is associated with some decrement in renal function but the majority of patients recover satisfactory renal function.

GFR-DECREASE AND PERSISTENT HYPERTENSION (PHT) AFTER TECHNICALLY SUCCESSFUL RENAL ARTERY STENOSIS REPAIR (RASR).
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Forty-two patients with renin activity(PRA) dependent renovascular hypertension were followed > 6 mo after RASR. Twenty potentially prognostic factors for development of PHT or decreased GFR following intervention were analyzed:

Age, sex, underlying disease, previous CVA or MI, ECG changes, number or drugs, use of ACE-inhibitors, known duration of hypertension, smoking, diastolic and systolic blood pressures, indications for treatment (hypertension / renal insufficiency),treatment method (percutaneous transluminal angioplasty [PTRA] / surgery), supine and standing PRA, initial GFR, proteinuria, uni- or bilateral stenosis, left ventricular hypertrophy (LVH).

9/42 had PHT. Organ involvement was prognostic for PHT: LVH on chest x-ray (8/9 with PHT vs 15/33, p=0.02) and low GFR (49 vs 71 ml/min, p= 0.04) Duration of disease tended to be longer in PHT (6 vs 11 years, p=0.07) and more drugs used (2.5 vs 2.9, p=0.09). 13/42 had a decrease in GFR. High PRA was prognostic for this (21.8 vs 7.5 mg/ml/min, p=0.007). There was also a tendency for more patients to have a decreased GFR following operation than PTRA (8/16 vs 5/26, p=0.04/0.08 with continuity correction).

Patients with hypertensive organ involvement, i e LVH or decreased GFR, or long-standing, difficult to treat hypertension run a high risk of PHT after RASR. Other factors such as age, sex, underlying disease, vascular complications and blood pressure levels lack prognostic significance. PTRA may be superior to operative repair, with regards to preservation of renal function.

URINARY FLOW RATE IS A RISK FACTOR FOR IDIOPATHIC UROLITHIASIS IN CHILDREN. F. Bruder Stapleton, and Leslie A. Miller. Department of Pediatrics and Clinical Research Center, Univ. of Tennessee, Memphis, TN.

Many risk factors for urinary calculi in children have not been examined. Urinary volume (fluid intake) has been implicated in the pathogenesis of urolithiasis in adults; we examined urinary (U) flow rate, U sodium concentration,U osmolality and creatinine clearance in 36 healthy children (24 males), 34 children (21 males) with urolithiasis and renal or absorptive hypercalciuria (HCU)(U Ca >4 mg/kg/d during an unrestricted diet) and 12 patients (8 males) with idiopathic Ca oxalate stones (normal UCa, U urate, U citrate, U oxalate, and U cystine excretion). All urines were collected as outpatients while ingesting a 300 mg Ca, 2.0 gm Na diet. Adequacy of urine collections was confirmed by creatinine excretion. Values are mean ± SEM. *=P<0.001 from other groups, **=P<0.02 from controls.

	Controls	HCU/ Stones	Idiopathic Ca. Oxalate
U Flow Rate, ml/kg/d	22.2±2.0	25.0±2.3	12.2±1.4*
U Na, mg/d	2601±226	1925±182**	1643±491
U Osm, mOsm/kg H2O	572±42	551±39	684±78
U Ca, mg/kg/d	1.7±0.2	3.6±0.3	1.6±0.2
Creat. Cl., ml/min/1.73m^2	129±8	136±5	128±18

U flow rate was similar between patients with renal HCU (25.8±2.4 ml/kg/d) and absorptive HCU (24.9±2.2 ml/kg/d) and did not vary with age in controls (r= -0.33, P<0.1). In 16 patients with HCU and hematuria without urolithiasis, U flow rate was 25.8±1.8 ml/kg/d compared to 25.0±2.3 ml/kg/d in HCU patients with urolithiasis. Lower U flow in idiopathic stones was not due to differences in Na intake or to hypotonic diuresis in other groups. We conclude that U flow rate is a risk factor in children with idiopathic urolithiasis, but does not explain why some patients with HCU have only hematuria and not overt urolithiasis. Increased fluid intake may be very beneficial for children with idiopathic urolithiasis.

TRYPSINOGEN (TRY) IS INSENSITIVE AS A MARKER OF PANCREATITIS IN RENAL DISEASE. William Steinberg*, Violet Habwe*, James Henry*, Paul L. Kimmel. George Washington Univ. Med. Ctr., Dept. of Medicine, Washington, D.C.

Previous studies suggested TRY is a more sensitive marker of pancreatitis than amylase (AMY) or lipase (LIP). The latter two are elevated in pts with chronic renal disease. In order to evaluate the utility of TRY as a marker of pancreatic disease in patients with renal disease, 45 pts on hemodialysis (HD), and 12 on CAPD had plasma (p), and 25 patients with chronic renal failure (CRF) and 10 normal controls had p and spot urines collected, and assayed for TRY, AMY, LIP, and creatinine. The fractional excretion (FE) of TRY, AMY and LIP were calculated. The upper limit of normal for TRY is 85 ng/ml, AMY 150 u/l, and LIP 208 u/l. (* p<.05 CAPD vs HD; # p<.05 CRF vs HD)

Mean Plasma Enzyme Concentration

	CRF	HD	CAPD
TRY	215+/- 160	278+/-180	139+/-119*
AMY	166+/- 109#	216+/-126	142+/- 69*
LIP	270+/- 179#	366+/-278	146+/- 97*

The % of pts with normal tests was analyzed by Chi^2 (# p<.01 TRY vs AMY, * p<.01 TRY vs AMY and LIP)

	CRF	HD	CAPD
TRY	4/25#	4/45*	5/12
AMY	12/25	18/45	7/12
LIP	10/25	14/45	8/12

The mean FE TRY in pts with CRF (42.7+/- 48) was higher (p<.01) than that of normals (4.4+/- 3.6). We conclude TRY is less sensitive than AMY or LIP as a marker of pancreatic disease in pts with renal disease. Peritoneal clearance of enzymes may contribute to their lower p concentration in CAPD. The high FE TRY in CRF suggests increased p TRY is due to extrarenal factors rather than reduced renal clearance.

THE LONGTERM RISKS OF UNILATERAL NEPHRECTOMY IN MAN: A CASE CONTROL STUDY C P Swainson and C Smith, Renal Unit, Royal Infirmary, Edinburgh, UK and Department of Medicine, Christchurch, New Zealand.

Follow up studies of renal donors, and of children with unilateral renal disease, who have had a unilateral nephrectomy have suggested that hypertension and proteinuria occur which are a consequence of "hyperfiltration injury". We report the preliminary results of a case control study of subjects who had a unilateral nephrectomy for renal disease 15-20 years ago. 119 patients (UN) aged 20-60 y were identified who had a unilateral nephrectomy in between 1966-1972. These were matched with 238 controls (C) for sex, age within 5 y and three levels of blood pressure who had other operations in the same period. 206 (87%) controls were traced. All subjects and controls were interviewed about there past medical history; blood pressure was taken sitting after 2 minutes and blood samples were checked for electrolytes and creatinine. Random urine samples taken for albumin, creatinine, microscopy and infection. 24h urines were checked in a subset of both subjects and controls.

48% of UN and 33% of C had died but when UN with malignancy were removed this was not significant. 1 UN and 3 C died from uraemia. There were no differences in deaths from CHD or in the proportions developing hypertension (25% vs 30%). 6 UN and 7 C patients have developed significant proteinuria. Plasma creatinine in UN was 0.18 mmol/l in UN and 0.12 mmol/l in C and only 3 UN had developed CRF from pre-existing disease. The risks of UN are not different from C in respect of hypertension and proteinuria.

NON-AMYLOIDOTIC FIBRILLARY GLOMERULOPATHY. B.C. Sturgill and W. Kline Bolton. Univ. of Virginia Med. Ctr., Charlottesville, Virginia.

Ten cases of non-amyloidotic fibrillary glomerulopathy were identified among 3,012 renal biopsies seen over a period of 14 years. Ages at the time of biopsy ranged from 39 to 67 years. M/F ratio was 4/6. Seven presented with nephrotic syndrome and all had some degree of proteinuria. Seven had hematuria. Five presented with chronic renal insufficiency. Two presented with acute renal failure. Associated conditions included cold urticaria (1), type 4 hyperlipidemia (1), rheumatoid arthritis (1), diabetes (2), and psoriasis (1). Histology of the renal biopsies was variable and non-specific (two had focal crescents) but ultrastructure and immunofluorescence studies were distinctive. Coarse fibrils with a diameter ranging from 10-20 nm expanded the mesangium and glomerular basement membranes extensively. Congo red stains were always negative. Immunofluorescence findings were variable but heavy deposits of both kappa and lambda light chains, IgG and C-3 were characteristic. Light deposits of IgM were found in seven cases, IgE in one, C1q in 5, IgA in 2 and IgE in one. None has developed evidence of systemic amyloidosis. Five are on chronic dialysis, one died 7 years after the biopsy and one year after beginning dialysis. One patient is two years post-transplantation and doing well. One patient treated with cyclophosphamide for severe destructive rheumatoid arthritis has had no progression of renal disease five years post biopsy. One patient had a marked reduction of proteinuria after treatment with captopril and one patient has normal renal function 3 years after biopsy.

RENAL RESERVE FILTRATION CAPACITY (RRFC) BEFORE AND AFTER KIDNEY DONATION. Piet M. ter Wee*, Adam M. Tegzess*, and Ab J.M. Donker* (intr. by L.W. Statius van Eps). Univ. of Groningen & Free Univ. Amsterdam, The Netherlands.

In 16 kidney donors RRFC was investigated before and 3 months after uninephrectomy. RRFC was tested by infusion of dopamine (1.5-2.0 µg/kg/min), by intravenous administration of an amino acid solution (Vamin-N^R), and by a combined infusion of Vamin-N^R and low-dose dopamine. After kidney donation the median value of the GFR was 65% of its initial value. The median value of the ERPF amounted to 70% of its value before uninephrectomy and FF fell from 0.26 to 0.24 ($p < 0.01$). Median values for dopamine-induced rises in GFR were 13.4% before and 4.7% after kidney donation ($p < 0.01$). Infusion of Vamin-N^R led to a rise in GFR of 10.6% before and 9.6% after donation (ns). During combined infusion these values were 18.4 and 10.8%, respectively ($p < 0.1$). Median changes in ERPF before and after kidney donation were 33.5 vs 23.2% during dopamine ($p < 0.02$), 7.8 vs 8.3% during Vamin-N^R (ns), and 36.2 vs 23.6% during combined infusion ($p < 0.02$). Since infusion of dopamine induces predominantly efferent vasodilation and RRFC tested by dopamine was significantly decreased after kidney donation, we conclude that glomerular hyperfiltration after kidney donation is due to a rise in glomerular blood flow as a result of predominently efferent vasodilation. Afferent vasodilation, resulting in a rise in glomerular capillary pressure and tested by amino acid infusion, seems to be less important to compensate for the loss of a kidney, as the amino acid-induced changes in GFR and ERPF were comparable before and after kidney donation.

PROGNOSTIC SIGNIFICANCE OF SERUM ANGIOTENSIN-CONVERTING ENZYME COMPARED TO THYROXINE IN ACUTE RENAL FAILURE. Kazutomo Ujiie*, Kimio Tomita, Akira Owada*, Yasuhiko Iino, Naoki Yoshiyama*, Noriaki Matsui*, and Fumiaki Marumo. Tokyo Med. & Dent. Univ., Tokyo. and Tsuchiura-Kyodo Hosp., Ibaraki, JAPAN.

Recent studies have shown that levels of serum angiotension-converting enzyme (ACE) and total serum thyroxine (T_4) are decreased in acute renal failure (ARF). Both ACE and T_4 are reported to be correlated with each other. We evaluated ACE and thyroid function to determine either of which is more important as a prognostic index of ARF.

Among 37 ARF patients, 24 patients survived (recovery group) and 13 patients died (lethal group). We measured ACE and thyroid hormone of venous blood samples that were taken from those patients at the progressive phase of ARF.

The reduction of T_4 level under normal range (normal range is from 4.6 to 12.6 μg/dl) was seen in 11 of 24 survived patients (5.3±2.6 μg/dl), and was seen in 11 of 13 died patients (3.0±1.4 μg/dl). The reduction of ACE under normal range (from 8.3 to 21.4 IU/l/37°C) was seen in 6 of 24 survived patients (11.7±3.6 IU/l/37°C), however, as many as 10 of 13 were decreased in died patients (7.0±2.8 IU/l/37°C). The reduction of ACE is a clinically useful index that distinguished between recovery group and lethal group ($p<0.03$), however the reduction of T_4 had no discriminary significance.

It is concluded that serum T_4 was depressed in both recovery group and lethal group, but the reduction of ACE was seen especially in lethal group. ACE activity is a specific and clinically useful prognostic index in ARF.

ACUTE RENAL FAILURE (ARF) IN AIDS PATIENTS (pts). A. M. Valeri* and A-J. Neusy. NYU Medical Center, NY, NY.

In a retrospective chart analysis of 449 AIDS pts (607 admissions) admitted to Bellevue Hospital from August 1983 to December 1986 examining etiology, course, and outcome of ARF, defined as an acute change in serum creatinine (Cr) > 0.3 mg%, there were 246 pts (55%) with 425 episodes of ARF. The causes included: volume depletion (VOL)- 38%, pentamidine (P)- 17%, amphotericin B (AB)- 11%, Bactrim (B)- 9%, sepsis ± shock (S/S)- 8%, unknown- 7%, contrast (C)- 4%, aminoglycosides (A)- 2%, and all others- 1% or less each, often the sequelae of treatment of certain infections: PCP (73% of pts had 1 or more cases), primary bacteremia (11%) and cryptococcal meningitis (6%). The mean peak Cr and its frequency distribution was not different among VOL, P, B, AB and C (range 2.1-2.6 mg%), and higher for S/S (3.7 mg%) and A (3.3 mg%). In surviving pts, complete recovery was: VOL (99%), C (100%), B (97%), P (90%), S/S (89%), A (80%) and AB (77%). Death before renal recovery was: VOL (8%), C (6%), AB (8%), B (13%), P (12%), A (50%) and S/S (80%). The mean duration of ARF was: VOL (5.5 days(d)), C (6.8 d), S/S (7.8 d), B (9.0 d), P (11.6 d), A (14.4 d) and AB (21.2 d). There were 21 cases in 17 pts of ARF with Cr ≥ 6.0 mg%; 7/21 were due to VOL. In 12 cases (9 pts), baseline renal insufficiency existed; VOL was responsible for ARF in 3. Four of 17 pts received dialysis; 9/17 died.

VOL represents the most common cause of ARF in AIDS pts and can be as severe as any other cause of ARF. This emphasizes the need for careful fluid management in these patients.

CLINICAL FEATURES OF AIDS-ASSOCIATED NEPHROPATHY (AAN). A. M. Valeri* and A-J Neusy. NYU Medical Center, NY, NY.

In a retrospective chart analysis of449 AIDS patients (pts) admitted to Bellevue Hospital from August 1983 to December 1986, we found 34 pts with evidence of AAN (persistent proteinuria and/or renal insufficiency (RI), defined as serum creatinine (Cr) > 1.3 mg%). Demographic features, similar to the general AIDS population at Bellevue, were: mean age 35.5 years; male:female 10:1; race 46% black, 35% Hispanic, 18% white; AIDS risk factor(s) 56% IVDA, 35% homosexual, 6% both and 3% other or unknown. At time of diagnosis of AIDS (DxAIDS), 35% had RI. AAN developed within (<) 1 yr from time of DxAIDS in all but 1 pt. Other clinical features included: RI (82%), proteinuria (100%), hypertension (12%), edema (35%), hematuria (6%), pyuria (24%), and a mean serum albumin of 2.5 gm%. Average 24-hour proteinuria, available in 17 pts, was 7.0 gms (12 were in the nephrotic range). ESRD (creatinine ≥ 6.0 mg%) developed in 11 pts, 9 in < 1 yr from the time of DxAIDS; 3 received dialysis. Nine pts died < 6 months from the time of ESRD (a mean of 25.5 days). By life table analysis, there was no difference in survival time of pts with AAN measured from the time of DxAIDS (50% survival at 10.5 months) compared to other AIDS pts (11.5 months, p> 0.05).

The incidence of AAN was 7.6% (3.0% per year). Survival from time of ESRD was short; however, overall survival time in AAN pts was no different from that of other AIDS pts.

ENDOTOXEMIA IN PYELONEPHRITIS. S.J.H.van Deventer*, I.de Vries*, L.W.Statius van Eps, H.R.Büller*, A.Sturk* and J.W.ten Cate *. Depts.of Internal Medicine, Slotervaart Hospital and Hemostasis and Thrombosis, Academic Medical Center, Amsterdam, The Netherlands.

Endotoxins are the most important bacterial factor for the induction of Gram-negative septicemia. Recently we developed a sensitive and specific test for the detection of bacterial endotoxins in blood (detection limit 5 pg/ml, assaytime 2 hours). This assay was used to determine the independent predictive values of Gram-negative septicemia and endotoxemia for the development of septicemia (based upon the following criteria: systolic blood pressure below 90 mmHg, thrombocytopenia, metabolic acidosis, or oliguria) in 80 consecutive febrile patients (body temperature 38.5°C) with pyelonephritis. The positive predictive value of the endotoxin test for septicemia was significantly higher than the positive predictive value of Gram-negative bacteremia (73% vs. 29%), whereas negative predictive values were similar (92% vs. 95%). Thus, an endotoxemic patient with pyelonephritis is at high risk for the development of septicemia. In addition, endotoxins induce enhanced uptake of aminoglycosides in renal tubules, and thereby may promote the nephrotoxicity of these frequently used drugs.

THE PROLONGED BLEEDING TIME (BT) IN RENAL FAILURE PATIENTS IS NOT DUE TO LOW PLASMA LEVELS OF HIGH MOLECULAR WEIGHT VON WILLEBRAND FACTOR (HMW vWF). Evan Vosburgh*, David B. Bernard, Mark J. Weinstein*. Boston University School of Medicine, Boston, Massachusetts.

To determine whether the prolonged BT of uremia is associated with abnormal vWF, we compared plasma vWF levels, ristocetin cofactor activity (RCof), and multimer distribution in hemodialyzed renal failure patients with BT < 8 min (n = 17) to those with BT > 8 min (n = 9). The vWF level in both groups of patients was twice that of normal pooled plasma, but the ratios of RCof to vWF antigen levels were unchanged, indicating that inactive forms of the protein were not present. vWF multimers were separated on SDS agarose gels, overlaid with ^{125}I-anti vWF, and quantified by densitometry of autoradiographs. HMW vWF have molecular weights greater than multimers at the optical density maximum of vWF on autoradiographs of normal pooled plasma, and are necessary for normal BT. Compared to a pooled plasma standard, the ratio of HMW vWF: total vWF in healthy subjects was 0.93 ± 0.1 (SD), n=49; in patients with BT < 8 min, 0.81 ± 0.17 ($p < 0.01$); and in patients with BT > 8 min, 0.81 ± 0.19 ($p < .05$). Thus, uremic patients have unusually high concentrations of plasma vWF and decreased levels of HMW vWF, but these abnormalities do not correlate with prolonged BT or give rise to changes in the RCof:vWF antigen ratio.

RELATION BETWEEN MICROALBUMINURIA AND BLOOD PRESSURE IN NORMOTENSIVE INSULIN DEPENDENT DIABETIC SUBJECTS. W.G. Walker, J. Hermann*, P. Duggal*, and R. Murphy*. Johns Hopkins Univ. Sch. of Med., Depts. of Med. and Ophthal., Baltimore, Maryland.

The relationship between microalbuminuria (μAlb) measured by radioimmunoassay and systolic arterial blood pressure (BP) in 90 subjects with IDDM was examined prior to the development of hypertension (mean BP 118 ± 14/76 ± 5 mmHg) and compared with similar data in 151 normal subjects (NOR). Mean μAlb in NOR yielded a mean value of 7.2 ± 6.7 (SD) μg/ml; for the 90 IDDM subjects, 228 ± 996 (SD) ($p<.0001$). In NOR, 95% were <21 μg/ml but only 57% IDDM fell in this range. Comparable data for μAlb/urine creatinine ratios were: NOR .010 ± .015 (SD) and IDDM .257 ± 1.08 (SD) μg/mg ($p<.0001$). Correlation between μAlb and BP was examined in both NOR and IDDM. For the IDDM group $r=+.35$; $p<.001$, but 8 of the 90 subjects had diastolic BP >90 mmHg. When these 8 subjects were excluded and the data reexamined, the significant correlation persisted ($r=+.25$; $p<.025$), but no correlation was demonstrated between BP and μAlb in the NOR ($p>.5$).

These data support a role for BP as an important determinant of the rate of evolution of diabetic nephropathy even before BP reaches the hypertensive range. The findings also raise the issue of benefit from earlier control of blood pressure at lower levels in IDDM. The clinical utility of μAlb measured sequentially in normotensive IDDM is evident from these data.

A DYNAMIC TEST TO EVALUATE FIBRINOLYTIC ACTIVITY IN NORMAL SUBJECTS AND PATIENTS WITH SLE AND LUPUS NEPHRITIS (LN). N.K. Wadhwa,* P. Glas-Greenwalt,* K.S. Kant, V.E. Pollak. SUNY, Stony Brook, N.Y., Univ. of Cincinnati Med. Ctr., Cincinnati, Ohio.

Fibrinolytic activity at rest does not reflect full fibrinolytic potential in patients predisposed to thrombosis. A dynamic test is desirable to evaluate fibrinolytic capacity in such patients. Fibrinolytic response to IV desmopressin (DDAVP) infusion (0.4 mg/kg) was studied in 20 normal subjects; 4 patients with LN and glomerular thrombi were studied twice at 1 year intervals. Functional tissue plasminogen activator (t-PA), t-PA inhibitor (PA-I) and α_2-antiplasmin (α_2-AP) were measured by standard fibrin plate methods. t-PA antigen was measured by ELISA, protein C (PC) and protein S (PS) by electroimmunodiffusion. Mean values before and 10 minutes after DDAVP infusion were:

	Normal Subjects (n=20)		Patients (No. Studies=8)	
	Pre	Post	Pre	Post
t-PA activity (IU/ml)	0.02	0.06***	0.01	0.07***
t-PA antigen (ng/ml)	4.3	12.1***	7.2	21.0*
PA-I (IU/ml)	1.32	1.23***	3.81	2.21**
α_2-AP (%)	107.2	92.4*	163.4	120.7**
PC (%)	102.4	86.9**	154.9	144.5*
PS (%)	90.3	102.1	134.9	128.0

*p <0.01, **p <0.001, ***p <0.0001

These data suggest that IV DDAVP infusion is a fast and reproducible dynamic test to evaluate fibrinolytic capacity.

EFFECT OF MAX-EPA IN PATIENTS WITH SLE. A DOUBLE-BLIND, CROSS-OVER STUDY. Gunnar Westberg and Andrej Tarkowski. Univ of Göteborg, Dept of Nephr and Rheum, Sahlgren's Hospital, Göteborg, Sweden.

Eicosapentaenoic acid, EPA, in large quantities, has been found to retard the development of renal disease, and in some studies to prolong the life-span of mice with SLE-like disease.

A double-blind, cross-over study was undertaken on the effects of EPA in patients with active SLE. The pats received identical capsules containing either MAX-EPA, 0.2 g/kg BW or the same amount of olive oil. MAX-EPA contains 17% of EPA and 17% of docosahexaenoic acid. After a 3 months run-in period, one of the substances was taken for 6 m, followed by 3 m wash-out, then the other substance was taken for 6 m. 20 pats entered the study, but 3 withdraw early and are excluded.

After 3 m on MAX-EPA, the clinical activity, defined as the number of signs and symptoms of SLE present, was lower than after 3 m on olive oil in 12 pats, higher in 3 pats and the same in 2 pats. Similarly the anti-DNA level was lower after 3 m on MAX-EPA than after 3 m on olive oil ($p=0.03$). These differences were caused by a deterioration after 3 m on olive oil (9 pats deteriorated and 2 improved, compared to 5 and 8 during 3 m on MAX-EPA) and had disappeared at 6 m. No clear difference was found in any other comparision, for ESR, albuminuria and hematuria, renal function, blood pressure or the amount of corticosteroids taken, after 3 or 6 months on either drug.

It is concluded that MAX-EPA, when given in this amount, corresponding to approx 15 capsules a day, and for 6 months, does not have a pronounced salutary effect on SLE patients.

EFFECT OF HUMAN ALBUMIN INFUSION ON MINIMAL CHANGE NEPHROTIC SYNDROME(MCNS). Ashio Yoshimura*, Terukuni Ideura*, Yasuki Hashimoto* and Shozo Koshikawa*, (Intr. by Kimio Tomita). Showa Univ. Fujigaoka Hosp., Dept. of Int. Med.,Yokohama,Japan

Human albumin is sometimes used for the treatment of MCNS. But this therapy is direct and rapid protein-overload for the patients. To assess the effect of human albumin infusion on the clinical course of MCNS, we compared MCNS patients with(ALB,N=16) and without(non-ALB,N=7) albumin administration, retrospectively. No differences were found between ALB and non-ALB in body weight increment, total protein level, serum albumin concentration, blood urea nitrogen, serum creatinine, creatinine clearance and amount of urinary protein excretion per 24 hours at admission. There were also no differences in the protein contents of diet and the initial dose of prednisolone between the two groups. All 7 cases of non-ALB showed complete remission within 20 days after the start of steroid therapy, but 9 patients of ALB took more than 20 days for complete remission. Significant differences was noted in the period for remission between ALB and non-ALB (73.4±18.6 vs. 13.3±1.4 days; $p<0.05$). Additionally, there were also positive correlation between the period for complete remission and the duration of albumin administration(3-86 days), ($p<0.01$) in ALB. The relapsing rate of nephrotic syndrome within two years was higher in ALB(11/16, 68.8%) compared to that in non-ALB(1/7.14.3%). These results suggested that administration of human albumin in MCNS attenuated the responsiveness to steroid therapy and increased relapsing rate.

HIGH INCIDENCE OF URINE ABNORMALITIES IN 1670 APPARENTLY HEALTHY GREEKS (AHG). Nicholas Zerefos, Dimitrios Dukakis and George Digenis.Univ. of Athens,Greece.

The goal of our study was to evaluate the incidence of urine abnormalities(UA)in representative samples of AHG. Morning midstream urines from 1670 AHG were examined for color,specific gravity,albumin,glucose,acetone,bile pigments,and microscopically for leucocytes,RBCs, casts,crystals, and microorganisms.Nine hundred of these samples were also tested for bacteria. The results can be summarized as follows:1) Abnormalities were observed in 11%, with a female preponderance (16.2%) over males (8.2%). 2) Males (M) presented an exacerbation of UA at ages < 10(3%),between 21-40 (11%), and after 60(20%),while most of the UA found in females (F) were observed at ages <10 (21,7%),with a decline during the following 5 years (9,5%), followed by a progressive increase thereafter that reached 38,8% in ages above 71%. 3) The lowest incidence of UA were observed in primary and high school male children (2.1 and 1% respectively), while the highest in soldiers,sailors and women enlisted in the Army(16%). 4) The most frequent abnormality was leucocyturia (5.9% in M and 11.8% in F),followed by bacteriuria (M=0.18%, F=4.75%), erythrocyturia (M=4%, F=3%) and proteinuria (M=0.74%,F=1.03%). We conclude that the high incidence of UA in a large number of apparently healthy individuals indicates the necessity of encouraging people to have their urines checked at regular intervals, particularly if they are:a) aged <10 and >60 (M or F), b)soldiers,sailors or women enlisted in the Army, c)men aged 21-40 and d) women of all ages.

CLINICAL APPLICATION OF PLASMA CRYOPRECIPITATION. A PRELIMINARY REPORT ON A NEW TECHNIQUE OF PLASMA EXCHANGE. Y. Yu* and L. Li* (intr. by R.W. Schrier). Dept. Nephrology, Jinling Hospital, Nanjing, China.

Six patients with severe nephrotic syndrome caused by systemic lupus erythematosus (4 cases), polyarteritis nodosum (1 case) and anaphylactoid purpura (1 case) have been successfully treated with a new technique of plasma exchange--plasma cryoprecipitation (PC). This method includes 4 procedures: collecting exchanged plasma, freezing (-30°C, 72h), centrifuging (-4°C, 2000g, 30min) and returning the supernatant to the patient after being warmed. By using this technique, donor's plasma was needed only once at the initiation of treatment and cryoprecipitated plasma was utilized thereafter. Plasma (1.2 to 2 l) was exchanged once a week for 3 to 10 weeks. After PC the cryoglobulins, circulating immune complexes and fibrinogen decreased; IgG and C_3 levels returned to normal; autoantibody titers decreased or disappeared. Clinically, all patients improved with decreased proteinuria and pulmonary infiltrates and improvement in renal function.

In summary: 1) PC effectively removes harmful substances such as cryoprotein, circulating immune complexes and autoantibodies. 2) The activity of clotting factors in plasma decreased after freezing and this may be beneficial in counteracting any hypercoagulative state. 3) After PC the exchanged patient's own plasma can be used as a substitute for donor's plasma. In conclusion, the clinical effects of PC are comparable to ordinary plasma exchange, but the cost is much less.

DETERMINANTS OF BLOOD PRESSURE (BP) DURING CHRONIC ERYTHROPOIETIN (EPO) THERAPY (Rx) IN HEMODIALYSIS (HD) PATIENTS. PA Abraham, JA Opsahl, LA McLain*, RW Asinger*, KJ Elsperger*, CE Halstenson*. Hennepin County Medical Center, and Univ. of Minnesota, Minneapolis, MN

Correction of anemia with EPO in HD pts may be associated with changes in systemic hemodynamics and/or worsening hypertension (HTN). We studied determinants of blood pressure in 8 (51±14 yrs) (mean±SD) anemic (Hgb ≤ 8.5 g/dL) pts whose BP was controlled during EPO Rx. HTN Rx was not added or increased but weight was adjusted to maintain BP control. BP (pre & post HD and 24 hr ambulatory), wt, plasma renin activity (PRA) and aldosterone, ^{51}Cr red blood cell mass (Cr-RBC), inulin space (ECFV), and cardiac output (CO) and systemic vascular resistance (SVR) by echocardiography were measured and plasma volume (PV) was calculated before and after 18±6 weeks of EPO Rx.

Hgb (g/dL)	Cr-RBC (mLs)	PV (mLs)	ECFV (mLs)	CO (L/min)	SVR (dynesecm^{-5})
6.7 ± 1.0	721 ± 288	2816 ± 898	15719 ± 3932	5.34 ± 1.61	1555 ± 821
11.3* ± 2.5	1294* ± 486	2397* ± 649	11103* ± 3358	5.13 ±1.10	1546 ± 376

BP (baseline pre-HD diastolic 82±14 mmHg), weight, PRA, and aldosterone did not change.

Thus, in these pts in whom PV and ECFV were significantly decreased, a 68% increase in Hgb was not associated with significant alterations in determinants of systemic BP.

DOES ERYTHROPOIETIN (EPO) TREATMENT CHANGE HEMODIALYSIS (HD) REQUIREMENTS? S.Acchiardo, L.Moore*, D.Miles*, J.Key*, L.Burk*, J.Sargent* University of Tennessee-Memphis, Dept of Medicine, Memphis, TN, and Quantitative Medical Systems, Emeryville, CA.

The elevation of Hct with EPO in HD patients (pts) has been considered a potential risk due to the decrease in solute specific blood water flow (Qe), especially for K^+ and Pi.

In 12 HD pts (8 High Flux, 4 conventional) treated with EPO, we measured dialysance corrected for Qe during the basal period and after the Hct had increased above 30%. We also determined the increase in Hct that occurred during dialysis.

	Hct	Hct	UF	Dialysance	ml/min	
	%	%Δ	% B Wt	BUN	K^+	Pi
BASAL	19.7	17	4.98	248	284	204
AFTER EPO	35.7*	12	5.14	221	276	188

*$p < 0.001$

After 6 w on EPO (mean dose 112 U/kg) there was a significant increase in Hct. No significant changes were observed in BUN (78 vs 83.4 mg/dl), serum K^+ (5.3 vs 5.2 mEq/L) or serum Pi (5.7 vs 6.6 mg/dl).The increase in Hct during dialysis was similar during the 2 periods. The UF measured as percentage of BWt was almost identical. Dialysance decreased 12.2% for urea, 3% for potassium and 8.5% for phosphorus, and none of these values were statistically significant.

At these levels of Hct the changes observed in dialysance are not clinically significant and they do not require altering the dialysis therapy.

HEMODYNAMIC CHANGES OF HEMODIALYZED PATIENTS BY ERYTHROPOIETIN(EP) TREATMENT. T. Akiba*, S. Kurihara*, H. Katoh*, H. Yoneshima*, and F. Marumo. Dept. of Int. Med., Tokyo Med. & Dental Univ., Tokyo, & Kasukabe Shuwa Hosp., Kasukabe.

The correction of renal anemia with the use of EP has been associated with the elevation of blood pressure in some regular dialysis patients(Pts). To assess this, 12 hemodialyzed uremic Pts were studied by Swan-Ganz catheter before and after 12 weeks of 1500-3000 units EP thrice a week(Tx). After Tx, hematocrit(Hct), blood viscosity(BV), and systemic vascular resistance(SVR) were increased and the cardiac index(CI) were decreased, significantly. The increase of SVR correlated with the increase of Hct. Pts were divided by the changes of mean blood pressure(MBP) into Group A(unchanged or decreased MBP, n=6) and B(increased MBP,n=6).

	Δ-Hct (%)	pre-MBP (mmHg)	Δ-MBP (mmHg)	pre-CI (1/min/m^2)	Δ-CI
Group A	10.0	121	-7	6.0	-1.20
(S.D.)	(3.8)	(10)	(5)	(0.6)	(.82)
Group B	9.4	100	19	4.7	-.16
(S.D.)	(1.9)	(14)	(18)	(0.2)	(.36)
p<	n.s	5%	1%	1%	5%

Pre and post SVR and BV were not significantly different in two groups. These results demonstrated that 1)the elevation of Hct was accompanied by the increase of SVR in all Pts, but the changes of SVR did not correlate with changes of MBP. 2)Pts with BP increase were characterized by a high pre-CI which was normalized by Tx, while Pts without BP increase were characterized by a lack of high pre-CI in spite of anemia. 3)High BP observed in Tx may reflect the presense of a decreased cardiac output response in anemic phase.

l-LACTATE VERSUS dl-LACTATE AS A BASE FOR HEMODIALYSIS. M. Ajam*, D.K. Gupta*, Z.M. Nawab*, V.C. Gandhi, T.S. Ing, and J.T. Daugirdas. Hines-Loyola Medical Center, Hines, IL.

Having previously assessed the use of dl-lactate as a hemodialysis base, we now examined the feasibility of using l-lactate. Nine patients were each dialyzed twice, once using 40 mM l-lactate, and once using 40 mM dl-lactate. Blood acid-base values, plasma l-lactate levels, and blood pressure and symptoms were monitored.

Acid-base changes were equivalent with the two forms of lactate. The plasma bicarbonate level fell slightly during the first several hours of dialysis, increased to baseline at the end of the 4-hour session, and then increased by 1 hour after dialysis (l-lactate: +2.25 ± 0.47 mM; dl-lactate +3.00 ± 0.43, p l vs dl, NS). Peak blood l-lactate levels were somewhat higher with l-lactate dialysis solution (7.8 ± 0.46 mM) than with dl-lactate dialysis solution (6.0 ± 0.73, p l vs dl < 0.05). With both forms of lactate, blood l-lactate levels were back close to baseline by 1 hour after therapy. Treatments were largely asymptomatic and blood pressure well maintained with both lactate solutions. The average intradialytic blood PO2 level compared to baseline with l-lactate was -4.2 ± 1.2 mm Hg, and with dl-lactate was -3.8 ± 2.4 mm Hg (p NS, l vs dl).

The results suggest that l-lactate is as suitable as dl-lactate as a dialysis solution base, and is theoretically preferable, given the reported adverse effects associated with d-lactate. However, use of lactate at a 40 mM concentration results in a slightly suboptimal correction of acidosis.

COMPARISON OF SINGLE (SND) AND DOUBLE (DND) NEEDLE TREATMENT EFFICIENCY WITH A SINGLE PUMP PRESSURE-PRESSURE CONTROLLED DEVICE. Howard J. Alfred, Gary Warns*, Patricia Powell*, Paula Deedy*, and Jon Morgenthaler*. Worcester Memorial Hospital, Worcester, Massachusetts, and COBE Laboratories, Lakewood, Colorado.

For patients with limited vascular access or with single-lumen indwelling catheters, hemodialysis must be performed with an appropriate SND device. A pressure-pressure controlled intermittently operating single pump system was evaluated. Six stable chronically hemodialyzed patients were treated using both DND (n=3 Rx per patient) and SND (n=6 Rx per patient), with hollow fiber dialyzers. Fluid balance was closely monitored for ultrafiltration (UF) accuracy. Blood was obtained pre and post dialysis for BUN. Treatment intensity (Keff and Kt/V) was determined by urea kinetic modeling techniques, and liters processed (LP) was calculated by actual blood flow (Qb) x treatment time (Td). Samples were obtained during dialysis for blood recirculation (R). Td, UF, and R were similar: Td, 3.3±0.4 hours (DND), 3.3±0.4 (SND); UF, 2.6±1 liters (DND), 2.6±1.4 (SND); R, 12.3±20% (DND), 6.3±2% (SND). Average blood flow (Qb) 264±23 (DND) and 230±26 ml/min (SND) (p<.001); Keff 166.4±14.8 (DND) and 141.1±16.8 (SND) (p<0.001); Kt/V, 1.04±0.26 (DND) and 0.86±0.21 (SND) (p<0.01): LP 52.0±5.7 (DND) and 43.6±7.2 (SND) (p<0.001). SND was less intense than DND due to lower average Qb, and not due to increased R. LP correlated well with Kt/V for each patient, providing a readily available measure of the adequacy of each dialysis treatment. UF accuracy is not affected by SND. We conclude that with careful monitoring and adjustment of Td, KA and Qb, SND is as effective as DND.

ACUTE TREATMENT OF HYPERKALEMIA IN HEMODIALYSIS (HD) PATIENTS WITH NEBULIZED ALBUTEROL. M. Allon, C. Copkney*, R. W. Dunlay* . Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, OK .

Beta-agonists induce an acute intracellular shift of potassium. They may, therefore, be useful in the acute treatment of hyperkalemia. The potassium-lowering effect of nebulized albuterol (a beta$_2$-agonist) was evaluated in 10 chronic HD patients with chronic hyperkalemia (K^+>5.0 mmol/L). Patients were studied in the fasting state 72 hours after the previous HD session. On 3 separate days, the patients received either albuterol (10 or 20 mg) or placebo (saline) inhaled over a 10 minute period. Blood samples were obtained at baseline, and every 30 minutes for two hours and vital signs were monitored. The changes in potassium from baseline (ΔK) during the 2-hour study were:

ΔK (mmol/L)	Albuterol 10 mg	Albuterol 20 mg	Placebo
30 min	-0.30±0.07*	-0.59±0.13*	+0.14±0.06
60 min	-0.45±0.1*	-0.86±0.16*	+0.30±0.12
90 min	-0.62±0.09*	-0.84±0.17*+	+0.36±0.12
120 min	-0.55±0.09*	-0.98±0.14*+	+0.24±0.11

* p<0.01 vs placebo (same time period)
+ p<0.05 vs 10 mg albuterol (same time period)

Nebulized albuterol had a significant potassium-lowering effect. The magnitude of the hypokalemic effect did not differ between diabetic and non-diabetic subjects (-0.50 ±0.15 vs -0.56±0.14 mmol/L, with 10 mg albuterol). Albuterol did not produce changes in blood pressure or heart rate. These results suggest that nebulized albuterol may be an important adjunct for acute treatment of hyperkalemia in HD patients.

EFFECT OF BETA BLOCKADE ON LV SYSTOLIC FUNCTION IN PATIENTS UNDERGOING HEMODIALYSIS. Andre K. Artis*, Martin A. Alpert*, John Van Stone, Zbylut J. Twardowski, Ramesh Khanna. University of Missouri Hospital and Truman VAMC, Columbia, Missouri.

To assess the effect of beta-blockade on left ventricular (LV) systolic function in patients (pts) undergoing hemodialysis (HD) we performed echocardiography just prior to and immediately following standard 4 hour HD on 19 pts receiving beta-blocking drugs in doses sufficient to reduce pre-HD resting HR to 50-65 beats/min (Group 1) and 19 pts not receiving beta-blocking drugs (Group 2). LV systolic function was assessed by calculating echocardiographic LV fractional shortening (LVFS). LV hypertrophy (LVH) was defined as increased echocardiographic LV mass. Pre-HD mean LVFS (%) and mean HR (beats/min) and the HD-related changes in these variables (± 1 SD) were as follows:

	Group 1			Group 2		
	LVH n=10	No LVH n=9	p	LVH n=10	No LVH n=9	p
LVFS	26±4	30±4	N.S.	27±4	30±3	N.S.
ΔLVFS	+0.6 ±1.5	+3.6 ±2.2	<0.01	+2.7 ±1.3*	+4.3 ±2.6	N.S.
HR	59±2	58±4	N.S.	91±8†	90±3†	N.S.
ΔHR	-2.0 ±1.6	-1.9 ±2.3	N.S.	-9.9 ±4.2†	-7.8 ±4.2†	N.S.

*p<0.05; † p<0.025 compared to analogous Group 1 variable. The results indicate that in pts with LVH (but not in pts without LVH) beta-blockade signficantly blunts the expected improvement in LV systolic function resulting from standard 4 hour HD.

AMBULATORY BLOOD PRESSURE (ABP) DURING INTERDIALYTIC (ID) PERIOD IN UREMIC PATIENTS TREATED BY HEMODIALYSIS (HD). KS. Ang,* P. Simon,* & G. Cam. (Intr. by G.S. Hill), Department of Nephrology, Hôp. La Beauchée, St. Brieuc, France

ABP was recorded in 60 HD patients classified according to pre and post HD BP (N1 diastolic BP ≤ 90 mm Hg): group A (n=32) with normal BP, group B (n = 18) with volume-dependent hypertension (VdHT) and group C (n = 10) with chronic hypertension (CHT). ABP was recorded during HD session and ID period (48 hours) at 15-20 minute intervals using a Delmar Avionic Pressurometer (PIV). Patients included in this study were between 23 and 72 years (mean age: 56 ± 17 yrs.) Some patients of group B and all of group C received antihypertensive drugs. Patients were dialyzed 3x4 hours a week. The following was observed: 1) During HD session BP dropped in pts of groups A and B and remained unchanged or paradoxically increased in patients of group C despite weight loss by ultrafiltration. 2) During ID period, hypertension was present less than 10 hours after the end of HD session in 9/18 pts classified as VdHT and circadian rhythm was lost in 18 of 32 pts (56%) of group A, 11 of 18 pts (61%) of group B and all patients of group C. 3) The effects of antihypertenstive drugs was inconstant or short. These preliminary data show that classic methods to identify HD pts with hypertension are insufficient and ABP monitoring is useful to recognize hypertension during the ID period in order to adjust antihypertensive drugs. They suggest that autonomic insufficiency associated with the uremic state could be responsible for circadian rhythm abnormality.

BETA 2 MICROGLOBULIN DEPOSITS IN UREMIC PATIENTS: SKIN STUDY. AGH Assounga*, S Bascoul*, B Canaud*, PA Bouya*, JP Vendrell*, JP Sciolla*, G Mourad*, P Baldet*, A Serre*, C Mion* (intr. by LB Melton) Dept of Nephrology, Centre Hospitalier, Univ., Montpellier, France.

Beta 2 microglobulin (B2M) tissue deposits have been demonstrated in uremic patients. Serum B2M are elevated in uremic patients. As the skin is much more accessible than internal organs, we examined skin biopsies of 15 uremic patients on dialysis for 1-19 years and 8 healthy controls by Congo red stain and by indirect immunofluorescence using an anti-B2M monoclonal antibody. The Congo red stain of the skin did not reveal any amyloid deposits in patients or controls. However, skin immunofluorescence showed B2M deposits in all patients and in 6 of the 8 healthy controls. The extent and intensity of deposits is greater in dialyzed patients than in the control group. All patients having massive B2M skin deposits have been dialyzed for at least 5 years and had presented a carpal tunnel syndrome. We noticed no difference between sexes, and no correlations between serum B2M and skin B2M deposits. Thus, immunofluorescence is more sensitive than Congo red stain in detecting B2M skin deposits. B2M skin deposits appear earlier than clinical manifestations due to internal organ deposits.

HIGH-FLUX DIALYSIS DOES NOT CAUSE DIALYSIS DISEQUILIBRIUM (DD) OR INCREASES IN INTRAOCULAR PRESSURE (IOP) IN WELL DIALYZED OUTPATIENTS. J.N. Austin*, M. Klein, J. Mishell, S.R. Contiguglia, J. Levy*, L. Chan, and J.I. Shapiro. Dept. of Med., Univ. of Colorado Med. Sch. Denver, CO.

DD occuring during conventional hemodialysis (CD) has been attributed to the establishment of an osmotic gradient generated between brain cells and the extracellular fluid causing cerebral edema and increases in intracranial pressure during CD. Increases in IOP occur concomminently and have been reported with CD in extremely uremic patients (Sitprijo et al, Invest Ophthal 3:273, 1964). Increases in IOP have not been observed during conventional hemodialysis in well dialyzed outpatients (Gofter et al, Nephron 40:74, 1985). As high-flux hemodialysis (HFD) causes more rapid osmolar shifts than CD, increases in the incidence of DD and increases in IOP might be anticipated. Observing approximately 50 patients converted from CD to HFD during the past 12 months, no clinical episode of DD has been observed. To further investigate this, 16 patients were evaluated with IOP determinations prior to and 2 hours into a HFD treatment using a Schiotz tonometer. Mean IOP prior to HFD was 15.7 $\pm$ 5.2 mmHg in the right eye and 14.1 $\pm$ 4.2 mmHg in the left eye. During treatment, intraocular pressure was 15.0 $\pm$ 4.5 mmHg in the right eye and 14.6 $\pm$ 3.9 mmHg in the left eye, values which are not different from the predialysis determinations. No patient had a baseline or intradialysis IOP greater than 21 mmHg. These data suggest that HFD is not likely to provoke either clinical or subclinical increases in intracranial pressure in well dialyzed outpatients.

HIGH BLOOD FLOW-LOW HEPARIN (Hep) HEMODIALYSIS (HD) WITH AN-69 MEMBRANE. RH Barth, S Sodden*, GM Berlyne. VA Medical Center and SUNY/Brooklyn, NY.

The relatively biocompatible AN-69 HD membrane has been reported to have low in-vitro thrombogenicity. We have sought to reduce HD Hep dosage using AN-69 hollow fiber (HF) dialyzers at 400-600 ml/min blood flow with volumetric ultrafiltration. During a 60-day period, 1431 HDs were performed, with mean (±SD) total Hep dose for AN-69 3339 ± 2205 U (n=948), for regenerated cellulose (RC) 4190 ± 1839 U (n=370), and for cellulose acetate (CA) 5150 ± 3517 U (n=113). In 504 HDs (35.2%) total Hep was 2000 U or less. Results for these low-Hep HDs were as follows:

Membrane:	AN-69	RC/CA	(P)
Treatments:	378	92/34	
Blood flow:	483±104	314± 56	$<.001$
Total Hep (U):	889±841	1283±863	$<.001$
Hep/kg/h (U):	3.9±3.7	5.1±3.8	$<.01$
Clotting:	1 (0.3%)	13 (10.3%)	$<.001$

191 Hep-free HDs were performed, with results below. All dialyzers were HF; blood compartments were pre-rinsed with 2000 U Hep in 1000 ml 0.9% saline. Periodic saline flushes were not used in AN-69 HDs.

Membrane:	AN-69	RC/CA	(P)
Treatments:	157	6/28	
Blood flow:	506±95	261±56	$<.001$
Clotting:	0 (0%)	6 (17.7%)	$<.001$

We conclude that the combination of high blood flow with AN-69 membrane allows marked reduction of HD Hep dose and safe routine use of Hep-free HD.

TOTAL CHOLESTEROL (TC) FALL WITH MONTHS ON DIALYSIS (MOD) AND ITS IMPACT ON ATHEROGENICITY. M.M. Avram, A.Lustig*, P.Goldwasser*, A.Antignani*, P.A. Fein, N. Mittman. The Long Island College Hospital, Brooklyn, New York

We have shown that hemodialysis pts with longer duration of ESRD tend to have lower TC. (ASN, 1987). We have expanded this survey to include 126 HD and 53 CAPD pts. Longitudinal values after 5 to 9 months were measured in 46 HD and 33 CAPD pts.

Analysis of covariance for all pts confirms the inverse correlation of TC ($p=.003$), HDL-C ($p=.01$) and its apolipoprotein (Apo) A-I ($p<.02$) with MOD. In addition, TC was higher in females ($p<.001$) and CAPD pts. ($p<.001$), and correlated with age ($p=.02$) and albumin ($p<.001$). HDL-C was higher in Blacks ($p<.001$) but not affected by gender, dialysis modality or albumin. Diabetic status, serum PTH and blood pressure (BP) were not significant variables for TC or HDL-C. The atherogenic risk ratio TC/HDL-C ($p<.001$) and Apo B (LDL- and VLDL-associated) ($p=.012$) were higher in Whites than in Blacks and correlated with BP ($p<.05$). Paired comparisons for individual pts after 9 months revealed lower TC in HD pts ($p<10^{-6}$), greater with longer MOD ($p<.04$). HDL-C decreased in HD ($p<.05$) but not CAPD pts during the study interval, but, interestingly, the risk ratio remained the same. In summary, TC falls with time on dialysis, more in HD than CAPD pts, possibly related to nutritional status. HDL-C and its Apo are lower with increasing MOD, probably explained by the fall seen in HD pts. Therefore, the impact of lower TC with MOD accompanied by stable risk ratios, cannot be directly linked to increasing atherogenesis. This data redefines overall cardiovascular risk and the presence of "accelerated atherosclerosis" in pts on dialytic therapy.

CONTINUOUS ON-LINE BIOIMPEDANCE TO MONITOR BODY FLUIDS AND CELL MEMBRANE STATUS DURING HD. G. Bazzato*, F. Scanferla*, A. Fracasso, Nephrology Div. Umberto I Hospital, Venice-Mestre, Italy.

In living organisms electrical conduction is related to water and electrolyte distribution in the biological conductor (cells). We have measured by a computerized integrated system (BIA 109, RJL AKERN) the changes of bioelectrical impedance deriving from a tetrapolar system working on 800 uA, 50 kHz constant current, in 15 uremic pts on RDT. Electrical resistance and reactance have been continuously monitored during HD session in each pt. We have observed a constant and univocal trend with linear increase of both resistance and reactance during HD treatment. Resistance changes resulted strictly correlated to dialytic ultrafiltration with $R>0.98$ in all of the subjects. Reactance showed a transient fall in corrispondence of 5 severe symptomatic hypotensive episodes. As reactance might be considered an index of cellular membrane function integrity, it could represent a reliable parameter of therapeutic efficacy in monitoring uremic patients on RDT. Furthermore the chance to control on-line bioimpedance is probably able to detect in advance the cell membrane mechanism alterations which are responsible for acute clinical events during HD. Our data are consistent with this hypothesis although further study is needed to confirm the usefulness of this apparatus in monitoring adequate HD treatment.

ANAPHYLATOXIN (A) MASS GENERATED BY CUPROPHAN MEMBRANE (CM) IS NOT INFLUENCED BY BLOOD FLOW RATE (Q_B). J.L. BELL* and L.W. Henderson. Nav Hosp, VA Med Ctr, Univ of Cal, San Diego, CA.

A generation by CM leads to adverse effects including leukopenia, hypoxemia and increases in pulmonary artery pressure. We investigated the effect of Q_B on A generation using an in vitro hemodialysis circuit by exposing standardized sera to CM 0.9 m^2 at various Q_B. C'3a and C'5a concentrations were measured at intervals from the serum reservoir. Negative control and background A were subtracted.

CUPROPHAN C'3a ug/ml (+/-sem) [a]n=6 all others n=3

ml/min	15 min	30 min	60 min	120 min
100	-1.70(.23)[a]	1.95(.41)[a]	6.15(.59)[a]	4.25(.99)
200	-0.42(.32)#	5.53(.84)+	8.59(.63)*	7.04(1.1)
300	0.97(.43)+	5.57(.36)+	6.98(.76)	3.85(1.1)

*p<0.05, #p<0.02, +p<0.001 vs 100ml/min

300++	1.61(.26)	5.27(.35)	7.47(.29)	6.70(.86)

++vol of sample reservoir doubled

p=n.s. vs 300 ml/min standard sample volume

C'5a generation by CM showed a similar Q_B dependence at 15 and 30 min which was not evident thereafter. Final C'5a concentration was also not different.

We conclude that: 1) A delivery from CM is Q_B dependent early in treatment only but the total A mass generated is Q_B independent and 2) the generation rate of A with time at a fixed Q_B is inversely related to the concentration of A and is independent of the mass of C'3 passing over the membrane. We postulate that the reactive sites on the membrane are maximally saturated early with C'3b and that A generation is dampened by regulatory proteins (I and H) with time.

EFFECT OF ERYTHROPOIETIN (EPO) ON BLOOD PRESSURE (BP) IN ANEMIC HEMODIALYSIS (HD) PATIENTS. A. Besarab, W. Gaughan, and F. Medina. Thomas Jefferson University, Division of Nephrology, Philadelphia, PA.

Neither the risk factors for worsening hypertension (HT) nor the direct effects of changing HCT on BP during EPO therapy are well defined. We enrolled 42 HD patients, the majority of whom had mild HT and required antihypertensives. Pre-enrollment severity of HT was assessed by a score (HTS) ranging from zero to 60 points. During EPO, changes in BP, interdialytic weight gain (IDWG), estimated dry weight (EDW), and Hct were recorded. Data were analyzed by multiple regression.

In the first 12 wks of EPO, mean EDW decreased 1.9 kg, Hct rose from 23 to 33 vol %, but mean BP did not change. Antihypertensives were increased in 10 patients. Changes in EDW correlated with initial Hct, weight losses occurring in severely anemic patients. HTS and age influenced pre and post dialysis BP. Pre dialysis BP was affected by IDWG and adjustments in EDW but not Hct. Diastolic blood pressures were weakly affected by Hct. Subsequently, B.P. remained stable or decreased on EPO therapy and antihypertensives could be decreased.

We conclude that (1) Increase in BP is common but can be controlled, (2) severely anemic patient carry excessive fluid; (3) older & previously hypertensive patients are at higher risk for hypertension; (4) BP returns to or below baseline once anemia is corrected but may take more than 10-12 weeks.

SERIAL ECHOCARDIOGRAPHIC EVALUATION OF CHRONIC HEMODIALYSIS (CHD) PTS DURING LONG-TERM CORRECTION OF ANEMIA WITH RECOMBINANT HUMAN ERYTHROPOIETIN (r-HuEPo). V. Berkley, F. Fouad, P. Currie, T.* Thomas, EP Paganini, The Cleveland Clinic Found., Depts. of Hypertension/Nephrology, Cardiology, and Heart & Hypertension, Cleveland, Ohio

Improvement in cardiac function has been reported in CHD pts with r-HuEPo correction of anemia. Temporal effects of this therapy, however were not described. To study serial changes in cardiac performance during therapy, 11 pts underwent echocardiographic and other hemodynamic testing immediately prior to, at target hematocrit (T-hct), and after one year of r-HuEPo therapy. Echos were independently read by two separate investigators, blinded to pt status.

While hct rose (pre: 21±2.7; T-hct: 36±3.1; 1 yr:35.5±1.9%;p<0.008), plasma volume(148±31.7 vs 109+29ml/cm, p<0.004) and blood volume (115±26 vs 102±26ml/cm, p<0.09) dropped at 1 yr, with pt wt unchanged. MAP rose at T-hct (98±18 vs 105±15 mmHg p<0.09) but returned at 1 yr to baseline value. There were no significant changes in heart rate, stroke volume (SV), % LV fractional shortening, LV end diastolic(ED) or end systolic(ES) volume(V) or diameter(D) throughout the study.

A subgroup (n=6) had their evaluations at a fixed time after HD at all study points. While there were marginal drops in EDD (5.4±1.1 vs 5.0±1.1cm, p<0.04), EDV(150±65 vs 124±56ml, p<0.08), and SV(80±26 vs 67±23ml, p<0.05) at 1 yr in this group, no changes were seen at T-hct.

A beneficial effect of correcting anemia on cardiac size and function was not readily demonstrable at either T-hct or 1 yr using echocardiography in this small pt group.

EFFECT OF ERYTHROPOIETIN (EPO)-INDUCED CHANGES IN HEMATOCRIT (HCT) ON DIALYSIS SOLUTE CLEARANCES(CL) Anatole Besarab, William Gaughan, Deborah Anzalone,* and Fani Medina.* Jefferson Medical College, Dept. of Nephrology, Philadelphia, PA.

Concern has been raised about the effect of changes in plasma flow rate (PFR) at constant blood flow rate (BFR) during EPO therapy in anemic dialysis patients. We performed 120 clearance measurements in 26 patients during EPO therapy as hematocrits (Hcts) increased from less than 25 to greater than 33 vol% (range 33 to 43). Mass balance for urea(N=60) and kinetic modeling(N=111) were performed. Multivariate step-wise data regression was performed to assess the effects of BFR, PFR, Hct, surface area (SA), and dialyzer type (plate or hollow fiber) with a p value < .05 accepted as significant.

Cl-urea was proportional to BFR and SA but independent of Hct and PFR. Parallel plate dialyzers had higher Cl-urea than hollow fiber dialyzers when factored by BFR and SA. Cl-Cr was proportional to SA and PFR but not BFR. Cl-Pi was independent of BFR or PFR of blood flows of 250-350 ml/min. Changes in BUN correlated with PCR. Serum-Pi increased by more than 25% om 1/3 of patients, was associated with dietary non-compliance (↑PCR) and required modification of Pi-binder regimens. Clinically, no significant changes in serum K^+, HCO_3^- or CA^{2+} were observed over the first 20 weeks of EPO therapy.

We conclude that EPO-induced changes in Hct, which influence PFR, produce clinically minor effects on solute clearance or blood chemistries with the exception of Pi. Dietary non-compliance develops in 1/3 of patients and requires modification of the dialysis prescription.

IMPACT OF HEMATOCRIT (Hct) ON SOLUTE TRANSPORT IN HEMODIALYSIS. Juan P. Bosch, Viroj Barlec*, Beat von Albertini*. George Washington Univ. Med. Ctr., Dept. of Medicine, Washington, D.C.

To determine the impact of Hct on solute transport during dialysis, clearance studies were performed in 4 patients on High Flux Hemodiafiltration. Mean blood and dialysate flow were 508 +/- 7 and 1003 +/- 39 ml/min, respectively. Ultrafiltration rates were comparable. 11 studies with whole blood urea mass balance error of less than 10% were used for analysis. Clearance was calculated from the solute mass recovered in dialysate.

Clearance (Mass Removed in Dialysate/ Plasma inlet concentration) was inversely related to Hct for Urea Nitrogen (UN) ($r = .80$, $p < .003$), creatinine (Cr) ($r = .79$, $p < .004$) and Phosphorus (Phos) ($r = .89$, $p < .001$). The Clearance/Plasma Flow ratio for UN was greater than 1 and was directly related to Hct ($r = .65$, $p < .04$). For Cr and Phos the ratios were .82 and .72, respectively and did not change with Hct.

We conclude, the clearance of UN, Cr and Phos is inversely related to the Hct ($p < .001$). For UN the decrease in clearance is less than the reduction in plasma flow observed with higher Hcts, suggesting urea transfer from red blood cells to plasma (equilibrium). For Cr and Phos, the reduction in clearance was proportional to the reduction in plasma flow, suggesting no transfer of solutes from red cells to plasma during the passage through the dialyzer (disequilibrium).

HIGH FLUX HAEMODIALYSIS: OPTIMAL TREATMENT FOR DIALYSIS ARTHROPATHY?
EA Brown, D Sethi, GD Perkin, RN Maini, PE Gower. Charing Cross Hospital, London UK. Introduced by DT Domoto.

It has been suggested that high flux haemodialysis (HFD), which eliminates β2-microglobulin (B2M), might be an effective treatment for dialysis arthropathy and carpal tunnel syndrome both of which are associated with B2M derived amyloid.

18 patients with dialysis arthropathy (4 female, 14 male, mean age 55±10 years, mean duration HD 12.6±3 years) were randomly allocated to either HFD (Polyacrylonitrile or Polysulfone) or cuprophane haemodialysis (HD) and underwent a single blind study lasting 6 months. Each patient had detailed rheumatological assessments, nerve conduction studies, biochemical and haematological tests before, during and after 6 months. Results showed a fall in pre-dialysis serum B2M of 21% ($p = 0.007$) in patients treated with HFD over 6 months but a rise of 8% (NS) in HD patients and no change in C-reactive protein in either group. The total range of movements of the shoulders improved from 72% at time 0, to 84% at 6 months ($p < 0.001$) in the HFD group alone. There was no significant improvement in walking time, grip strength, fist closure, Ritchie Articular Index or pain perception (Visual Analogue Scale). Serial conduction studies of the median, ulnar, sural and lateral popliteal nerves did not show any difference between the groups over the time studied.

Thus despite a large fall in serum B2M, our results have demonstrated an objective improvement only in the shoulder movements of patients with dialysis arthropathy after 6 months on HFD.

SERUM NON-TRANSFERRIN BOUND IRON (NTBI) IN HEMODIALYSIS (HD) PATIENTS. P. Brissot,* P.Simon,* AM. Barthel,* KS. Ang,* and G. Cam,* (Intr. by G.S. Hill), Hôpital La Beauchée, Saint-Brieuc, Hôpital Pontchaillou, Univ. Rennes, France.

Hepatic siderosis is a potential hazard in HD patients receiving multiple blood transfusions. The mechanism responsible for the deposition of iron in other tissues (bone, myocardium) could be due to the existence of an abnormal amount of NTBI in the serum of HD patients. In this study, serum NTBI level was assayed before and after blood transfusions in 16 HD patients, 5 of whom had laboratory evidence of iron overload (IO, defined as ferritin level > 400 ng/ml) and 2 pts with aluminum overload (AO, defined as Al after DFO test > 150 ug/ml) and 5 pts treated by CAPD. Variations in serum NTBI were also assayed during DFO test in 5 pts with IO. The following results were obtained: 1) In 9 HD and 5 CAPD pts without IO or AO, serum NTBI level was normal (< 1.5 umol/l). 2) In 2 HD pts with AO, serum NTBI level increased transiently one to two weeks after blood transfusions. 3) In 3 of 5 HD pts with IO, serum NTBI level was continuously higher than normal (2.5 to 4.5 umol/l), increased during DFO test (> 150% of basal serum level) and decreased significantly at the end of the HD session. In 2 of 3 pts with IO and high NTBI, iron was histochemically demonstrable in a bone biopsy. These preliminary results show that some HD patients receiving multiple blood transfusions have an abnormal serum level of NTBI. They suggest it has a role in the occurrence of iron deposits in bone.

EFFECTS OF ORAL BASE THERAPY ON PLASMA IONIZED CALCIUM (ION CA), PHOSPHOROUS (PHOS), AND PTH IN CHRONIC HEMODIALYSIS PATIENTS. HT Campbell, RJ Caruana, RS Weinstein*, BA Chaudhary*, KL Smith*, KM Kurunsaari*. Renal Division, Dept Medicine, Med Col GA, Augusta, GA.

Oral base supplements are often prescribed for chronic hemodialysis patients; the effects of oral base on bone mineral metabolism and PTH are unknown. We studied 20 chronic hemodialysis patients before and after one month's treatment with base. Oral dose of base was adjusted to achieve midweek predialysis plasma bicarbonate (bicarb) levels at 22-26 mEq/l (mean daily dose 1.5 ± 0.9 mEq/kg/d). Results shown are means ± SD:

	off base	on base	diff
art pH	7.35 ± .03	7.39 ± .04	$p < .005$
bicarb	18.6 ± 2.9	22.5 ± 4.0	$p < .0005$
P_aCO_2	35.0 ± 5.3	37.3 ± 5.4	$p < .005$
ion Ca	5.03 ± .37	4.83 ± .34	$p < .01$
phos	6.0 ± 1.5	6.1 ± 1.3	N.S.
iPTH	24.2 ± 20.0	25.8 ± 22.7	N.S.

(bicarb and ion Ca in mEq/l, P_aCO_2 in mm Hg, phos in mg/dl, midregion iPTH in ng/ml).

No significant changes were observed in plasma total Ca, albumin, BUN, potassium, alkaline phosphatase, blood pressure, and interdialytic weight gain.

Conclusions: Oral base therapy in hemodialysis patients raised plasma bicarb, but the expected increase in arterial pH was blunted by an increase in P_aCO_2. Ionized Ca levels fell, but no changes in phos or iPTH were seen. This short-term study does not demonstrate benefit from oral base therapy in patients on chronic hemodialysis.

EPOPROSTENOL (E) VERSUS HEPARIN (H) FOR ANTICOAGULATION IN CHRONIC HEMODIALYSIS (HD). RJ Caruana, JC Hall*, JW Crow*, A Cato*, MC Smith and D Clyne. Med. Coll. of GA, Augusta, GA, Burroughs Wellcome Co, Research Triangle Park, NC, Community Dial. Ctr., E. Cleveland, OH and VAMC, Cincinnati, OH.

Short term studies have demonstrated the efficacy and safety of E-HD in patients at high risk of bleeding. The purpose of this study was to compare the long term efficacy and side effects of E-HD to H-HD. Thirty one chronic, stable HD patients received either E or H (15E, 16H) as the sole anticoagulant for 24 consecutive dialyses. Two centers re-used hollow fiber artificial kidneys (HFAK) and 1 did not. Data from 325 E dialyses and 374 H dialyses were analyzed for HFAK function, HFAK clotting and adverse patient effects.

Mean E infusion rate was 4.1 ± 0.7 ng/kg/min and mean H dose was 5895 ± 1364 U/dialysis. HFAK function as reflected by intradialytic decrements in BUN, creatinine, potassium, and body weight were similar with E and H. Significant clotting requiring termination of dialysis or changing of HFAK or lines occurred in 37/325 E dialyses and 0/374 H dialyses. Mean fiber bundle loss was greater with E than H but averaged less than 10% in both groups. Dialyzer re-uses averaged 2.7 with E and 6.6 with H. Intradialytic hypotension and somatic complaints (nausea, vomiting, headache, cramps) were more frequent with E than H but rarely dialysis limiting with only 10/325 E dialyses terminated prematurely because of adverse effects. No tachyphylaxis or cumulative toxicity were noted with E.

E provides adequate dialysis anticoagulation with an acceptable level of side effects in chronic HD patients.

SEROPREVALENCE OF ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN PATIENTS TREATED BY MAINTENANCE HEMODIALYSIS (MH). K. Chirgwin*, T.K.S. Rao, S.H. Landesman* and E.A. Friedman. Department of Medicine State University of New York Health Science Center at Brooklyn, Brooklyn, N.Y.

To assess the prevalence of HIV seropositivity in patients and staff in an inner city hemodialysis unit at the epicenter of the AIDS pandemic, we offered voluntary HIV antibody testing to both the 86 patients underging MH, and the staff caring for them. The distribution of risk factors for HIV (intravenous narcotic drug addiction, Haitian origin, male homosexuality and blood transfusions), was similar among the 70 consenting patients and 16 who declined testing. Of the 70 patients tested, 27 (38.6%) were seropositive for HIV by both ELISA and Western Blot methods There were no false positive ELISA reactions. In all of the 27 positive patients, a known risk factor for HIV could be identified (intravenous narcotic drug use in 23, homosexuality and Haitian origin in 1 patient each, and blood transfusions in the remaining two). Mean duration of MH before HIV testing in HIV positive patients was 7 months (range 1-255 months). Retrospective testing of frozen sera at initiation of MH was also positive in each of 14 patients with available sera.

All dialyses were performed using CDC guidelines for protection against hepatitis and AIDS without isolating individual HIV positive patients. No patient without a recognized risk factor for HIV infection initially was or subsequently became seropositive. All 24 of the 25 staff members tested were found to be seronegative for HIV.

None of the seropositive patients manifested signs or symptoms of AIDS/ARC. All 16 positive patients had a reduction in the number of absolute circulating T4 (CD4) cells (mean 225, range 12-500), as compared to a mean of 964 (range 285-1492) in seronegative patients. After 9 months of follow up no patient has developed clinical AIDS.

We conclude that nosocomial transmission of HIV in a hemodialysis unit employing CDC AIDS guidelines is an unlikely event. HIV seropositivity is associated with a reduction in T4 cells in patients treated by MH. The rate of development of clinical HIV disease in these MH patients and its long-term prognosis are unknown.

POLYACRYLONITRILE (PAN) MEMBRANES BIND A GREATER AMOUNT OF C3a THAN CUPROPHAN (Cu). Alfred K. Cheung, Charles J. Parker*, Linda Wilcox*, Jarmila Janatova*. VA Med. Ctr. and Univ. of Utah, Salt Lake City, Utah.

Conventionally, complement (C) activation by dialysis membranes is determined by quantifying fluid phase C3a. Based on such measurements, Cu have been classified as strong C activators while PAN are considered to be weak activators. In previous studies, however, we have observed that PAN binds C3a. Thus, if a relatively large portion of the C3a that is generated during hemodialysis remains bound to PAN, quantitation of fluid phase C3a alone would not be a valid measure of C activation. A method for determining both fluid phase and membrane bound C3a was therefore devised.

Pieces of PAN and Cu were incubated with C3-deficient serum that had been repleted with radiolabeled C3. Subsequently, the supernates and the membranes were separated. To quantify membrane associated C3a, proteins were eluted from Cu and PAN by SDS-PAGE. Simultaneously, the corresponding supernates were also subjected to SDS-PAGE. The amount of C3a in each track was determined by counting the radioactivity of the gel slice that contained C3a (the position of the C3a in the gel was marked using purified C3a). The results of these experiments are presented in ng (*p<0.005 vs PAN):

	Supernate	Membrane	Total
PAN	29.0 ± 2.6	210.8 ± 4.8	239.8 ± 4.4
Cu	167.4 ± 9.6*	2.1 ± 0.3*	169.5 ± 9.7*

These studies demonstrate that in some instances determination of fluid phase C3a alone is not an adequate measurement of C activation by dialysis membranes. The pathophysiological significance of membrane bound anaphylatoxins remains to be determined.

CHANGING DEATHS PATTERNS WITH TIME ON CHRONIC DIALYSIS (CHD) - INFECTION IS THE SPECIFIC LETHAL COMPLICATION OF CHD. A.Collins, A.Umen*, I.Odar-Cederlöf*, B.Hylander*, CM.Kjellstrand. Depts of Med:, Hennepin Co Med Ctr and Karolinska Hosp Stockholm, Sweden.

Controversy exists about what is the specific dialysis complication that causes death.

We followed 2004 patients starting CHD since 1966. The patients were divided into age younger and older than 50 and prospectively classified as having (WIC) or not having (NOC) 1 or more of 10 complicating diseases at start of CHD.

Of 778 classifiable deaths, 45% were due to cardiovascular disease, 18% due to stopping dialysis, 16% due to infections and 19% to other causes. Cardiovascular diseases decreased from 50% of all deaths the first year to 15% beyond 9 years on dialysis, while infections increased from 10% to 25% and stopping dialysis from 15% to 30%. Beyond 6 years of dialysis, infections were the most common cause of death in <50 years NOC (75%), in >50 NOC (35%) and <50 WIC (30%) (all differences p=<0.05). In >50 WIC stopping dialysis was most common beyond 6 years (25%). Infections increased in all groups while cardiovascular disease decreased in <50 WIC and < 50 NOC and stabilized in >50 WIC.and increased in >50 NOC.

Infection is the specific dialysis complication that causes most deaths followed by stopping dialysis that is particularly common in older patients with complications. Contrary to belief, cardiovascular deaths are not caused by dialysis but decrease with time on dialysis and in young patients is probably caused by pre-existing vascular disease. If the older patients live long enough on dialysis they will, similar to nondialyzed patients, develop degenerative cardiovascular deaths. To improve the specific dialysis complication of infection, studies and correction of the immune-defect of dialysis and improvement and cleaner use of vascular access are necessary.

IMPACT OF ERYTHROPOIETIN (EPO) THERAPY (RX) ON RAPID HIGH EFFICIENCY HEMODIALYSIS (RHED). A.Collins, P. Keshaviah *, R. Berkseth, J. Opsahl and P. Abraham. Hennepin County Medical Center and University of Minnesota, Minneapolis, Minnesota.

It has been speculated that EPO Rx will complicate the administration of RHED. We studied 11 patients on chronic RHED: 3 mo. pre-EPO (C, Hct=23%), 1.5 mo. into EPO Rx at the target Hb of 12.5 gm/dl (T, Hct = 35 %), and after 2 mo. of stabilization at this Hb (S, Hct = 35 %). Outcome parameters included pre-dialysis hypertension (BP > 160/90), symptomatic hypotension (systolic < 90 with symptoms), intra-treatment complications, blood circuit pressures, small solute removal, and post-rinse dialyzer appearance:

Parameter	C	T	S
Hypertension (%)	23	41	24
Sym. Hypotn.(%)	13	8	10
Nausea (%)	9	6	5
Cramps (%)	9	15	10
(KT/V) urea	1.45	1.43	1.43
Wt. Loss (kg)	2.8	3.0	2.6

Hypertension and cramps increased significantly (C to T), but returned to baseline levels during S with changes in medications and 'dry' weight. Pre-dialyzer pressures increased from 328 to 380 mm Hg with EPO, but did not prevent the achievement of high blood flows (~ 400 ml/min), short treatment times (~180'), desired weight loss, or prescribed KT/V. 92 % of dialyzers were rated as "clean rinsebacks" during C, compared to 74 % during EPO Rx.

RHED can be successfully applied to patients on EPO with appropriate technical procedures and medical management.

DEPRESSANT EFFECT OF ACETATE IN ISOLATED CARDIAC TISSUE. J.T. Daugirdas, X. Wang*, C. Nutting*, V. Swanson*, and A. Agrawal*. Hines-Loyola Medical Center, Maywood, IL.

It has been suggested that acetate may have cardiodepressant properties in vitro, but previous studies have not separated out effects of acetate from possible effects of osmolality, sodium concentration, or calcium complexing. We studied the effects of isosmotic acetate substitution (at a constant Ca^{2+} level) on contractility in isolated rat atrial tissue and in an isolated perfused non-working whole heart preparation.

In spontaneously contracting right atrial tissue, acetate induced dose-dependent inhibition of peak isometric tension in the clinically important dose range of 4 to 16 mM. Bath acetate levels of 4, 16, or 64 mM reduced peak tension to 78% ± 2.7 (SEM), 56% ± 2.5, or 61% ± 4.6, respectively, of control values ($p<0.01$ all). Because the Ca^{2+} levels of the acetate baths were set equal to that of the control bath, calcium complexing by acetate could not have accounted for acetate's cardiodepressant effect. The inhibitory action was demonstrable with both isosmotic and hyperosmotic acetate solutions. Acetate had no effect on the spontaneous rate of atrial contraction. Inhibition of contractility in the whole heart was also seen at bath acetate levels of 8 and 16 mM: %change +dP/dt max: control: -1.4% ± 3.0; 8 mM Ac: -22.7% ± 3.3 ($p<0.01$); 16 mM Ac: -24.4% ± 3.1 ($p<0.01$).

The results suggest that acetate has a myocardial depressant effect in vitro at concentrations achievable in the plasma during hemodialysis. The myocardial depressant action is not dependent on calcium complexing or on changes in bath osmolality or sodium concentration.

COMPARISON OF ELECTROMAGNETIC BLOOD FLOW DEVICE (EBF) VS. HEMATOCRIT (HCT) METHOD FOR DETERMINATION OF CONTINUOUS THERAPY BLOOD FLOW (Qb). F. Cosentino, E. P. Paganini, S. S. Nakamoto, S. Swann*, D. Kennedy*, M. Magdenic.* Cleveland Clinic Foundation, Dept. of Hypertension/Nephrology, Cleveland, Ohio.

In vitro circuitry blood flows (Qb) during continuous ultrafiltration (SCUF) and postdilution CAVH were simultaneously measured using a new electromagnetic blood flow device (EBF, Biomedicus) and standard Hct determinations. Known pulsatile pump flows (25,50,75,100,150 cc/min) were generated by a volumetrically calibrated BSM 20 blood pump (Hospal).

EBF measurements were obtained in the pre filter position while simultaneous paired Hct determinations were drawn from pre and post filter sampling ports. Hct Qb was calculated by the formula: (UF rate) $(Hct_V)/(Hct_V-Hct_A)$

	SCUF		CAVH	
Std Qb	Hct Qb	EBF Qb	Hct Qb	EBF Qb
25 cc/min	32±25	23±7	46±66	36±12
50 cc/min	59±19	52±8	*22±5	66±10
75 cc/min	88±71	75±6	*28±7	87±7
100 cc/min	115±74	100±7	*34±8	115±6
150 cc/min	214±233	148±9	*53±8	163±18

*p<0.05

In all modes while EBF Qb vs. Std Qb were equal ($r=0.99$), Hct Qb vs. Std Qb were disparate ($p<0.05$). This data suggests that the EBF device accurately measures Qb at the low rates found in continuous therapy. However the Hct method requires multiple determinations in order to approach only marginal precision.

DOES PARATHYROID HORMONE (PTH) CHANGE ACUTELY DURING HEMODIALYSIS (HD)? C. L. Davis, SW Med Center at Dallas, and Dallas VAMC, Dallas, TX.

An increase in cardiac contractility during HD has been associated with an increase in the ionized calcium concentration (Ca^{++}). However, a decrease in PTH could also affect cardiac function. To evaluate the rapidity of a change in PTH during HD, we serially measured Ca^{++} and PTH (intact molecule, RIA) during HD in 11 chronic dialysis patients. Dialysate Ca^{++} was 1.0 mM for the first 60 min of dialysis and 1.75 mM thereafter.

	Pre	15min	60min	120min
Ca^{++} (mM/L)	.94	.91	.83	1.07#
PTH (pg/ml)	375	422	422	196*

*$p<0.05$, #$p<.001$ versus 60 min

Ca^{++} decreased and intact PTH increased slightly on the 1.0mM bath. After the dialysate Ca^{++} was increased, PTH levels decreased sharply and significantly. Intact PTH was not detected in the dialysate. Therefore, intact PTH levels decrease rapidly during HD in response to increasing Ca^{++}. Thus, it is possible that PTH may influence cardiac contractility during HD, a question deserving further study.

CLINDAMYCIN (C) AND TOBRAMYCIN (T) CLEARANCE IN CONTINUOUS ARTERIOVENOUS HEMOFILTRATION (CAVH): AN IN VITRO MODEL. Gary M. Davis and John Cleary*, Univ. of Mississippi Med. Ctr., Jackson, MS.

The pharmacokinetics of C and T were evaluated in an in vitro model of CAVH using a single compartment. C or T was added to a 3 liter reservoir of porcine blood that was maintained at a constant temperature of 37.5° C and pumped through a polysulfone membrane (Amicon Dialfilter 20) at a constant blood flow rate of 100 ml/min for each 3 hr study period. Arterial (A), venous (V), and ultrafiltrate (U) collections were used to calculate a seiving coefficient (SC): SC = 2 x U/(A+V). The average ultrafiltration rate (UFR) for all studies was 290 cc/hr and was used to calculate ultrafiltration clearance (Cl_{UF}): Cl_{UF}= SC x UFR.

DRUG	MW	PB	SC	Cl_{UF}(ml/min)
C	461	90%	0.08±0.04	0.4
T	467	< 10%	0.94±0.05	4.5

While the molecular weight (MW) of C and T are nearly identical, the ClUF for T is markedly greater, suggesting that this difference is related to protein binding (PB).

The SC for C has not to our knowledge been previously reported. However, the SC of T with our model is comparable to the SC obtained in vivo (.83±.05). This suggests that this in vitro model may be useful for determining pharmacologic clearance data for drugs administered to patients undergoing CAVH.

SEVERE DIALYZER DYSFUNCTION DURING REUSE. J. Delmez, C. Weerts*, R. Hasamear*, and D. Windus. Chromalloy Kidney Center, Washington Univ. School of Med., St. Louis, MO.

It is generally accepted that careful monitoring of total cell volumes (TCV) and ultrafiltration rates (UFR) will ensure adequate reused dialyzer function. We noted the percent of patients with clearances of less than 200 ml/min (derived from urea kinetics) unexpectedly increased from 5% to 48% despite adherence to these dialyzer rejection parameters. There was no relationship between the number of reuses and clearance. All patients were switched to new dialyzers. In those (N=48) with initial urea clearances less than 200 ml/min the clearances rose from 150 ± 34 (S.D.) to 256 ± 43 ($P<0.001$) whereas those (N=51) above 200 did not change (241 ± 29 vs. 240 ± 30). Injection of dye into the dialysate port revealed non-uniform flow through channels in the severely affected dialyzers (channelling). In vitro conductivity studies revealed that clearances of one lot of TAF 12 dialyzers declined progressively with reuse treatment despite normal TCV and UFR. This was not seen in a different lot.

	Lot #1		Lot #2	
#reuse	Q_B=200	Q_B=300	Q_B=200	Q_B=300
0	176.9±3.4	217.9±11.2	176.5±2.4	223.1±1.0
5	126.1±40.1	144.6±54.9	179.2±1.6	223.7±2.4
10	103.7±42.4	114.9±54.8	177.0±4.1	226.2±4.7

We conclude: 1) TCV and UFR do not ensure adequate dialyzer function, 2) Channelling and dialyzer failure may occur in some dialyzers subjected to reuse. Thus, inadequate dialysis, usually attributed to recirculation or errors in dialysis prescription, may result directly from dialyzer malfunction.

THE PHARMACOKINETICS OF DESFERRIOXAMINE (DFO) AND ITS CHELATED COMPOUNDS FERRIOXAMINE (FO) AND ALUMINOXAMINE (AlO) IN DIALYSIS (D-) PATIENTS. Marc E. De Broe*, Patrick C. D'Haese*, Gert A. Verpooten*, Ludwig V. Lamberts* (intr. by R.P. Wedeen). Univ. of Antwerp, Dept. of Nephrology-Hypertension, Antwerp, Belgium.

DFO has proven to be useful in the treatment of severe iron and aluminum overload in D-patients. Recently, life threatening infections with non-siderophore producing microorganisms have been reported in D-patients receiving DFO treatment. We studied the pharmacokinetics of free DFO and its chelates (FO and AlO) in D-patients with or without hemosiderosis (HS) combined or not with liver damage (LVD). An indirect atomic absorption method was developed for the selective measurement of the three compounds. Plasma half-lifes ($T_{\frac{1}{2}}$) of DFO (i.e. sum of free DFO, FO and AlO) was determined using a monocompartimental pharmacokinetic model. Preliminary results are represented in table below.

group	N	D	HS	LVD	$T_{\frac{1}{2}}$ (± SD)	FO peak ± SD
°(i)	2	-	-	-	98±13* min	8.0±1.6 umol/l
(ii)	3	+	-	-	596±47**	6.6±1.9
(iii)	4	+	+	-	918±125***	29.4±8.5
(iv)	6	+	+	+	1417±329****	36.4±9.2

° normal renal function
* vs **: $p<0.1$; ** vs *** and *** vs ****: $p<0.05$

It is suggested that the increased $T_{\frac{1}{2}}$ of DFO together with the elevated FO levels observed in D-patients with HS combined or not with LVD might play a role in the increased risk of infections with non-siderophore producing germs.

POSSIBLE RELATIONSHIP BETWEEN VITAMIN C AND GLUCOSE HOMEOSTASIS IN PATIENTS WHO UNDERGO REGULAR HEMODIALYSIS. T.S. Dharmarajan, N.Ramasubramanian, D.Salort and E.P.Norkus, Our Lady of Mercy Medical Ctr., Depts. of Nephrology, Nutrition and Biomedical Research, Bronx, NY.

Glucose and dehydroascorbic acid (DHA), the form of vit.C transported across cell membranes, share a common membrane transport system. Thus, hyperglycemia may decrease the supply of vit.C to tissues by competitively inhibiting DHA transport. In addition, hemodialysis removes vit.C from the body due to diffusion of this water-soluble vitamin into dialysate during treatment. Because of these concerns we determined serum vit.C levels in 20 subjects with chronic renal failure who had similar vit.C intakes (10 diabetics and 10 non-diabetics). A single hemodialysis treatment reduced circulating vit.C in all subjects by about 40%. However diabetics had significantly greater pre- and post-dialysis serum levels of DHA than non-diabetics (pre-dialysis, 0.42±0.18 mg/dl vs. 0.23±0.11 mg/dl, $p<0.001$ and post-dialysis, 0.46±0.13 mg/dl vs. 0.13±0.10 mg/dl, $p<0.001$). These data suggest that tissue uptake of vit.C may be impaired in diabetics, particularly if their hyperglycemia is poorly controlled. Additional studies are needed to determine if tissue vitamin C levels are low in diabetics undergoing regular hemodialysis.

IN VITRO MULTILINEAGE INCREASE OF BONE MARROW HAEMATOPOIETIC PROGENITORS FROM HAEMODIALYSED PATIENTS TREATED WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEPO). P.Dosquet*, F.Haddoum*, P.Sporer*, B.Viron*, C.Baillou+, A.Najman+, P.Scigalla**, F.Mignon* (intr. by C. Le Grimellec). * Hôpital Tenon, +Hôpital Saint-Antoine, Paris, France.**Boehringer-Mannheim Pharma, Francfort, FRG.

The correction of haemodialysed patients anemia with rHuEPO is now well established, but few in vitro studies of haematopoietic bone marrow progenitors (HBMP) are available.Semi-solid bone marrow cultures (BMC) were realised in 8 haemodialysed patients before and after 2 weeks treatment with 80 U/kg rHuEPO thrice weekly.We studied erythropoietic progenitors (early progenitors,i.e. BFU-E, and late progenitors,i.e. CFU-E), and also megacaryocytic progenitors (CFU-Mk) and granulo-monocytic progenitors (CFU-GM).

In all patients, an increase of hemoglobin level (7.18±1.25 vs 6.29±1.17 g/dl; $p<0.05$), reticulocyte count (104±35 vs 57±27 10^9/l; $p<0.05$) and platelet count(234±77 vs 194±65 10^9/l; $p<0.05$) was observed.White blood cell count was unchanged.BMC demonstrated an increase of BFU-E(1.24±0.31 fold;$p < 0.05$), CFU-E(1.47±0.33 fold;$p<0.05$), CFU-Mk(1.52±0.76 fold;$p<0.05$) and CFU-GM(1.34±0.43 fold;NS for one value with W test) .These in vitro results seem to show a non selective stimulation of HBMP after 2 weeks in vivo administration of rHuEPO.This broad effect could be due either to an unexpected action of rHuEPO on a common early progenitor or to a stimulation of non-erythroid progenitors by an indirect unknown mechanism.

DISPOSITION AND PHARMACODYNAMICS OF FOSINOPRIL SODIUM (FS) AND ITS DIACID IN HEMODIALYSIS (HD) PATIENTS. K. Duchin, K. Kripalani*, P. Kramer*, D. Sica, E. R. Squibb & Sons and Medical College of VA, Princeton, NJ and Richmond, VA.

The kinetics and dynamics of SQ 27,519 (SQ) the active diacid of FS, a long-acting angiotensin converting enzyme (ACE) inhibitor, were evaluated in HD patients. In healthy subjects (HS), SQ is eliminated equally by renal and non-renal routes. In a crossover manner, 6 HD patients (age 23-49 yrs) post-dialysis received ^{14}C-FS (10 mg) orally or an equimolar dose (7.5 mg) of SQ-^{14}C intravenously. Plasma samples collected up to 96 hr were analyzed by thin-layer radiochromatography. The mean kinetic values for SQ were: total clearance (Cl) (11.3 mL/min), elimination $t_{½}$ (28.3 hr), Vd-steady-state (11 L) and plasma protein binding (95%-98%). These parameters were similar to those in HS, except for a 50% reduction and increase in Cl and $t_{½}$, respectively. The mean values for FS absorption (36%) and SQ bioavailability (29%) were similar to values in HS. FS was hydrolyzed completely; about 75% of the radioactivity in plasma was SQ and the remainder consisted mostly of a β-glucuronide conjugate of SQ. After FS, serum ACE activity was >90% suppressed for 24 hr and returned to 50% of baseline at 96 hr. The maximal increase in plasma renin activity (104%) and decrease in plasma aldosterone levels (35%) occurred at 24 hr. Six hr after FS, blood pressure decreased by 22/13 mm Hg and returned to baseline at 24 hr. These data indicate that in HD patients FS lowers blood pressure and the initial dose of FS should be about half of that given to patients with normal renal function.

KINETICS OF ERYTHROPOIESIS IN HEMODIALYSIS PATIENTS (HD pts) ON RECOMBINANT ERYTHROPOIETIN (rHuEpo) TREATMENT. T. Drüeke*, B. Zins*, A. Moynot*, C. Naret*, F. Deschryver*, B. Varet*, C. Jacquot*, Y. Najean* (intr. by D.A. McCarron). Dept Néphrologie, Höp. Necker; Sce de Médecine Nucléaire, Höp. St. Louis; Sce d'Hématologie, Höp. Cochin; Centres AURA Bessin and E. Rist, Paris, France.

Recent clinical trials have confirmed the expected beneficial effect of rHuEpo in the anemia of HD pts. In the present work, we explored the mechanisms underlying the hormone's action. RBC and plasma volumes, ^{59}Fe kinetics, and red cell survival were measured in 9 transfusion-dependent HD pts before and 6 months after start of rHuEpo treatment (100 IU/kg thrice weekly).

Mean hemoglobin levels increased from 6.4±1 to 10.6±2 g/dl ($p<0.01$). Although total blood volume remained unchanged, RBC volume increased in all cases (from 13.7±2.5 SD to 24.2±4.9 ml/kg, $p<0.01$). In keeping with this, a quantitative improvement of red cell production was noted in all cases: ^{59}Fe renewal +50 hr, from 3.99±0.66 to 1.49±0.53, $p<0.01$; percent ^{59}Fe incorporation at day 14, 35.8±13.9 vs 75.6±13.6, $p<0.01$. Sacrum/liver ^{59}Fe uptake ratio increased from 0.42±0.17 to 1.60±0.51, $p<0.01$. Qualitative defects of erythropoiesis appeared also to be repaired, whereas RBC 1/2-life was not modified.

In conclusion, rHuEpo treatment is associated with a long-lasting improvement of RBC production and thus leads to an increase in circulating red cell mass. It has, however, no effect on total blood volume or RBC survival rate.

HEMODIALYSIS SURVIVAL IN A SINGLE INSTITUTION: A RISK FACTOR APPROACH. Francis Dumler, Gerard Zasuwa*, Edward Peterson*, and Nathan W. Levin. Departments of Medicine and Biostatistics, Henry Ford Hospital, Detroit, Michigan.

Analysis of factors influencing the survival of dialysis patients is important in delineating both public policy and individual care of patients. We have assessed the effect on survival of a wide variety of covariates using a Cox proportional hazards general linear model. The study population of 604 patients was 63% black and 51% male; 22% were diabetic. Mean age was 59±15 years and mean time on hemodialysis 117±86 months. Sex, age, and income had no significant impact on survival. Race, diabetes mellitus, the amount of hemodialysis delivered (Kt/V), and protein intake (PCR) were found to be important predictors of survival. Relative risks were as shown below:

Variable	Relative Risk	P-Value
Diabetes mellitus	2.17	0.001
Race: black vs white	1.66	0.011
Kt/V:		
<.8	1.74	0.003
.8-1.2	1.00	-
>1.2	1.18	0.503
PCR (mg/kg/day):		
<.8	1.50	0.023
.8-1.2	1.00	-
>1.2	0.62	0.065

These results define a population at risk that may be targeted for prospective intervention. The important role of protein intake in a group of long term dialysis patients and the need for optimizing the hemodialysis prescription are emphasized.

A SURVEY OF IRON OVERLOAD IN HEMODIALYSIS PATIENTS(PTS). Enia G,Sicuso C,Postorino M,Maggiore Q,Marzolla O*,Cantafio S**. Renal Units of Reggio Calabria,Melito PS (*)and Lamezia T (**)(ITALY).

To identify which proportion of pts is affected from iron overload we determined,by double antibody RIA,serum ferritin(FE)in 159 unselected Pts of 3 dialysis units. Pts' charts were reviewed for blood transfusions and iron treatment in 1985-1987. FE levels lower than 15ng/ml and higher than 400ng/ml were considered to be suggestive of iron deficiency and of overload respectively. Iron overload was found in 27 (17%) Pts, 10 of whom had serum FE levels higher than 700ng/ml(range 725-1542). Pts with high FE had a more pronounced anemia and had received more units of blood than those with normal FE(Table). FE correlated well with units of blood received by Pts (r=0.42 p<0.001),and weakly with parenteral iron administered (r=0.22 p<0.02),but did not correlate with the total amount of oral iron (r=0.08).Carpal tunnel syndrome was more prevalent among pts with iron overload than in those with normal FE (33 vs 6.6 % p<0.01);age, duration of treatment , prevalence of parathyroidectomy and bilateral nephrectomy were similar.

FE ng/ml	<15	15-400	>400<700	>700
Pts n	24	108	17	10
Hb g/dl*	8.1	9.5	7.9	6.5
Blood units*	1.1	1.3	3.1	7.7

*Mean values

TOTAL SOLUTE EXTRACTION VERSUS CLEARANCE IN THE MEASUREMENT OF RAPID DIALYSIS EFFICIENCY.

Aldo Fabris, Mariano Feriani, Massimo Milan, Giuseppe La Greca and Claudio Ronco (Intr.by Paul Kimmel) Dept of Nephrology, St.Bortolo Hosp.Vicenza,Italy.

We compared the efficiency of standard HD (t= 240 mins,Qb=300 ml/min,Qd=500 ml/min) with that of rapid HD (t=150 mins, Qb=500 ml/min, Qd=700 ml/min). The study was carried out in eleven patients in two subsequent dialysis sessions utilizing the same dialyzers (Cuprammonium rayon 1.5 m^2 or Cellulose Acetate 1.7 m^2) and starting with identical predialytic blood chemistries and weight gain. Each session was carried out after a two days interdialytic period. In Rapid HD,as expected, the clearances(K) of BUN, Creatinine (Cr) and Phosphate (P)were significantly higher than in standard HD (KBUN=331 vs 225, KCr=286 vs 193 and KP=231 vs 176 ml/min). The Kt product did not differ significantly in the two techniques (49.6 Liters vs 54.0 Liters); No significant differences in the total BUN extraction (measured in the total amount of exhausted dialysate) were found. As regarding other solutes,, in spite of higher clearances, the total extractions in rapid dialysis resulted significantly lower (Cr=-16% p 0.05 and P=-21% p 0.05). This fact could be explained by the curves of solute concentration in the patient's systemic blood during dialysis. In conclusion, in the evaluation of the efficiency of rapid dialysis, K can be adequate only for small molecules, while for larger solutes other parameters such as extraction should be carefully considered.

CORRECTION OF ANEMIA WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo) ENHANCES THE QUALITY OF LIFE OF HEMODIALYSIS (HD) PATIENTS (PTS). RW Evans*, B Rader*, J Egrie*, JW Adamson*, JW Eschbach. Univ. of Washington, Seattle, WA, and for the Multicenter Epo Clinical Trial Group.

When the anemia of HD pts is corrected with rHuEpo, pts report increased well-being and activity. To quantitate this, a questionnaire similar to that used in the National Kidney Dialysis and Kidney Transplantation Study was completed by 130 pts at baseline (B), after a mean of 5.6 months (mo) (I) and after a mean of 9.7 mo (II) of rHuEpo treatment. The following was reported:

Parameter	B	I	II
1. Hematocrit	23.7	34.2*	33.9*
2. No complaints	25.9%	44.5%*	43.5%*
3. Very active	19.8%	37.3%*	35.5%*
4. Very energetic	25.9%	45.4%*	48.1%*
5. Low/no energy	46.2%	23.2%*	22.2%*
6. Energy level limitation score†	47	31.5*	17.7*

(*=p<0.01 compared to B. †=data obtained by Nottingham profile which assesses limitations on energy level. No limitations=0; completely limited=100. pts 3 mo after kidney transplant=20).

Conclusion: energy and activity levels increased significantly in patients receiving rHuEpo and there was improvement in overall health and life satisfaction.

HYPERLIPIDEMIA IN CHRONIC HEMODIALYSIS PATIENTS - LACK OF PREDICTIVE FACTORS SUGGESTS AN UNDERLYING PATIENT PREDISPOSITION Eli A. Friedman, Mariana S. Markell* and Clinton D. Brown* SUNY Health Science Center at Brooklyn, Dept. of Med., Bklyn, N.Y.

Hyperlipidemia, specifically increased triglyceride (Type IV hyperlipidemia) with or without increased total cholesterol (Types IIa or IIb), has been noted in chronic hemodialysis patients. It is unclear whether this reflects an underlying predisposition in the patient or whether it is related to some aspect of end-stage renal disease or the hemodialysis procedure itself (or an interaction of both).

101 chronic dialysis patients were screened for fasting hyperlipidemia. Factors which might have contributed to the development of hyperlipidemia were analyzed, including presence or absence of hypertension, length of time on dialysis, age, sex, weight, original renal disease, diabetes, cigarette smoking, diuretic or beta-blocker use, "uremia" as assessed by pre-dialysis BUN/creat., Hct., PO4, nutritional status as assessed by albumin and total protein, and uric acid. There were 75 patients with total cholesterol values less than 240 (range 85-230), while the remaining 26 were hypercholesterolemic (range 245-484). Significant differences between the two groups were noted for: triglyceride (TG, mean 384±378 vs. 194±125, p<0.05), LDL (mean 183±35 vs. 98±28.5, p<0.001), but not HDL, with TC/HDL ratio (value >4.5 a possible risk indicator of cardiovascular disease) of 4.4±1.4 for the low cholesterol group vs. 6.05±2.5, p<0.05 for the high cholesterol group. No other factor was significant.

59 patients had normal fasting TG (range 33-180) and 42 had TG>200 (range 220-1805). Significant differences between these groups included TC (189±49 vs. 234±74, p<0.001), HDL (higher for the LC group, 49±19 vs. 41±15, p<0.05), VLDL (26±8 vs 53±10, p<0.0001) and TC/HDL ratio with the low triglyceride groupmean of 4.22±1.5 and the high triglyceride group-mean 5.78±2.02, p<0.0001. No other factor achieved significance in this analysis. These data suggest that hyperlipidemia is a substantive problem in chronic hemodialysis patients and probably reflects a genetic predisposition in the specific patient rather than a global effect of end-stage renal disease and its management.

EFFECT OF PERITONEAL DIALYSIS SOLUTIONS ON THE ACTIVITY OF ANTIBIOTIC AND DISINFECTING AGENTS AGAINST STAPHYLOCOCCUS EPIDERMIDIS BIOFILMS.
R.F. Gagnon, G. Obst, J. Prentis and G.K. Richards (intr. by P. Somerville). Montreal General Hospital, Montreal, Canada.

We examined the modifying effect of fresh and spent peritoneal dialysis solutions (1.5% dextrose) upon the activity of a number of antimicrobial agents against standardized S. epidermidis biofilms. Antimicrobial effect was judged by assessing residual metabolic activity of the exposed bacterial biofilms. Rifampin (an antibiotic) and RenNew-P (a chemical disinfectant, Alcide Corp.), demonstrated superior activity in their class of antimicrobial agents. Results are expressed as the minimal exposure time required by the antimicrobials to effect killing of the biofilms, antibiotics at a concentration of 10 µg/ml and RenNew-P at 1/2 dilution:

		Dialysis solutions	
	Control	Fresh	Spent
Rifampin:	10 hr*	6 hr	>24 hr
Vancomycin:	>24 hr	>24 hr	>24 hr
RenNew-P:	1 min	1 min	>24 hr

*Occasional resisters

These results illustrate the divergent influence of peritoneal dialysis solutions on the activity of antimicrobials against S. epidermidis biofilms.

INFLUENCE OF $KT/_V$ ON HEMODIALYSIS MORBIDITY (DM).
Nabeel Ghabra* and Rasib Raja. Albert Einstein Medical Center, Kraftsow Division of Nephrology, Philadelphia, Pennsylvania.

Analysis of National Cooperative Dialysis Study (NCDS) suggested that DM was related to $Kt/_V$. It was proposed that $Kt/_V$ of <0.8 delivers inadequate therapy, $0.8 < Kt/_V < 1.0$ represented transition from high to low DM and >1.0 ensured adequate therapy. Although direct evidence of urea as a toxin is lacking, $Kt/_V$ is commonly used as guide to dialysis therapy. With the recent advent of high efficiency dialysis, following $Kt/_V$ has been advocated to be mandatory. We studied 26 pts prospectively with urea kinetics and $Kt/_V$ determination every 6-12 months. Each pt was given 3 hrs thrice wkly therapy and T_D was changed only if clinically indicated. $Kt/_V$ ranged from 0.7-1.69. Pts were divided into those requiring dialysis related hospitalization (A) and those never hospitalized (B). The results are:

	PTS	$Kt/_V$	FOLLOW-UP YRS/PT	BUN mg/dl
A	9	1.11±0.3	4.37±0.4	79.2±6
B	17	1.05±0.2	3.30±0.2	72.6±7

$Kt/_V$ did not correlate with hospitalizations, Hct, blood transfusions, HCO_3^-, Ca++ and phosphorus ($P > 0.05$). These data suggest that $Kt/_V$ may not be good indicator for adequacy of hemodialysis in individual pts. Pts both with high (> 1.2) and low (< 0.8) measured $Kt/_V$ may have similar morbidity. Non-dialytic factors as psychosocial, age, BP may play an important role in DM. A search for a better index for dialysis adequacy should be continued.

COLON CANCER SCREENING IN DIALYSIS PATIENTS.
V.C. Gandhi, M. Ajam*, L. Ramanujam*, D.J. Leehey, T.S. Ing, T. Schnell*, J.T. Daugirdas. Hines-Loyola Medical Center, Hines, IL.

The benefits of colon cancer screening using stool guaiac testing have been established in large control populations, but not in dialysis patients. In seventy-two asymptomatic dialysis patients (51 treated with hemodialysis [HD], and 21 with peritoneal dialysis [PD]) who underwent outpatient stool guaiac testing, the test was positive in 11 (16%) patients (8 HD and 3 PD). Eight of the 11 were investigated further by colonoscopy; when deemed necessary by the treating physician, esophagogastro-duodenoscopy and/or barium enema were also performed.

A site of active bleeding was identified in 3 of the 8 patients (hemorrhoids, telangiectasia, ulcerative colitis). In each of the 5 other patients, potentially bleeding lesions were identified: colonic polyps (two malignant and two benign) in 4 patients, Barrett's esophagus in 1, diverticulosis in 2, and colonic vascular deformities in 2.

In a large outpatient control group undergoing the same screening protocol, we have to-date evaluated 5,500 patients, of which 250 (4%) had positive tests. Thus, although the baseline incidence of positive guaiac tests may be higher in dialysis patients than in non-uremic controls, our results suggest that stool guaiac testing of dialysis patients may not only be useful in detecting colonic polyps, but may also identify other previously unsuspected causes of gastrointestinal bleeding.

IMPROVED QUALITY OF LIFE WHILE RECEIVING RECOMBINANT ERYTHROPOIETIN (r-HuEPO).
S.D. Gibilaro*, B.G. Delano, R.Quinn*, A.P. Lundin, R. Galonsky* and E. A. Friedman. SUNY Health Science Center, Brooklyn. Bklyn, N.Y.

Thirty seven patients, (13 men, 24 women) age ranges 24-80, undergoing maintenance hemodialysis for chronic uremia and receiving r-HuEPO for the concomitant anemia had their quality of life assessed by questioning the patients and their assigned nurse. A modified Karnofsky score was calculated pre and post study drug. Of the 51 patients started on r-HuEPO, 37 were evaluated for this study, 5 received a kidney transplant, 4 were excluded for non-compliance, 4 died, and 1 patient had a worsening of a pre existing myeloproliferative disorder. The mean hematocrit went from 19.8 vols% (range 14-26) to 31.5 vols% (range 19-42). The Karnofsky score (mean 76 pre, 86.6 post) improved in 29, remained the same in 6 and decreased in 2 of the patients. Potency returned in 62%. Ninety five percent avoided any transfusions during the study period. Six patients (16%) returned to work. Appetite improved in 81%. Eighty four percent had an increased sense of well being. In 78% exercise tolerance improved subjectively, and was objectively better in all 11 patients in whom it was tested. Seventy percent participated in more social activities. Sleep improved in 68%, hair texture improved in 30%, and skin color was better in 51% of the patients. Negative effects included iron deficiency (32%), clotted vascular access (11%), hypertension and hyperkalemia (16% each), volume overload (3%), and body aches (8%). We conclude that for most patients treatment with r-HuEPO improves the quality of life without substantial negative effects.

SURVIVAL OF IVDA-HIV POSITIVE AND NEGATIVE PATIENTS ON DIALYSIS. J. Jorge Gordinho,* Mimi Weaver,* Betty P. Whaley,* and Norman Lasker. UMDNJ, Div. of Nephrology, Dept. of Medicine, Newark, NJ.

The dialysis unit at University Hospital - UMDNJ - is situated in Newark, NJ, a high IVDA area. In view of the reports of low survival rates on dialysis of IVDA and AIDS patients, we have analyzed our survival data for 48 patients who were cared for in our dialysis unit from Jan. 1, 1986 through July 31, 1988. Results are summarized as follows:

		#	Survival Mean Mos.	Death
IVDA	HIV +	22	14	8
	HIV -	9	21	2
	Not Tested (Clinical AIDS)	4	27	4
	Not Tested (No Clinical AIDS)	8	45	0
Non IVDA	HIV +	5	24	5

Four of the non-IVDA patients contacted AIDS by sexual means. One patient was on dialysis for a total of 80 months. He was infected by a blood transfusion prior to 1986. Another patient with clinical AIDS survived for 20 months on dialysis.

IVDA patients who refused to be HIV tested showed better levels of rehabilitation and shared needles less often than the tested group.

HIV positive patients may have a prolonged survival on dialysis. Many of them achieve acceptable levels of rehabilitation. IVDA patients who are HIV positive have a shorter survival than the HIV negative group.

Hemodialysis improves Carbohydrate Metabolism in Polymorphonuclear Neutrophils (PMN) Marianne Haag-Weber, Michael Hable, Peter Schollmeyer, and Walter H. Hörl:(intr. by Thomas Lüscher) Univ. of Freiburg, Dept. of Medicine, Freiburg, FRG

Abnormalities of carbohydrate metabolism have been reported, e.g. increased plasma glucose levels, insulin resistance, decreased glucose uptake of PMNs after stimulation with PMA.
Therefore, we investigated the carbohydrate metabolism before and 3h after HD. PMNs were isolated from EDTA-blood according to Bass et al (Proc Natl Acad Sci,1977). Glycogen (G) in PMNs was determined according to Solling et al (Anal Biochem,1975), glycogensynthetase (GS) according to Thomas et al (Anal Biochem,1968). Hexose uptake of PMNs was measured without and with stimulation with FMLP according to McCall et al (Biochem Biophys Res Comm 1985).
There was a significant increase of glycogen of PMN during HD from 10.2 ± 1.4 to 14.8 ± 1.3 $\mu g/10^{-6}$ cells. There was also a significant increase of GS from 7.1 ± 1.1 before HD to 8.8 ± 0.4 $mU/10^{-6}$ cells after 3h of HD. After 3h of HD we found the same values for G and GS as by healthy controls. There was only a 3.1 ± 0.9 % increase of hexose uptake by PMNs after stimulation with FMLP before HD, whereas the increase was 10.0 ± 1.5 % after HD. In healthy controls we found an increase of 25.4 ± 3.8 %.

These data show, that glycogen, activity of GS and hexose uptake by PMNs after stimulation improves significantly during HD. It seems that low molecular weight factors are removed, which are responsible for these effects.

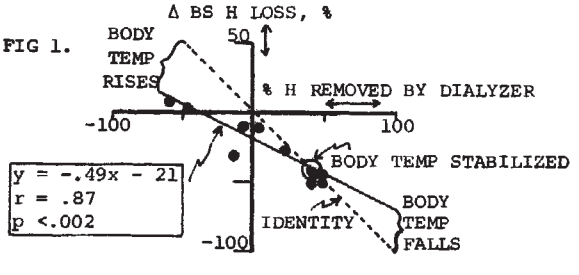
HEAT KINETICS IN HEMODIALYSIS (HD)
Frank Gotch, Davies Medical Center, San Francisco, CA.

Lowered dialysate temp (Td,°C) is known to improve vascular stability (VS) in HD; body temp (T) ↑'s in HD when T=Td but body heat production (H, Kcal/min) has been shown constant in chronic HD. A kinetic model of heat balance was formulated for analysis of these phenomena:

$W(dT/dt) = H - Ks(T-Te) - Kd(T-Td)$

where W=body wt., Kg, with heat capacity 1 Kcal/Kg/°C;Ks & Kd are body surface (BS) and dialyzer heat transfer coefficients, Kg/min; Te is environmental temp. In vitro studies showed Kd=QB, QB=.1 to .4 L/min, with both .9 & 1.8 m^2 dialyzers. The model was solved for Ks and used to analyze 10 reported studies with H estimated = .02(W). The results in Fig 1 indicate: 1. When T=Td, Ks ↓'s 21% resulting in ↑ T; 2. ↓'d Ks likely reflects ↓'d BS blood flow (SBF) compensating ↓ blood volume due to Qf & other factors; 3. when T ↑'s, a stimulus for ↑ SBF results in ↓ VS; 4. to prevent ↑ T & ↓ VS, ~40% of H must be removed by the dialyzer. It is concluded that kinetic modeling of heat balance should be useful to guide clinical dosing of heat flux with thermal control systems in HD.

FIG 1.



PHAGOCYTIC FUNCTION OF NEUTROPHILS (PMN) IN DIALYSIS (HD) PATIENTS: INFLUENCE OF RECURRENT COMPLEMENT (C) ACTIVATION BY DIALYSIS MEMBRANES. Raymond M. Hakim, W. Stone, W. Green and A. Gung. Vanderbilt University Medical Center, Division of Nephrology and Pathology, Nashville, Tennessee.

Bacterial infection remains a major cause of morbidity and mortality in HD patients and may increase in prevalence with years on HD. PMN phagocytic activity plays a major role in host defense mechanism. C activation by Cuprophane membranes leads to activation of PMN and subsequent release of granulocytic enzymes as well as production of toxic reactive oxygen species.

We examined intradialytic (pre, 1 hr., 3 hrs.) and interdialytic PMN phagocytic function in 8 chronic dialysis patients, during 2 weeks of HD using C activating cuprophane membranes and 2 weeks of non-complement activating polymethylmethacrylate (PMMA) membranes. The study was prospective, cross-over, randomized. Phagocytic function was determined by flow cytometry, comparing the DCF-DA fluorescence of isolated PMN in response to Staphylococcus aureus (woods) at ratios of 10:1 and 20:1 (bacteria to PMN).

The phagocytic capacity of PMN decreased during dialysis with C activating membranes. At 3 hours following initiation of dialysis it was than 72% of its predialysis capacity ($P<0.01$). In contrast, during dialysis with PMMA membranes, phagocytic function at 3 hours was 194% of predialysis value ($P<0.01$). After 2 weeks of dialysis with Cuprophane membranes, predialysis phagocytic function was also significantly reduced (181 fluoresence units at the beginning of 2 weeks, 123 at the end of 2 weeks ($P<0.01$)). In contrast, using PMMA membranes, predialysis phagocytic function improved after 2 weeks (138 fluoresence units at the beginning of 2 weeks, 338 at the end of 2 weeks ($P<0.001$)).

We conclude that recurrent C and neutrophil activation may result in a decrease of phagocytic function of PMN and may be a factor in the pathogenesis of bacterial infection in HD patients.

ENDOTOXIN TRANSPORT ACROSS HIGH FLUX CAPILLARY FIBER DIALYSIS MEMBRANES - AN IN VITRO STUDY

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A potential complication of high flux dialysis is an increased incidence of pyrogenic reactions resulting from backfiltration of contaminated dialysate. The purpose of this study was to determine a) the sieving coefficient of endotoxin (ET) for polysulfone, polyacrylonitrile and cellulose triacetate high flux CF membranes and b) the potential for backfiltration of non-ET, bacterial cell derived, IL-1 inducing substances. ET was prepared by sterile filtration of an 8 day suspension of P. aeruginosa ATCC 17648 grown in bicarbonate based dialysate. This solution, containing 804 ± 180 EU/ml endotoxin, was recirculated through the dialysate path of miniaturized CF dialyzers (100 cm^2 surface area) at 50 ml/min. Membranes were pretreated with pooled normal human serum to minimize nonspecific membrane binding. Filtrate collected from the blood path after 15, 30,and 60 min. was assayed for ET by a chromogenic Limulus amebocyte lysate method (Baxter). Sixty minute filtrates were also coincubated with human buffy coat cells for 24 hr., followed by freeze thawing and measurement of IL-1 in supernatent by [^{3}H] thymidine incorporation into C3H/HeJ thymocytes in the presence of sub-optimal doses of PHA. ET was not detected in any filtrate, except for positive controls, revealing a sieving coefficient of <0.0002 for all membrane types. Furthermore, IL-1 inducing substances were not detected in any filtrate. These data indicate that backfiltration of environmental endotoxin in high efficiency dialysis is improbable.

THE EFFECTS OF COCURRENT AND COUNTERCURRENT DIALYSIS ON BACKFILTRATION AND IN VIVO CLEARANCES USING HIGHLY PERMEABLE MEMBRANES.

S. Hyver* and J. Petersen, Palo Alto VAMC and Stanford Univ., Stanford, CA.

Backfiltration (BF) may occur during hemodialysis using highly permeable membranes, with the potential for unsterile dialysate fluid to enter the blood compartment. We have measured pressures determining BF across a large surface area polysulfone hollow fiber dialyzer under varying conditions of ultrafiltration (0.8 - 2.25 L/h) and co or countercurrent dialysate flow rates. BF occurs during countercurrent hemodialysis when the venous pressure is less than the dialysate compartment inlet pressure + the plasma oncotic pressure. This occurs with ultrafiltration rates less than 1.2 L/h. BF occurs during cocurrent hemodialysis when venous pressure is less than the dialysate compartment outlet pressure + the plasma oncotic pressure. This does not occur at any ultrafiltration rate. However, urea clearances were significantly reduced from countercurrent values. Increasing cocurrent dialysate flows using a dialysate recirculation system (flows 800 - 1500 ml/min) did not return these clearances to countercurrent levels.

These studies indicate that BF may occur during dialysis with large surface area, highly permeable membranes. In situations where the sterility of the dialysate cannot be assured, BF can be abolished by cocurrent dialysis. As solute clearances are compromised in these circumstances, an in-line bacterial filter may be a preferable alternative.

BICARBONATE BUFFERED SUBSTITUTION SOLUTION FOR CONTINUOUS ARTERIO-VENOUS HEMOFILTRATION (CAVH): IN VITRO AND IN VIVO EVALUATION.

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In a substantial number of patients with acute renal failure (ARF) CAVH with lactate buffered substitution solution is contraindicated due to lactic acidosis. Hence, a bicarbonate buffered solution was evaluated in vitro and in vivo.In plastic bags 4.5 L of the solution A containing (in mmol/L): Na+109, Ca++ 1.78, Mg++0.52, Cl-114.2, lactic acid 3, glucose 5, and pH 3.4, were mixed with 160 ml of 1 M Na-bicarbonate. pH, pCO2, HCO3, Na+, and Ca++, were measured 1,4, and 24hr after mixing.Values are mean±SD.N=8 bags

Time	pH	PCO2	HCO3	Na+	Ca++
		mmHg		mmol/L	
1 hr	7.32±0.01	64±3	31±1	141±5	1.72±0.02
4 hr	7.29±0.03	59±1*	30±1	139±1	1.73±0.01
24 hr	7.33±0.05	55±3*	30±1	140±1	1.72±0.02

* indicates $p < 0.05$ vs 1hr. After mixing pH and HCO3- remained constant; pCO2 decreased only slightly. In comparison to solution A Ca++ decreased not more than expected by dilution. This excludes precipitation of calcium-carbonat. In vivo, solution A was mixed with 160 ml 1M Na-bicarbonate immediately prior to use and was given to 7 patients with ARF treated by CAVH. The substitution rate was 19±6 L per 24 hr. The substitution time was 6 days per patient.The serum Ca++ (2.15±0.13 mmol/l), Mg++ (0.93±0.25), HCO3- (23±2.5 mmol/l), pH (7.36±0.07),and lactate (3.0±1.4 mmol/l) did not change significantly. The bicarbonate buffered solution was stable,safe to administer,and well tolerated. CAVH is no longer contraindicated in lactic acidosis.

INTRAOPERATIVE (IHD) VS ROUTINE (RHD) HEMODIALYSIS IN ESRD PATIENTS UNDERGOING OPEN HEART SURGERY.

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Hyperkalemia, metabolic acidosis and volume overload are common in ESRD patients following open heart surgery, and many require early post-operative HD to correct these hemodynamic and metabolic derangements. We compared IHD vs. RHD to determine whether early post operative HD could be delayed in these hemodynamically unstable patients. Of 13 chronic hemodialysis ESRD patients undergoing open heart surgery, 7 received IHD using a high flux artificial kidney (1.7m^2 surface area) during cardiopulmonary bypass (average time 84 min.) and 6 received RHD. Values are mean ± SEM:

	IHD n = 7		RHD n = 6	
	Pre-op	Post-op	Pre-op	Post-op
[K^+]	4.76±0.15	4.36±0.25*	4.78±0.19	5.43±0.23*
[HCO_3^-]	22.7±1.1	21.2±1.2	23.7±1.5**	19.5±0.8**

* p=.01 ** p=.04, two-tailed T test

Post-operatively, 0/7 IHD patients vs. 4/6 RHD required parenteral bicarbonate therapy (chi square, p<.01), 2/7 IHD vs. 2/6 RHD received Kayexalate (NS) and 0/7 IHD vs. 2/6 RHD received glucose/insulin for hyperkalemia (NS). 2/7 IHD vs. 2/6 RHD were judged clinically to be in CHF post operatively (NS). RHD patients required hemodialysis 1.02±0.36 days before surgery and 1 day after surgery, whereas IHD patients were hemodialyzed 2 days before surgery (p=.009) and 2 days after surgery (p=.009).

IHD lessened post-operative hyperkalemia and metabolic acidosis and delayed post-operative hemodialysis by an additional day.

COMPARISON OF HEMODYNAMIC EFFECTS OF BLOOD TRANSFUSION AND ERYTHROPOIETIN IN CHRONIC HEMODIALYSIS PATIENTS.

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To elucidate the effects of increase in red cell mass on hemodynamics, we have used blood transfusion or recombinant erythropoietin (EPOCH, CHUGAI). In six chronic hemodialysis(HD) patients (4 f, 2 m), 1-2.4 L(mean 1.4) of blood was transfused to obtain 10% increase in hematocrit (Hct),18.6 to 29.6%, over 6 weeks(W). Six to 8 W after the blood transfusion(Bl-t) study, when Hct decreased to the pre-treatment level, EPOCH(1500-4500 units, 3X/W) was given to the same patients over 6 W to obtain the similar increase in Hct(18.0 to 30.3%). Cardiac output was measured by dye dilution method, indocyanine green, cuvette. Total peripheral resistance index(TPRI) was calculated as mean blood pressure (MBP)/CI. Blood volume(BV) was measured by I-131,RISA. Data are expressed as mean±SEM.[B;before, A;after]

	MBP(mmHg)	CI(L/min/m^2)	TPRI(U)	BV(L)
B	Bl-t 87.5±5.8	4.04±0.28	22.6±2.8	3.95±0.24
A	Bl-t 95.9±6.2	3.22±0.25	31.5±4.6	3.92±0.18
B	EPO 86.8±6.4	3.94±0.36	23.8±3.7	3.93±0.19
A	EPO 87.0±5.4	3.51±0.11	24.8±1.4	3.99±0.24

Comparing to significant($p<0.01$) changes in hemodynamics(MBP,CI,TPRI) in blood transfusion, EPO caused no hemodynamic responses despite similar increase in Hct. These results may suggest either the existence of factor(s) other than increase in red cell mass or the difference in the autoregulatory response in EPO treatment.

THE EFFECT OF HEMODIALYSIS USING CALCIUM FREE DIALYSATE ON CRITICAL HYPERCALCEMIA INDUCED BY MALIGNANT TUMORS.

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The effect of hemodialysis using Ca free dialysate (Ca free HD) on reducing serum calcium levels was evaluated,as treatment of critical hypercalcemia induced by malignant tumors. 7 patients were studied:4;adult T cell leukemia(ATL), 1;multiple myeloma,1;primary parathyroid tumor,1;uterus cancer. The constitution of Ca free dialysate was:Na;135,K;2.5, Mg;1.5,Cl;103,HCO_3 30mEq/l. Ca free HD for 3 hours reduced serum Ca levels significantly from 15.1±2.5 to 11.6± 1.3mg/dl. The reduction rate was the highest at the first one hour. Hypercalcemic symptoms disappeared markedly. 46.2±2.6mEq of Ca was dialyzed. Serum Ca levels in 3 ATL,however,did not decrease down to normal range in spite of prolonged time of HD. The amount of Ca dialyzed,however,kept increasing during therapy. The serum Ca levels in all patients returned to the former levels after 24 hours. These results suggest that(1)Ca free HD is the most effective therapy for critical and urgent hypercalcemic patients.(2)As this effect is temporary,however,radical therapy against original diseases is necessary. (3)A new feedback mechanism to maintain hypercalcemia in ATL will be considered.

CRITICAL VALUES OF HEMATOCRIT IN EXTRACORPOREAL BLOOD FILTRATION.

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In order to understand the nature of high hct blood purification, we performed a theoretical analysis of extracorporeal blood filtration. This has revealed the existence of critical values of venous return hct. Above the first critical value, the pressure on the blood side of the filter increases. Above the second critical value of the venous hct, no steady state is possible. In pumped systems the pressure on the blood side of the filter rises to very high levels. In CAVH systems these pressures rise and UF and Q_B drop down to zero. We have also studied this phenomenon by perfusing in-vitro 0.7 M^2 polysulfone hemofilters and tubing with bovine blood using a roller pump. Hct was 26, and Q_B was 125 ml/min. As the hct increased above 36 the pressure on the blood side of the filter began to increase above the no-filtration value. When the venous hct reached 55, pre and post-filter pressures abruptly rose to levels requiring the run to be terminated (400-1100 mmHg). The results were repeatable with the same and other hemofilters. No clotting occurred. The theoretical analysis predicted that the pressure excursion would occur at a hct of 53 to 55. The experimental results are in good agreement with the theoretical analysis for the pumped case. We have observed the same phenomenon with in-vitro and in-vivo CAVH systems as the analysis predicts. Critical hct values depend on the shape of the hct vs. viscosity curve and system design parameters. This work shows conditions which could lead to failure of the extracorporeal system.

ADSORPTION OF ANAPHYLATOXINS C3a AND C5a ON DIALYZER'S POLYVINYLCHLORIDE BLOOD LINE - IN VIVO STUDY.

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In the studies of complement activation during hemodialysis, blood sampling points from dialyzer's blood lines have usually not been precisely defined. In this prospective study, adsorption of C3a and C5a on dialyzer's polyvinyl-chloride blood line in 8 patients during regular hemodialysis was investigated. Blood samples were taken simultaneously at two points on the output blood line of cuprophan dialyzer 340 cm apart. Blood flow was 200 ml/min throughout the study. C3a and C5a plasma concentrations were determined by radioimmunoassay. The most relevant results are presented in the table ($\bar{X}$±SD).

	min HD	proximal pt.	distal pt.	p
C3a ng/ml	15th	9416±3501	6775±2406	< 0.02
	60th	4089±1014	3656± 356	NS
	240th	2490±1120	2043± 406	NS
C5a ng/ml	15th	63.1±39.8	59.7±37.5	NS
	60th	32.7± 3.6	26.1± 5.2	< 0.01
	240th	14.3± 5.3	15.0± 3.6	NS

Results suggest that significant adsorption of C3a and C5a on polyvinylchloride blood line occurred in 15th and 60th min of hemodialysis, respectively. This adsorption should be taken into account in assessing dialyzer's membrane generation and adsorption of both anaphylatoxins.

A COMPARATIVE STUDY OF HEMODIALYSIS (HD) AND HEMODIAFILTRATION (HDF). P. Keshaviah*, A. Collins, G. Hanson*, J. Ebben * and R. Berkseth. Regional Kidney Disease Prgm, Hennepin Co. Med. Cntr. Minneapolis.

Improved cardio-vascular stability and solute removal efficiency are advantages claimed for HDF. A crossover study was undertaken in 11 chronic dialysis patients : 3 mo HD(C1), 4 mo. HDF (E) & 3 mo. HD (C2). Cellulose Acetate (CA) membranes were used for HD and 2mo. of HDF (E1). Polysulphone and polyacrylonitrile membranes were used during the 2 other mo. of HDF (E2). Treatment durations were 2.5 hr. Bicarbonate dialysate was used for both HD & HDF. Substitution fluid for HDF (7.5 L / run) was prepared on-line by ultrafiltering dialysate using a modified SPS-450 machine.

PARAMETER	C1	E1	E2	C2
Sym.Hypotension(%)	9	9	10	7
Pre MAP (mmHg)	96	95	94	96
Post MAP (,,)	83	83	83	83
23% Saline (ml/run)	14	12	12	13
Wt. Loss (kg)	2.2	2.2	2.4	2.4
BUN Removal (%)	71	71	67	69

The data indicate no significant differences between HD & HDF with respect to blood pressure stability and hypotension The incidence of other intra-therapy complications (nausea, vomiting & cramps) was similar in HD & HDF. Efficiency of small solute removal was similar in HD & HDF with the CA membrane but lower in HDF with the synthetic membranes.

We were unable to document any advantages of HDF over HD, or of synthetic membranes over the CA membrane with respect to cardiovascular stability and small solute removal. From a technical standpoint, HD was simpler than HDF.

PROLONGED CONTINUOUS ARTERIOVENOUS HAEMODIALYSIS IN CRITICALLY ILL PATIENTS. J.C. Kingswood, S.L. Cohen, S. Machin, R. Miller. Intr. by A. Watson. University College Hospital and Middlesex Hospital, London.

CAVHD is a recognised treatment for acute anuric renal failure (ARF) in critically ill patients in Intensive Care Units (ITU). We describe two patients in whom anuric ARF was treated by CAVHD for respectively 42 and 84 days.

A 56 year old man underwent choledochoduodenostomy for ascending cholangitis with pancreatitis. Postoperative complications included septicaemia, intra-abdominal bleeding, Pneumona, adult respiratory distress syndrome (ARDS). He was treated with 42 days CAVHD, mechanical ventilation, parenteral nutrition and has since recovered.

A 55 year old man became speticaemic and developed ARDS following a perforated gastric ulcer complicated by repeated haemorrhage and ARF. He underwent 7 further surgical procdures and was treated with CAVHD for 84 days together with assisted ventilation, parenteral nutrition and antibiotics. Bleeding persisted, he developed brain stem signs and died on the 84th day.

In both cases CAVHD was performed via Scribner shunts using a Hospal 0.43 m^2 polyacrylonitrate parallel plate haemodialyser (HOSPAL AN69S)Hospal UK, heparinised by a continuous infusion of 800 units/hour to the dialyser. Mean P. Urea was 21 & 31 mmol/l, and mean P. Creatinine 257 & 190 µmol/l in the two patients respectively during CAVHD. Creatinine clearances up to 32ml/min were achieved across the dialyser. In the second case thrombocytopenia $59x10^9$/L was noted in the last day of life but heparin induced antibodies were not found. Their postop ARF was controlled by CAVHD.

COMPARISON OF CALCULATED CHANGES IN PLASMA VOLUME DURING ROUTINE HEMODIALYSIS USING MEASUREMENT OF HEMATOCRIT, PROTEIN, AND COLLOID OSMOTIC PRESSURE. Barry Kirschbaum, M.D., Medical College of Virginia, Dept. of Medicine, Richmond, Virginia.

A decrease in blood volume is expected during routine dialysis since most patients gain fluid weight during the interdialytic period and require ultrafiltration to reach their target weight. Large declines in plasma volume may account for some of the hemodynamic instability that characterizes hemodialysis. A simple measure that accurately permits estimation of the shrinkage of plasma volume may provide useful management information. We compared changes in plasma volume (Δ PV) based on pre and post dialysis hematocrits (HCT), total protein concentrations (TP) measured by refractometry, colloid osmotic pressure (COP) determined by osmometry, concentrations of albumin and IgG determined by bromcresol green and DAKO immunoturbidimetric assay respectively. During 22 studies, patients lost an average of 3.07 $\pm$ 1.11 kg (range 0.7 to 5.8 kg). Changes in plasma volume were -9.4, -9.4, and -10.7 percent based on HCT, TP, and COP respectively which were not significantly different from each other and demonstrated a high level of correlation. Nonetheless, in some individuals, a large degree on non-identity among the three values was observed. We could not show any gain or loss of protein from the vascular compartment as a consistent feature. ΔPV did not correlate with the change in body weight expressed as percent of total body weight or total body water which was measured by total body impedance analysis.

DETERMINATION OF 17-HYDROXYCORTICOSTEROIDS IN DIALYSATE SOLUTION AS A MEASURE OF ADRENOCORTICAL FUNCTION IN HEMODIALYSIS PATIENTS. Yukimasa Kohda,* Ryoji Hiramatsu,* Kuniharu Kuwahara,* Tatsuo Sato* (intr.by James E Balow). Kumamoto Univ. Third Dept. of Int. Med., Kumamoto, Japan.

Measurement of urinary 17-hydroxycorticosteroids (17-OHCS) is used to evaluate adrenocortical function. However, this approach is not applicable to patients with chronic renal failure since they usually show anuria. In the present study, the dialysate content of 17-OHCS in patients with chronic renal failure receiving hemodialysis was studied as a potential measure of adrenocortical function. The concentration of 17-OHCS in dialysate was determined by a spectrophotometric method after hydrolysis with β-glucuronidase. The total content of dialysate 17-OHCS was calculated by multiplying dialysate 17-OHCS concentration by the volume of dialysate solution (150 L). Dialysate 17-OHCS in the basal control state after a two day-interval of hemodialysis in 11 patients was 5.8 $\pm$ 0.5 mg (Mean $\pm$ SE). Dialysate 17-OHCS increased in 4 patients after iv injection of 40 mg cortisol by 6.9 $\pm$ 0.9 mg. Overnight 1 mg dexamethasone (Dex) suppressed plasma cortisol in 5 of 7 patients. The dialysate 17-OHCS in the Dex-non-suppressible patients were 7.1 and 5.2 mg, which were not statistically significantly greater than the mean of the dialysate 17-OHCS in the Dex-suppressible patients (5.5 $\pm$ 1.2 mg). Dialysate 17-OHCS was suppressed by a standard Dex suppression test in a patient non-suppressible after the overnight 1 mg Dex. These results suggest that determination of dialysate 17-OHCS content is a useful technique for evaluation of the adrenocortical function in hemodialysis patients.

SLEEP DISORDER IN HEMODIALYSIS PATIENTS: THE EFFICACY AND EFFECTS OF TRIAZOLAM. Stuart J. Kolner*, Richard G. Christiansen. Univ. Illinois Coll. Med. at Rockford, Rockford, Illinois.

Sleep disorder is a common complaint among patients on hemodialysis, but its prevalence has not been reported in the literature. Our research in an in-center hemodialysis population of 87 patients found the prevalence of major sleep disorder to be 52.9%, significantly higher than in the general population.

Triazolam is the hypnotic used most frequently in our patient population because of its short half-life and our concern regarding accumulation. Our dialysis staff has observed over several years that most patients using a hypnotic drug are not helped significantly, and many appear to be adversely affected neurologically. To test this hypothesis, hemodialysis patients with sleep disorder who were not already taking a hypnotic were selected for a double-blind, placebo-controlled trial of triazolam. Patients using triazolam (n=6) nightly for seven days did not find significant improvement in their sleep when compared to the placebo group (n=6); however, they did not report an increased frequency of adverse effects over the placebo. The triazolam group performed as well as the control group in pre- and post-trial objective neurological assessments. Serum triazolam assays showed no significant accumulation of the drug.

We conclude that 1) the prevalence of sleep disorder in hemodialysis patients is significantly greater than that in the general population; and 2) although our data do not support the efficacy of triazolam in hemodialysis patients, there is no evidence of adverse effects or accumulation of the drug in the dialysis patient's serum.

EFFECT OF PLASMA ALUMINUM (Al) ON PARATHYROID HORMONE (PTH) SECRETION. Mark S. Kramer, Stephen J. Goldstein, Solomon Epstein,* Maria Mendez, Rasib M. Raja, and Sidney Kobrin.* Albert Einstein Medical Center, Kraftsow Division of Nephrology, Philadelphia, Pennsylvania.

Al toxicity is a syndrome frequently recognized in chronic renal failure patients (pts). The pathogenesis of diminished PTH secretion, one of the manifestations of this syndrome, is unclear. Suggested mechanisms include Al infiltration of parathyroid glands or negative feedback suppression by elevated plasma Al levels. We studied the effect of varying plasma Al levels on PTH secretion. Five maintenance hemodialysis pts and 2 chronic ambulatory peritoneal dialysis pts underwent standard desferoxamine (D) stimulation tests. Blood samples for measurement of ionized Ca++, Mg++, middle molecule component of PTH, Bone Gla Protein (BGP), and plasma Al were collected immediately before and 48 hours after D administration.

Results (Mean ± Standard Error of the Mean)

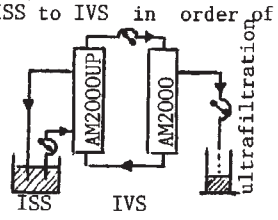
Time	Al MCG/L	PTH ng/ml	BGP ng/ml	Ca++ mg/dl	Mg++ mg/dl
Pre	72±20*	14.5±6.4	163±43	4.4±0.1	2.1±0.1
Post	224±78*	14.0±60	149±60	4.3±0.1	2.0±0.1
Δ	152±74	-0.5±2.1	-8±37	-0.1±0.1	-0.1±0.1

* $P < 0.04$ Δ = change

Plasma Al increased significantly after D while PTH, BGP, Ca++ and Mg++ were not significantly changed. These data suggest that an increase in plasma Al does not play a role in the disordered parathyroid gland function present in pts with Al toxicity. The pathogenesis of suppressed PTH secretion may be related to Al infiltration of parathyroid glands.

INTERCOMPARTMENTAL SHIFT OF BETA-2-MICROGLOBULIN (B2M) DURING HEMODIALYSIS(HD). K.Kumano,* M.Nanbu* K.Yoshida,* T.Sakai* (intr. by Dr.Y.Tsukamoto) Kitasato Univ., Kidney Ctr., Sagamihara, Japan

According to our previous studies, an rise in plasma B2M after HD could not be explained by hemoconcentration or by enhanced synthesis of B2M or by enhanced release from circulating blood cells. This study was designed to clarify the mechanism for this rise of B2M during HD. Post HD plasma levels of B2M, myoglobin and alpha-1-microglobulin were compared to predialysis levels, which were significantly increased by 7-16 % even if corrected for hemoconcentration. This may indicate that these rises in plasma levels are due to transitional shift of low molecular weight proteins(LMWPs) from interstitial space(ISS) to intravascular space(IVS). To prove this hypothesis, in vitro experiment was done usig CAPD drainage fluid as dialysate simulating interstitial fluid while CAPD drainage fluid with 5 % albumin passed through AM 2000UP, a cuprophane dialyzer capable of filtering B2M(S.C.: 0.4), simulated IV fluid(see figure). Fluid removal from IVS was achieved by isolated ultrafiltration usig AM 2000, a cuprophane dialyzer without sieving B2M, which also forced fluid shift from ISS to IVS. This fluid shift was accompanied by the movement of all all the three LMWPs from ISS to IVS in order of their sieving coefficients with concomitant rise of of their concentrations in IVS. The above observation confirms our hypothesis and this phenomenon must be considered in B2M kinetics during HD.



CHANGE OF HEMATOPOIETIC RESPONSE TO RECOMBINANT ERYTHROPOIETIN (r-HuEPO) IN HEMODIALYZED PATIENTS. S. Kurihara*, T. Akiba*, M. Kawabe*, H. Yoneshima*, and F. Marumo, Kasukabe Shuwa Hospital, Kasukabe, Tokyo Medical and Dental University, Tokyo, Japan.

The change of hemopoietic responses to r-HuEPO after several weeks of withdrawal of r-HuEPO has not been studied. We compared the hematocrit(Hct) increases in 1st and 2nd treatments(Tx) with 38-63 IU/kgBW r-HuEPO three times a week in four chronic hemodialysis patients(Pts). All Pts were discontinued 1st Tx of r-HuEPO when Hct elevated to a mean of 29.7% and resumed with the same dose of 1st Tx when the Hct fell down to the level before Tx.

Case	start Hct(%) 1st Tx	2nd Tx	Interval (wks)	Increase in Hct(%/week) 1st Tx	2nd Tx
1	17.0	17.0	21	0.5	0.3
2	15.9	16.8	32	1.7	1.2
3	19.8	20.0	29	1.4	0.8
4	21.1	20.5	13	1.6	0.9
mean	18.5	18.6	23.8	1.30	0.80
S.D.	(2.4)	(1.9)	(8.5)	(0.54)	(0.37)

In no patients, the antibodies against r-HuEPO were detected by RIA assay. Serum Fe, TIBC, ferritin, aluminium, creatinine and blood urea nitrogen showed no significant changes between 1st and 2nd Tx periods. In conclusion, hemopoietic responses to r-HuEPO were decreased in 2nd Tx comparing with that of 1st Tx in all four Pts. This decreased response may suggest the deterioration of sensitivity to r-HuEPO of bone marrow cells and/or shortning of of the half life of administered r-HuEPO.

RAPID VANCOMYCIN REMOVAL DURING HIGH FLUX HEMODIALYSIS NECESSITATES SUPPLEMENTATION TO MAINTAIN THERAPEUTIC LEVELS. D.M. Lanese,* P.S. Alfrey*, and B.A. Molitoris. VAMC, Denver, CO.

Since middle molecule clearance is increased with high flux dialysis (HFD) we questioned whether HFD increased vancomycin clearance (VC). Vancomycin removal was studied in a paired fashion comparing the Travenol 12 · 11 cuprophan and Fresenius polysulfone membranes. Arterial and venous samples were obtained simultaneously at 0-4 hours and vancomycin levels were determined using the Abbott TDxTM assay system. Vancomycin removal was consistent with a one compartment model. Plasma VC increased markedly and the intradialytic half life (ID $t_{\frac{1}{2}}$) of vancomycin decreased with use of the polysulfone membranes (table, *p <0.01; Q_B250 Q_D500 ml/min).

	cuprophan	F-40	F-60	F-80
VC (ml/min)	9.6±2.9	44.7±6.7*	73.0±5.0*	85.2±7.0*
ID $t_{\frac{1}{2}}$ (min)	35.1±11	11.8±5.6*	6.7±1.0*	4.5±0.F*

Vancomycin levels decreased to 51.7±2.5% of pre-dialysis values over 4 hr with the F-80. VC correlated with membrane surface area in the Fresenius series (r=0.91, p 0.05). VC was also blood flow dependent as increasing Q_B from 200 to 400 increased VC from 80.6 ± 0.8 to 101.7 ± 9.7 ml/min using the F-80. These data indicate that clinically significant vancomycin removal is achieved using the polysulfone F-40-80 membranes. We recommend that with use of the Fresenius polysulfone F-40-80 dialyzers vancomycin be supplemented post-dialysis to maintain therapeutic levels.

MACROCYTOSIS IS A COMMON OCCURRENCE IN HEMODIALYSIS PATIENTS CHRONICALLY RECEIVING RECOMBINANT ERYTHROPOIETIN (EPO). Michael C. Laver, E. Paul Mac Carthy, David H. Clyne & Victor E. Pollak. University of Cincinnati Medical Center, Cincinnati, Ohio.

Of 18 patients receiving EPO for a period of >12 weeks, 7 developed macrocytosis, with MCV values as high as 119 and MCV increases as much as 24.3. The table shows the mean values of MCV, Hb, Hct and reticulocyte count pre EPO, at 6 weeks and 12 weeks of EPO therapy, and the mean maximum values observed.

	Baseline	6 Weeks	12 Weeks	Maximum
MCV	91.9	101.4	101.4	105.4
Hb	7.33	8.40	8.84	10.71
Hct	22.4	26.8	27.7	32.6
Retics.	0.99	2.39	1.51	6.33

The increase in MCV was not due to the reticulocytosis, as in most patients it occurred weeks after the peak reticulocyte response, and persisted long after the reticulocyte count returned to normal. Serum folate and Vitamin B 12 levels were normal. 3 patients had evidence of transfusion related iron overload, but none had evidence of significant hepatic dysfunction.

The cause of these changes in erythrocyte size is not clear. It seems unlikely to be a direct pharmacologic effect of EPO, as it is not seen in all patients and is not related to EPO dose. Folic acid deficiency seems unlikely in view of the normal serum levels and the occurrence despite supplementation. Similarly, Vitamin B 12 deficiency is not likely because of normal serum levels and the presence of considerable hepatic stores. In one patient intramuscular B 12 therapy over 6 months has failed to normalize the MCV.

RECOMBINANT HUMAN ERYTHROPOIETIN (EPO) AND PHLEBOTOMY (PLB) IN THE TREATMENT OF IRON OVERLOAD (IO) IN CHRONIC HEMODIALYSIS (HD) PATIENTS (PTS). J. Michael Lazarus, Raymond M. Hakim, Judy Newell,* and Mahamane Maiga.* Brigham & Women's Hospital/Harvard Medical School, Boston, MA and Vanderbilt Medical Center, Nashville, TN.

Five pts with evidence of IO were selected for treatment with EPO to increase the hematocrit (hct) allowing repeated PLB. The dose of EPO was 300 U/kg/HD. PLB was done after HD to maintain the hct at 35%. Hct was done each HD; CBC, SMA 20, & serum ferritin-monthly; CAT scan for liver density (den)-every 6 months (mos). Data are after 14 mos. of treatment except Pt#5 who was transplanted after 9 mos.

	Pt#1	Pt#2	Pt#3	Pt#4	Pt#5
Years on HD	14.3	10.5	14.0	7.5	5.8
#Transfusions	238	369	163	147	130
Units PLB	57	27	41	57	47
Ferritin-(Pre)	9620	13,520	3700	7400	7820
Ferritin-(Post)	3846	4735	273	415	5769
Ferritin-(Change)	5774	8785	3427	6985	2051
Liver den-(Pre)	105	92	67	90	91
Liver den-(Post)	85	75	60	67	71

*Normal= 8-480 ng/ml

*Normal= 50±5 Hounsfield units at 125 KV

One pt had repeated clotting of the AV fistula requiring lowering of maximum hct to 28% and use of Coumadin. Two pts had transient arthralgia, myalgia and night sweats with the 300 U/kg/HD dose but responded to dose reduction and time. There were no sequaela from PLB. Plan is to continue until serum ferritin is persistently in normal range. This appears to be a safe and effective method of reducing IO in HD pts.

EFFECTIVENESS OF TOPICAL BETADINE OINTMENT (B) AGAINST HEMODIALYSIS (HD), SUBCLAVIAN LINE (SCL) RELATED SEPSIS. A. Levin,* A.J. Mason,* I.W. Fong,* K.K. Jindal,* M.B. Goldstein. Univ. of Toronto, Toronto, Ontario, Canada.

109 HD patients were prospectively randomized to study the impact of exit site B application on SCL related infections. Patient demographics were not different between the 2 groups. Nasal cultures were taken weekly. Cultures of exit site (ES) and blood (BC) were obtained as clinically indicated. All available SCL tips (T) were cultured. SCL were removed if line sepsis was suspected, and non functioning catheters were removed over a wire preserving the site. Mean duration of site (39 vs 33 days), catheter number per site (1.4 vs 1.2) and insertion difficulty were comparable in both groups.

	Positive Cultures				
Patient Group	n	ES	BC	n	T
Betadine (B+)	49	3	2*	60	11•
No Betadine (B-)	60	11	11	66	25

*p <.02 •p <.03 B+ vs B-

Of the staph aureus (SA) nasal carriers, none who received B (B+) (n=6) had evidence of SA infection (BC, ES, or T), whereas in the B- group (n=20), SA positive cultures were: BC 7, ES 3, and T 11*. Conclusion: Topical Betadine ointment appears to have an important role in the prevention of both staph aureus and staph epidermidis hemodialysis subclavian line related infections.

MORTALITY IMPACTS OF SHORTER DIALYSIS PROCEDURES. Nathan W. Levin, Henry Ford Hospital, Detroit, MI, Philip J. Held, The Urban Institute, Washington, D.C.*, Louis H. Diamond, Georgetown Univ., Washington, D.C.*, Randall R. Bovbjerg, The Urban Institute, Washington, D.C.*, and Mark V. Pauly, Univ. of Pennsylvania, Philadelphia, PA*.

Length of dialysis procedures was analyzed for its relation to patient mortality in 1984-85, before the advent of high-efficiency dialysis. A national sample of 651 Medicare hemodialysis patients, clustered in 35 dialysis units, was randomly selected from the master listing. The study collected detailed medical record data, including a sample of up to 18 dialysis treatments during a six-month period, patient biographical information, and each dialysis unit's policy on dialyzer reuse and kinetic modelling. Patient mortality and other outcome information as of 12/31/1987 (approx. 2.5 years later) were added from the Medicare Information System.

Mortality was shown to be associated with length of dialysis, using Cox multivariate analysis, with other patient and unit covariates held constant. Patients with average blood urea nitrogen and with an average procedure length of less than 3 hrs. and 30 minutes were found to have a relative risk of 2.0 ($p<0.01$) compared to patients with longer dialysis procedures. Units' use of kinetic modelling, however, was associated with lower mortality. Medical as well as economic factors were shown to be associated with the likelihood of a patient receiving shorter dialysis procedures.

DIRECT INHIBITORY EFFECT OF CALCITRIOL ON PARATHYROID FUNCTION (SIGMOIDAL CURVE) IN DIALYSIS PATIENTS. F. Llach, R. Dunlay*, M. Rodriguez, A. Felsenfeld, C. Williams*, and J. Pederson. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, Okla.

Calcitriol (CTR) directly inhibits in vitro parathyroid hormone (PTH) secretion. The present study evaluates the effect of 2 mcg intravenous (IV) CTR on parathyroid function in 6 chronic dialysis patients with secondary hyperparathyroidism. CTR, 2 mcg, was administered after each dialysis thrice weekly for 10 weeks. Parathyroid function was assessed by inducing hypo- and hypercalcemia with low Ca (1.5 mEq) and high Ca (4.0 mEq) dialysates prior to and after 10 weeks of CTR. To avoid hypercalcemia during CTR, the dialysate Ca concentration was 2.5 mEq/L. Values of PTH (pg/ml) from dialysis-induced hypo- and hypercalcemia were plotted against serum Ca and the sigmoidal relationship between PTH and Ca was evaluated. Basal PTH levels fell from 862±128 prior to CTR to 451±101 after therapy ($p>.01$). The sigmoidal relationship Ca-PTH was displaced to the left after CTR. Maximal PTH decreased from 1334±169 to 865±107 ($p<.001$). Lowest PTH value induced by hypercalcemia was 235±50 before CTR and 157±11 afterwards ($p<.05$). All changes in PTH function occurred in the absence of significant increases in serum Ca during CTR therapy.

In summary, IV CTR decreased PTH secretion and shifted the sigmoidal curve to the left, independent of changes in serum Ca. This suggests a direct inhibitory effect of CTR on parathyroid function in dialysis patients with secondary hyperparathyroidism.

ALTERED BETA$_2$-MICROGLOBULIN IN AMYLOID DEPOSITS OF PATIENTS ON LONG-TERM HEMODIALYSIS. Reinhold P. Linke, Manfred Eulitz, Hannelore Hampl and Hartmut Lobeck (intr. by Juan Bosch). Univ. and GSF of München, Heimdialysezentrum and Inst. of Pathol., FU, Berlin, Germany

Pathologic bone fractures occur in patients on maintenance hemodialysis. Histologic examination of the responsible lesion revealed in many cases the presence of amyloid. Chemical analysis of the amyloid deposits showed β_2-microglobulin (β_2m) as the major amyloid fibril protein (BBRC 136, 665, 1985). Since increased concentrations of β_2m, that are usually found in renal failure, do not induce amyloid formation alone, and passive adsorption of β_2m to the tissues (as it is believed today) does not explain the restriction of amyloid deposition to defined anatomical sites, we set out to examine the chemical and antigenic structure of amyloid of β_2m-origin (AB-amyloid) in more detail. Using 60-80% formic acid in water and size exclusion HPLC, besides β_2m with an intact N-terminus molecules lacking the N-terminal 6 or 19 amino acids were also found. Antigenic studies using immunoprecipitation in agarose revealed a strong reaction of AB-proteins with anti-AB and likewise of β_2m with anti-β_2m, but only a weak reaction with the non-homologous combination, as has been described for immunoglobulin-derived amyloid (J. Immunol. 111, 10, 1973). These data strongly indicate, that β_2m is altered by limited proteolysis in cases investiated. This alteration may be accompanied by a conformational change as compared to the native β_2m, which is indicated by the immunoprecipitation studies showing an antigenic alteration of the β_2m-complexion in amyloidogenesis

PASSAGE OF CYTOKINE-INDUCING E.COLI FRAGMENTS (CEF) THROUGH VARIOUS HEMODIALYSIS (HD) MEMBRANES.

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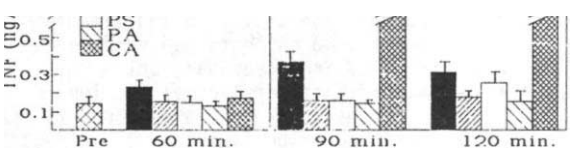
Bacterial products, which are potent inducers of cytokine production, may contaminate standard dialysate. We have demonstrated that CEF may penetrate tight cellulosic HD membranes (CU) under the conditions of in vitro dialysis with plasma present in the blood side. To determine to what extent CEF penetrate open cellulosic or synthetic high flux HD membranes, the dialysate side of a closed loop system was perfused with pyrogen free tissue culture medium (MEM) for 60 min. and then challenged for further 60 min. with E.coli filtrate (1 ug/ml endotoxin). The blood side contained 10% plasma in MEM. In comparison with CU, cellulose triacetate (CA), acrylonitrile (AN), polysulfon (PS), and polyamide (PA) dialyzers were tested in 5 parallel experiments. Samples drawn from the blood side after 60 (sterile dialysate), 90 and 120 minutes (E.coli challenge) were assayed on mononuclear cells for the induction of tumor necrosis factor (TNF). TNF was measured (RIA) in supernatants after 18 hrs of mononuclear cell culture at 37°C. Results (mean±SEM) are shown in the figure.



These results demonstrate that CEF penetrate CU membranes at 90 min. of in vitro dialysis. However, compared to CU, CA was much more permeable for CEF. When testing PS, small amounts of CEF appeared on the blood side at 120 min. Since CEF were not detected on the blood side of AN and PA dialyzers, adsorption to these synthetic membranes may prevent the passage of CEF from the dialysate to the blood side in our model. However, during high flux in vivo HD, back filtration may facilitate transmembranous passage of CEF and thus may override membrane adsorption.

$CaCO_3$ AS THE SOLE PO_4 BINDER WITH LOW CALCIUM (CA) DIALYSATE IN HEMODIALYSIS PATIENTS (HDP): POTENTIAL ELIMINATION OF ALUMINUM (AL) INDUCED COMPLICATIONS.

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A potential benefit of $CaCO_3$ compared to $Al(OH)_3$ as a PO_4 binder for HDP is a reduction of Al accumulation. Therefore, 194 HDP were switched from $Al(OH)_3$ to $CaCO_3$ as the sole PO_4 binder without Vit D therapy and observed over a 6 month period. In order to minimize the incidence of hypercalcemia (↑Ca), dialysate Ca was lowered from 3.5 to 2.5 mEq/L. Monthly serum total Ca, ionized Ca, alkaline phosphatase (AP), and PO_4 were measured. 1,25 dihydroxy vitamin D, iPTH, and Al were measured every 3 months. Desferoxamine (DFO) stimulation tests (40 mEq/kg) were performed initially and after 12 months to allow adequate time for Al redistribution. Mean ± SD ($P < 0.001$* vs. control) was analyzed by ANOVA of transformed data over the study period.

	Total Ca	Ionized Ca	AP	PTH	Vit D	PO_4	Al**
Control	8.9 ± 1.1	2.19 ± 0.33	200 ± 194	64 ± 58	8.3 ± 6.3	6.2 ± 2.3	87 ± 119
6 mon	8.9 ± 1.5	2.27 ± 0.40*	193 ± 189	60* ± 58	8.9 ± 1.5	5.9 ± 2.4	30.5* ± 17

** After 12 mon $CaCO_3$

DFO Stimulation (n = 49):

	Control	12 mon
Pre	65 ± 32	37 ± 21*
Post	225 ± 108	71 ± 43*

The incidence of ↑ Ca was 2.5% in $Al(OH)_3$ treated patients and was 3.6 to 5.7% (NS) in $CaCO_3$ treated patients.

Conclusions: 1) $CaCO_3$ is as effective as $Al(OH)_3$ in controlling serum PO_4 in HDP. 2) Al concentrations decrease dramatically after discontinuation of $Al(OH)_3$, DFO stimulation tests confirm markedly lower total Al levels. 3) We conclude that $CaCO_3$ with low Ca dialysate is the PO_4 binder of choice in HDP.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODY IN PATIENTS UNDERGOING CHRONIC HEMODIALYSIS.

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In June 1986, CDC began a multicenter study to estimate the prevalence and incidence of HIV antibody in chronic hemodialysis patients. Dialysis centers from throughout the US voluntarily enroll in the study. For each patient who consents to participate, epidemiologic data and serum for HIV-antibody testing are collected. Patients complete a confidential questionnaire self-reporting other risks for HIV. As of June 30, 1988, initial data and specimens had been received from 1291 patients in 27 dialysis centers in 10 states. The mean patient age was 55 years; 48% were female; 63% were black, 32% white, and 3% Hispanic. History of blood transfusion was recorded in 61% of the patients' hemodialysis records; the average number of units transfused per patient was 15. Receipt of transfusion since 1978 was self-reported by 7/% of the patients, and sharing needles for injection of drugs by 2%. HIV-antibody tests performed on the 1291 patients identified 18 (1.4%) whose serum specimens were reactive by EIA, and 10 of the 18 were Western blot-positive (seropositivity rate=0.77%). The mean age of these 10 patients was 40 years; 5 were male. All 10 have received blood transfusions; 5 reported self-injection of IV drugs since 1978. The HIV-seroprevalence rate in hemodialysis patients in this study is 0.77%; all 10 infected patients had recognized risk factors for HIV infection.

CALCULATION OF KT/V FROM THE POST:PRE DIALYSIS PLASMA UN RATIO (R): INCREASED ACCURACY WITH INCLUSION OF DIALYSIS TIME, POST-WEIGHT, AND UF VOLUME.

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For auditing purposes, there is a need to be able to determine exactly the KT/V based on simple parameters. In 240 maintenance dialysis patients, (360 modeling sessions) we compared the KT/V as derived from standard 3-blood sample modeling with the KT/V as estimated from various formulae based on the ratio of postdialysis to predialysis plasma urea nitrogen levels (R). We studied whether incorporation into the formulae of dialysis time in hours (T), ultrafiltrate volume (U), and postdialysis weight in kg (W), could increase the accuracy of estimating KT/V from R.

The formulae tested were:

(1) $KT/V = -\ln(R)$

(2) $KT/V = -\ln(R - 0.005 \cdot T - U/W)$

(3) $KT/V = -\ln(m \cdot R + i)$

In equation 2, T, U and W were as described above. In equation 3, m and i were functions of T, U, and W which were derived from iterative computer solutions of the urea modeling equations.

The resulting KT/V values were compared by regression, with y = KT/V from each of the three formulae, and x = KT/V from standard modeling.

FORMULA	R VALUE	SLOPE	INTERCEPT	S.E. ESTIMATE
(1)	0.978	0.900	-0.029	0.041
(2)	0.991	0.951	-0.008	0.026
(3)	0.996	0.970	0.022	0.020

The results suggest that the problems in predicting KT/V from R alone can be largely solved if T, U, and W are taken into consideration.

EFFECT OF DIALYSATE BASE COMPOSITION UPON OXALATE REMOVAL AND OXALATE CLEARANCE BY HEMODIALYSIS

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To determine the effect of bicarbonate (HCO_3) and acetate (OAc) dialysis upon oxalate removal (ROx), we performed two paired studies using HCO_3 or OAc dialysate in two hemodialysis patients with primary hyperoxaluria. These features were kept constant for each paired study: dialyzer (cellulose-based), dialysis time (3½, 4 hr), whole blood (WB) flow rate (275 ± 25 ml/min), dialysate flow rate (533 ± 20.9 ml/min) and plasma flow rate (210 ± 14.4 ml/min). Plasma oxalate (POx) was measured before (pre-HD) and at the end of dialysis (post-HD). Dialysate was collected, weighed, and analyzed for oxalate to determine ROx. Arterial and venous samples were obtained for POx after 1 hr of dialysis to determine oxalate clearance (COx). Total body water cleared (TBW Cl) of oxalate during dialysis was determined by dividing ROx by pre-HD POx. Results are shown below. (Values = Mean ± SEM; N=4).

	POx(uM)		COx(ml/min)		ROx	TBW Cl
	PreHD	PostHD	Plasma	WB	(umoles)	(L)
OAc	96+ 13.5	41+ 5.3	127 ± 18.5	166+ 19.1	1780+ 320.2	18.6+ 2.1
HCO_3	83+ 19.1	35+ 10.3	132+ 15.8	172+ 17.4	1895+ 291.0	24.1+ 2.8

There were no significant differences between HCO_3 and OAc dialysis for these acute studies. There was a tendency for HCO_3 dialysis to provide higher COx, ROx, and TBW Cl. This may be important for the long-term treatment of hemodialysis patients to prevent or reverse oxalosis.

DIET PRIOR TO INITIATION OF HEMODIALYSIS AND SUBSEQUENT RISK OF EARLY MORTALITY. William McClellan and Elbert Tuttle. Department of Medicine. Emory University School of Medicine, Atlanta, Georgia.

We examined the association between predialysis diet and mortality. We estimated the average daily consumption of protein, calories, total fat, monounsaturated, polyunsaturated and saturated fat, and carbohydrates using a semiquantitative food frequency questionnaire. A cohort (N=239) of patients beginning hemodialysis therapy was followed for a mean of 479 days; 32 (13.4%) of the cohort died. No differences were found in group means for consumption of macronutrients.

	Cal. Kcal	Prot. Gm	Tot. Fat Gm	Carbo Gm
Surv.	2008	79.4	79.5	246.6
Nonsurv.	2179	82.4	87.4	271.5

Individuals with protein consumption, .81 gm/Kg/d or greater were more likely to die (RR=3.27, p=.049), as were individuals with high total fat consumption, .83 gm/Kg/d or more, (RR=3.45,p=.039). After controlling for age, sex, race, primary cause of renal failure and comorbidity, these association were strengthened.

We conclude that high dietary fat consumption prior to initiation of hemodialysis may be associated with risk of early death and low levels of protein consumption are not associated with increased risk of early mortality.

COMPARISON OF EFFECT OF RECOMBINANT HUMAN ERYTHROPOIETIN (rHEPO) IN PATIENTS ON HIGH FLUX (HF) VS CONVENTIONAL (C) HEMODIALYSIS. Ravinder Mohini, Robert Michaels*, Cecilia Trost*, Donna Gron*, Gerard Zasuwa*, Francis Dumler, Nathan W. Levin. Division of Nephrology, Henry Ford Hospital, Detroit, Michigan.

The concomitant use of rHEPO and high flux short time dialysis presents possible problems of safety and efficacy. To study this question, 34 clinically stable patients treated with rHEPO were randomly allocated to HF hemodialysis (polysulfone membranes: Kd-urea 277±13 ml//min, dialysis time 133±4.8 min; n=16), and conventional hemodialysis (cellulosic membranes: Kd-urea 186±8 ml/min, dialysis time 170±7.8 min; n=18). rHEPO was administered intravenously post dialysis 3/week. Mean maintenance doses were 100 u/kg and 106 u/kg for HF and C, respectively.

At 6 months, no significant differences were observed in hematocrit (33.8% - HF vs 33.6% - C), BUN, creatinine, serum phosphate, Kt/V, PCR, pre and post systolic and diastolic blood pressure, body weight and interdialytic weight gain. Heparin requirements increased in both groups compared to baseline. Dialyzer reuse remained higher in HF vs C. Adverse reactions and hospitalizations were not greater in HF.

It is concluded that rHEPO can be administered safely and efficaciously to patients on HF hemodialysis at the hematocrits achieved.

IMPLEMENTATION OF ROUTINE CLINICAL HEMODIAFILTRATION (HDF). JH Miller, S Jönsson*, PW Gardner*, JH Shinaberger. Med. Svc. West L A VA Med. Ctr. & Gambro AB, Lund, Sweden

The goal of HDF is to combine the efficient diffusive removal of small molecules of hemodialysis with the much greater convective removal of large molecules and vascular stability of hemofiltration (HF). In HDF the high convective flux (18 L/Rx) prevents potentially harmful back-filtration of dialysate as may occur in high flux dialysis (HFD) but requires infusion of sterile replacement fluid (RF). Prior investigation of HDF was limited by lack of a commercially available system. The Gambro Multi Purpose System (MPS-10) with required lines, bacterial and pyrogen filters and modified polyamide hemodiafilters (suitable for HDF, HFD and HF) make clinical studies and application of HDF practical. The dialysate and RF withdrawn from it are rendered consistently sterile and LAL apyrogenic on line by integral, well characterized pyrogen filters. The MPS was used in HDF vs HFD studies in 8 pts including the maintenance of 2 pts on HDF for 9 pt mos without adverse effects. In 143 studies (92 HDF) volume control in either mode was accurate. In HDF, at QRF's of 60 to 100 ml/min, clearances (when corrected for QD) were improved over HFD by the approximate amount (QU + QRF)(1 - K(0)/QBi), K(0) = clearance at 0 QU and QF. In paired studies of 3 hr HFD vs HDF at QB = 417, QD = 550 - QRF, and QRF = 0 (HFD) and 60 (HDF), all ml/min, HDF yielded improved pre-post drop in serum BUN, creatinine and PO4 levels and greater solute mass in dialysate of about 10%. At QRF = 90 ml/min, marked improvement in vascular stability and well being compared to HFD occurred. HDF is simplified and offers clear advantages in solute removal and patient comfort.

POLYACRYLONITRILE DIALYSIS MEMBRANES SCAVENGE C_3A des ARG IN MAN. Brooke Moore*, John Walsh,* Gerald Schulman, Leonard Arbeit. Division of Nephrology, SUNY-Stony Brook, NY.

In five chronic hemodialysis patients cuprophane (C) dialysis membranes activated the complement cascade as evidenced by increased C_3A des Arg (C_3A) levels. Polyacrylonitrile (PAN) membranes did not increase C_3A levels. In further studies we dialyzed these same patients against C and PAN membranes connected in series. During one dialysis PAN was attached to the arterial line while C was attached to the venous line (PAN→C). Dialysis tubing connected the two kidneys creating a circuit. In a subsequent dialysis the membrane order was reversed (C→PAN). The net increase in C_3A at 15 minutes produced by C alone (13,400 ng/ml ± 4,700) was markedly reduced in the PAN→C circuit (1,820 ng/ml ± 510), although C_3A was significantly increased over baseline C_3A values (530 ng/ml ± 120, P<.05). In the C→PAN circuit a net increase in C_3A over baseline at 15 minutes was completely prevented (520 ng/ml ± 190 to 1,270 ng/ml ± 700, P=NS).

There was no significant decline at 15 minutes in the white blood cell count (WBC) with PAN alone. WBC declined to 80% of baseline with C, PAN→C, and C→PAN.

These data demonstrate in man that PAN both inhibits activation of the complement cascade (PAN→C) and removes activated complement components (C→PAN). Indeed, when PAN is placed in series after C (C→PAN), it completely prevents the increase in C_3A. However, despite its ability to scavenge the activated complement components, PAN does not protect dialysis patients from the neutropenia caused by C. This suggests that other factors besides complement may be important in dialysis membrane bioincompatibility.

THROMBOSIS PREVENTION DURING HEMODIALYSIS OF PATIENTS WITH HIGH HEMORRHAGIC RISK (HHR) BY THE VERY LOW MOLECULAR WEIGHT HEPARIN (VLMWH) CY 222, VERSUS THE RINSING PROCEDURE WITHOUT HEPARIN (RPWH) A RANDOMIZED COOPERATIVE TRIAL .

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CY 222 from Choay is a VLMWH (2 500 Daltons) with a much lower antithrombin activity than unfractionned heparin (25 IU/mg using the IVth International Heparin standard) suggesting that it induces a lesser hemorragic risk. Previous studies have determined the optimal dose for patients with HHR as 150 anti Xa institut Choay Units per kg of body weight in a single bolus intravenous injection at the beginning of the 4 hours dialysis session. At this dose, no bleeding and only 4% of massive clotting were observed in a study of 650 hemodialysis sessions. Therefore the present randomized study was performed to compare CY 222 to RPWH during the hemodialysis of 100 patients with HHR, i.e. with hemostasis disorders (PT< 70%, APTT > 40 sec ; non heparin induced thrombocytopenia) and/or medical, surgical or traumatic hemorrhages which had occured in the 4 previous days. None of these patients were given antiaggregant or anticoagulant therapy. Efficacy was assessed on the prevention of massive clotting and safety on the occurence of hemorrhage worsening in the following 24 hours.

	CY 222	RPWH	X2	P
massive clotting (yes/no)	5/49 (9%)	19/27 (41%)	14	.0001
hemorrhage worsening (yes/no)	3/51 (6%)	1/45 (2%)		

Conclusion : CY 222 allows a more effective prevention of thrombosis of the dialysers than the RPWH without inducing a greater hemorrhagic risk. Since it does not put so much strain on the nurses as RPWH does use of CY 222 represents an optimal way to prevent thrombosis during hemodialysis of HHR patients.

THE VASORELAXANT EFFECTS OF ACETATE IN THE RAT CAUDAL ARTERY ARE NOT MEDIATED BY ADENOSINE.

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The presently accepted theory of how acetate causes vasodilatation is the so-called "metabolic theory" of Liang and Lowenstein: metabolism of acetate by thiokinase generates AMP; the AMP is degraded to adenosine; and it is the adenosine that would cause the vasorelaxation.

To test this hypothesis, we compared the effects of adenosine and acetate on relaxation and cyclic AMP generation in the rat caudal artery. We also evaluated the effects of inosine, adenosine deaminase (ADA), 8-phenyltheophylline (8-PT; an adenosine receptor blocker), and oxypurinol, on acetate-induced vasorelaxation.

Adenosine per se had no vasorelaxant effect in the range of 10^{-8} to 10^{-2} M; in fact, a variable, transient, exhaustible, constrictor effect of adenosine was demonstrable. Preincubation of tissues with ADA or with 8-PT increased, rather than blocked the vasorelaxant effect of acetate: Relaxation to 16 mM acetate -- control: 48% $\pm$ 5.5; Ac + ADA: 82% $\pm$ 3.7 ($p < 0.01$); Ac + 8-PT: 59% $\pm$ 3.6 ($p < 0.05$). Inosine and oxypurinol had no effect on acetate relaxation. Whereas acetate increases cyclic AMP in this tissue, 10^{-3} M adenosine, 10-3 M AMP, or 10-6 M PIA had no effect on tissue cyclic AMP levels: control 13.1 $\pm$ 2.5 pmol/mg protein; 4 mM acetate 24.2 $\pm$ 4.6 ($p<0.001$); adenosine 15.2 $\pm$ 2.6 (p NS); AMP 12.8 $\pm$ 2.9 (p NS); PIA 12.8 $\pm$ 1.8 (p NS).

Our results suggest that adenosine does not mediate the vasorelaxant effects of acetate in the rat caudal artery.

BRAIN FUNCTION IMPROVES in CHRONIC HEMODIALYSIS (CHD) PATIENTS (PTS) AFTER RECOMBINANT ERYTHROPOIETIN (r-EPO).

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Brain event-related potentials (ERP) can be used to measure CNS functional status in CHD pts. We measured ERP in 17 pts. before (T1), after 12 weeks (T2) and at least 1 year (T3) of treatment with r-EPO in order to determine whether anemia contributes to uremic brain dysfunction. Latency (LAT) and amplitude (AMP) of one ERP measure, the P3 wave are sensitive measures of information processing (IP). Studies were performed 24 hours following a routine HD. Mean HCT was 23% at T1 and had risen to 37% by T2 and remained stable thereafter. No change in P3 LAT was found from T1 to T3 indicating no improvement in the speed of IP. The AMP of the P3 wave, however, increased significantly from T1 to T3 ($p<.03$) with a 19%, 63%, and 160% improvement in parietal, vertex and frontal brain areas respectively. This has been associated with increased attention span, memory and efficiency of cognitive function. Therefore, correction of anemia with r-EPO leads to significant improvement in brain function in CHD pts. In addition, part of the uremic syndrome is attributable to the effects of anemia on the CNS.

ON THE BEHAVIOR OF VASO-CONSTRICTOR HORMONES (VCH) DURING ISOLATED ULTRA-FILTRATION (UF) AND ISOLATED DIALYSIS (DI).

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Hypertension is the most common and dangerous side effect of DI. Studies of single VCH during DI have been contradictory.

We studied the behavior of 7 VCH: neuro-peptide Y(NPY), noradrenaline (NA), adrenaline (A), dopamine (DA), renin (REN), angiotensin-II (ANG) and vasopressin (VP) during UF and DI. Concentrations (conc) and changes(Δ) in VCH were studied as dependent variables of Δ-weight and Δ-osm and values andΔ in systolic blood pressure (SYS) as dependent variables of conc and Δ in VCH.

	NPY	NA	A	DA	REN	ANG	VP
Pre UF	41.5	3.2	0.5	0.4	1.6	19.7	8.3
PostUF/PreDI	38.5	3.9	0.4	0.4	2.5	26.4	9.1
PostDI	39.7	2.6	0.3	0.3	2.2	22.1	8.1

All pre-UF levels were normal. Only Δ-DA correlated with Δ-osm no Δ-VCH with Δ-weight. No Δ-VCH correlated significantly with Δ-SYS but NPY correlated with pre- and post-dialysis-SYS.

Patients on chronic hemodialysis have normal predialysis VCH, respond appropriately to volume decrease with a rise in vaso-constrictor hormones but inappropriately to the chemical changes of dialysis with a fall in VCH. However, the UF rate as such, is not the stimulus to increases in VCH, nor does dialysis efficiency, globally measured as Δ-osm, predict hormonal dysfunction. Other factors, such as biocompatibility, hemoconcentration, refill rate or changes in electrolytes or specific toxins must be responsible.

BONE DEPOSITION OF BETA-2 MICROGLOBULIN (B2M) IN HEMODIALYSIS PATIENTS. Shuhei Onishi*, Dennis L. Andress, Norma A. Maloney*, Jack W. Coburn, Donald J. Sherrard. Nephrology Divisions, V.A. Medical Center and University of Washington, Seattle, WA, and the Wadsworth V.A. Medical Center, Los Angeles CA.

The unique amyloid that is found in dialysis patients deposits in a variety of tissues including bone. Using Congo red to screen and B2M-specific antibody to confirm, we assessed previously obtained bone biopsies to determine the prevalence of B2M in bone. We found B2M in 0/45 patients on dialysis $\leq$ 5 years, 3/98 on dialysis 6-10 years and 14/82 on dialysis > 10 years. B2M was present in 7/7 patients with fractures (5 femoral, 1 tibia, 1 vertebra) and in 15/231 iliac crest biopsies. In the iliac crest, B2M was found primarily in the periosteum (11/12 patients). B2M was also found in the synovium, cartilage, marrow and bone mineral. Among the 11 patients with B2M in the iliac periosteum, 7 (64%) had femoral neck fractures while only 1 of 25 matched patients without B2M at this site had a femoral fracture ($p < 0.001$). Evaluation of bone histology in relation to B2M revealed that 50% of patients with B2M had osteitis fibrosa while only 26% without B2M had this lesion ($p < 0.04$).

In conclusion: 1) Bone B2M was absent in patients with < 5 years of dialysis time but was present in 17% who dialyzed longer than 10 years 2) the high prevalence of femoral neck fracture in patients with B2M in iliac periosteum suggests this site for diagnostic purposes 3) the association of bone B2M and osteitis fibrosa suggests that B2M may promote high bone turnover.

THE NATURAL HISTORY OF CONGESTIVE HEART FAILURE (CHF) IN DIALYSIS PATIENTS. P.S. Parfrey, S.M. Griffiths, J.D. Harnett, R. Taylor, A. King, J. Hand, P.E. Barre. Memorial Uni, St. John's, and McGill Uni, Montreal, Canada.

To determine the outcome of CHF and the disorders with which it is associated 150 nondiabetic dialysis patients were followed clinically and with serial echocardiograms for 3-5 years. On entry into the study 10% (N=15) had CCF and had been on dialysis for 2.9 $\pm$ 0.5 S.D.years. 53% (N=8) of CCF had dilated cardiomyopathy and 47% (N=7) had hypertrophic hyperkinetic disease. Compared to patients without CHF, patients with CHF had a significantly higher mortality (2 years survival 33% vs 80%). 17% (N=24) of total group had dilated cardiomyopathy, 57% of whom had the disease diagnosed at or within 2 years of starting dialysis. Dilated cardiomyopathy never evolved from hypertrophic hyperkinetic disease and was still present in 90% after 32 $\pm$ 17 months followup. Left ventricular end diastolic diameter was unchanged but end systolic diameter increased significantly from 4.5 $\pm$ 0.7 cm to 5.0 $\pm$ 1.0 cm. Mortality was significantly worse than that of 30 patients with normal echocardiograms (2 year survival 67% vs 90%). 11% of the total group had hypertrophic hyperkinetic disease. This disease was present on starting dialysis in 78% and persisted in all patients. 2 year survival was 30% vs 90% in those with normal echocardiograms. We conclude that congestive heart failure in dialysis patients is caused by dilated and hypertrophic cardiomyopathy, both of which occur quite frequently, are often present on starting ESRD, persist, and have a poor prognosis.

RETROVIRAL INFECTIONS IN PATIENTS ON CHRONIC HEMODIALYSIS. C. Ortiz,* G. Perez, M. de Medina,* J. J. Bourgoignie. University of Miami and VAMC, Miami, FL.

The frequent administration of blood transfusions and the sizeable number of patients with intravenous drug use and with HIV nephropathy in urban dialysis units suggest that retroviral infections may be prevalent in such centers. We evaluated 129 patients undergoing maintenance hemodialysis in 1986 and 1987 for HIV-1 and HTLV-I infection. Viral antigen based ELISA's (Abbott Labs) were used for screening IgG antibodies to each virus. Each positive ELISA was confirmed by the appropriate Western blot, RIPA and p24 antigen RIA. Viral cultures confirmed HTLV-I infection in 6 living patients. Overall prevalence of retroviral infection was 30/129 or 23.2%. Twenty-two patients were infected by HIV-1 alone (17.1%), 4 by HTLV-I alone (3.1%) and 4 intravenous drug users had dual infections (3.1%). The 8 patients infected by HTLV-I included 6 men and 2 women, ages 33-61. All were black. All received blood transfusions. Five were intravenous drug users; one was born in the Caribbean; one lived in Japan. The 4 patients infected solely with HTLV-I are asymptomatic. Two patients with dual infection are asymptomatic and 2 are dead (1 AIDS, 1ARC). Ten patients infected with HIV-1 alone are dead (6 AIDS, 1 ARC). This survey indicates that, in addition to HIV-1, the prevalence of HTLV-I is high (6.2%) in urban dialysis centers. Blood transfusion is a likely way of contamination for HTLV-I.

COMPLEMENT ACTIVATION BY HEMODIALYSIS MEMBRANES: C3b BOUND TO CUPROPHAN MEMBRANES (CuM) IS PROTECTED FROM INACTIVATION. Charles J. Parker*, Alfred K. Cheung, Linda A. Wilcox*, Jarmila Janatova*. Veterans Administration Med. Ctr. and Univ. of Utah, Salt Lake City, Utah.

Compared to cellulose acetate membranes (CAM), hemodialysis with CuM is associated with greater activation of the alternative pathway of complement (APC). In order to characterize the factors that determine APC activity on hemodialysis membranes, pieces of CuM and CAM were incubated with serum. Subsequently, membrane associated proteins were eluted and analyzed by SDS-PAGE and Western blot (using anti-C3, anti-factor B and anti-factor H as the primary antibodies).

The CAM eluate contained >20 times more protein than the CuM eluate. Virtually all of the C3 present in the CAM eluate, however, was in the form of inactive degradation products (C3c and C3dg). In contrast, the functionally active form of C3 (C3b) was a prominent constituent of the CuM eluate. In addition, the portion of factor B present in its enzymatically active form (Bb) was greater in the CuM eluate. Consonant with these observations, factor H (inhibitor of APC C3 convertase activity) was seen in CAM but not CuM eluates.

When CuM and CAM that had been previously incubated with serum were exposed to isolated C3, CuM subsequently activated 3 times more C3 than CAM. These studies suggest that C3 convertase (C3bBb) activity persists on CuM because a portion of the membrane bound C3b is protected from inactivation. In contrast, C3b that binds to CAM is rapidly degraded.

Thus, the complement activating capacity of hemodialysis membranes appears to be determined, at least in part, by biochemical properties that modulate the interactions of the APC regulatory proteins with membrane associated C3b.

CONSTANT NONINVASIVE AV FISTULA BLOOD GAS ANALYSIS DURING DIALYSIS. J.A. Pederson, M. Jackson*, C. Williams*, and F. Llach. Univ. of Okla. HSC and VAMC, Okla. City, Okla.

Critical control during dialysis usually requires frequent blood sampling. In line, noninvasive analysis could facilitate control. Photochemical technology by Cardiovascular Devices, Inc. (CDI) is presently used to constantly monitor pH, pCO_2, HCO_3 and pO_2 during cardiac surgery. This study evaluates the CDI device during clinical hemodialysis.

A CDI flow-through sensor was inserted in the inlet blood line prior to dialysis. Treatment variations used standard proportioning and sorbent systems to deliver both acetate and bicarbonate dialysate. Observations through 210 minutes were made during 17 treatments in 10 stable, male patients.

There were no statistical differences in CDI and standard blood gas analysis (BGA) data between treatment groups. The composite data are shown in linear regression form (Y = a+bX) below.

TABLE 1: CDI vs BGA DURING HEMODIALYSIS.

Y		a		b	X	r
CDIpH	=	-2.2	+	1.3	BGA	0.99
CDIpCO_2	=	17.3	+	0.4	BGA	0.10
CDIHCO_3	=	-18.4	+	2.0	BGA	0.90
CDIpO_2	=	-345.5	+	4.5	BGA	0.77

In summary, pH and HCO_3 increased during dialysis while pO_2 decreased. There was good correlation between CDI and BGA values. No significant changes in pCO_2 occurred at the sampling intervals.

In conclusion, CDI technology for BGA is feasible and promising for care of dialysis patients.

INCREASED LEVELS OF ß2-MICROGLOBULIN FOLLOWING CUPROPHAN HEMODIALYSIS ARE A FUNCTION OF ECF REDUCTION. J. Petersen, I Yeh * and R Rajiv*. Palo Alto VAMC and Stanford Univ., Stanford, CA.

ß2 microglobulin (ß2-M) levels are elevated following dialysis with cuprophan membranes. It has been suggested that this may be the result of de novo generation. We, therefore, examined pre and post dialysis levels of ß2-M (MW 11,800) and myoglobin (MW=18,000, MYO), a protein which should be similarly restricted during cuprophan dialysis. Post dialysis levels of ß2-M and MYO were corrected for estimated loss of ECF by the formula of Bergstrom and Wehle (1987). Results, in 12 patients, * p< 0.05 vs pre, were :

	Pre	Post	Post corrected
ß2-M	43.5	55 *	44
(mg/L)	±5	±6	±5
MYO	161	187 *	150
(µg/L)	±19	±19	±17

Levels of ß2-M and MYO increased proportionately following dialysis so that their ratios remained unchanged (0.28 vs 0.30, p = ns). Levels of MYO and ß2-M, when corrected for ECF loss, were not elevated over predialysis values. The ECF content of ß2-M similarly remained unchanged after cuprophan dialysis (640 ± 87 pre vs 625 ± 85 mg post , p = ns).

Thus, changes in ß2-M parallel those of myoglobin, a protein for which there is no evidence for dialysis induced production. We conclude increased levels of ß2-M following cuprophan dialysis are a result of fluid removal rather than increased production.

A PATIENT-CENTERED ELECTRONIC MEDICAL INFORMATION SYSTEM (MIS): AN EXPERIMENT IN MEASUREMENT AND CONTROL OF CHANGES IN DIALYSIS TECHNOLOGY. Victor E. Pollak, Univ. of Cincinnati Medical Center and Dialysis Clinic, Cincinnati, Ohio.

From 10/85 to 4/87 changes in hemodialysis were made under operational conditions: (1) negative pressure (NP) to ultrafiltration control (UF) dialysis machines, and (2) cuprophan to high-flux polysulfon dialyzers. The MIS was used to determine whether observations could be satisfactorily controlled during the change. Sixty-five patients (with most problems during dialysis sessions, 43% diabetic) changed from NP to UF; 85 dialyzed only with NP (17% diabetic). They served as contemporaneous controls, and were analyzed in 2 time periods; an arbitrary "date of change" (NP1-NP2) was assigned by random computer ID number. Results were:

	NP	UF	NP1	NP2
HD sessions	7162	6297	5320	4817
	Symptoms/100 HD Sessions			
All symptoms	92.3	78.0	91.0	84.1
Hypotension	39.3	33.0	35.9	36.5
Cramps	12.9	10.8	13.4	10.9
Nausea/vomiting	12.7	7.7	12.8	10.5
	Hospitalizations/Yr			
Admissions	106	113	58	71
Days	545	624	303	371

With contemporaneous controls, patients did better with UF machines; symptoms during HD decreased further with UF machines and high-flux dialyzers, particularly hypotension, nausea and vomiting. Controlled observations on the effects of changes in therapy and operational conditions were possible using an electronic MIS.

RESPONSE TO ERYTHROPOIETIN (EPO) IN PATIENTS (PTS) ON CONVENTIONAL (CD) AND HIGH FLUX DIALYSIS (HF). Paul Quinn*, Sergio Acchiardo, Scarlett Cockrell*, Joye Key*. Univ. of Tennessee-Memphis, Dept. of Med., Memphis, TN.

We studied 20 hemodialysis pts, 10 on CD and 10 on HF. The pts were assigned at random to EPO or placebo. Twelve pts were on EPO, 7 on HF and 5 on CD. In the control group 3 pts were on HF and 5 on CD. The pts were treated for 12 w. Most of the pts were black,14 males and 6 females. Mean age was similar in all the groups.

	#pts	Hb g	K mEq/L	BUN mg/dl	B Wt kg	EPO U/kg
HF EPO pre	7	6.2	5.0	67.8	75.0	114
post		10.0*	5.4	75.2	74.6	
CD EPO pre	5	5.8	5.0	76.8	88.2	110
post		10.3*	4.9	88.4	86.2	
CONTROL	8	5.9	5.1	78.0	74.8	0

*p<0.001

No differences were observed in any of the parameters in the control group. Two pts on placebo received blood transfusions. All pts on EPO on CD or HF responded with significant elevation of their Hb levels. The mean dose required for the response was 114 U/kg, and the mean time of the initial response was 6 w. BUN also increased in these pts. No changes were observed in BWt, potassium, creatinine, coagulation studies, and cardiovascular performance evaluated by Echocardiogram. No changes were observed in blood pressure, except for a pt that developed severe headaches and hypertension and decided to discontinue the study. No differences were observed in clotting of the vascular access or the dialyzers. Dialyzers were only reused in HF pts and the number of reuses did not change in pts on EPO. An assessment of their functional activity did not show any significant change.

Pts responded well to EPO independent of the type of dialysis. The only complication observed was hypertension in one pt that subsided after discontinuation of the drug.

INFLUENCE OF FILTER DESIGN ON PERFORMANCE IN CONTINUOUS ARTERIOVENOUS ULTRAFILTRATION (CAVH). Rasib M. Raja, Nabeel Ghabra,* and Sidney Kobrin.* Albert Einstein Medical Center, Kraftsow Division of Nephrology, Philadelphia, Pennsylvania.

Clotting of hemofilter is a frequent problem during CAVH. It has been suggested that decreasing filter fibre length and increasing number of fibres may reduce clotting. Ultrafiltration (UF) during CAVH may be maximum in the inflow (I) side and decrease towards the outflow (O) as the oncotic pressure in the filter increases and equals hydrostatic pressure. Studies on filter of same surface area and membrane with varying fibre length are not available. This study compares CAVH with two Amicon-20 filters in parallel (P) and in series (S) at Q_B=100 ml/min and outlet pressure=25 mmHg in-vitro with perfusate containing albumin 4.0 Gm/dl and 8.0 Gm/dl and urea nitrogen (UN) 80 mg/dl and Cr of 8.0 mg/dl. The results are:

		UF(mil/min)	I/O ALB	I/UF UN	I/UF Cr
4% ALB	P	22.7*	0.82	0.96	0.96
	S	24.9	0.79	0.93	0.87
8% ALB	P	8.3*	0.93	0.94	0.96
	S	9.9	0.92	0.94	0.94

* $P < 0.05$ (P vs S)

These data suggest: 1) UF may be higher in ultrafilters with equivalent surface area with higher fibre length than large number of fibres, 2) even with 8% ALB, equilbration of oncotic pressure with hydrostatic pressure did not decrease UF in series design with increased fibre length. This may be due to higher pressure and resistance in the filters in series and 3) UN and Cr in UF may be higher in UF than I due to lack of proteins and not transfer from RBC's as proposed earlier.

PROGNOSIS OF THE HIV POSITIVE (POS) DIALYSIS PATIENT (PT). Seymour Ribot,*Margaret Saavedra,Melvin Goldblat,*Charles Crane. Renal Div., Newark Beth Israel Med. Ctr. and U.M.D.N.J., Newark, N.J.

The course of 27 HIV pos. dialysis pts. treated between 4/82 and 5/88 was examined to determine prognosis. The diagnosis in 9 pts. with AIDS and renal failure was suspected clinically and confirmed by Elisa and Western Blot (WB) testing. These pts. ranged in age from 21 to 42, mean 37.9 yrs. Male/Female(M/F) was 7/2. Identified predisposing causes (IPC) were: Intravenous drug abuse (IVDA) 5, Homosexual(HS) 2, IVDA and HS 1, F sexual partner of M IVDA 1. All 9 died between 1 and 17, mean 6.1 mos. Only 1 survived more than 1 yr. Hepatitis B surface antigen and antibody (HBs Ag and Ab) status indicated 4 HBs Ag and Ab neg, 4 HBs Ab pos, and 3 not tested. In May 1987 HIV testing for current and new Pts. was instituted. Pos. Elisa tests were confirmed with WB. This identified 18 pts. with AIDS related complex (ARC). These pts. ranged in age from 27-71, mean 41 yrs. M/F was 5/13. IPC were IVDA 14, Blood transfusion 3, F sexual partner of M IVDA 1. Six pts. died and had been treated with dialysis from 18-120 mean 39 mos. 12 surviving pts. continue treatment from 2-103, mean 49 mos. Four were HBs Ag pos. All AIDS and ARC pts. were black. B/W Non-AIDS dialysis pts. was 67/42. Our data is in agreement with the reported poor prognosis for the dialysis-dependent AIDS pt. Survival for the dialysis dependent ARC pt. was much longer than previously reported. IVDA was the most frequent predisposing cause for HIV infection for the AIDS and ARC pts. HBs Ab was more prevalent in our HIV pos. than in our HIV neg. dialysis pts.

CALCULATION OF PCR FROM THE POST:PRE DIALYSIS PLASMA UN RATIO (R) AND THE PREDIALYSIS PLASMA UN LEVEL. L. Ramanujan*, F.J. Manahan*, M. Ajam*, T.S. Ing, V.C. Gandhi, and J.T. Daugirdas. Hines-Loyola Medical Center, Hines, IL.

For auditing purposes, there is a need to be able to estimate the PCR of maintenance hemodialysis patients based on simple parameters. In 240 such patients (360 modeling sessions), we compared the PCR as derived from standard 3-blood sample modeling with the PCR as estimated from R and the predialysis plasma UN level. The KT/V was first calculated from R, the dialysis time, the post-weight, and the UF volume as described in an accompanying abstract. The PCR was then derived from KT/V and the predialysis plasma UN level using the Gotch nomogram. In those few patients with residual renal function, this was taken into account in determining total KT before applying the Gotch nomogram.

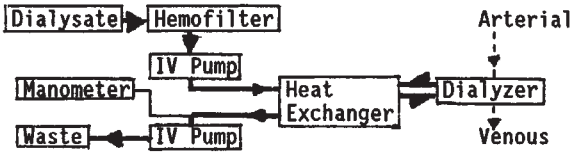
The PCR values derived using standard modeling averaged 1.01 ± 0.29 (SD) g/kg/day (range 0.29 - 2.02). The PCR values derived from R and the predialysis plasma UN level averaged 0.98 ± 0.25 (range 0.34 - 1.83). There was an excellent correlation between the two PCR determinations: (y = PCR new method, x = PCR standard modeling)

GROUP	N	R	SLOPE	INTCPT.	S.E. ESTIMATE
Kr-all	360	0.89	0.76	0.203	0.116
Kr=0	269	0.90	0.77	0.19	0.106

The results suggest that, in stable hemodialysis patients, the PCR can be reliably estimated solely from a pre and post-dialysis plasma UN level (as long as the dialysis time, post-weight, and UF volume are also known).

A NEW SYSTEM FOR CONTINUOUS ARTERIO-VENOUS HEMODIALYSIS (CAVHD). M.Roberts, V.M.Stephens, M.Trott, A.Bodt, A.J.Montez, and D.B.N.Lee. UCLA San Fernando Valley Program, Sepulveda VAMC, Sepulveda, CA

The new system is illustrated in the figure. Sterile, non-pyrogenic bicarbonate dialysate (DST) is prepared on-line by passage through a hemofilter, thus eliminating the need for the more expensive, lactate-containing peritoneal DST. In addition to the first IV pump which regulates DST inflow (16.6ml/min), a second pump is used to control DST outflow, and thereby the ultrafiltration rate. To prevent pressure buildup in the DST compartment, the traditional recourse to electromechanical devises is preempted by the use of a fail-save water manometer which displays and requlates DST pressure by overflowing at a pre-set level. Drip chambers proximal to the 2 pumps minimize air bubble-induced alarms. An additional innovation is a simple counter-current heat exchanger which allows the fresh, inflow DST to capture the heat from the used, outfow DST. This system was developed in conjunction with regular hemodialysis in ESRD patients and has functioned trouble-free, providing urea and creatinine clearance of 15.6 and 14.8ml/min respectively at 0 ultrafiltration.



THE SET POINT OF PARATHYROID HORMONE (PTH) IS HIGHER IN OSTEITIS FIBROSA (OF) THAN ALUMINUM BONE DISEASE (ABD). M. Rodriguez, A. Felsenfeld, R. Dunlay*, and F. Llach. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, Okla.

Dialysis patients (pts) with low turnover ABD have lower baseline and stimulated PTH levels than OF pts. However, a comparison of the serum calcium (Ca)-PTH curve and the set point of PTH has not been done. Excluding PTX pts, 17 ABD and 10 OF pts were studied during a low and high Ca dialysis. The following parameters were evaluated: basal PTH (PTH_b), maximum PTH (PTH_{max}), minimum PTH (PTH_{min}), basal serum Ca (Ca_b), serum Ca at maximum PTH (Ca_{max}), serum Ca at minimum PTH (Ca_{min}), and the set point for PTH, defined as the serum Ca concentration required to reduce maximum PTH secretion by 50% (Ca_{50}).

		ABD	OF	P value
PTH_b	(pg/ml)	151±38	857±122	<.001
PTH_{max}	(pg/ml)	442±90	1897±494	<.001
PTH_{min}	(pg/ml)	59±20	349±97	<.001
Ca_b	(mg/dl)	9.42±.21	9.45±.33	NS
Ca_{max}	(mg/dl)	7.73±.09	8.81±.23	<.001
Ca_{min}	(mg/dl)	10.41±.11	10.75±.20	NS
Ca_{50}	(mg/dl)	8.83±.11	9.60±.24	<.02

In summary, compared to ABD, pts with OF have 1) higher basal, and maximally stimulated and suppressible PTH levels; 2) an increase in Ca_{max} and Ca_{50}; and 3) similar basal Ca levels. In conclusion, 1) in OF as compared to ABD, the parathyroid gland is more sensitive to changes in serum Ca as manifested by a shift to the right of the set point of PTH and serum Ca-PTH curve; 2) non-suppressible PTH levels are present in dialysis pts; and 3) as previously shown, PTH secretion is decreased in ABD.

CLINICAL IMPACT OF A NEW DESIGN HOLLOW FIBER FOR CONTINUOUS ARTERIOVENOUS HEMOFILTRATION.
Claudio Ronco, Luciano Fecondini, Alessandra Brendolan and Giuseppe La Greca (Intr. by J.P.Bosch). Dept. of Nephrology, St.Bortolo Hosp. Vicenza, Italy

In vitro and in vivo studies were carried out to test characteristics and performances of a new polysulphon hollow fiber especially designed for CAVH. The main feature of the fiber is the increased inner diameter (250 micra). With this approach a theorical decrease of 60% in the overall resistance of the filters would be expected. In vitro tests were performed to compare pressure profiles, resistances and filtration rates in filters with conventional and new fibres. The same filters were tested on 6 patients to evaluate the clinical impact of the new fiber under conditions of non pumped circulation. In Vitro, the blood flow at a given pressure was significantly higher with the new fiber (from 180 conventional to 320 ml/min new at 100 mmHg) and the resistance calculated by the end-to-end pressure drop showed a reduction of 54.6% in the new fiber. As a consequence, the obligate filtration at a given blood flow was lower with the new fiber thus reducing filtration fraction. This hydraulic behaviour had a remarkable impact in vivo where the reduction of the resistance of the filters, allowed us to treat 6 patients severely hypotensive (MAP= 70mmHg) operating with blood flows higher than 50 ml/min and filtration rates higher than 10 ml/min. The new fiber represents a further step in the optimization of continuous renal replacement therapies.

WATER AND SOLUTE KINETICS WITH LOW FLUX SYNTHETIC POLYSULPHON MEMBRANE: IN VITRO AND IN VIVO STUDY.
Claudio Ronco, Aldo Fabris, Stefano Chiaramonte, Giuseppe La Greca (Intr. by Paul Kimmel). Departm. of Nephrology, St.Bortolo Hospital, Vicenza, Italy.

New synthetic membranes have been developed to reduce the UF coefficient(Kf) maintaining an adequate permeability to medium-large solutes and a good biocompatibility. Three filters($1.25m^2$) with a new polysulphon low flux membrane have been tested in vitro at different blood flows and transmembrane pressures to evaluate hydraulic properties of the membrane and solute sieving, while the same parameters were tested in vivo on nine filters during hemodialysis with different schedules.

In vitro solute sievings resulted near 1 up to 5000 Daltons while in vivo they were slightly reduced but still high (Inulin S=0.9). Kf resulted 5.4 in vitro while in vivo a certain variability was observed(shear and polarization effect). The end-to-end pressure drop in the fibres was quite low showing a reduced resistance of the device and its adequacy to be used at high blood flows. BUN Clearances varied from 207 ml/min at Qb=300 to 298 ml/min at Qb=500 ml/min. This value guarantee in a 70 Kg patient a KT/V index = 1 in a dialysis session of less than 180 mins. Beta 2 microglobulin clearances varied from 35 to 50 ml/min depending on UF rate and a significant adsorption on the membrane was detected. This membrane seems to join the advantages of other synthetic membranes reducing the risks of backfiltration and the need for UFC systems.

ALUMINUM (AL)-CITRATE (C) INTERACTION IN END STAGE RENAL DISEASE (ESRD). David Rudy*, Domenic Sica, Thomas Comstock*, Judy Davis*, John Savory*, Anton Schoolwerth. Depts. of Med. and Pharm., Medical College of Virginia, Richmond, VA and Dept. of Path., Univ. Virginia, Charlottesville, VA.

It has been suggested that the concomitant ingestion of AL with C results in elevations in blood, tissue and urine AL levels. Thus, we examined the influence of C on AL absorption in 6 patients undergoing hemodialysis (HD) in a 4 limb study: (I-1600 mg of oral $Al(OH)_3$ gel; II-1600 mg of oral $Al(OH)_3$ gel + 30cc Bicitra containing 3 gms Na citrate dihydrate and 2004 mg citric acid monohydrate; III- 30cc Bicitra; IV-1600 mg of oral $Al(OH)_3$ gel and 2400 mg oral $NaHCO_3$) on an off-dialysis day. Al samples obtained over the next 24 hours were measured by graphite furnace atomic emission spectrometry and are reported as mean ± SEM (* = $p < 0.05$).

LIMB	AUC_{0-8} (ug*hr/L)	Al_0 (ug/L)	Al_{24} (ug/L)
I	16 ± 30	32 ± 11	29 ± 10
II	73 ± 23*	34 ± 10	54 ± 11
III	-27 ± 14	71 ± 33	58 ± 30
IV	6 ± 22	63 ± 31	61 ± 34

We conclude that Al absorption is significantly increased in the presence of C. In addition, increments in serum Al appear to persist longer when C has been previously ingested. The ingestion of C-containing products with Al preparations appears to be contraindicated in ESRD patients.

VASCULAR VACCESS FOR HEMODIALYSIS IN GERIATRIC PATIENTS, S.A. Sadjadi, R.M. Shah, M.P. Dhakal, VA Medical Center, Wilkes-Barre, PA.

Geriatric patients usually have atherosclerotic vessels and a high incidence of Diabetes Mellitus. Due to this, construction of an A-V fistulas is usually difficult and the established fistulas do not last long. Over the past 2 years we have treated 40 elderly patients with hemodialysis. During this time 65 Femoral, 52 Subclavian, and 2 internal jugular vein catheterizations have been performed. Two patients had 8 catheterizations of the subclavian veins. No pneumothorax or catheter insertion site infection was noted. Catheter tip culture was positive in one patient and he improved promptly upon the subclavian catheter removal. No patient developed clinical evidence of subclavian vein stenosis or thrombosis. Patients were able to go home and we were able to wait until a vascular access is matured or in cases of thrombosed accesses, until a new access is constructed. The subclavian catheters were functional for 4-6 weeks and the femoral catheters for 7-10 days.

Conclusions:
1) Acute vascular access can be easily established in elderly patients.
2) Subclavian vein access is easy to establish and is most convenient for the patient.
3) Femoral vein access is most suitable for emergencies and not so good for long term use.
4) Internal jugular vein catheters are easy to insert but are inconvenient for long term use.

QUANTITATIVE MEASURES ARE NEEDED TO ASSURE QUALITY PATIENT CARE IN DIALYSIS. John A. Sargent. Quantitative Medical Systems, Emeryville, CA

This project determines in representative facilities if prescribed dialysis is delivered and if it is adequate. BUN drop from expected treatment (duration T; diayzer clearance K) is analyzed with urea kinetics to calculate urea volume (Vk) for 180 patients in 28 dialysis centers across the US. Vk is estimated (Ve) from height, weight and sex. If $|(Vk-Ve)/Ve| \geq .2$ it is concluded that there is substantial difference between the calculation parameters (BUNs, T, or K) and the actual values. The BUNs consistent with success in the National Cooperative Dialysis Study (NCDS) are compared to the BUNS experience with these patients to assess the adequacy of the patient's treatment (whether delivered or not). In 18% of facilities there was no treatment compromise. In the rest, 44% (18) (sd) of treatments (range 17% to 82%) were not delivered as expected. In 14% of the units all treatments were within the adequacy range of the NCDS. In the rest, 38% (22) of treatments were outside the NCDS adequate range. Of these, half corresponded to "non-delivery" of treatment; 26% were due to high protein intakes not matched by dialysis; 23% were for other reasons with half for patients with low protein intakes and presumed shorter treatments due to inappropriate interpretation of low BUNs.

Large numbers of dialyses are not delivered as intended although the percent varies from 0 to 82 for the facilities analyzed. Similar numbers of treatments are out of NCDS adequacy ranges. With current emphasis on reduced time dialysis and rapid blood flow, the reproducible delivery of the desired treatment is essential. For reliable quality assurance in dialysis, quantitative methods must be employed.

FACTORS AFFECTING MODALITY CHOICE FOR ACUTE DIALYSIS. Stephen Sandroni, John Crump*, Kathryn Koch*. University Hospital, Jacksonville, Florida.

New modalities such as CAVH are often considered options in treating acute renal failure. Factors favoring these modalities include hemodynamic instability and reduced dependence on dialysis nurses and machines. Potential disadvantages include use of more blood vessels, prolonged use of blood vessels, and reduced flexibility for transport of patients requiring multiple procedures. We reviewed our acute dialysis experience at a single center that supports several intensive care units (ICU), cardiac surgery and a regional trauma service. Of 195 acute treatments with high-efficiency membranes and bicarbonate bath only three could not be completed due to hypotension. Pressor dependence did not predict difficulties with hypotension during dialysis. Central venous or arterial catheters (exclusive of dialysis lines) averaged two per patient. In the medical ICU setting only 9% of patients had two or more catheters simultaneously but 30% of trauma patients did at the time of dialysis. Average duration of non-dialysis central venous or arterial catheters was 3.5 days--an important issue for surgical ICU patients with renal failure, who averaged 20.3 days in the ICU, with multiple changes of catheter sites. Surgical ICU patients averaged 2.6 surgical procedures during their ICU stay, with attendant transportation needs. We found CAVH, CAVHD, or VVH to be most useful in cardiac patients with renal failure but that contemporary hemodialysis using a single double-lumen catheter is well tolerated in most patients and logistically preferable for many surgical ICU patients.

LONG TERM EFFECT OF CONVENTIONAL AND HIGH FLUX MEMBRANES ON ELECTROPHYSIOLOGIC (EP) INDICES IN HEMODIALYSIS PATIENTS. Boutrous Sawaya*, Robert Provenzano*, Robert Simkins*, Gregory Barkley*, and Nathan W. Levin. Departments of Medicine and Neurology, Henry Ford Hospital, Detroit, Michigan.

Cognitive central nervous system (CNS) abnormalities are characteristic of uremia. These changes are related to the uremic milieu and are only partially reversed by conventional dialysis. The advent of new membranes permitting a substantial flux of compounds of molecular weight up to 20,000 has facilitated comparison with conventional membranes. We compared the effect of saponified cellulose ester and polysulfone membranes using the Sieger Neuroscope utilizing flash visual evoked potentials (VEP) as a measure of dialyzer efficacy.

Eight stable hemodialysis patients were randomly assigned to each dialyzer group. VEP studies were performed at baseline, after 1, 2 and 4 weeks and every 12 weeks thereafter for 18 months. Investigators were blinded to the type of dialyzers used.

	Wave 4 Latency (msec)	
	Baseline	Change at 18 months
Conventional	124.5±15.6	+5.4±5.1
High Flux	134.7±9.7	-5.3±6.2*

*(P=0.039 when compared to conventional)

We conclude that high flux dialysis improves EP response while deterioration occurs with conventional membranes. This provides an insight into the CNS manifestations of uremia.

IMPROVED SEXUAL FUNCTION IN UREMICS ON ERYTHROPOIETIN. A POSSIBLE ROLE FOR PROLACTIN

Roland M. Schaefer[1], Franczicek Kokot[2], August Heidland[1] (intr. by S.G. Massry); Dept. of Internal Med., Universities of [1]Würzburg, FRG and [2]Katowice, Poland.

As it was reported that correction of anemia in long-term dialysis patients by recombinant human erythropoietin (r-HuEPO) is associated with improved sexual function, we conducted the present study to further dilineate the underlying mechanism(s). Serum levels of prolactin, testosterone and parathyroid hormone (PTH) were followed by radioimmunoassays during 4 months of r-HuEPO therapy in 16 dialysis patients (7 males, 9 females). Within 4 months of treatment hematocrit levels rose from 24 ± 1 to 36 ± 0.2 %, while hemoglobin values increased from 7.3 ± 0.3 to 11.3 ± 0.4 g/dl. In parallel, serum prolactin values decreased from 66.9 ± 9.3 to 9.6 ± 2.6 ng/ml ($p < 0.001$) in females and from 39.5 ± 10.5 to 10.3 ± 1.0 ng/ml ($p < 0.001$) in male patients. Testosterone concentrations were in the lower normal range in males and remained unchanged during r-HuEPO therapy. Baseline PTH values were elevated (1,880 ± 220 pg/ml) in patients of both sexes and declined to 1,410 ± 180 pg/ml (n. sign.) after 4 months of r-HuEPO. Sexual function improved in 4 out of 7 males and 5 out of 9 female patients started to have regular menstruations again. It appears that the striking reduction of serum prolactin values may have contributed to improved sexual function in dialysis patients treated by r-HuEPO. Other factors like lower serum PTH values or an increased sense of well-being could also be of clinical relevance.

BICART DIALYSIS - A NEW AND SAFER WAY TO PERFORM BICARBONATE HEMODIALYSIS.

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Bicarbonate (HCO_3) dialysis (HD) has attracted much attention in the recent past, especially for critically ill patients (pts). On the other hand, it has repeatedly been reported that HD fluid containing HCO_3 was found to be contaminated with bacteria, these resulting in side-effects such as fever or shock, especially when more open membranes were applied. In contrast, BiCart HD is performed using a polypropylene column containing HCO_3 powder which allows for on-line production of liquid HCO_3 concentrate. Besides the fact that the HCO_3 cartridge is "cleaner" than the normally used liquid HCO_3 concentrate, we were impressed by the ease with which this device can be handled. In addition, several clinical studies (n = 70 per parameter) revealed perfect operation of the BiCart. In 7 pts the following blood data were recorded: ph before HD (b): 7.37 ± 0.10; after HD (a): 7.48 ± 0.06; HCO_3: 22.7 ± 3.2 mmol/l (b); 28.1 ± 2.0 mmol/l (a); pCO_2: 38.7 ± 4.3 mm Hg (b); 37.7 ± 4.5 mm Hg (a); pO_2: 74.0 ± 13.5 mm Hg (b); 76.6 ± 16.1 mm Hg (a). Data obtained from the HD fluid remained constant during the treatment: ph: 7.24 ± 0.02; HCO_3: 36.4 ± 1.2 mmol/l; pCO_2: 81.9 ± 9.1 mm Hg.

In conclusion: 1) BiCart dialysis represents a new, safer and easier way to perform HCO_3 HD.
2) The cartridge operates without problems and should reduce the side effects of HCO_3-HD related to bacteriological contamination.

SOLUBLE INTERLEUKIN-2 RECEPTOR (IL-2R) SERUM CONCENTRATIONS IN ENDSTAGE RENAL FAILURE.

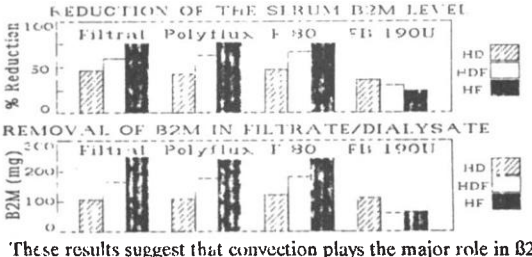
A Schwarz*, U Kunzendorf*, G Walz*, O Josimovic-Alasevic*, G Offermann* (intr. by TB Strom), Dept. of Internal Medicine and Immunology, Free University of Berlin, FRG.

Long-term hemodialysis (HD) results in a low-grade inflammatory process manifested by activation of the complement system and activation of macrophages and T lymphocytes. Activated T-cells express and secrete (soluble) IL-2R, the 55 kD IL-2 binding protein. Elvated serum concentrations of IL-2R were detected in 58 of 68 hemodialysis patients (ELISA). A simultaneous rise of IL-2R and B2-microglobulin serum concentrations occurred during hemodialysis (cuprophan) ($p<0.05$), suggesting that dialysis, per se, incites shedding of both surface proteins. 42 HD patients were vaccinated against hepatitis B. A significant increase of anti-HBsAG antibodies correlated with low IL-2R serum concentrations ($p <0.05$). 16 HD patients with past or present carpal tunnel syndrome evidenced significantly elevated IL-2R serum concentrations ($p <0.005$), when compared to 49 HD patients without a detectable dialysis-associated amyloidosis. The presence of activated T-cells (assessed by IL-2R serum concentrations) may contribute to impaired immunity in uremia and dialysis-associated amyloidosis.

HIGH-FLUX SYNTHETIC VERSUS CELLULOSIC MEMBRANES FOR REMOVAL OF ß2-MICROGLOBULIN (ß2M) DURING HD,HDF OR HF

S.Shaldon*, J.Floege*, C.Granolleras*, G.Deschodt*, G.Baudin*, KM.Koch* (Intr.by C.Olbricht). Depts.Nephrology, Univ.Hospitals Hannover,FRG, Nimes and Montpellier,France.

Efficient removal of ß2M in ESRD patients is a continuing preoccupation, as the incidence and severity of dialysis associated amyloidosis are increasing. To evaluate comparative ß2M removal we studied 6 stable ESRD patients during 3hr hemodialysis (HD), hemodiafiltration (HDF; UF rate 130ml/min), and hemofiltration (HF; UF rate 170ml/min) using the capillary devices FILTRAL 1.6m² (acrylonitrile, Hospal), POLYFLUX 1.6m² (polyamide, Gambro), HF80 1.8m² (polysulphone, Fresenius) and FB 190U 1.9m² (cellulose-triacetate, Nipro). Bloodflow was 450±35ml/min and equal in all modalities. Dialysateflow was 500ml/min (HD) and 400ml/min (HDF). Weight loss was not different during the three treatment modalities. ß2M levels were measured (RIA) in pre- and post-treatment serum samples and in the pooled ultrafiltrate/dialysate. ß2M adsorption as measured by recirculation during sham dialysis prior to treatment averaged 95mg (FILTRAL) and <50mg (POLYFLUX, F80, FB 190U). Further results are shown in the following figures.



These results suggest that convection plays the major role in ß2M removal when high flux synthetic membranes are used. In contrast, using high flux cellulosic membranes, ß2M removal is diminished when UF-rates are increased. To achieve negative balance (assuming normal ß2M generation 20mg/kg/week) HF or HDF (at equivalent UF rate) and high flux synthetic membranes will be needed rather than HD and high flux cellulosic membranes.

DISADVANTAGES AND RISKS OF NORMAL HEMATOCRIT (HCT) HEMODIALYSIS (HD).

J. H. Shinaberger, J. H. Miller, P. W. Gardner*. West L A VA Medical Center and UCLA Depts. Med. Los Angeles, CA

Human recombinant erythropoietin is expected to produce normal or elevated Hcts in those dialysis pts receiving it. Our studies indicate that whole blood clearances (KD), calculated from whole blood flows (QB) and plasma solute concentrations produce a discrepancy between KD and actual mass removed unless the relative volume of distribution of RBC solute equals that of plasma H_2O (PH_2O) and the diffusion of RBC solutes into PH_2O is instantaneous. This discrepancy is not apparent without dialyzer mass balance studies. Of clinically relevant solutes, these conditions are approximated only by urea, currently used in modeling adequate treatment. For the critical solutes K+ and PO_4 only the use of QPH_2O in clearance calculations results in dialyzer mass balance. The relation between dialyzer perfusion flow rates and clearance is such that at KoA 690, QB 300 ml/min, increasing Hct from 20% to 40% decreases K+ removal at least 19% and already poor PO_4 removal 10% during apparently urea adequate dialysis. Thus, unexpected hyperkalemia and worsened hyperphosphatemia may result from Hct increase. During 3 hr. high efficiency dialysis and UF initial systemic Hct is further increased by loss of PH_2O (15% / 5% body weight loss, r = .82). Intradialyzer Hct and viscosity elevation are more radical, increasing blood compartment pressures so that most dialysate machines cannot provide adequate positive dialysate pressure to maintain low UFRs at the high QB's required. Pre-existing diffuse and segmental vascular disease, post-dialysis hypotension and hyperviscosity may produce disastrous syndromes of organ ischemia, thrombosis or infarction. As pt Hct rises above 36% extreme caution and adjustment of dialysis regimen is recommended.

CHANGES IN PLASMA ATRIAL NATRIURETIC PEPTIDE (ANP) DURING HEMODIALYSIS (HD).

Nobuhiro Sugino, Kazuo Kubo, Akira Nishida, Hiroshi Nihei. Tokyo Women's Medical College, Department of Medicine, Kidney Center, Tokyo, Japan

It is generally known that plasma immunoreactive ANP levels are elevated in patients with endstage renal failure. The reduction of plasma ANP levels are also observed during conventional HD, probably due to either the loss of ANP though the artificial membrane or the decrease in ANP production secondary to volume contraction of the body fluid. In order to clarify the mechanism, the protocol of the sequential ultrafiltration (UF) and HD are set up. PMM membranes are used both for UF and HD. Average plasma ANP level of 8 patients undertaking longterm stable HD was 63.6 pg/ml before UF or HD. After 2-hour UF, plasma ANP level was decreased by 44.3 % ($p<0.01$) in association with 4.3 % decrease in body weight (BW) and 10.2 % reduction of mean blood pressure (MBP). In contrast, there were no significant changes in plasma ANP levels, BW and MBP following 2-hour HD without UF. However, simultaneous 2-hour HD with UF induced 22.1 % decrease in plasma ANP level ($p<0.05$) in association with 2.1 % decrease in BW but no change in MBP.

Therefore, it is concluded that the major factor of decreasing plasma ANP levels in longterm hemodialysis patients is a rapid volume contraction of body fluids.

LONG-TERM EFFECTS OF $CaCO_3$ AND 2.5 mEq/L Ca DIALYSATE ON MINERAL METABOLISM IN HEMODIALYSIS PATIENTS.

E. Slatopolsky, C. Weerts*, K. Norwood*, K. Giles*, P. Fryer*, J. Finch*, D. Windus and J. Delmez. Washington Univ. School of Medicine, Dept. of Medicine, St. Louis, MO. 63110.

Many investigators have shown that $CaCO_3$ is an effective phosphate-binder which also prevents the potential disabling effects of aluminum accumulation. However, hypercalcemia may develop in a substantial number of patients. Thus, to control serum PO_4 and prevent hypercalcemia, we performed studies in 21 patients on maintenance hemodialysis in which, in addition to the oral administration of $CaCO_3$, the concentration of calcium in the dialysate was reduced from 3.25 to 2.5 mEq/L. The studies were divided in three periods. I. Control, on Al-binders (one month), II. No Al-binders (one month), III. $CaCO_3$ 3.75 to 18 gms/day (seven months). Blood was obtained three times/week before dialysis.

Period	I	II	III
	n = 252	n = 252	n = 1092
PO_4	5.03 ± 0.07	7.29 ± 0.11	4.95 ± 0.06
Ca	8.86 ± 0.08	8.65 ± 0.07	9.19 ± 0.07

During $CaCO_3$ administration, serum Al decreased from 64.2 ± 8.5 to 37.1 ± 3.6 and 25.1 ± 3.0 µg/L (at three and seven months respectively). Serum PTH decreased by 20%. In summary: lowering dialysate Ca to 2.5 mEq/L allowed the long-term administration of large doses of $CaCO_3$. This corrected the hyperphosphatemia without the development of hypercalcemia or worsening of the secondary hyperparathyroidism. Al levels returned toward normal. Thus, by utilizing this maneuver aluminum binders are unnecessary in almost all patients on maintenance hemodialysis.

AUGMENTATION OF PLASMA RENIN ACTIVITY(PRA) IN PATIENTS UNDER MAINTENANCE HEMODIALYSIS(HD).

N Takahashi*, G Kimura, Y Kawano*, M Imanishi*, T Sanai*, S Kojima*, T Inoue*, M Kuramochi*, T Omae*. National Cardiovascular Center, Osaka, Japan

Although renin secretion is believed to continue even after the renal excretory function has deteriorated, yet there are no data available about long-term follow up of PRA in HD patients.

Mean arterial pressure (MAP) and PRA were followed up for 8-10 years after introduction to HD in 25 patients. PRA was significantly increased from 3.0±0.5 to 5.8±0.8 ng/ml/hr during follow up period, while MAP reduced. Increase in PRA was strongly correlated with reduction in MAP ($r=0.63$, $p<.001$). PRA immediately before the introduction to HD had negative relationships with increase in PRA ($r=-0.46$, $p<.05$) and with reduction in MAP ($r=-0.40$, $p<.05$). PRA was increased and MAP was markedly reduced in patients whose PRA immediately before the introduction was lower than approximately 6 ng/ml/hr, while PRA was decreased and MAP was not altered in patients whose PRA was higher.

Our results show renin secretion continues even after disuse atrophy of the kidney and almost complete deterioration of its excretory function. Increase in renin secretion, despite depression of sympathetic nerve activity in long-term HD patients, may be due to stimulation of baroreceptor mechanism, since increase in PRA was correlated with reduction in MAP, suggesting renin plays an important role in blood pressure regulation on maintenance phase of HD therapy. Based on PRA before the introduction to HD, blood pressure response to maintenance HD seemed predictable.

PSYCHOSOCIAL ADJUSTMENT IN FULL-CARE VERSUS SELF-CARE HEMODIALYSIS. Thomas E. Talley, Jaine Strauss*, Edward Deci*, Steven Freilich*, Beth Martin*, and Sheila Williams*. University Of Rochester and Regional Kidney Services Centers, Rochester, New York.

This study was designed to learn wether the features associated with self-care dialysis (SC) (autonomy, choice, competence, and control), in themselves aid patient (pt) adaptation to chronic illness and even augment pt psychosocial and adaptive mechanisms in life situations outside the treatment setting.

Thirty full-care (FC) and thirty SC hemodialysis (HD) pts. participated. All had been on HD for at least 5 months. The two groups did not differ in sex, race, marital status, socioeconomic status, primary ESRD diagnosis or months on HD. There was a marginally significant difference in terms of age.

Psychosocial outcome was assessed using the Center for Epidemiologic Studies Depression Scale and the Social Adjustment Scale-Self-Report. In addition, the Ward Atmosphere Scale-Hemodialysis Version, the Multidimensional Health Locus of Control Scale, the State-Trait Anxiety Inventory and a measure of coping style (COPES) were used.

The WAS and MHLC confirmed that SC pts. saw themselves as more autonomous in terms of their dialysis and general health than did FC pts. However, the two groups did not differ on depression or social adjustment but expressed the full range of adjustment. Prospective tests may offer the identification of the optimal treatment modality for each individual pt.

ABSENCE OF Al+++ OSTEOMALACIA AFTER DEFEROXAMINE (DFO) THERAPY DESPITE POSITIVE DFO INFUSION TESTS, Charles E. Thomas*, Charles J. Diskin, Opelika Nephrology Referral Center, Opelika, Alabama, Bruce A. Julian, Marie E. Beckner, Univ. of Alabama at Birmingham, Birmingham, Alabama.

Increased serum Al+++ after DFO stimulation is widely used to detect Al+++ associated osteomalacia in patients with renal failure. We assessed bone histology in patients who had positive DFO infusion tests after a therapeutic course of DFO chelation. Ten patients on chronic dialysis exhibited clinical features suggestive of Al+++ toxicity and had positive DFO infusion tests. $Al(OH)_3$ containing medications were discontinued and dialysate Al+++ was less than .005 mg/L. The patients were treated with DFO chelation (34±5 (SE) gm over 3 to 15 months). One week after the last DFO dose DFO infusion tests were repeated. Serum Al+++ increased from 290±7 to 482±8 ug/L ($p<.05$) (Biotrace, Denver, CO). Shortly thereafter trans-iliac bone biopsies were performed after double tetracycline labeling. All biopsies had less than 1% trabecular surface staining for Al+++. Mixed renal osteodystrophy was found in 7 specimens and osteomalacia in 3. The "diagnostic" DFO infusion tests did not predict Al+++ associated osteomalacia in these patients. Thus, positive DFO infusion tests are not a sufficient basis to continue chelation therapy.

EFFECT OF HIGH FLUX HEMODIALYSIS ON SERUM BETA 2 MICROGLOBULIN (B2M) CONCENTRATION IN PATIENTS WITH AND WITHOUT RESIDUAL RENAL FUNCTION. Jukaku Tayeb*, Gerard Zasuwa*, Francis Dumler and Nathan W. Levin. Division of Nephrology, Henry Ford Hospital, Detroit, Michigan.

Predialysis serum B2M concentrations were measured monthly in 63 patients undergoing high flux hemodialysis (HFHD) using polysulfone membranes over an 8-month period. Measurable renal function (2.0±2.2 ml/min) was present in 25 patients. The results in patients with (RRF+) and without (RRF-) residual function and in the combined group (RF+/-) were as follows:

	B2M Concentration (mg/L)		
Patient Group	Baseline	1 Month	8 Months
RRF- (n=38)	44±12*	38±8*	38±13
RRF+ (n=25)	34±17	28±10	35±15
RF+/- (n=63)	40±15	33±10	37±13

(*$p < 0.05$ when compared to RRF+)

Compared to conventional hemodialysis (baseline) there was an initial decrease in B2M in all groups, although statistical difference (P .01) was attained only in the combined group. However, over the period of the study B2M increased in RRF+ patients without significant change in the RRF- group. It is concluded that high flux dialysis results in an immediate improvement in B2M concentration but that subsequent changes must be interpreted in relationship to residual renal function.

ADVERSE DRUG EFFECTS OF RECOMBINANT HUMAN ERYTHROPOIETIN (r-HuEPO). F. Valderrabano*(intr: by F. Llach). Univ. Complutense. Hospital General Gregorio Maranon. Madrid, Spain.

The adverse effects of r-HuEPO in the treatment of anemia in 150 maintenance hemodialysis pts were analyzed in a multi-center European trial. Pts were stable for a period of at least 3 months. Hemoglobin (Hgb) and hematocrit were lower than 30% and r-HuEPO was given thrice weekly at escalating doses of 24,48, 96, and 192 units/kg i.v. until Hgb increased to > 2 g/dl, and subsequent steps of 24 units/kg until Hgb was > 10 g/dl. Pain, pyrexia or chills occurred in 20 pts (13.3%). Seven pts had bone pain, and 5 had associated back pain (flu-like syndrome). Hypertension, requiring start or adjustment of antihypertensive therapy was observed in 31 pts (20.7%); 17 hypertensive episodes were observed in pts already on therapy at the time of the event. One pt was discontinued from the study due to intractable hypertension. Thrombosis of the vascular access requiring surgical or thrombolytic therapy was observed in 14 pts (9.3%). Pruritus occurred in 14 cases (9.3%), and severe allergic reaction in 1 (0.7%). Headache was observed in 18 pts (12%) and 5 pts (3.3%) had problems maintaining "ideal" weight. One pt had a mild petechial purpura and another, acute abdominal pain. Antibodies to r-HuEPO were not detected. Three pts died of intracerebral bleeding, myocardial infarction and cardiac dysrhythmia.

In summary, adverse side effects of r-HuEPO were not infrequent and may be related to the increase in hematocrit.

PREDICTING IRON STATUS IN PATIENTS RECEIVING ERYTHROPOIETIN FOR DIALYSIS-ASSOCIATED ANEMIA. DB Van Wyck,* J Stivelman, L Kirlin,* J Ruiz,* MA Katz and DA Ogden. Univ. of Arizona Dept. of Med., and VA Med. Ctr., Tucson, Arizona.

We developed a method to quantify iron stores in anemic hemodialysis patients receiving acute erythropoietin therapy (rHuEPO, Amgen; 150-300 mg/kg thrice weekly). We calculated projected iron needed for new hemoglobin (Hb) synthesis from the difference between initial and target Hb concentrations; initial iron reserves from the logarithm of initial serum ferritin levels; and net projected surplus or deficit from the difference between needs and reserves. Among 27 patients, despite pretreatment indices of iron sufficiency (normocytosis, transferrin saturation >20%, ferritin >100 μg/l), we predicted 22 would develop iron deficiency (mean projected deficit 268±70 mg; mean±sd); in fact, 20 showed evidence of exhausted iron stores (transferrin saturation <16% or ferritin <30 μg/l) before reaching target Hb; 2 predicted to become deficient (projected deficit <100 mg) did not; and all 5 predicted to avoid iron deficiency (mean projected surplus 177±20 mg) remained iron replete. During acute rHuEPO therapy we calculated that net body iron balance remained neutral in patients receiving no iron supplements and increased 5 mg/d in patients prescribed oral ferrous sulphate. Moreover, in patients given iv iron dextran, less than 50% of elemental iron administered became measurable as iron stores or usable for Hb synthesis. Thus, though iron deficiency is common during acute rHuEPO therapy, simple formulae can be used to quantify projected iron deficits and guide iron supplementation.

ENHANCEMENT OF NEUTROPHIL (PMN) OXIDATIVE BURST BY DIALYSIS MEMBRANES IS COMPLEMENT DEPENDENT. R.A. Ward, S.R. Wellhausen,* E.A. Hagan,* and M.K. Tanner.* Dept. Medicine, Univ. Louisville, Louisville, Kentucky.

Exposure of blood to dialysis membranes activates complement. Concomitant stimulation of PMN oxidative burst has also been reported. An enhanced oxidative burst may lead to endothelial damage in dialysis patients. We hypothesized that stimulation of PMN oxidative burst results from complement activation. We tested our hypothesis using an alloy of polysulfone and polyvinylpyrrolidone as a model membrane. Membrane was incubated in vitro with normal blood components for 60 min at 37C. Complement fragments, C3a and C5a, were measured by RIA. Oxidative burst was assessed by using flow cytometry to measure H_2O_2 production by PMN after phagocytosis of Staphylococcus aureus. Incubation with plasma increased C3a and C5a to 2.98 ± 1.83 μg/ml and 33.5 ± 8.4 ng/ml, respectively, relative to blank controls. H_2O_2 production increased to 178 ± 42% of the blank control value after incubation with whole blood. Incubation with isolated PMN in buffer (5x 10^6 PMN/ml) caused no increase in H_2O_2 production relative to blank controls. Finally, incubation with cells resuspended in heat-inactivated serum (56C for 60 min) caused no increase in H_2O_2 production relative to blank controls. In all PMN experiments, incubation with LPS enhanced H_2O_2 production, demonstrating the functional integrity of the PMN. Our data show that membrane enhancement of PMN oxidative burst is mediated by a heat-sensitive, serum component. Since the membrane activated complement and C5a stimulates PMN, we suggest that the component is complement.

INCREASE OF HDL TRIGLYCERIDES IS RESPONSIBLE FOR THE ANTIKETOGENIC ACTION OF 'HIGH-DOSE' L-CARNITINE IN HEMODIALYSIS PATIENTS. Christoph Wanner, Heinrich Wieland, Peter Schollmeyer, and Walter H. Hörl: (intr. by Thomas Lüscher). Univ. of Freiburg, Dept. of Medicine, Div. of Nephrology, Freiburg, FRG.

The ketogenic and antiketogenic actions of L-carnitine therapy in hemodialysis (HD) patients have not fully been elucidated. Clofibrate exerts its hypotriglyceridemic effect, in part, through the activation of hepatic peroxisomal, mitochondrial and microsomal carnitine acyltransferases.

Therefore, we investigated the effect of L-carnitine (15 mg/kg body weight at the end of each HD) alone (n=15) or in combination with Beclobrate (n=15), a new hypolipidemic agent related to the "fibrate"-group (100 mg/day), on serum lipid profile in 30 patients with hyperlipidemia type IV during a 3 months trial. Plasma free and esterified carnitine were determined with a modified radiochemical-enzymatic assay, cholesterol and triglycerides (TG) by fully enzymatic methods after isolation of VLDL by ultracentrifugation.

Markedly increased plasma free and esterified carnitine levels were not different in both groups of patients after treatment. L-carnitine caused significantly elevation of HDL-TG (35.9 ± 4.0 vs 60.3 ± 9.1 mg/dl; p<0.01) after 3 months. The same effect was observed when combination therapy was used (35.6 ± 6.0 vs 55.2 ± 6.4 mg/dl; p<0.01). However, VLDL-TG significantly decreased in 7 and increased in 8 patients under L-carnitine. LDL-TG remained unchanged in both groups.

The increase of HDL-TG after L-carnitine blunts the ketogenic response of VLDL-TG. Further improvement of hypertriglyceridemia could not be achieved through the stimulation of carnitine transport systems. Therefore, it is unlikely that antiketogenic responses to L-carnitine depend on carnitine acyltransferases rather than on other metabolic disorders in uremic patients.

RECOMBINANT ERYTHROPOIETIN (r-EPO) IMPROVES COGNITIVE FUNCTION (CF) AND QUALITY OF LIFE (QL) OF CHRONIC HEMODIALYSIS (CHD) PATIENTS (PTS). D.L. Wolcott*, S. Schweitzer*, A.R. Nissenson, UCLA School of Medicine, Los Angeles, Ca.

Anemia may impair QL and CF in pts. on CHD. In order to test this hypothesis we studied 17 pts. prior to EPO treatment (T1), after normalization of hematocrit 3-6 months later (T2) and 10-15 months after study entrance (T3). Variables included biochemical status, CF, subjective health status, mood state, dialysis-related stresses, self esteem, vocational function, and social/leisure activity participation. Pts. were predominantly Caucasian (77%), non-diabetic (82%), averaged 42.1 years old, and had been on dialysis an average of 8 1/2 years. At T2 and T3 pts. had a significant rise in Hct (P<.001), a progressive and significant improvement in Simmons self esteem scale scores, and mood disturbance and general dialysis stress scores. There was improvement in CF scores including symbol-digits modality and trailmaking. The psychological benefits of r-EPO treatment appear to be significant and progressive, and CF appears to also be improved with normalization of HCT. Chronic anemia may therefore contribute to uremic encephalopathy as well as impair QL and this can be improved with r-EPO.

HEMODYNAMIC INSTABILITY (HI) OF HEMODIALYSIS (HD) IS NOT MEDIATED BY ANAPHYLATOXINS (A) OR IMPAIRED NOREPINEPHRINE (NE) RELEASE. M.G. Ziegler*, S.D. Fox*, L.W. Henderson. Univ. of Calif. and VA Med. Ctr., San Diego, CA.

Previous studies of hemodynamic responses to fluid removal during HD and HF have verified the improved vascular stability with HF. HI during HD has been attributed to inadequate increase of systemic vascular resistance (SVR) and impaired NE release. We examined hemodynamic changes during HD and HF in a cross-over study of 9 patients using impedance cardiography (IC), at matched fluid removal rates. Acetate, high sodium (144 meq/L) and PAN (Hospal) membrane were used in all treatments (Rx). Hemodynamic parameters and NE were determined serially during each Rx and clearances (C) of urea and NE were measured. Hemodynamic changes were examined in relation to changes in NE and osmolality. Results (% change pre- to end-Rx), $\bar{x}$ (SEM):

	MAP	CI	HR	SVRI	NE
HF	-4(3)	-3(6)	+25(5)	+3(7)	+60(21)
HD	-10(4)	+28(8)	+48(8)	-27(6)	+170(71)
p*	NS	<.001	<.01	<.001	NS

*paired t-test. End-Rx NE, [Na], osmolality, anion gap, and bicarbonate were not statistically different, whereas plasma calcium was significantly ($p<.01$) higher at end-HD. NE C were similar despite greater urea C end-HD ($p<.001$). We conclude that: 1) HI during HD can be dissociated from changes in NE, osmolality, or differences in A generation; 2) Hemodynamic parameters obtained by IC compare favorably with previously reported invasive determinations. Our data are consistent with convective clearance of an abnormal vasodepressor macromolecule.

TUMORAL CALCINOSIS IN HEMODIALYSIS PATIENTS (HD PTS): ROLE OF ALUMINUM. B. Zins*, T. Petitclerc*, C. Basile*, P. Ureña*, T. Bardin*, J. Zingraff*, T. Drüeke* (intr. by D.A. McCarron). Dépt. Néphrologie, Höp. Necker; Sce de Rhumatologie, Höp. Lariboisiere, Paris, France.

Extraskeletal calcifications (C) are often seen in uremia. Among them, periarticular tumoral C (TC) represent multiloculated calcium-phosphate (Ca-P) deposits with a diameter > 3 cm. We report here the analysis of 12 cases of TC in HD pts (all males) observed since 1970. Moderate (4), mild (2), and severe (1) osteitis fibrosa was observed in 7/11 bone biopsies. TC disappeared in only 1/8 pts after parathyroidectomy (PTx). TC occurred in 1 pt after PTx. Hyperphosphatemia was constant in all pts and increased Ca-P product was found in 75%. Serum alkaline phosphatase activity was normal in all pts. Evidence of aluminum (Al) intoxication was found on the basis of bone histology showing moderate (4), mild (2), and severe (2) osteomalacia, bone histochemistry (+ Aluminon staining in 9/11), bone Al content (increased in 6/6) and increased serum Al levels either at the basal state or after a deferoxamine test (+ in 4/7). The different treatment strategies were unable to modify notably the course of TC, including PTx which was successful in only 1 case. Thus secondary hyperparathyroidism is not a necessary prerequisite for TC development in HD pts. PTx must not be performed on the sole indication of TC. We hypothesize that a pre-existing mineralization defect such as that due to Al intoxication may be associated with other factors inducing hyperphosphatemia in order to generate TC. Al intoxication must be carefully looked for in the presence of TC.

EFFECT OF DIALYSATE COMPOSITION ON THE MUSCLE RESPONSE TO L-CARNITINE (C) SUPPLEMENTATION DURING HEMODIALYSIS (HD). Gaston Zilleruelo, Pedro L. Ferrer, Milan Novak, Carolyn Abitbol, Jose Strauss. Univ. of Miami Sch. of Med., Dept. of Pedi., Miami, Fl.

Cumulative C losses through dialysis membranes may affect muscle function during long-term HD. However, the role of C supplementation in improving skeletal muscle or myocardial performance remains controversial. We have compared in a randomized, double-blind study the effect of dialysate buffer composition (acetate or bicarbonate) on muscle function in 9 patients ($\bar{x}$ age 19 years, range 14-23) undergoing HD. Voluntary muscle function was evaluated quantitatively with timed-stand tests and a questionnaire on muscle related symptoms. Left ventricular (LV) performance was evaluated by multiple parameters obtained by M-mode echocardiograms. Measurements were obtained at baseline and after receiving 2 g L-carnitine or placebo added to dialysis bath for 3 months. Patients on acetate and bicarbonate HD had a significant increase in plasma carnitine levels after C supplementation. Both supplemented groups had a significant improvement in lower extremities strength and in muscle related symptomatology. Patients on bicarbonate HD had better LV systolic function than acetate HD. Although C supplementation improved LV systolic function in both groups, effect was greater on acetate HD. These results suggest that C supplementation may be beneficial for muscle related symptomatology in patients on HD. Effects on myocardial function appeared influenced by dialysate buffer.

TRANSFER FROM CAPD TO HEMODIALYSIS:ONE CENTER'S NINE YEAR EXPERIENCE. H.B. Carey*, A.S. Kliger, K. Cooper, S. Santacroce*, J. Rifkin*, N. Brennan*, F.O. Finkelstein. Yale Univ. Dept. of Medicine, Hospital of St. Raphael, New Haven, CT.

Transfer to hemodialysis, TSFR, remains the major factor in limiting technique survival on CAPD (death excluded). The reasons for TSFR and length of time on CAPD were reviewed for our 93 TSFR patients of a total 294 patients starting CAPD between 1979-88. Life-table analysis of our patients' mortality and transfer rates parallels that of the National Registry.

and (%) TSFR Patients by Cause and Time on CAPD

	P	PS	E	OT
<1 yr	15(38%)	12(30%)	5(12%)	8(20%)
1-2 yr	15(54%)	6(21%)	2(7%)	5(18%)
2-4 yr	6(46%)	3(23%)	--	4(31%)
>4 yr	10(83%)	1(8%)	--	1(9%)
Totals	46(49%)	22(24%)	7(8%)	18(19%)

P-peritonitis PS-psychosocial E-elective OT-other

The OT category includes tract infections, hernias, loss of ultrafiltration or clearance, and leaks. No single category in OT accounted for more than 5% of the total TSFR. PS related TSFR rates were similar for each time period less than 4 years on CAPD. After 4 years PS related TSFR rates declined and P related TSFR rates increased. The total percentage of PS related TSFR(24%) is greater than generally reported. It is concluded that P and PS are the major reasons for TSFR from CAPD. Analysis of PS apsects of CAPD may represent a key factor in improving technique survival, especially in the early years.

LONGITUDINAL STUDY OF HUMERAL IMMUNE FACTORS IN CAPD EFFLUENT. John Chapman*, Clifford Holmes*, and Fred Aono* (intr. by S.K. Webster). Baxter Healthcare, Round Lake, Illinois.

A 6 month longitudinal study was performed to assess variability of and differences between selected antibody and complement components of peritoneal dialysis effluent in 4 low peritonitis incidence patients (1 episode/90.5 months) and 4 high incidence patients (1/5.7 months). Overnight dwell effluents were assayed for total IgG, specific IgG and IgM and C3b deposition to Staphylococcus epidermidis (SE). Overall variability of total IgG was 41%. Specific IgG was consistently detected in all patients at <0.2% of normal human serum (NHS), except for patient G.B. The latter had >3% of NHS values, corresponding to a recent history of multiple SE peritonitis. Specific IgM was seen only intermittently in 5 of 8 individuals and ranged fromm 0.01-0.4% of NHS. C3b binding occurred in 6 of 8 patients ranging from 0-2.8% of NHS values. Patient G.B. also displayed elevated complement fixation as compared to the total population (1.3->5% of NHS). Comparison of high to low peritonitis patients showed:

	High	Low	P value
IgG (mg/dl)	10.43±4.8	4.87±1.9	<0.05
spIgG(%NHS)	0.11±0.05	0.09±0.02	ns
spIgM(%NHS)	0.01±0.13	0.05±0.08	ns
C3b (%NHS)	0.44±0.14	0.09±0.06	<0.01

We conclude that, in this study population, low IgG, specific IgG and IgM and C3b binding to SE were not significantly associated with a high incidence of peritonitis and would not be useful indicators per se of high risk individuals.

PD IN A CENTER CITY (CC) POPULATION. BH Cohen, S Alexander,* R Gerepka,* M Somerstein, T Sudhakar, and M Raza, Helene Fuld Med. Ctr., Trenton, N.J.

Although PD remains an accepted form of ESRD RX, about 35% of pts. transfer to HD in 2 yrs, the main cause due to peritonitis (P). Many established HD units still remain reluctant to start a PD program, particularly in CC areas where additional psychosocial risk factors for P exist (education, income, inadequate housing, poor hygiene and limited family support). Our CC PD program was established in 1984 with our P rates noted below:

Category	Pt. Mo.	Incidents	Rate/Mo.
Overall	1026	78	1:13.15
IV Drug Use	123	20	1:6.15
Over 60 yrs.	468	21	1:22.2
Diabetics	316	24	1:13.16
CAPD	910	64	1:14.2
CCPD	56	11 (6=1 pt.)	1:5
IAPD	102	3	1:34

As noted, the overall P rate is better than the national (NIH 1:8.6). Although worse, the IV Drug Use P rate continues to improve supporting PD as a viable RX for pts. who present multiple problems for HD. The Intermittent Ambulatory PD (IAPD) pts. who do 4 exchgs/day, 6 days/wk (one "off day") have the best P rate. Our success is due largely to: a committed team approach, an RN whose sole responsibility is pt. assessment and teaching, an established med/surg unit for PD admits, ongoing education for professional staff in ICU/CCU and the med/surg unit, no cross coverage to PD from HD and the use of Delmed's Safelock System which simplifies technique. From our experience, we feel home PD is an acceptable RX for ESRD in a CC population.

RANDOMIZED CLINICAL TRIAL (RCT) COMPARING PERITONITIS RATES AMONG NEW CAPD PATIENTS USING THE Y SET DISINFECTANT SYSTEM (Y) TO STANDARD SYSTEMS (S). Canadian CAPD Clinical Trials Group. Presented by D.N. Churchill, St. Joseph's Hospital, McMaster University, Hamilton, Ontario.

Sixty-one new CAPD patients were allocated to Y and 63 to S in an RCT addressing peritonitis rates in 8 CAPD programs in 6 Canadian cities. S was a standard System I or II (Baxter Corporation); Y used a Y set tubing with a hypochlorite (Amuchina) flush. The groups were not different for age, sex, race, marital status, social support, eye-hand coordination, exit site care, proportion adding medication to dialysate or proportion with diabetes. Exit site infections occurred in 22/61 Y and 23/63 S. Accidental infusions of Amuchina occurred in 15/61 Y on 22 occasions or 1 infusion per 2500 exchanges. There were no serious clinical sequelae. There were 21 episodes of peritonitis in 15 Y patients (1 every 21.5 patient-months) and 47 episodes im 30 S patients (1 every 9.9 patient months). The probability of developing peritonitis by 6 months was 35% for S and 20% for Y; the probability by 12 months was 74% for S and 53% for Y (p=0.002). The risk reduction for peritonitis for Y was 60% (95% C.I. 30-80%). Skin organisms were responsible for peritonitis in 8/21 (38%) of Y and 31/47 (66%) of S (p=0.038). There is a clinically important and statistically significant decrease in peritonitis rates for Y as compared to S, primarily due to a decrease in infections caused by skin organisms.

HIVAg IN DIALYSIS FLUID (DF) OF CAPD PATIENTS. R. Correa-Rotter*, S. Saldivar*, L.E. Soto*, F. Ojeda*, S. Ponce de León*, G.M. Ruiz-Palacios*, J.C. Peña. Inst. Nac. de la Nutrición Salvador Zubirán, Mexico City, Mexico.

Patients with ESRD infected with HIV have increased in recent years, mainly due to multiple blood transfusions with contaminated blood or to AIDS-related nephropathy.

CAPD is one of the therapeutic options of these patients. Hepatitis B virus has been demonstrated in DF and it is possible that HIV may be also present in DF.

The presence of HIV Ag in DF was investigated in patients with ESRD undergoing CAPD. Group I included three patients with positive HIV serum Ab and Group II seven patients used as controls with negative HIV serum Ab. The p24 HIV Ag was determined by a double-sandwich ELISA (Organon-Teknika) and the Ab with a double-sandwich ELISA (Abbott). Cell counts and bacterial cultures were done in all DF samples. Aliquots were run blind and in duplicate.

Of the three patients with serum HIV Ab (Group I), p24 Ag was positive in DF in only two patients. In one, serum Ab and Ag were present while in the other only serum Ab was detected. In Group II, p24 Ag was not found in sera and DF of any of the seven patients. The number of red and white cells in DF was not different between groups. All bacterial cultures were negative.

We conclude that DF of HIV infected patients may contain the Ag and is therefore potentially infective. Health care providers and relatives of such patients should be alerted to handle DF with special care and precautions.

THE PERITONEOSCOPIC PLACEMENT OF A POLYURETHANE ACCESS DEVICE (DERMAPORT®) FOR PERITONEAL DIALYSIS. Cosme Cruz, Mark A. Faber, Alma Melendez Henry Ford Hospital, Detroit, Michigan.

Eight Dermaport devices have been implanted peritoneoscopically on eight ESRD patients (7 males, 1 female, mean age 46 yr) using local anesthesia, preoperative antibiotic prophylaxis (Cefazolin 500 mg IV) and a modification of the Y-TEC method (Medigroup, North Aurora Il) which permits the visual inspection of the abdominal cavity and the atraumatic positioning of the subdermal flange.

In all cases the device was used immediately following the implantation without bleeding, outflow obstruction or leakage. After a mean follow-up period of 4.5 months there has been excellent dermal/epidermal incorporation of the skin button requiring minimal exit site care. Four patients have experienced transient stress related leakage of dialysate 2 to 4 weeks after implantation and 1 experienced a recurrence associated with treated peritonitis 5 months after implantation.

This is a practical alternative to the surgical method of implantation for the Dermaport® being simple, eliminating the incision of the parietal peritoneum, allowing the inspection of the abdominal cavity, immediate use, good patient acceptance and lower costs. This refinement in catheter placement techniques may represent a significant improvement in the care of the CAPD patient.

ABSORPTION OF RECOMBINANT HUMAN GROWTH HORMONE (rgGH) FOLLOWING INTRAPERITONEAL (IP) INSTILLATION. Richard N. Fine, Shawney E. Fine* & Barry M. Sherman*. UCLA Ctr. for Health Science, Dept. Ped. Div. Neph., L.A., CA. & Genentech, Inc., S. San Francisco, CA.

rhGH in the dose of 0.250 mg/kg, 0.125 mg/kg & 0.050 mg/kg was instilled into the peritoneum of 6 children undergoing continuous cycling peritoneal dialysis (CCPD). Adequate absorption was obtained with all 3 doses. Peak serum growth hormone (GH) levels were at 4 & 8 hrs. following IP instillation & were 424 ng/dl, 37 to 49 ng/dl & 21 to 37 ng/dl respectively for the 3 dosages used. By 18 to 24 hrs. following IP instillation & after 10 hrs. of CCPD, the serum GH levels returned to baseline values. In one pt., both the 0.125 mg/kg & 0.050 mg/kg doses were used. Adequate absorption with a peak serum GH level of 49 ng/dl was obtained with the 0.125 mg/kg dose whereas a flat absorption curve with a peak serum GH level of $\leq$ 10 ng/dl was obtained with the 0.050 mg/kg dose. These data indicate that pharmacokinetic studies should be undertaken prior to initiating clinical studies using IP rhGH in individual pts. Since daily rhGH has been shown to enhance growth velocity in GH deficient children, it would seem appropriate to utilize a daily dosage schedule when treating children undergoing CCPD. The data included in this report indicate that daily IP rhGH is a feasable approach to improve the growth velocity of children undergoing peritoneal dialysis. Two pts. are currently being treated with such a protocol & 6 mo. data will be available at the time of presentation.

CLEARANCE OF APOLIPOPROTEIN (Apo) AND ITS IMPACT ON RISK FACTORS IN CAPD. P. A. Fein, A. Antignani*, N. Mittman, P. Goldwasser*, M. M. Avram. The Long Island College Hospital, Brooklyn, New York

Protein loss is one of the disadvantages of CAPD. To assess the impact of CAPD on serum Apo levels, we measured serum HDL-cholesterol (HDL-C), total cholesterol (TC), Apo A-I and Apo B in 16 medical clinic controls and 10 CAPD pts (6 cases without peritonitis (NP)), and dialysate (D) levels of Apo A-I and Apo B. Apo levels were determined immunoturbidometrically. D was concentrated by ultrafiltration. In CAPD pts, mean serum values were (mg%±SD): HDL-C 39±8, TC 187±51, Apo A-I 101±8.4, Apo B 70±22, and 0.37±0.08 for HDL-C/Apo A-I ratio. In controls, the respective values were: 48±16, 212±31, 101±20, 82±30 and 0.47±0.08 (p=.01 vs CAPD for HDL-C/Apo A-I ratio). PD to serum Apo concentration ratios correlated with dwell time in 6 NP cases: r=0.95 for Apo A-I, r=0.54 for Apo B. In NP cases, mean clearances (C) were 0.03±0.007cc/min for Apo A-I and 0.02±0.01 for Apo B and the mean ratio of C_{A-I} to C_B was 1.6±0.7. This ratio correlated strongly with serum TC/HDL-C ratio (r=0.95). In peritonitis (n=4), PD clearances rose to 0.12±0.12 (Apo A-I) and 0.14±0.15 (Apo B) and the ratio of the clearances was 1.0±0.2. We conclude that peritoneal clearance of apolipoproteins is compatible with those reported for other macromolecules. The Apo A-I content of HDL was significantly lower in CAPD than in normals. Interestingly, the CAPD pts with the highest atherosclerosis risk ratio (TC/HDL-C) also had the highest peritoneal C of Apo A-I (which is associated with HDL) relative to Apo B (associated with LDL and VLDL). This new finding may be of significance in the atherogenesis of ESRD pts on CAPD.

BIDIRECTIONAL PERITONEAL TRANSPORT AND LOCAL TISSUE DEPOSITION OF IGG. Michael F. Flessner,* James C. Reynolds,* Ronald G. Blasberg,* Robert L. Dedrick.* (intr. by Mark A. Knepper). NHLBI, Dept. of Nuc. Med., Div. of Res. Services, NIH, Bethesda, Maryland.

We studied the simultaneous bidirectional transport of monoclonal antibody (MAb) 96.5, labelled with two isotopes, between the blood and peritoneal cavity in anesthetized, non-tumor bearing athymic rats. Experiments included an iso-osmotic and a hyper-osmotic dialysate and dialysis periods of 20 minutes and 200 minutes. Plasma, peritoneal, and tissue concentrations were measured for both isotopes of the MAb. Plasma concentrations were unaffected by the type of dialysis solution. However, the peritoneal concentrations were inversely proportional to the volume in the cavity. After iv injection, no differences were noted between the 20 and 200 minute tissue concentrations for a given dialysis solution, with the highest levels in heart, lung, and liver. For a given duration, significantly higher tissue concentrations (40-70%) were observed with the hyper-osmotic solution, but the total deposition in peritoneal tissues was nearly identical for both solutions (37-38%). After ip injection, the tissue concentration increased significantly with time in those tissues adjacent to the peritoneal cavity, and 50-60% of the MAb absorbed from the cavity was found in the local tissue, with the highest concentrations in the diaphragm and anterior abdominal wall. Neither tissue concentration nor total deposition was influenced by type of dialysate. We conclude that MAb deposition is affected by the route of administration but not by the dialysis solution.

IS TUMOR NECROSIS FACTOR (TNF) PRODUCED IN THE PERITONEAL CAVITY OF ASYMPTOMATIC CAPD PATIENTS ?

J.Floege*, G.Lonnemann*, S.Shaldon*, G.Deschodt*, A.Slingeneyer*, C.Mion*, KM.Koch* (Intr.by C.Olbricht). Depts.Nephrology, Univ.Hospitals Hannover,FRG, Nimes and Montpellier,France.

CAPD long term survival depends upon the permeability of the peritoneal membrane. TNF is the most potent inflammatory mediator and stimulates fibroblast proliferation. In view of this we decided to study the possibility of local TNF production in the peritoneal cavity of 18 long term CAPD patients without clinical, cellular or bacteriological evidence of peritonitis. A serum and drain fluid sample (last liquid after a 4 hr dwell of 2L DIANEAL containing 3.86% glucose) were obtained. The samples were processed immediately by centrifugation to minimize in vitro artifact cytokine production and drain fluid was sterile by culture. TNF and myoglobin were measured by RIA, albumin by rocket immune electrophoresis and urea by autoanalyzer. Results (mean±SD) are expressed as the ratio of drain fluid concentration to serum.

These results suggest that the peritoneal membrane and/or lymphatics were either transporting TNF (17 kD) at the same rate as urea (0.06 kD) or that TNF is produced locally in the apparently asymptomatic CAPD patient. Further work will be required to elucidate potential inducers in CAPD infusion fluid in order to minimize or prevent local TNF production if it is occurring.

PARIETAL PERITONEUM (PP) IS THE PREDOMINANT SOLUTE TRANSPORT SURFACE DURING PERITONEAL DIALYSIS (PD).

S.D. Fox*, J.K. Leypoldt*, L.W. Henderson. VA Med. Ctr. and Univ. of Calif., San Diego, CA.

Anatomical studies suggest that visceral peritoneum (VP) is the major transport surface during PD. Recent work by others suggests, however, that PP predominates in solute exchange. We determined the contribution of VP by comparing diffusive solute exchange in control (C) and eviscerated (E) rabbits. Preliminary studies in C (n=5) and E (n=5) demonstrated a 3-fold reduction in the permeability-area product (PA) following E, consistent with loss of 2/3 of transport area. This observation could be due to either removal of VP or inadequate dialysate-PP contact following E. Further studies in C and E were performed using abdominal compression (AC) to maximize dialysate-PP contact. Consecutive isotonic exchanges with dialysate volumes (DV) of 30 and 100 ml/kg were performed. Creatinine and dextrans (D) were infused by vein as test solutes. Results, $\bar{x}$(SEM):

				PA (ml/min)*	
Group	DV	n	Creatinine	D(15Å)	D(35Å)
C	30	5	1.31(.14)	0.15(.03)	0.04(.01)
	100	5	1.63(.18)	0.18(.07)	0.05(.02)
E	30	6	1.17(.18)	0.12(.02)	0.04(.01)
	100	5	1.47(.06)	0.14(.03)	0.05(.01)

*p = NS for all values. These results cannot be explained by differences in measured fluid egress following E. We conclude that: 1) the contribution of VP to diffusive solute exchange during PD is small; 2) AC improves solute exchange following E presumably by improving dialysate-PP contact. Our results may explain the disparity between anatomical and functional estimates of peritoneal membrane area during PD.

A PROSPECTIVE EVALUATION OF ELDERLY (E) PATIENTS FOR PERITONEAL DIALYSIS (PD) PREDICTS SUCCESS OF THERAPY.

C.J. Foulks, Dallas VAMC, J.L. Holley, U. of Pittsburgh.

We prospectively evaluated the suitability of E patients for PD (CAPD/CCPD) training. All patients over the age of 60 (EPD) (n=34) treated with PD over a period of 74 months were chosen for study and were compared to patients under age 60 (YPD) (n=61) treated with PD over this same period.

The evaluation examined visual acuity, manual dexterity, personal hygiene, family support, adaptability to change, learning ability, motivation, compliance, preference for home dialysis, and rehabilitation potential. EPD pts had 1.3 exit site infections per year vs 2.1/pt-yr for YPD ($p<0.05$) and 11 hernias requiring surgery vs 7 for the YPD ($p<0.05$). The EPD pts score was 2.2± 0.61 for those who were successful on therapy vs 3.2±0.42 for those EPD pts who were not ($p<0.02$).

Transfer to HD, death, peritonitis and catheter loss were not different between groups. Only patient motivation and preference for PD predicted success. The other criteria were useful in tailoring the PD training program. We feel that PD represents an excellent form of therapy for elderly patients and that patient motivation and preference predict success of therapy.

ROUTINE ABDOMINAL ECHOGRAPHY (E) IN THE EARLY DIAGNOSIS OF SCLEROSING ENCAPSULATING PERITONITIS (SEP)

Agostino Fracasso and Giorgio Bazzato* Nephrology Div. Umberto I Hospital, Venice-Mestre, Italy.

SEP is still considered the most serious complication on long term PD. Usually, when the disease reveals itself is in a stage too advanced for seaving the life to the patient.

In this study the AA have evaluated the role of abdominal E and computerized tomography (CT) for early diagnosis of SEP.

Since 1979, 182 pts entered our CAPD program and only one ascertained case of SEP was observed. In this patient both abdominal E and CT showed a marked thickening of the visceral peritoneum confirming the clinical diagnosis. Since there is not knowledge about the real stimuli (genetic infectious, chemical and others) responsible for the "evolution" from the aspecific peritoneal sclerosis to SEP, only an early diagnosis might be helpful in the prevention of this syndrome. For this reason our PD program includes an abdominal E, once a year. An early detection of PM thickening with atypical signs of bowel malfunction may be an index of evolution into SEP. Thus 36 patients (19.7%) who showed echographically evidence of PM thickening with intestinal mobility alterations were transferred to HD and followed in the subsequent years. None of this group of patients have so far, developed a feature of SEP. However double blind large study to confirm our data is needed.

CHRONIC RENAL FAILURE (CRF) AND CAPD PATIENTS EXHIBIT ELEVATED RBC CALCIUM (Ca). U. Gafter*, T. A Malachi*, H. Barak*, T. Weinstein*, and J. Levi. Nephrology, Hasharon Hosp. Petah-Tikva; Tel-Aviv Univ. Med. Sch. and The Biology Inst., Ness-Ziona, Israel.

RBC Ca homeostasis is abnormal in hemodialysis patients. In the present study RBC Ca homeostasis was studied in 18 patients with CRF (creatinine 6.2±0.6mg/dl), 9 CAPD patients and 12 controls. RBC Ca was 5.47±0.71 μmol/l cells in controls, it was markedly elevated in CRF patients 30.58±6.82 ($p<0.02$) and CAPD patients 23.56±6.67 μmol/l cells ($p<0.02$). To evaluate Ca extrusion basal and calmodulin stimulated activity of CaATPase was measured in RBC ghosts and expressed as nmol Pi/mg protein/h. A substantially reduced basal activity was found in CRF ($p<0.001$) and CAPD ($p<0.01$) patients. A similar pattern was found with calmodulin stimulated activity.

	Control	CRF	CAPD
Basal	850.7±66.7	504.9±34.4	618.2±47.3
+Calmodulin	1034.3±89.0	593.5±41.7	735.5±55.6

A negative correlation was found between RBC Ca and CaATPase activity in CRF patients ($r=-0.935$, $p<0.005$), CAPD patients showed a similar trend ($r=-0.675$, $p<0.10$). Ca influx in ATP-depleted RBC of controls was 4.61±0.39 nmol/ml cells/h, it was increased in CRF 12.00±1.34 ($p<0.001$) and CAPD patients 13.60±1.73 nmol/ml cells/h ($p<0.001$).

In summary: 1.RBC Ca was elevated in CRF and CAPD patients due to decreased CaATPase activity and increased Ca influx, similarly to hemodialysis patients. 2.Decreased Ca extrusion was the major cause for increased RBC Ca. 3.CAPD improved only slightly CaATPase activity and did not change Ca influx.

EFFECT OF HEMATOCRIT (Hct) ON SOLUTE REMOVAL IN CAPD. Violet Habwe*, Susie Q. Lew, Joan Watson*, Susan Early* and Juan P. Bosch. George Washington Univ. Med. Ctr., Dept. of Medicine, Washington, D.C.

It is not known whether Hct affects solute removal in CAPD. To determine the effect of Hct on solute removal 8 patients on maintenance CAPD, with Hct ranging from 22-36%, were studied during a 4hr dwell using 1.5% Dextrose dialysate. Blood and dialysate were sampled at 1, 2, 3 and 4 hrs, and assayed for urea nitrogen (UN) creatinine (Cr) and phosphorus (Phos). Dialysate/Plasma ratios for the solutes studied were calculated for each hour and correlated with the respective Hcts. Results: (* = $p<.05$)

Correlation coefficients (r) for Dialysate/Plasma ratio (y) vs Hematocrit (x) at hourly intervals

	1st Hr	2nd Hr	3nd Hr	4th Hr
UN	.787*	.918*	.286	.141
Cr	.856*	.841*	.766*	.856*
Phos	.958*	.881*	.829*	.752*

The significant relationship between Dialysate /Plasma ratio and Hct demonstrate an important effect of hematocrit on solute removal during CAPD. For UN, the Hct has a significant effect on solute removal during the first 2 hrs. During the 3-4 hrs as equilibrium is near, solute removal is independent of Hct. For Cr and Phos, equilibrium is never reached and Hct influences solute removal throughout the procedure. Our studies suggest a reduction of +/- 45% in solute removal for Cr and Phos when Hct increases from 20 to 35%. These results suggest that higher Hcts in CAPD patients will significantly affect the plasma creatinine and phsphorus concentrations.

BICARBONATE-BUFFERED PERITONEAL DIALYSIS SOLUTION IMPROVES POLYMORPH SUPEROXIDE PRODUCTION. D.K. Gupta*, F.J. Manahan*, F.Q. Zhou*, S.A. Zahir*, I. Pal*, J.T. Daugirdas, T.S. Ing. Hines-Loyola Medical Center, Hines, IL.

Superoxide produced by polymorphs plays an important role in the killing of bacterial pathogens. We studied the effect of lactate- and bicarbonate- containing peritoneal dialysis solutions on polymorph superoxide production, as measured by the amount of superoxide dismutase-inhibitable reduction of cytochrome c.

Two solutions were compared, one ultimately containing 24 mM lactate; the other 24 mM bicarbonate. Both solutions also contained in mM: Na 132, Ca 1.75, Mg 0.25, Cl 96, dextrose 83. HCl (2.4 mM) was added to the bicarbonate-buffered solution to achieve a pH of 7.4-7.6. The pH of the lactate-containing solution was 5.0-5.2. Polymorphs from 8 normal subjects were incubated in either solution containing added cytochrome c. Results were calculated as nanomoles of superoxide produced per million cells.

With the lactate-containing solution, mean superoxide generation was only 8.1 ± 4.3 (SD). With the bicarbonate solution, superoxide production was 24.4 ± 6.2, ($p < 0.01$).

Our results suggest that bicarbonate-buffered solution improves polymorph superoxide production; an effect which may be related to its more physiologic pH.

ANTIMICROBIAL ACTIVITY OF RIFAMYCIN ANTIBIOTICS AGAINST STAPHYLOCOCCUS EPIDERMIDIS BIOFILMS IN A CAPD ENVIRONMENT MODEL. A. Harris, G. Obst, R.F. Gagnon, J. Prentis and G.K. Richards (intr. by P. Somerville). Montreal General Hospital, Montreal, Canada.

We have demonstrated that rifampin has exquisite antimicrobial activity against standardized S. epidermidis biofilms. To see whether this unique property is shared by the entire class of rifamycin antibiotics, we compared the activity of rifampin (R) with two analogs (A1:CGP029861 and A2:rifapentine) and the parent compound rifamycin SV (RSV). The tests were performed in peptone water (PW) and peritoneal dialysis (PD) solutions, fresh (F) spent (S), and fresh with foetal calf serum (FCS) or buffered to pH 7 with HEPES. Values are hours of exposure to the antibiotics (10 μg/ml) required for killing of the biofilms:

		PD solutions (1.5%)			
	PW	F	S	F+FCS	F+HEPES
R	12*	6	>24	10	>24
A1	12*	8	24	10	10
A2	12*	8	24	10	>24
RSV	18*	10	>24	>24	>24

*Occasional resisters.

Results indicate that several members of the rifamycin antibiotics share potent antimicrobial activity against bacterial biofilms. The divergent influences of fresh and spent PD solutions may be ascribed primarily to pH.

QUANTITATIVE METHOD OF MEASURING ANTIVIRAL ACTIVITY (AVA) OF PERITONEAL DIALYSIS EFFLUENT (PDE). Z.Korzets, A.Pomerantz, O.Smetana, J.Bernheim. Depts. of Nephrology and Microbiology,Meir General Hospital and Sackler School of Medicine,Kfar-Saba, Israel. (Intr. By J. Levi).

We have previously reported the presence of AVA in PDE of patients treated by intermittent or continuous ambulatory peritoneal dialysis.AVA was determined be recording the inhibition of the cytopathic effect (CPE) of various viruses on Vero cells tissue culture plate in a semi quantitative manner using observer's subjective judgment.Although PDE was constantly seen to inhibit CPE, we sought a quantitative method to measure such AVA.To this end the CPE of para-influenza virus (Para-3) on Vero cells was evaluated.The CPE of this virus is manifested by distinct plaques which can be easily counted after staining the tissue culture plate. 83 samples of PDE (0.5ml) were added to Vero cells after which Para-3 was added. As control Para-3 on Vero was used. The number of plaques obtained by virus control was taken as 100% CPE. Inhibition of CPE by PDE was measured as a percentage of the CPE shown by virus control. It ranged from zero to 13% ($p < 0.001$ vs control). In addition PDE was diluted down to 1/2 and 1/64 and the inhibitory effect determined as before. The results of undiluted 1/2, 1/64 PDE samples were respectively 18.11±24.2, 33.57±18.16, 77.93±17.4% ($p < 0.01$ between 1/2 and 1/64 PDE). These data demonstrate a direct relationship between the concentration of PDE and the degree of inhibition of CPE. In conclusion Para-3 allows a quantitative measure of the AVA of PDE.

PERITONEAL CLEARANCES AND RESPONSIVENESS OF PERITONEAL MICROCIRCULATION TO NITROPRUSSIDE IN DIABETIC PATIENTS. Hi Bahl Lee, Min Sun Park*, Kyung Soo Kim*, Seung Duk Hwang*, Dept. of Internal Medicine, Soon Chun Hyang Univ. Hosp., Seoul, Korea.

Diabetic patients with endstage renal disease may have significant peritoneal microvascular disease and may have decreased peritoneal clearances of solutes limiting the efficacy of peritoneal dialysis. In order to evaluate peritoneal membrane function in diabetic patients on CAPD, we obtained peritoneal clearances of urea(Curea) and creatinine (Ccr), and protein concentration in drained dialysate (DPC) before and after nitroprusside (NP:4.5mg /L) addition to the dialysate in 7 diabetic patients (DM:age 54.4±8.7) who were just trained for CAPD and the results were compared to those of 18 non-diabetics (Non-DM:age 46.2±9.9) equally new on CAPD. Dialysate used for hourly clearance studies contained 1.5% glucose. Curea was not different between DM and Non-DM either before (19.5±3.9 vs. 16.7±4.0ml/min) or after NP(18.2±3.1 vs. 18.4±2.8). Ccr was significantly higher in DM than in Non-DM both before (14.8±3.3 vs. 10.8±2.6, P=0.004) and after NP(19.6±4.4 vs. 13.9±3.0, $P < 0.001$). Ccr increased significantly after NP in both DM (P=0.02) and Non-DM (P=0.01). DPC was significantly higher in DM than in Non-DM before (43.2±21.6 vs. 21.1 ±11.4mg/dl, P=0.03) but not after NP (62.0±32.5 vs. 43.0±18.2, P=0.07). NP addition caused significant increase in DPC in both DM (P=0.05) and Non-DM ($P < 0.0001$).

The results suggest that peritoneal protein loss is larger in DM but that peritoneal clearances of urea and creatinine and responsiveness of peritoneal microcirculation to NP are not reduced in adult DM when compared to Non-DM.

INTRAPERITONEAL (IP) CISAPRIDE (CIS) IS EFFECTIVE IN THE TREATMENT OF DIABETICS (DM) WITH GASTROPARESIS (GP) AND END STAGE RENAL DISEASE (ESRD). Andrew I. Lazarovits and Denis Page. Nephrology Services of University Hospital, London and Ottawa General Hospital, Ottawa, Ontario, Canada.

GP is a frequent complication of DM and ESRD. CIS stimulates gut motility by facilitating acetylcholine release. Oral CIS may not be efficacious in the treatment of GP. We present 2 patients with DM, GP and ESRD on CAPD who failed therapy with oral CIS, domperidone and metoclopramide (1 patient was admitted 9 times in 8 months). The intractable vomiting responded to intravenous (IV) CIS, only to recur a few weeks after discharge on oral CIS. Since many drugs may be given IP we measured plasma and PD fluid CIS levels following oral, IV and IP administration by HPLC. Plasma levels of CIS were equivalent whether the dose was 10 mg IV Q6H (mean pre - 24.3 ng/ml, mean post - 54.3 ng/ml) or 10 mg IP Q6H with each exchange (mean pre - 28.2 ng/ml, mean post - 52.8 ng/ml). Both patients have responded dramatically to continuous IP CIS. One has not been admitted for 4 months and has gained 4 kg in dry weight - no side effects have been noted. Thus CIS may be given continously IP to DM with GP and ESRD who are resistant to other forms of therapy.

FUNGAL PERITONITIS COMPLICATING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD): SUCCESSFUL TREATMENT WITH FLUCONAZOLE, A NEW ORALLY ACTIVE ANTIFUNGAL AGENT. Jerrold S. Levine*, David B. Bernard, Beldon A. Idelson, Holly Farnham*, Carol Saunders*, Alan M. Sugar*. Evans Memorial Department of Clinical Research, Boston University School of Medicine, Boston, MA.

The new antifungal agent fluconazole should be an ideal agent for the treatment of fungal peritonitis in patients on CAPD, because it is nearly completely absorbed after oral administration, has excellent penetration into the peritoneal dialysate, is apparently free of toxicity, and has a broad spectrum of antifungal activity. We report the first use of fluconazole in 2 patients with Candida peritonitis. Both patients received an oral loading dose of 200 mg. of fluconazole followed by a single daily dose of 100 mg. Simultaneous measurements of serum and dialysate concentrations of fluconazole demonstrated significant entry of fluconazole into the peritoneal fluid; the ratio of dialysate to serum concentration was greater than .6 for both patients. Despite this, symptoms persisted as long as the catheter was in place (4 and 12 days respectively), but resolved in each patient within 48 hours of catheter removal. Both patients received fluconazole for a total of 6 weeks, at which time a new peritoneal dialysis catheter was placed. Both were cured of their Candida peritonitis and have successfully resumed effective and peritonitis-free CAPD for periods of 4 and 9 months, respectively. Ease of administration and a seeming lack of toxicity make fluconazole an exciting new antifungal agent worthy of further study.

COMPARISON OF PERITONEAL WBC PARAMETERS FROM CAPD PATIENTS IDENTIFIED AT HIGH OR LOW RISK FOR PERITONITIS. Sharon Lewis,* Clifford Holmes,* and Winnie Kubey.*(intr. by Kenneth Gardner Jr.) Univ. of New Mexico, Dept. of Pathology, Albuquerque, N.M. and Baxter Healthcare, Round Lake, Illinois.

The purpose of this study was to determine if there was variability over time in peritoneal WBC obtained from CAPD patients or differences between patients at high or low risk for peritonitis. WBC were obtained from peritoneal dialysis effluents (overnight dwell) which were collected at monthly intervals for 6-8 months. Our results demonstrated that there was no significant difference in the mean total WBC yields or absolute WBC counts between the two patient groups. WBC differentials remained stable over time for an individual patient and there was no significant difference among patients or between the two patient groups. WBC receptors involved in normal host defense mechanisms were analyzed by flow cytometry using fluorescein-labeled chemotactic factors(C5a and fmet-leu-phe) and monoclonal antibodies specific for Fc receptors and complement receptors CR1 and CR3. Although there was a trend toward increased expression of all of these receptors in the high-risk peritonitis patients, there was no significant difference in the percentage or fluorescence intensity of peritoneal neutrophils or macrophages that expressed these receptors among patients or between the two patient groups. If altered host defense mechanisms exist in CAPD patients at risk for peritonitis, they could not be identified in the WBC parameters evaluated in this study.

THE ROLE OF DIALYZABLE SOLUTES IN THE MEDIATION OF UREMIC ENCEPHALOPATHY IN THE RAT INDEXED BY ELECTROENCEPHALOGRAPHY AND REGIONAL CEREBRAL GLUCOSE UPTAKE. **J J Lipman, P L Lawrence, D K DeBoer, M O Shoemaker, D Sulser, S Tolchard,and P E Teschan. Vanderbilt University, Div. of Nephrology, Nashville, Tennessee, USA, 37232.**

Uremic encephalopathy (UE) in humans and rats is indexed by slowing of the quantitative electroencephalogram (Q.EEG) demonstrated by an increase in the theta:alpha power ratio (TAR) of the background rhythm. In totally nephrectomized (Nx) rats, TAR increases with increasing abnormality of serum solutes from control levels of 1.57±0.29 to 2.87±0.3 (n=37, p<0.001) at 66h post Nx when BUN is 200±45 mg/dl. Therapeutic peritoneal dialysis (T-PD, 1.5% Inpersol, 8 x 30 ml x 30 min exchanges) significantly attenuates BUN elevation (to 98±23 mg/dl) and maintains TAR within normal limits. To test the hypothesis that one or more of the commonly measured serum solutes removed by TPD engenders UE, rats were dialysed with a "mock" uremic dialysate containing; (mg/dl) urea N 262; creatinine 3.7; Pi, 15.9; Ca, 8.8 and (MEq/l); K, 8.5; Mg, 2.38; HCO_3, 11. This solution was not as efficacious as T-PD, inducing a partial reversal of UE; (66h TAR = 2.41±0.18). A comparable effect was found when the solution's only abnormality was Pi, 15.9 mg/dl. Nx-induced changes in regional cerebral glucose uptake (rCGlu) corresponding to the Q.EEG changes were found in the geniculate bodies and substantia nigra. We conclude that a component of the dialysis reversible UE indexed by Q.EEG and rCGlu is independent of the removal of commonly measured serum solutes, the dependent component being accounted for by the elevated Pi concentration.

THE INFLUENCE OF MACROMOLECULAR CHARGE ON PERITONEAL TRANSPORT (PT) IS NOT DUE TO TISSUE BINDING. **J.K. Leypoldt* and L.W. Henderson. VA Med. Ctr. and Univ. of Calif., San Diego, CA.**

Our previous studies using fluorescent-labeled (FL) dextrans showed that the addition of positive charge, but not negative charge, reduces diffusive macromolecular transport during peritoneal dialysis. Other transcapillary transport studies have reported larger distribution volumes for cationic macromolecules that may result from tissue binding to anionic constituents of the interstitium. We determined whether reduced PT for DEAE dextran (D+) was due to only positive charge or to tissue binding. PT of FL D+ was determined during an isotonic exchange in rabbits either: 1) alone (n=7); 2) after passage of D+ through an anion exchange column (n=3); or 3) in the presence of excess unlabeled (UL) D+ (n=3). PT was assessed by the permeability-area product (PA), calculated from the increase in the dialysate concentration with time. Mean PA values (SEM) for D+ of 20 Å Stokes radius (R):

	Group 1	Group 2	Group 3
PA (ml/min)	.106(.014)	.072(.014)	.100(.038)

No differences in PA were observed for 15 < R < 40 Å. Values of PA from additional experiments using FL and UL neutral dextran (D) were indistinguishable, confirming that the addition of the FL is not important. Data summary for FL D+ and D:

	R (Å)	D+ (n=13)	D (n=9)
PA (ml/min)	20	.099(.013)*	.222(.034)
PA (ml/min)	35	.023(.007)*	.135(.030)

***lower than for D (p < .05). We conclude that: 1) addition of positive charge can reduce diffusive PT and 2) this reduction is not the result of tissue binding.**

THE PHARMACOKINETICS OF RECOMBINANT ERYTHROPOIETIN IN CAPD PATIENTS. I.C. Macdougall*, G.A. Coles*, J.D. Williams* (Intro. by R.B. Sterzel). KRUF Institute of Renal Disease, Royal Infirmary, Cardiff, Wales, UK.

Recombinant erythropoietin (EPO) is currently being used to treat the anaemia of haemodialysis patients. There is, as yet, no information on its role in CAPD subjects. We have therefore studied the pharmacokinetics of EPO in stable CAPD patients.

Eight patients (4 males, 4 females; mean age 62 yrs, range 43-76 yrs), who had been on CAPD for more than 6 months, received 120 units/kg of EPO as an IV bolus. Blood samples were taken at frequent intervals over 24 hours. PD samples were taken from the bags used during this period. EPO levels in both blood and dialysate were measured by radioimmunoassay.

The plasma disappearance rate had a mean half-life of 8.2 hours, range 6.2 to 10.2. Analysis of the results suggested that EPO was distributed in a single compartment during the first 24 hours after injection, the mean apparent volume of distribution being 0.033 l/kg (range 0.021-0.063). The mean plasma clearance rate was 0.047 ml/min/kg (range 0.032-0.085). Losses in the dialysate represented 2.1% (range 1.3 to 2.7%) of the administered dose during the first 24 hours.

Thus the half-life of IV EPO in CAPD patients is similar to that reported for HD subjects. Peritoneal losses are insignificant. The volume of distribution was of the same order as plasma volume as reported in previous animal investigations. Studies on the absorption of EPO from the peritoneal cavity are in progress.

EFFECT OF VOLUME (V) ON MASS TRANSFER IN PERITONEAL DIALYSIS (PD). Bruce Z. Morgenstern, Goeffrey A. Patrissi, Brenda Owings and James Brown. USAF Medical Center Keesler, Keesler AFB, MS

Increased dialysate V in PD results in augmented small solute removal with no change in removal of larger solutes. Previous data in rats suggest this may be due to an increase in mass transfer area coefficients (MTAC) for small solutes. Seventeen New Zealand white male rabbits underwent two 4-hour PD study exchanges using, in random sequence, a small volume (SV, 40 mL/kg) or a large volume (LV, 80 mL/ kg) of commercially available 2.5% dextrose dialysate. C^{14} Dextran was used as a V marker. MTAC's and reflection coefficients (RC) were determined in each exchange for urea (U), creatinine (C), Vit. B_{12} and total protein (TP). Paired data were compared. As expected, the LV exchange removed more urea than the SV (43 ± 16 mg vs 28 ± 10 mg, $p < 0.004$), with no difference in TP removal (375 ± 139 mg vs 550 ± 306 mg, $p > .1$). The MTAC's for the solutes (SV vs LV, mL/min) were: U: .51 ± .21 vs .53 ± .27, C: .57 ± .41 vs .65 ± .42, B_{12}: .16 ± .17 vs .09 ± .1, TP: .001 ± .01 vs .001 ± .03. The RC's (SV vs LV) were: U: .19 ± .01 vs .18 ± .02, C: .33 ± .17 vs .43 ± .29, B_{12}: .52 ± .02 vs .52 ± .02, TP: .99 ± .007 vs .99 ± .009. There were no significant differences in membrane properties for any solute. The increased small solute removal in LV exhanges is due to the additive effects of increased convection due to increased ultrafiltration (SV 30 ± 12 mL, LV 42 ± 15 mL, $p < 0.02$) as well as prolongation of the transmembrane concentration gradient.

ABDOMINAL COMPRESSION (AC) REDUCES FLUID ABSORPTION DURING PERITONEAL DIALYSIS (PD) IN THE RABBIT. S.N. Okamoto*, S.D. Fox*, J.K. Leypoldt*, L.W. Henderson. VA Med. Ctr. and Univ. of Calif., San Diego, CA.

Peritoneal absorption of fluid and macromolecules during PD is considered to occur primarily through subdiaphragmatic lymphatics. Increases in intra-peritoneal pressure (IPP) augment observed peritoneal fluid loss rates (Fo) presumably via this route. Recent work, however, reveals a disparity between Fo and lymphatic flow rates (LFR), implicating convective fluid loss into adjacent visceral or parietal tissues. We compared data from 3 animal models of PD to assess factors modifying Fo. Rabbits underwent 80-240 minute isotonic exchanges under different conditions: Awake (Gp 1); anesthetized (Gp 2); anesthetized with external AC to increase IPP (Gp 3). Dextran (2000 kd) was used as IP volume marker. Fo was calculated as: dextran indicator dilution volume at end-dwell - [drain volume + residual volume] divided by dwell time. Results:

Group	n	IPP(mm Hg)	*Fo (ml/min), $\bar{x}$ (SEM)
1	23	-	0.33(.03)
2	9	2-2.2	0.43(.10)
3	9	4-5	0.12(.06)

*1 vs. 3 ($p < .02$); 2 vs. 3 ($p < .05$)

These results cannot be explained by differences in respiration or body weight. AC leads to a fall in Fo despite increased IPP (Gp 3) and most likely alters fluid absorption into adjacent tissues, not LFR. Our results suggest a potential role for the application of AC to increase net ultrafiltration volumes during PD.

MODULATION OF ANTIBIOTIC ACTIVITY AGAINST STAPHYLOCOCCUS EPIDERMIDIS BIOFILMS BY THERAPEUTIC DRUGS ADDED TO PERITONEAL DIALYSIS (PD) SOLUTIONS. G. Obst, R.F. Gagnon, J. Prentis, G.K. Richards (intr. by P. Somerville). Montreal General Hospital, Montreal, Canada.

We previously reported the exceptional antimicrobial activity of rifampin against standardized S. epidermidis biofilms. To extend the relevance of this observation to CAPD, we examined the interrelated effects of 1.5% dextrose PD solutions and various therapeutic drug additives on the susceptibility of the biofilms to antibiotic action. In a panel of commonly used antibiotics, only rifampin was able to produce killing of the biofilms. Amongst nine non-antibiotic drugs added to fresh PD solution, five strikingly antagonized the antimicrobial effect of rifampin. Heparin and insulin were included in these five. Fresh and spent PD solutions had markedly divergent effects on rifampin activity. Fresh PD solution acted in a synergistic manner whereas spent PD fluid was profoundly antagonistic, an effect largely attributed to alkalinity. These findings have significant implications for the antibiotic management of CAPD peritonitis.

NUTRITIONAL STATUS OF CAPD PATIENTS: AN INTERNATIONAL STUDY. D.G.Oreopoulos, Toronto, Canada; H. Anderson, Toronto, Canada; J. Bergstrom, B. Lindholm, Huddinge, Sweden; A. Brownjohn, G. Young, Leeds, U.K.; A. DeVecchi, Milan, Italy; J.D. Kopple, Torrance, CA; K. Nolph, Columiba, MO; C. Algrim, L. Martis, K. Serkes, E. Vonesh, Round Lake, IL.

Since there has been concern that CAPD patients may become malnourished, we examined the nutritional status of 224 patients from 6 centers in Europe and North America. Nutritional status was assessed as normal, mildly malnourished or severely malnourished based on history and physical examination (Subjective Global Assessment), serum chemistries and anthropometry. Based upon this data, 18 patients (8.0%) were severely malnourished, 73 (32.6%) mildly malnourished and 133 (59.4%) normal. Serum albumin (mean±SD) in the three groups was 3.0±0.6, 3.5±0.5 and 3.7± 0.4 g/dl, respectively. Since there were intercenter differences, multivariate statistical analyses were used to develop objective scoring techniques. Nutritional parameters that correlated with the degree of malnutrition included albumin, mid-arm muscle circumference, history of weight loss, muscle wasting and loss of subcutaneous fat. We conclude that the nutritional status of most CAPD patients is within the normal range, a substantial number (33%) exhibit mild malnutrition and a small percentage (8%) are severely malnourished.

EFFECT OF CATIONIC MACROMOLECULES ON PERITONEAL PERMEABILITY. Irena Pietrzak,* Przemyslaw Hirszel, Robert E. Lee,* Eva K. Chakrabarti* and John F. Maher, Dept. of Med., Uniformed Services Univ. Hlth. Sci., Bethesda, MD and Walter Reed Army Med. Cen., Washington, DC.

The peritoneal transport barrier contains abundant anionic charges that could restrict solute and water passage. To examine their importance anionic charges were blocked during experimental 60 min peritoneal dialyses in 7 rabbits by adding 15.4±.3 mg/Kg of poly-l-lysine HCl(L) and comparing the results to those of control dialyses. Osmotically induced net ultrafiltration (436 µl/Kg/min) was not increased by L ($p>0.1$) nor was urea clearance, 738 µl/Kg/min ($p>0.1$) but protein clearance rose from 21 to 99 µl/Kg/min with L ($p<0.01$). The dialysate absorptive rate was unchanged. To assess whether charge repelling alone explains the protein transfer increment, the transport of neutral dextran (T 40) and of smaller ions were examined. Dextran clearance increased from 14 to 25 µl/Kg/min with L ($p<0.02$) suggesting increased pore size. Salicylate clearance rose from 435 to 658 µl/Kg/min with L ($p<0.08$), potassium clearance rose from 1.29 to 1.61 ml/Kg/min ($p<0.09$) and phosphate clearance increased from 351 to 520 µl/Kg/min ($p<0.06$). The data suggest that neutralization of membrane anions lessens a transport restriction on charged solutes but it also increases effective pore size, both mechanisms contributing to increased protein loss.

A RECOVERY INDICATOR METHOD FOR THE INVESTIGATION OF INTACT BACTERIAL BIOFILMS. G.K. Richards, G. Obst, J. Prentis and R.F. Gagnon (intr. by P. Somerville). Montreal General Hospital, Montreal, Canada.

Bacterial biofilms are associated with CAPD catheters and are suspected of playing a major role in the development of peritonitis. A standardized technique was developed for forming uniform plane biofilms from a slime-producing strain of Staphylococcus epidermidis. A recovery indicator method assessing residual metabolic activity permits the examination of the effects of nutrition, exposure time, temperature, pH, antibiotic and disinfecting agents on the viability of bacterial biofilms. Microscopy allows the direct estimate of morphological change and metabolic function of individual bacterial cells in situ.

This is an ideal system for the study of comparative resistance of bacterial biofilms to antimicrobial agents.

BAGLESS CAPD IMPROVES CATHETER INFECTION RATE. Beth M. Piraino, Judy Bernardini*, Michael I. Sorkin. Univ. of Pittsburgh and VAMC, Pgh., PA

The purpose of the study was to evaluate the effect of the bagless CAPD system (Ultraset) on catheter infection (CI) and peritonitis (P) rates.

We matched 37 study patients on the bagless system with 37 control patients on the standard spike system for sex, age, insulin dependent d.m., and time on CAPD. Mean time on CAPD was 22 mo./patient for both groups. The study patients' experience on the bagless system (study bagless) was compared to their previous experience on the spike system (study spike) and to the control patients on the spike system (control spike). The study patients were on the standard spike system a mean of 15 mo./patient, followed by 7 mo./patient on the bagless system. Cumulative CI and P rates (as episodes/y) were:

	Control spike	Study spike	Study bagless
CI	1.2	1.8	0.7
P	0.8	0.7	0.5

The micro-organisms causing CI and P were the same with the spike and bagless systems.

We conclude that the bagless system is associated with very low rates of catheter infections as well as P episodes. The low P rate with the bagless system, previously demonstrated by others, may be due not only to the drain prior to infusion technique, but also due to the decrease in CI rates. We attribute the latter result to a decrease in trauma to the exit site that results from the absence of a bag attached to the catheter.

OXALATE METABOLISM IN PERITONEAL DIALYSIS PATIENTS RECEIVING VITAMIN C SUPPLEMENTS. E.A. Ross, G.M. Shah, J. Costello, H. Bhagavan*, A. Sabo* and M. Pichon*. Long Beach VA-UCI Program, Long Beach, CA, Allegheny-Singer Research Institute, Pittsburgh, PA & Roche Vitamins, Nutley NJ.

Oxalate (OX) metabolism was examined in 6 men receiving peritoneal dialysis (5 CAPD. 1 CCPD). All vitamin supplements were discontinued for 4 weeks, followed by daily oral administration of 100 mg ascorbic acid (AA) for an additional 4 weeks. Plasma (P) and dialysate OX levels were measured by a double enzyme method in which recoveries were monitored with ^{14}C OX. AA levels were assayed by a modified HPLC procedure after deproteinizing samples with 12% m-phosphoric acid.

POX levels were 457±107 (mean±S.E.) and 439±82 ug/dl (normal 11.25±0.88) at weeks 3 and 4 of the supplement-free period, respectively. The corresponding PAA levels were 1.1±0.2 and 1.0±0.2 mg/dl (nml 0.8-1.0) on a diet containing 84±16 mg/d vitamin C (140% US RDA) and 113±58 mg/d OX. Oxalate removal in peritoneal dialysate was 24±4 mg/d and in urine 16±4 mg/d. After 4 weeks of AA supplements, the POX levels increased by 25%, and PAA levels by 23%.

We conclude that (1) Peritoneal dialysis patients have severe hyper-oxalemia, which occurs despite normal PAA levels and substantial peritoneal and urinary OX removal; (2) With adequate dietary vitamin C intake, AA supplements should be avoided because they further worsen POX levels.

WHICH PERITONEAL MEMBRANES PARTICIPATE IN ULTRAFILTRATION ? J Rubin, Q Jones, A Planch. Dept. of Medicine, University of Mississippi Medical Center, Jackson, Mississippi.

The membranes involved in ultrafiltration during peritoneal dialysis are unknown. The peritoneal cavity is conceptualized as two distinct groups of membranes: a) intestinal viscera with attached mesentery and b) diaphragm, liver, and parietal lining. This investigaton was carried out to define their contribution to ultrafiltration.

Two groups of rats were studied: a control group - sham operated - and an eviscerated group - esophagus to rectum. Rats received i.v. saline at 10 ml/hr starting 1 hr prior to study. At time 0, abdominal closure, 30 ml of a solution, 1200 mOsm/L, containing 5500 mg/100 ml glucose and 5600 mg/100 ml essential amino acids (Travasol, Baxter) was infused. After 120 min the peritoneal cavity was opened and aspirated into a syringe. Any rat failing to maintain a mean BP of 95 mmHg was rejected. Glucose and nitrogen content of the dialysate (Kjeldahl technique) were assayed.

Glucose and nitrogen uptakes from the peritoneal cavity were similar for both groups (mg sd): Controls - glucose 1068 ± 102 (n=14) , nitrogen 147 ± 11 (n=7); Eviscerated - glucose 923 ± 84 (n=11) , nitrogen 148 ± 12 (n=7). Ultrafiltration was greater (P<0.01) in Controls (ml ± sd) - Controls 18 ± 5 (n=14), Eviscerated 5 3 (n=11).

We conclude that the intestinal viscera - esophagus to rectum and mesentery - accounted for 2/3 of ultrafiltration. All other peritoneal structures contributed 1/3 of ultrafiltration.

ENHANCED CALCITRIOL DELIVERY AFTER INTRAPERITONEAL ADMINISTRATION. IB Salusky, JS Adams*, R Horst*, M Holloway*, JW Coburn, WG Goodman, Depts of Peds & Med, UCLA and USC Sch of Med, LA, CA.

Serum (S) calcitriol (1,25) levels are higher for the first 3 hrs after intravenous (IV) than after oral (PO) or intraperitoneal (IP) doses (KI 33:250, 1988). Repeated IV injections of 1,25 are not feasible, however, in CAPD/CCPD patients. Thus, alternatives to enhance the IP delivery of 1,25 were evaluated. Since plastics are widely used in PD systems, the potential for 1,25 adherence to plastic components was examined in vitro using trace amounts of ^{3}H-1,25 (10^6 cpm) added to 2 ug of 1,25 in 500 ml bags of Dianeal 1.5% (Baxter). Results are stated as means of determinations in triplicate samples.

^{3}H-1,25 recovered from:	N	cpm x 10^{-3}	%
Vials of 1,25 (glass)	3	960.6	100
Glass syringes	3	904.2	94.2
Plastic syringes	3	820.0	85.4
Dialysate effluent	21	435.1	45.3
^{3}H-1,25 bound to:			
Dialysate bag	6	286.8	30.0
Dialysate tubing	9	8.8	1.0

Because 1,25 binds to plastic, S-1,25 levels were measured in pts given 4.0 ug/Kg BW of 1,25 orally (n=5) or by direct IP instillation in 20 ml of dialysate (n=6). Values ($\bar{x}$±SD, pg/ml) did not differ before IP (6.0±1.4) and PO (2.5±3.0, NS) doses, but they were higher 1 hr, 103±15 vs 33±33, p<0.01, and 2 hrs, 114±17 vs 50±44, p<0.05, after IP than after PO 1,25; the levels at 3 hrs were not different. 1,25 binds to the plastic in PD systems, and this may reduce the bioavailability of the sterol. Alternative methods of IP 1,25 administration may counteract this effect and enhance the systemic delivery of 1,25.

ASCORBIC ACID REQUIREMENTS IN PERITONEAL DIALYSIS PATIENTS. G.M. Shah, E.A. Ross, A. Sabo*, M. Pichon*, W. Gebreselassie* and H.N. Bhagavan*. Long Beach VA-UCI Program, Long Beach, CA & Roche Vitamins, Nutley NJ.

Ascorbic acid (AA) requirements were investigated in 6 men receiving peritoneal dialysis (5 CAPD, 1 CCPD). All vitamin supplements were discontinued for 4 weeks, followed by daily administration of ascorbic acid supplements orally for an additional 4 weeks. Plasma (P) and dialysate ascorbic acid levels were assayed by a modified HPLC procedure after deproteinizing the samples with 12% m-phosphoric acid.

Without oral supplements, dietary intake of vitamin C was 84 ± 16 mg/d (mean ± S.E.) which was 140% of the US RDA. At week 1 of the supplement-free period, PAA levels were 1.3 ± 0.2 mg/dl (normal 0.8-1.0 mg/dl), which decreased by 23% to 1.0 ± 0.2 mg/dl at week 4 ($p > 0.05$). The PAA did not correlate with dietary consumption of vitamin C ($p > 0.05$). The peritoneal removal rate of AA was 53.6 ± 9.1 mg/d (89% of the US RDA), and correlated with PAA levels ($r = 0.9$, $p < 0.02$). Mean peritoneal AA clearance (which was 84% of peritoneal creatinine clearance) was 3.6 ± 0.3 ml/min. After 4 weeks of vitamin C supplements (100 mg/d), PAA increased by 40% and peritoneal removal rate by 37%.

We conclude that: (1) in spite of substantial peritoneal removal, the PAA levels remain within normal limits in peritoneal dialysis patients; (2) vitamin C supplements resulted in a moderate increase in PAA and peritoneal removal rate of AA; and (3) peritoneal dialysis patients do not need daily vitamin C supplements if their dietary intake of vitamin C is adequate (i.e., ≥ 100% US RDA).

PROTAMINE SULFATE INDUCES ENHANCED PERITONEAL PERMEABILITY TO PROTEINS

A Shostak, P Galdi, J Jaichenco, L Gotloib, Dept of Nephrology and Kornach Laboratory for Experimental Nephrology, Central Emek Hospital, Afula, Israel

In previous studies we demonstrated the presence of anionic sites (AS) in both the mesothelium and the subendothelial microvessels. We now present a direct link between neutralization of AS by intraperitoneal protamine (P) and an increase in the passage of negative macromolecules to the peritoneal cavity under conditions of isosmotic (Hartmann's lactated Ringer's) peritoneal dialysis in rabbits.

Each experiment included two 1 hour exchanges. In group A(control) no drugs were included during the second hour. In group B,P(50umg per ml) was added to the dialysate in the second exchange volume. In group C,P and heparin (H) (50 IU per ml) were added to the dialysate. In group A (control) and C (P+H), slopes of appearance curves observed during the first (control) and the second (experimental) hour failed to show statistically significant variations. In group B, differences for urea, glucose and uric acid were not significant; where as they were highly significant for protein ($p < 0.001$ on an increase of 100%).Therefore, we found that the transperitoneal passage of albumin is substantially enhanced by P.This increase is prevented by neutralization of P with H; showing that the availability of AS appears to be the crucial limiting factor. P did not significantly change peritoneal permeability for small neutral molecules.

FOSINOPRIL SODIUM (FS) PHARMACOKINETICS AND PHARMACODYNAMICS IN CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). Domenic Sica, Judy Davis*, Itaf Fakhry*, Julia Nunley*. Dept. of Med., Medical College of Virginia, Richmond, VA

FS is a prodrug angiotensin converting enzyme (ACE) inhibitor that is hydrolyzed to the pharmacologically active diacid, SQ 27,519 (SQ). In normal volunteers (NV), SQ is eliminated equally by renal and non-renal routes. The kinetics and dynamics of SQ were determined after administration of FS (10 mg) to 5 CAPD patients (mean age=46 yr). Serum and dialysate samples collected up to 48 hr post-dose were assayed for SQ by radioimmunoassay. Comparative serum data from 12 NV given 10 mg FS are presented (mean age=29 yr). Serum parameters (mean ± SEM) for SQ are given below.

PARAMETER	CAPD	NV
Cmax (ng/mL)	202 ± 32	133 ± 13
Tmax (hr)	4.8 ± 0.7	3.4 ± 0.2
AUC (ngxhr/mL)	3187 ± 963	1243 ± 113

The mean peritoneal clearance (PC) of SQ (0.09 ± 0.03 mL/min) was lower than that of urea (5.3 ± 0.7 mL/min). The maximal plasma aldosterone decrease was 45 ± 6% and plasma renin activity increased 135 ± 145%. Serum ACE levels declined to 98 ± 3% of baseline at 1 hr and remained 50% suppressed at 72 hr. Maximum blood pressure (8/9 mm Hg) decrease occurred at 6 hr. Based on the limited PC and higher serum levels of SQ, the initial dose of FS should be halved in hypertensive CAPD patients, compared to patients with normal renal function.

PERITONEAL TRANSPORT KINETICS IN THE ANEPHRIC MICROPIG. Sarah K. Webster and Kenneth E. Burhop. Baxter Healthcare Corporation, Round Lake, Illinois

The successful treatment of ESRD patients with peritoneal dialysis is dependent on the peritoneal transport properties of individual patients and the availability of a variety of regimens to optimize therapy in each case. These studies were designed to characterize factors which affect peritoneal transport in an anephric animal model with the ultimate goal of evaluating techniques which may contribute to optimization of therapy.

Anephric micropigs with chronically implanted peritoneal catheters were maintained on peritoneal dialysis throughout the study. Peritoneal equilibration tests were performed to determine the rates of ultrafiltration and equilibration of solutes.

The kinetic values obtained compared favorably with those values obtained in humans*: Mass transfer-area coefficients (ml/min) for urea, 23±6 vs 22±1 in humans; for creatinine, 11±3 vs 11±2 in humans; and for glucose, 9±2 vs 10±1 in humans. Lymphatic uptake from the micropig peritoneum was 0.95±0.19 ml/min, a rate similar to that observed in humans. Peritoneal clearance of urea normalized for body weight was 0.30±0.03 ml/min/kg in micropigs vs 0.27±0.02 in humans; and for creatinine was 0.27±0.02 in micropigs vs 0.18±0.02 in humans.

The dialysate to plasma solute ratios mimicked "high-perm patients". Increasing the intraperitoneal volume to 90 ml/kg increased the ultrafiltration rate by decreasing the rate of dissipation of the osmotic gradients and resulted in equilibration curves that reflected "normal permeability".

In summary, the anephric micropig may provide valuable information for testing various factors to improve dialysis efficacy in ESRD patients.

*Spencer, et al. in Peritoneal Dialysis (1985) p581

GENERATION OF EICOSANOIDS BY HUMAN PERITONEAL MACROPHAGES IN RESPONSE TO PHAGOCYTOSIS OF STAPHYLOCOCCUS EPIDERMIDIS. J.D. Williams*, R. Mackenzie*, M. Petersen*, G.A. Coles*. (Intr. by R.B. Sterzel). KRUF Institute of Renal Disease, Royal Infirmary, Cardiff, Wales, U.K.

The present study examines the response by isolated human PMØ to stimulation by a peritoneal isolate of Staph. epidermidis. PMØ phagocytosed bacteria in a dose and time dependent manner. Cell viability as assessed by trypan blue exclusion was >95% over this period. Unstimulated cells demonstrated a consistent background generation of PGE_2 and TxB_2 whereas there was no generation of leukotrienes. Stimulated PMØ generated LTB4 and LTC4 in a dose and time dependent manner. In contrast there was a dose dependent inhibition of cyclooxygenase metabolites. This decrease did not represent a change in substrate availability since the addition of exogenous arachidonic acid over a wide range of concentrations failed to reverse the inhibition, nor was there any evidence of increased cyclooxygenase product metabolism. However, the addition of exogenous LTB4 to cultures of human peritoneal macrophages caused an inhibition of background cyclooxygenase activity demonstrating a negative feedback effect of this 5-lipoxygenase product on the cyclooxygenase system.

The generation and release of LTB4 by monocytes under these circumstances is consistent with a rapid influx of PMN into the peritoneal cavity. In addition the immunoreactivity of inflammatory cells may be greatly influenced by a decrease in the immunosuppressive PGE_2 generated.

ATRIAL PEPTIDE-INDUCED INHIBITION OF DNA SYNTHESIS IN RAT MESANGIAL CELLS. R.G. Appel. Dept. of Med., School of Med., East Carolina U., Greenville, NC.

Atrial peptide (AP) binding sites exist in numerous tissues that may not be involved in volume homeostasis. We studied the effect of AP on proliferation of rat mesangial cells (MC) in culture. Monolayers approaching confluence were made quiescent by a 3-4 day incubation in serum-free media. Cells were reactivated by exposure to a serum-free defined media (DM) containing insulin, bovine serum albumin and soybean lipids. DNA-associated [^{3}H] thymidine incorporation was measured as a means of assessing MC proliferation. Quiescent counts were low at 8.5 ±1.1 cpm/µg prot. Reactivated counts (DM) were 46.0±8.4 cpm/µg prot. Counts in the presence of DM plus 0.001, 0.01, 0.1, 1, and 10nM AP were 44.3±7.9, 35.8±6.5, 24.4±4.5*, 27.2±3.4*, and 24.5 ±5.3* cpm/µg prot. respectively (mean ± SEM of triplicates in from 4-8 cell strains; *$p<0.05$ vs. DM alone). Addition of fibroblast growth factor (FGF) to DM increased counts by 30% above DM alone. Addition of AP to the DM plus FGF-stimulated cells inhibited [^{3}H] thymidine incorporation by 49% (n=6; $p<0.05$, DM+FGF+AP vs. DM+FGF) The inhibitory effect of AP on [^{3}H] thymidine incorporation did not reflect inhibition of active transport of the thymidine through the cell membrane, since in the presence of DM plus AP there was no reduction in total cell-associated counts compared to DM alone (5966 ± 606 vs. 5454 ± 645 cpm/well). Only DNA-associated counts fell (810±93 vs. 1558±184 cpm/well), arguing for an inhibitory effect of AP on DNA synthesis. Thus, AP may be a multifunctional peptide, consistent with a number of peptide growth factors.

THE SYMPATHETIC NERVE MODULATES GLOMERULAR ANP RECEPTORS WHICH ARE LINKED TO THE FILTRATION FUNCTION. M Awazu*, V Kon, R C Harris, T Imada*, T Inagami* and I Ichikawa. Vanderbilt University School of Medicine, Nashville, TN.

To investigate the mode of interaction between ANP and the sympathetic nervous system, we examined the characteristics of the ANP receptors in rat glomeruli isolated from subacutely (~4 days) denervated (DNX) and non-denervated (non-DNX) kidneys of normal rats (NL) and rats subjected to water deprivation for 48 hrs (WD). Plasma ANP level of WD rats (39±9 pg/ml, n=8) was significantly lower than NL level (132±21, n=10). Results [Mean±SE; P<0.05 vs non-DNX NL (†); vs non-DNX ($); vs non-DNX WD (¶)] include:

	non-DNX NL	non-DNX WD	DNX WD		DNX NL	
			non-DNX	DNX	non-DNX	DNX
Ro	250±13	613±89†	469±40	898±79§¶	276±17	281±20
KD	.39±.05	.83±.15†	.50±.14	.63±.11	.51±.11	.49±.09

Thus, both receptor density (Ro, fmol/mg prot) and KD (nM) of non-DNX WD rats (n=6) were significantly higher than those of non-DNX NL (n=6). Within WD rats (n=6), Ro of DNX kidneys was markedly elevated over that of contralateral non-DNX kidneys, while this difference between DNX vs non-DNX kidneys was absent in NL rats (n=6). Exogenous infusion of ANP (4 µg/kg/hr, n=4) increased whole kidney GFR significantly (from .49±.05 ml/min to .68±.09) in DNX WD kidneys, whereas GFR remained unchanged in contralateral non-DNX WD kidneys (.52±.10 ml/min vs .59±.09, n=4). Micropuncture measurements in DNX WD kidneys (n=5) revealed that, in association with its vasodilative influence on afferent (RA) and efferent (RE) arteriolar resistances, ANP increases SNGFR, glomerular plasma flow rate (QA) and glomerular ultrafiltration coefficient (Kf), and decreases glomerular capillary pressure (PGC), contrasting the absence of these effect of ANP on Kf in normal kidneys. (** P<0.05 vs baseline)

	SNGFR	QA	PGC	RA	RE	Kf
	-------nl/min-------		mmHg	min·mmHg/nl		nl/min·mmHg
Baseline	23±2	100± 8	62±1	2.4±.4	1.9±.1	1.2±.4
ANP	32±3**	147±24	53±1**	2.2±.6	1.3±.2**	2.3±.5**

We conclude that renal sympathetic tone modulates the density of intraglomerular ANP receptors, which is linked to the regulation of filtration function.

POSITIVE COOPERATIVITY BETWEEN GLOMERULAR (G) ATRIAL NATRIURETIC PEPTIDE (ANP) BINDING SITES. Barbara J. Ballermann. Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

In rat G three ANP binding sites can be distinguished by ^{125}I-ANP affinity cross-linking/SDS-PAGE (reducing conditions). Their respective molecular weights are 130 kDa (ANP-R1), 180 kDa (ANP-R2) and 64 kDa (ANP-BP). The receptors, ANP-R1 and ANP-R2, are coupled to guanylate cyclase, whereas the binding protein, ANP-BP, is not. This study sought to clarify the function of ANP-BP.

1 µM dANP, an ANP analog that binds selectively to ANP-BP, competed for 69±6% of specific ^{125}I-ANP binding in G (n=3). The remainer of ^{125}I-ANP binding was inhibited by 10 nM native ANP.

Affinity crosslinking of ^{125}I-ANP to intact G in the presence of increasing concentrations of ANP or dANP revealed that ANP inhibited labeling of ANP-R1, ANP-R2 and ANP-BP, whereas dANP inhibited labeling only of ANP-BP and markedly enhanced labeling of ANP-R1 and ANP-R2 (n=3).

In two experiments 0.1 µM ANP (+1 mM IBMX) raised G cGMP levels from 0.8 and 0.7 pmol/mg prot. to 14.3 and 13.1 in the absence of dANP, and to 19.9 and 22.9 in the presence of 1 µM dANP. 1 µM dANP alone raised cGMP levels only to 1.2 and 1.2 pmol/mg prot.

Thus, selective occupancy of ANP-BP with dANP augments binding of ^{125}I-ANP to ANP-Rs, and potentiates ANP-stimulated cGMP accumulation in G. These effects of dANP could be explained by increased availability of ANP to ANP-Rs or a molecular interaction between ANP-BP and ANP-Rs.

ALTERATIONS IN THE CENTRAL ANF-SYSTEM OF RATS WITH RENAL HYPERTENSION

Udo Bahner, Helmut Geiger, Miklós Palkovits*, Detlev Ganten**, Klaus Schafferhans and August Heidland
University of Würzburg, Dept. of Medicine, D-8700 Würzburg, *Semmelweis University, Med. School, H-1450 Budapest, **University of Heidelberg, Dept. of Pharmacology, D-6900 Heidelberg (introduced by R.W. Schrier).

Atrial natriuretic peptides (ANF) in the brain are involved in the regulation of blood pressure (B.P.) and electrolyte and fluid homeostasis (EFH). To investigate a possible role of the central ANF-system in renal hypertension we have measured the concentration of ANF by radioimmunoassay in 18 selected brain areas of the volume-dependent 1K1C - and the renin-dependent 2K1C - renal hypertension and their controls (1K-C resp. 2K-C).

The most important results are summarized in the table:

Brain areas	1K-C	1K1C	2K-C	2K1C
OVLT	83	20**	107	39
Preoptic periv. nucl.	360	460*	350	134***
Supraoptic nucl.	20	55*	32	65*
Paraventric. nucl.	145	226***	154	174
Med. eminence	171	359***	154	92**
Locus coeruleus	69	122**	81	82
NTS	74	20**	54	53

Values are pg ANF/mg protein.*=p 0.05,**=p 0.01,***=p 0.001

The alterations of ANF-concentration in various brain areas known to be involved in BP- and EFH-regulation strengthen the idea that ANF is an important modulator in these physiological mechanisms. The fact that ANF is changed in the opposite direction in 1K1C- and 2K1C-hypertensive rats indicates a certain correlation with the renin-angiotensin system.

AORTA AND V. CAVA ARE SIGNIFICANT SOURCES OF NON-CARDIAC ATRIAL NATRIURETIC PEPTIDE (ANP) IN CALVES AND RATS. R. L. Baranowski, G. Burns,* D.B. Olsen,* R. Brownley,* C. Kablitz, C. Westenfelder. Univ. of Utah and VA Med. Ctr., Div. of Nephrology and Artif. Organs, Salt Lake City, UT

We observed that complete replacement of native atria and ventricles (to eliminate all cardiac ANP) by an artificial heart caused marked salt retention and only a small decrease in plasma ANP in calves. In the present study, the origin and molecular characteristics of this non-cardiac ANP were determined. Acetic acid extract (O.1 M) of calf atria contained 40 µg ANP/gm wet wt. (by RIA, anti-hANP), whereas that of aorta contained 100.0 ng and that of v. cava 2.0 ng/gm wet wt. Reversed phase HPLC confirmed that circulating non-cardiac ANP co-chromatographed with hANP-(99-126). Neither acute volume expansion with 6 L NS nor a 50% reduction or elevation in cardiac output altered plasma ANP levels. In comparison, extract of rat atria contained 371 µg ANP/gm wet wt., that of aorta 3.0 µg and that of v. cava 2.2 µg/mg wet wt.. Conclusion: 1) large vessels, especially the aorta, contain significant quantities of ANP; 2) circulating non-cardiac ANP is identical to ANP-(99-126); 3) release of ANP from these sites is not regulated by alterations in volume, which may explain why calves with total artificial hearts retain salt.

ATRIAL NATRIURETIC FACTOR (ANF) AND SODIUM NITROPRUSSIDE (SNP) ANTAGONIZE ANGIOTENSIN II (ANG) STIMULATED INOSITOL PHOSPHATE (IP) RELEASE AND CA EFFLUX IN RAT MESANGIAL CELLS (MC). R. Barnett, S. Blaufox,* S. Singer,* P. Ortiz* and L. Ramsammy*. Department of Medicine, SUNY Stony Brook, Stony Brook, NY.

We have previously demonstrated that ANF antagonizes ANG-mediated IP_3 release in MC. These results may relate to ANF inhibition of the ANG-stimulated rapid rise in MC Ca determined by fluoroprobe methods. Since ANF (10^{-7}M) action may result from enhanced cGMP synthesis, we compared it to the SNP(10^{-4}M) modulation of ANG ($5x10^{-7}$M) induced total IP generation and ^{45}Ca efflux. Confluent first subcultures were used in all experiments. MC were prelabelled with [^{3}H] myo-inositol and pre-incubated for 10 minutes ± agent prior to ANG exposure. Results expressed as CPM/100μg protein indicate that ANF ($p<.02$) and SNP ($p<.004$) significantly inhibited ANG-mediated [^{3}H] IP release. ANF or SNP alone were not different from control.

Agent	Control	ANG	ANG+Agent
ANF(n=6)	3127±480	5071±597	3763±549*
SNP(n=6)	2103±460	5544±488	2540±425*

An effect on phosphoinositide (PI) metabolism could effect MC Ca. Neither ANF nor SNP altered basal Ca efflux. ANG promoted a four fold increase in ^{45}Ca efflux peaking at 60 sec. Both ANF and SNP inhibited this effect (n=6 for each, $p<.001$). Since both ANF and SNP induced a comparable 40 fold increase in MC cGMP accumulation, our data indicates that this second messenger may alter vasoreactivity by effects on PI metabolism and Ca regulation.

KINETICS AND PHARMACODYNAMICS OF ATRIAL NATRIURETIC PEPTIDE FOLLOWING BOLUS AND INFUSION ADMINISTRATION IN THE ISOLATED PERFUSED RAT KIDNEY. Michael E. Brier*, George B. Harding*, and George R. Aronoff, Department of Medicine, University of Louisville and VAMC, Louisville, KY.

The kidney is a site of action and elimination of atrial natriuretic peptide (ANP). Since ANP's effect and elimination are receptor mediated, the method of administration may influence these processes. We used the isolated perfused rat kidney (IPK) to test the hypothesis that the kinetics and dynamics of ANP are altered by the method of administration. We perfused 31 rat kidneys in 6 the groups shown below. Kidneys were perfused for 90 minutes. Calculated ANP clearance (Cl), elimination rate constant (Ke), and urinary sodium excretion (UNaV) are shown below (mean,sd).

Bolus (ng)	Cl(ml/min)	Ke(min^{-1})	UNaV(mmol)
45	3.27(0.51)	0.040(0.014)	0.101(0.072)
180	2.89(1.02)	0.032(0.007)	0.193(0.041)
450	2.28(1.29)	0.046(0.018)	0.253(0.172)
Infusion (ng/min)			
0.5	4.49(0.72)	0.086(0.022)	0.140(0.108)
2.0	3.40(1.48)	0.036(0.005)	0.270(0.095)
5.0	0.97(0.21)	0.012(0.004)	0.308(0.088)

Increasing the ANP dose decreased ANP Cl in both groups, and decreased Ke in the infusion group. Increasing ANP dose increased UNaV in both groups. Although ANP exposure was less in the infusion group, it resulted in a consistently greater natriuresis. We conclude that ANP Cl is saturable. This effect is greater when ANP is given by infusion. Infusion results in a more efficient natriuretic response. These effects may be related to the rate of presentation of ANP to its receptor and resulting receptor down regulation.

ATRIAL NATRIURETIC PEPTIDE MODULATES CALCIUM CHANNEL CURRENTS IN ADRENAL GLOMERULOSA CELLS. P. Q. Barrett, C. M. Isales*, R.T. McCarthy*. Yale Sch. Med., Depts. Med.& Cell Biology, New Haven, CT.

Both T- and L-type Ca++ channels, which differ in their response to small changes in extracellular K+ (3-15 mM), have been previously identified in bovine adrenal glomerulosa (BAG) cells (PNAS, 85: 2412, 1988). Ca++ influx through these channels regulates aldosterone secretion. Since atrial natriuretic peptide (ANP) potently inhibits K+ stimulated aldosterone secretion, we examined the role of T and L channels in ANP inhibitory action using whole cell patch clamps and aequorin measurements of $[Ca^{++}]_i$ in BAG cells.

Raising K+ from 3.5 to 10 mM stimulated the rate of aldosterone secretion to a maximal 9-fold increase at 10 mM K+ which declined at 15 mM K+, consistent with voltage dependent inactivation of T channels (173 to 100 pg/min/10^6 cells, $p<0.001$). Although 10 nM ANP inhibited at all [K+], the secretory rate at 15 mM K+ was 29% greater than at 10 mM K+ (40 vs 31 pg/min/10^6 cells, $p<0.025$).

ANP inhibited a rapidly inactivating component and enhanced a slowly inactivating component of inward Ca++ current. Tail current analysis revealed that ANP inhibited T channels shifting, by -10mV, the voltage dependence of inactivation without altering activation characteristics. In contrast, at relatively depolarized potentials where T channels are largely inactivated, ANP enhanced the L channel component of the Ca++ tail current (+75%: Vh -65 mV). In weakly depolarized cells (8 mM K+) ANP reduced $[Ca^{++}]_i$ only if L channels had been previously inhibited by nitrendipine (1nM) whereas in strongly depolarized cells (12 mM K+) ANP elevated $[Ca^{++}]_i$.

These studies indicate that at physiologically relevant potentials ANP decreases the number of T channels available for opening (via inactivation) while increasing the contribution of L channels to net Ca++ flux . This dual modulation of channel activity by ANP accounts for the changes observed in $[Ca^{++}]_i$ and in aldosterone secretion from the ANP inhibited cell.

CHARACTERIZATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) RECEPTORS IN FRESHLY ISOLATED RABBIT RENAL PREGLOMERULAR MICROVESSELS (RMV). A. Chaudhari, A. Pedram*, M. Tran*, M.A. Kirschenbaum. Nephrology Section, VA Medical Center and UCI-LB Medical Program, Long Beach, CA.

ANP has potent natriuretic, diuretic, and vasorelaxant activity and infusions of ANP *in vivo* result in a dose-dependent vasodilatation of arcuate and interlobular arteries and afferent arterioles. In view of these observations, we have examined for the presence of specific ANP binding sites in rabbit RMV. Interlobular arteries and afferent arterioles were isolated by methods described by this laboratory. A membrane preparation (MP) was obtained by centrifuging a homogenate derived from freshly isolated RMV at 30,000xg for 25 min. Routine incubations consisted of 50 mM Hepes buffer (pH 7.5), 5 mM $MgCl_2$, 0.2% BSA and 0.1% bacitracin at 0° C, ^{125}I-labeled rat 1-28 ANP (25-60 pM) and MP (50-100 μg prot). Non-specific binding was determined in the presence of 0.1 μM unlabeled ANP. Bound ^{125}I-ANP was separated from free ligand by filtration. A time-dependent increase in binding was observed which reached equilibrium by 120 min (all subsequent incubations were performed for 120 min). In the absence of unlabeled ANP, 5-10% of added total counts were specifically bound to RMV membranes. The binding of radioligand (diluted with unlabeled ANP) was saturable. Scatchard analysis of saturation binding studies revealed a single class of binding sites. The K_d and B_{max} values for the binding sites were 0.47 nM and 375 fmol/mg protein. These data indicate the presence of specific ANP binding sites in freshly isolated rabbit RMV and suggest a role for ANP in the regulation of renal microvascular resistance.

RESPONSE OF ATRIAL NATRIURETIC PEPTIDE (ANP) TO ACUTE SALINE VOLUME EXPANSION (VE) IN YOUNG RATS. R L Chevalier, B A Thornhill*, R A Gomez, M J Peach*, R M Carey*. University of Virginia Departments of Pediatrics, Pharmacology, and Medicine, Charlottesville, Virginia.

Plasma ANP levels ([ANP]) are elevated in the fetus and neonate, and decrease postnatally. To study the effects of age on the [ANP] response to VE, saline (3% body weight) was infused in anesthetized Sprague-Dawley rats pre-weaning (PW, 15 to 20 days, N=6) and weanling (W, 25 to 35 days, N=8). Rats were weaned at 21 days. The following were measured in control periods (C) and after VE: [ANP] (pg/ml), urine flow (V, ul/min/g KW), sodium excretion (UNaV, uEq/min/g KW), and cyclic GMP excretion (UcGMPV, pM/min/g KW). Results (mean±SEM, #p<0.05):

	--[ANP]--		---V---		---UNaV---		--UcGMPV--	
	C	VE	C	VE	C	VE	C	VE
PW	140 #	236	4.5 #	34	0.30 #	5.11	6.0 #	17.5
	±27	±34	±.8	±6	±.07	±1.60	±3.1	±5.4
	#		#	#	#			#
W	34 #	291	1.7 #	8	0.07 #	1.39	8.9 #	31.4
	±15	±84	±.3	±1	±.01	±0.35	±1.8	±5.7

Lower control Hct (26±1, PW vs. 39±1, W), and higher [ANP], V and UNaV suggest relative basal VE in PW rats. Although [ANP] after acute VE was similar at both ages and UcGMPV was greater in the W group, V and UNaV were over three-fold greater in the PW than W group. Thus, while ANP is stimulated by acute VE in both age groups, greater V and UNaV in PW rats is dissociated from [ANP], and may be due to altered physical factors resulting from a basal VE state.

DIFFERENCES IN THE TIME COURSE BETWEEN RENAL HEMODYNAMIC AND NATRIURETIC EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN MAN. D. de Zeeuw*, WMT Janssen*, GK van der Hem*, PE de Jong* (Intr. by LW Statius van Eps). Univ. Hosp. Groningen, the Netherlands.

We studied the (time-course) relation between the ANP-induced changes in GFR, ERPF and sodium excretion (UNaV) in 9 healthy subjects upon a 2-hour infusion of low dose ANP (1μg/min). GFR rose and ERPF fell already in the first infusion hour (table). The change in ERPF reflects a fall in true renal blood flow (RBF), since after correction for hematocrit (rose from 38.5±1.5 to 40.6±1.5%, p<0.001) and hippuran extraction (rose from 77.8±1.1 to 79.1±1.1% ,p<0.02), RBF fell from 1065±57 to 988±44 ml/min, p<0.02). Since blood pressure did not change, renal vascular resistance rose. Urinary osmolality (Uosm) fell in the first hour. Despite these changes, UNaV did not change significantly the first hour. Only the second hour natriuresis occurred. Interestingly, the Uosm change in the first hour correlated with the UNaV change (r=-0.71, p<0.05), and with the rise in ANP levels (r=-0.78, p<0.05). ANP rise correlated also with UNaV increase (r=0.90,p<0.01).

	Control	ANP(1h)*	ANP(2h)*	Recovery
ERPF (ml/min)	507±27	464±21*	445±22*	491±19
GFR (ml/min)	117±3	120±3*	121±3	115±3
Uosm(mOsm/kgH20)	311±70	179±32*	237±42*	370±43
UNaV (mmol/h)	8.7±1.4	10.1±1.0	13.3±1.6*	9.1±0.9

In conclusion, 1. ANP is the first saliuretic with renal vasoconstrictor properties, 2. hemodynamic ANP effects precede the natriuresis. This may indicate that the ANP induced natriuresis is in part due to an increase in filtered sodium load into a washed-out medullary interstitium.

AGE AND DOSE RELATED DIFFERENCES IN RESPONSE TO ATRIAL NATRIURETIC PEPTIDE IN MAN. BA Clark*, D Elahi*, FH Epstein, Dept. of Medicine, Beth Israel Hospital, Harvard Medical School, Boston,MA

Seven young (20-39 yr) and 5 old (65-83 yr) healthy, normotensive, non-obese males were studied on 200mEq/day sodium (Na) diets. Synthetic human atrial natriuretic peptide (ANP,1-28) was infused for 60 min at rates of 1) .05 μg/kg/min (high dose) in 7 young and 5 old and 2).005 μg/kg/min (low dose) in 6 young and 4 old. Old compared to young had higher basal ANP levels (44±14 vs 10±1 pg/ml, p<0.025), achieved higher plasma levels with low dose infusion (114±14 vs 42±7 pg/ml, p<0.001) and had a longer half life (7.6±0.8 vs 4.3±0.5 min, p<0.001) suggesting decreased catabolism in the old. Despite these age related differences in ANP levels there was no difference in Na excretion rates. During high dose infusions there was no difference in natriuresis or peak ANP levels in old vs young (429±32 vs 398±19 pg/ml). In both groups, after the low dose infusion ended and despite the return of plasma levels to baseline within 30 min, a sustained natriuresis (2 fold above control) was observed for 3 hr. In contrast, the high dose infusion produced a transient natriuresis (2.5 fold above control) lasting only the duration of the infusion. In conclusion, 1) in the elderly, there appears to be diminished clearance of ANP along with renal resistance at physiologic levels, and 2) the biologic action of ANP (natriuresis) persists longer than the increase in circulating plasma levels at low doses (physiologic range). This effect may be overcome at high doses (pathologic range) secondary to activation of counter-regulatory hormones or other systemic effects.

WATER INDUCED NATRIURESIS AND ANF SECRETION: MODULATION BY THE SODIUM DIET. Marie-France Debiève*, Joëlle Nortier*, Elie Cogan*, Philippe Demaeyer*, Piedad Calderon*,Jared J. Grantham and Maurice Abramow*. Dept.of Physiol. and Pathophysiol., Free Univ. of Brussels (U.L.B.), Belgium and Univ.of Ks. Med.Ctr., Dept.of Med. Kansas City, KS.

We have previously shown that an oral acute water load (AWL:20 ml bottled*water/Kg body weight) induced an increase in plasma ANF (pANF) and in urinary sodium excretion (UNaV). In order to investigate the physiological role of ANF in the volume adaptation to water expansion, we performed AWL after high Na+ intake (HNa, n=14; 300 mEq Na+ intake/day during 4 days) or after slight Na+ depletion induced by 40 mg furosemide followed by low Na+ intake (LNa, n=11; <30 mEq Na+ intake/day during 4 days). Plasma ANF and Aldosterone (pAldo), serum and urine electrolytes and urine cGMP were determined at the basal state and hourly (4 times) after AWL. After LNa, pANF and UcGMP decreased respectively from 4.88 ±0.96 to 3.18±0.42 fmoles/ml (p<0.05) and from 455.2±55.97 to 317.8±45.31 pmoles/min (p<0.05). In contrast, after HNa neither pANF nor UcGMP were significantly changed. AWL induced a transient increase in pANF after HNa (from 5.67±0.79 to 7.07±1.18 at 1 hr, p<0.05), but not after LNa. A sustained increase of UNaV (μEq/min) was found in both groups after AWL, with a peak at the 1st hour (HNa: from 215±24.5 to 372.2±46, p<0.001; LNa: from 4.02±0.97 to 15.84±3.33, p<0.001). The increase in Na excretion during the 4 hrs after AWL (ΔUNaV, mEq/4hrs) was markedly higher after HNa than after LNa (19.1±4.9 versus 2.34±0.6, p<0.01). In HNa, but not in LNa, a significant linear relationship (r=0.53,p<0.05) between ΔUNaV and the maximum pANF after AWL was found.

We conclude that after slight sodium depletion, the volume expansion induced by AWL is not high enough to stimulate ANF secretion. We suggest that the daily sodium intake may modulate the contributory role of ANF in the volume adaptation following an acute water expansion.

*Spa Reine®, Belgium (Na content: less than 0.13 mmoles/L)

IMPAIRED RESPONSE OF ATRIAL NATRIURETIC FACTOR TO HIGH SALT INTAKE IN HYPERTENSION-PRONE MAN. Claudia Ferrier*, Ralph Hollmann*, Roland Dietler*, Sidney Shaw*, and Peter Weidmann. Med. Poliklinik, Univ. of Berne, Switzerland.

The genesis of hypertension could theoretically involve a deficiency in a natriuretic factor. To test this hypothesis, we studied plasma atrial natriuretic factor (ANF) and other variables in normotensive sons of normotensive (SN) or essential hypertensive parents (SEH) (each group N=10; age ± SEM, 25 ± 1 yr). After mild dietary sodium (Na^+) restriction (70 mmol/d x 4 d), body surface (1.86 ± .04 vs 1.91 ± .03 m^2), supine mean blood pressure (BP) (95 ± 2 vs 94 ± 2 mmHg), plasma ANF (26 ± 2 vs 27 ± 4 pg/ml) and 24 h urinary Na^+ (54 ± 9 vs 66 ± 8 mmol) did not differ between SN and SEH. On high Na^+ intake (350 mmol/d x 4 d), mean BP (95 ± 2 vs 96 ± 3 mmHg), exchangeable Na^+ (3.12 ± .09 vs 3.04 ± .09 mol), and 24 h-urinary Na^+ (276 ± 22 vs 334 ± 38 mmol) were similar in SN and SEH; plasma ANF increased in SN ($P < .001$) but not in SEH (to 55 ± 5 vs 35 ± 5 pg/ml). Subsequent administration of furosemide produced in SN and SEH a similar natriuresis (170 ± 8 vs 174 ± 14 mmol within 2 h and 411 ± 34 vs 429 ± 49 mmol within 24 h) and restored plasma ANF to control values (32 ± 3 vs 29 ± 3 pg/ml at 2 h and 24 ± 3 vs 26 ± 3 pg/ml at 24 h after furosemide). Conclusions: A diminished response of circulating ANF to high Na^+ intake may occur as a familial disturbance in SEH. Considering the high Na^+ intake of modern man, impaired activation of the potentially vasodilator-natriuretic ANF could at long term favor the development of hypertension.

OPPOSITE EFFECTS OF NITRENDIPINE AND ENALAPRIL ON RENAL ANF RESPONSE IN MAN. CA Gaillard, HA Koomans, AJ Rabelink, and EJ Dorhout Mees. Dept. of Nephrology, Univ. of Utrecht, Utrecht, The Netherlands.

We investigated the effect of calcium entry blockade (CEB) with nitrendipine (4 days; 20 mg b.i.d.) and of converting enzyme inhibition (CEI) with enalapril (4 days; 20 mg b.i.d.) on renal effects of ANF (25 μg bolus followed by 0.03 $\mu g.kg^{-1}.min^{-1}$ for 90 min) in 6 healthy men taking 300 mmol sodium daily. In the clearance study without CEB or CEI, natriuresis rose from 239±38 to 605±137 umol/min ($p<0.05$). During CEB natriuresis increased from 330±53 to 943±152 μmol/min ($p<0.05$), and during CEI from 236±22 to 344±39 μmol/min (ns). ANF induced a rise in maximal free water clearance, inulin clearance, and in the excretion of multiple electrolytes except potassium. Fractional lithium reabsorption fell. In general, these effects were stronger during CEB and blunted during CEI. Para-aminohippurate clearance tended to decrease during control study (ns), remained constant during CEB, and decreased significantly when ANF was infused during CEI ($p<0.05$ vs control). During treatment with CEB and CEI mean arterial pressure (MAP) was lowered by, respectively, 3 mmHg and 7 mmHg). ANF had no additional effect on MAP except for a shortlasting drop during CEB. From these data we conclude that, in healthy humans, the effects of ANF on natriuresis and renal sodium handling are enhanced by CEB and blunted by CEI. The clinical implication of these data might be that CEB can be used to enhance the actions of endogenous ANF (heart failure), and CEI to attenuate action of endogenous ANF (hyperfiltration).

BIOLOGICAL RECEPTORS OF ANF IN CULTURED RENOMEDULLARY INTERSTITIAL CELLS (RMIC). B.M.A. Fontoura*, K.A. Pelton*, E.Townes-Anderson*, T. Maack and D. R. Nussenzveig*. Dept. of Physiology, Cornell Univ. Med. Coll., New York, N.Y.

To further evaluate the distribution and function of renal biological (B) and clearance (C) receptors of ANF we tested for their presence in cultured RMIC from the rat. A homogenous population of cells was obtained from a primary culture and was studied during the 3th-6th passage. Sudan black staining and electron microscopy of these cultured cells showed the typical and unique morphological features of RMIC, including large lipid inclusions. Equilibrium saturation binding curves with ^{125}I-ANF1-28 showed a high density of high affinity specific binding sites for the endogenous form of ANF (Kd = 70 pM, Bmax = 40 fmoles/million cells, app. 23000 sites/cell). There was only minimal specific binding of $^{125}I(^3Y)$C-ANF4-23, a specific ligand of C-ANF receptors. ANF1-28, but not C-ANF4-23, completely displaced specifically bound ^{125}I-ANF1-28 (Ki = 256 pM). ANF1-28, but not C-ANF4-23, led to a marked dose-dependent increase in cGMP with an ED50 = 3 nM. At a maximal concentration (1μM) ANF1-28 increased cGMP by >60-fold above basal levels to 120 pmoles cGMP/2min/million cells. Both density of B-ANF receptors and maximal ANF-induced increase in cGMP were greater than in any other type of renal cell tested to date. The results demonstrate that cultured RMIC contain a high density of B-ANF receptors and a minimal density of C-ANF receptors. Thus, RMIC are major target cells for ANF in the renal medulla and may mediate some of its known functional effects in this kidney region.

PARATHYROID HORMONE (PTH) IS INVOLVED IN THE RELEASE OF ATRIAL NATRIURETIC PEPTIDE (ANP) DURING ACUTE VOLUME EXPANSION (VE). Helmut Geiger*, Udo Bahner*, Shaul G. Massry, Marianne Meibner*, Michael Kristein*, Roland M. Schaefer*, August Heidland*. Univ. Wuerzburg, Med Clinic, Dept. Nephrol, Wurzburg, FRG, and Univ. So. Calif., Los Angeles, CA.

The secretion of ANP is calcium dependent and is influenced by cytosolic calcium. PTH has ionophoric action and may thus, influence release of ANP. We examined whether atrial ANP and its blood levels during acute VE are modulated by PTH. Acute VE in normal rats produced by infusion of 15 ml of NaCl/Kg over 2 minutes was associated with a significant rise in blood ANP (114±23 to 702±86 pg/ml; $p<0.01$) and a significant rise in ANP content of both left and right atrium. In contrast, the same degree of acute VE in parathyroidectomized normocalcemic rats, ANP in both atria did not increase and plasma ANP was (217±38 pg/ml) significantly lower than in the intact rats (702 ± 68 pg/ml), $p<0.01$. Infusion of calcium chloride, 1-84 PTH or 1-34 PTH for 2 hours to normal rats without VE did not cause a significant increment in ANP levels in plasma when compared to rats receiving the same small volume of infusate in the form of NaCl only (0.2 ml/hr). The results show that hypercalcemia or PTH infusion in animals without volume expansion do not affect plasma levels of ANP. However, the presence of PTH is required for the augmented ANP release during acute volume expansion.

Acute renal failure (ARF) in the intensive care unit: Are there any benefits of atrial natriuretic factor (ANP) in Dopamine/Furosemide resistant acute renal failure?

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Previous animal studies have shown that ischemic ARF (early phase) was ameliorated by ANP. Therefore, we investigated the effect of ANP in critically ill patients with protracted dopamine and furosemide resistant ARF. 13 pts. of the intensive care unit (10 male, 3 female) with protracted ARF were investigated. 7 pts. were anuric, 6 pts. non-oliguric (> 1500 ml/d). ANP (1-28 hANaP, Bissendorf, W.-Germany, 2.5 µg/min) was infused for 3 hours. Following parameters were measured: urine volume, creatinine, electrolytes, osmolality in serum and urine, plasma catecholamines, plasma renin activity (PRA), ANP-levels, and glomerular filtration rate (GFR, Cr-EDTA) before and after ANP-infusion.

In anuric pts. ANP was not able to reverse anuria. In non-oliguric pts. urine volume increased more than 50 %. GFR did not change significantly both in the anuric group (6.2 + 1.5 ml/min) and in the non-oliguric group (19.8 + 8.5 ml/min). Sodium excretion (FENa) increased by about 40 % in non-oliguric pts. All pts. showed a slight elevation of hemoglobin and hematocrit. A decrease of PRA was observed in both groups, an elevation of plasma-norepinephrine only in the non-oliguric states.

These preliminary data suggest that ANP was not able to ameliorate protracted, dopamine and furosemide resistant ARF in intensive care unit pts. If there is any benefit of ANP in severe ARF, it appears to be crucial to administer ANP early before more severe anatomical and functional damage develops.

INHIBITION OF ATRIAL NATRIURETIC PEPTIDE (ANP) RELEASE IN THE ISOLATED ATRIUM BY VITAMIN D_3.

A.Halabe,* N.L.M.Wong, E.F.C.Wong,* R.L.A.Sutton

Calcitriol[1,25$(OH)_2D_3$] the active metabolite of Vitamin D_3 has been reported to alter mRNA transcription of Calcitonin and Parathyroid hormone. We have previously shown that patients with idiopathic hypercalciuria have lower plasma (ANP) and higher plasma Calcitriol levels than normal control subjects. In the present study in rats we investigated the effect of acute and chronic Calcitriol administration on spontaneous ANP release from isolated atria using a modified Langendorff apparatus.In the chronic studies 0.5mcg Calcitriol was administered i.p. every other day during 4 weeks. Plasma ANP was significantly depressed in Calcitriol treated rats compared to the control group (59±4 vs. 98±5 pg/ml $p<0.001$).In the acute studies, male wistar rats (200-250 gm) were given i.p. Calcitriol at a dose of 1, 5, 10, 50, 100, and 150 ng 3 hours prior to the experiments. The right atrium was quickly excised and perfused with tyrode's solution for 100 min. at 0.5 ml/min. The effluent was collected with a fraction collector every 5 min. for the determination of IR-ANP. ANP release (expressed in pg/ml/mg tissue) showed a significant dose dependent suppresion in response to Calcitriol from 8.61±0.46 (n=12) in controls to 3.57±0.28 (n=6), 3.34±0.24 (n=6) and 1.30±0.03 (n=6) after 1,5 and 10 ng of Calcitriol respectively.

These studies show that Calcitriol may play a regulatory role in ANP release.

BRAIN NATRIURETIC PEPTIDE(BNP) ACTS VIA ATRIAL NATRIURETIC PEPTIDE(ANP) RECEPTORS ON PARTICULATE GUANYLATE CYCLASE(PGC) TO INHIBIT NA^+ TRANSPORT IN INNER MEDULLARY COLLECTING DUCT(IMCD) CELLS.

M. Gunning,* B.M. Brenner, P. Silva, M.L. Zeidel, Harvard Medical School, Boston, MA.

BNP, a novel peptide distinct from but with some homology to ANP, isolated from porcine brain causes a natriuresis, diuresis, and a fall in blood pressure when infused in rats. We have examined the mechanism of natriuretic action of BNP on a nephron segment known to respond to ANP, the IMCD. In suspensions of IMCD cells BNP (1 µM) completely displaced ^{125}I-ANP binding; the peptide exhibited affinity (IC_{50}=120pM) similar to ANP_{5-28}, greater than ANP_{5-25}, and less than ANP_{1-28} (IC_{50}=60pM). At micromolar concentrations, BNP and ANP gave equivalent stimulation of cGMP accumulation in IMCD cells (7.4±2.6 vs 6.1±1.6 pmol/mg) and gave similar stimulation of PGC activity (315±35 vs 315±40 pmol/mg/min). However, PGC was less sensitive to BNP (half-max. stim. at 1000 pM) than to ANP (half-max. stim. at 60 pM). The Na transport effects of BNP on IMCD cells were examined using QO_2 and ^{22}Na uptake. BNP inhibited QO_2 by 18±1%; inhibition was non-additive with ouabain and reversed by amphotericin B, indicating inhibition of Na entry. In addition, BNP reduced conductive Na uptake by 57±16% (n=5) at 30 sec.

We conclude that BNP acts on IMCD cells in a manner similar to ANP, by binding to ANP receptors, stimulating PGC and inhibiting conductive Na^+ uptake. Since IMCD cells possess a single class of 130 kD PGC-linked ANP receptors, our results demonstrate that BNP interacts with this receptor, albeit at lower affinity than ANP.

DETERMINANTS OF THE RENAL MICROVASCULAR RESPONSIVENESS TO ATRIAL NATRIURETIC PEPTIDE (ANP): LACK OF EFFECT ON THE MYOGENIC RESPONSE OF THE AFFERENT ARTERIOLE.

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ANP, a potent vasodilator of the afferent arteriole (AA), is reported to have little effect on renal autoregulation. Since pressure-induced (myogenic) pre-glomerular vasoconstriction contributes to renal autoregulation, we compared the effects of nifedipine (NIF) and ANP on the myogenic response of the AA. We previously demonstrated that ANP completely reverses norepinephrine(NE)-induced vasoconstriction of this vessel (J.P.E.T. 246:522-528,1988). Chronic unilaterial hydronephrosis was induced in rats to facilitate direct visualization of the renal microvessels. Hydronephrotic kidneys were excised and perfused in vitro. AA diameter, measured by videomicroscopy, decreased as renal arterial pressure (RAP) was increased (CONT). This myogenic response of the AA was prevented by NIF (1.0 µM), but not by ANP (100 nM).

RAP mm Hg	80	100	120	140	160	180
AA-CONT	18±1	18±1	17±1	16±1	15±1	14±1
AA-NIF	20±1	20±1	20±1	20±1	21±1	22±1
AA-ANP	18±1	17±1	16±1	15±1	15±1	14±1

(AA diameters in µm, mean ± SE, n=6)

These findings are consistent with the observations that renal autoregulation is abolished by calcium antagonists but is preserved during ANP administration, and support the view that the myogenic response of the AA plays a prominent role in autoregulation. Furthermore, since we previously found ANP to reverse NE-induced AA vasoconstriction, the lack of effect on ANP on the myogenic response of this vessel indicates that the renal microvascular actions of ANP vary depending on vasoconstrictor stimuli.

RESISTANCE TO THE EFFECTS OF BRAIN NATRIURETIC PEPTIDE (BNP) IN CONCIOUS RATS WITH CONGESTIVE HEART FAILURE.
Aaron Hoffman* and Harry R. Keiser* (intr. by John R. Gill Jr)
National Heart, Lung and Blood Institute, NIH, Bethesda, MD

A new natriuretic 26 amino-acid polypeptide extracted from porcine brain was described recently. Reportedly it has similar hemodynamic and renal excretory effects as the atrial natriuretic peptide (ANP) even though there are 8 amino-acid residues replaced. We have shown high levels of plasma ANP together with a markedly blunted response to synthetic ANP in rats with congestive heart failure (CHF), so it was of interest to study the effects of BNP in this model.

In Wistar-Kyoto rats we placed a sutureless infrarenal aortocaval fistula, 1.2±0.2 mm long, to produce high output CHF. Seven to ten days later, under halothane anesthesia, the rats and normal controls were cannulated and prepared for urine collections. After 4 hours of recovery, baseline mean arterial pressure (MAP) and renal parameters were determined and a bolus of 20 ug/Kg synthetic BNP was given intravenously. Measurements (Mean±SEM) for 1 hr and an additional recovery period of 1hr were:

		Baseline	BNP	Recovery
	MAP (mm Hg)	140±5	121±4*	135±8
Control	$\dot{V}$ (µl/min)	28±5	40±4*	14±5*
(n=6)	$UNa\dot{V}$(µeq/min)	1.4±0.2	3.7±0.6*	2.1±0.1*
	MAP (mm Hg)	115±7**	115±8	115±7
CHF	$\dot{V}$ (µl/min)	7.1±3.1**	8.5±3.4	10.5±7.7
(n=6)	$UNa\dot{V}$(µeq/min)	0.26±0.14**	0.23±0.13	0.17±0.09

($\dot{V}$-urine volume, $UNa\dot{V}$-urinary sodium excretion, *=p<0.05 vs. Baseline, **=p<0.05 vs.Control)

Whereas BNP elicited considerable diuretic, natriuretic and hypotensive responses in control rats, there was total resistance to these effects of BNP in rats with CHF. This resistance may be due to the same mechanisms operating in the resistance to ANP and may be a contributing factor to the sodium retention in CHF.

ATRIAL NATRIURETIC FACTOR RELEASE AFTER RAPID CARDIAC PACING IN HYPOPHYSECTOMISED RATS.
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Spontaneous or paced tachycardia is a strong stimulus for atrial natriuretic factor (ANF) release from the atria, independent of right atrial pressure. This stimulus was chosen to test the hypothesis that the hypophysis is involved in modulating ANF release by a specific factor. The validity of this hypothesis was challenged by the demonstration of normal ANF release in hypophysectomised (Hypx) rats in congestive heart failure, a model of chronic stimulation for ANF release.

Twelve anesthetised Sprague-Dawley rats, Hypx or sham operated by Taconic Farm were used. Pacing at a constant rate of 500 beats/min. for 5 minutes was done by a bipolar wire introduced into the right atrium via the jugular vein and connected to a Grass stimulator. Mean arterial blood pressure (MAP) and heart rate (HR) were recorded continuously and blood was drawn for plasma ANF (pANF) determinations before and at the end of the pacing period. pANF was determined by a radioimmunoassay commercial kit (Peninsula Lab). Results were:

	Sham (6)		Hypx (6)	
	MAP	pANF	MAP	pANF
Baseline	120±7	101±19	78±7	60±12
Pacing	109±7*	241±49*	45±10*	237±57*

(Mean±SEM, *=p<0.05,MAP in mmHg, pANF in pg/ml)

In Hypx rats the pANF increased fourfold even though they suffered severe hypotension at the time of the pacing. In contrast MAP decreased only slightly in the control rats and their pANF doubled. The absolute levels of pANF reached were similar in both groups. These results suggest that no specific hypophyseal factor is required for ANF release in rats after rapid cardiac pacing.

THE ANTIHYPERTENSIVE EFFECT OF A 5 DAY INFUSION OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN MAN.
WMT Janssen, Dick de Zeeuw, GK van der Hem, PE de Jong (Intr. by LW Statius van Eps). Univ. Hosp. Groningen, The Netherlands.

To date only relative short-term ANP infusions have been evaluated in man : at high dose salidiuretic as well as blood pressure lowering effects are observed, whereas at low (more physiological) dose only salidiuretic effects are present. We therefore studied the effects of a 5 day very low dose (0.2µg/min) ANP infusion on mean arterial pressure (MAP) and sodium balance (NaB) in 6 essential hypertensives (EH) in metabolic ward conditions. Patients were without medication for 6 weeks, in balance on 150 mmol/day sodium intake, and kept bedrest during 3 equilibration, 3 control, 5 ANP-infusion, and 3 recovery days. MAP and pulse were measured every 15 min, ANP and NaB every 4 hours (h). Table shows the 24h mean ± sem.

	control	ANP 1st	5th-day	recovery
ANP (pg/ml)	35±6	81±11	73±3	35±8
MAP (mmHg)	104±3	101±3	92±2	98±4
Pulse (bpm)	64±4	69±4	72±4	67±4
NaB (mmol)	0±10	-70±11	-72±15	-3±52

ANP caused an immediate rise in sodium excretion, levelling after 24h (range NaB: -32 to -139 mmol). MAP only started to fall after 12h to level off after 40h. The fall in MAP was 10.9±1.0% (7.7-13.8 %). Plasma ANP levels and NaB recovered to baseline within 24h, whereas MAP slowly returned towards baseline values over 3 days. The data show that in EH chronic low dose ANP infusion causes a negative sodium balance followed by a slower decrease in blood pressure with a new equilibrium after about 2 days. Thus, ANP-like substances may become usefull antihypertensive drugs.

Atrial natriuretic peptide (ANP) is not a major determinant of the renal response to head-out water immersion.
H.A. Koomans, A.J. Rabelink, C.A. Gaillard, E.J.Dorhout Mees. Dept.of Nephrology. University Hospital Utrecht, The Netherlands.

In order to appraise the role of ANP in the natriuresis induced by head-out water immersion (HOI) we compared during clearance studies the effect of 3h HOI with an equally natriuretic 3h infusion of ANP (0.01 $ug.kg^{-1}.min^{-1}$ human ANP 99-126) in 7 healthy individuals taking a 100 mmol sodium diet. Since we found previously that converting enzyme inhibition with enalapril inhibits the natriuresis after ANP, studies were also performed during enalapril (20 mg bid). The main findings were: 1) HOI induced a similar natriuresis as ANP infusion with an about 5 times smaller rise in plasma ANP; 2) while both maneuvers tended to increase glomerular filtration rate (inulin clearance), effective renal plasma flow (PAH clearance) was increased by HOI and decreased by ANP; 3) maximal free water clearance was increased and fractional lithium reabsorption decreased by HOI, whereas ANP had no effect on these variables but increased minimal urine osmolality; 4) in contrast to earlier studies with higher dosages, ANP reduced potassium excretion. HOI, however caused a initial kaliuresis. 5) enalapril virtually abolished the natriuresis after ANP, but did not affect natriuresis after HOI, even though during enalapril HOI caused a large fall in blood pressure (-15 mmHg). These data indicate that the mechanism of natriuresis of these two equally natriuretic maneuvers is essentially different, and that ANP plays no major role in the natriuresis of HOI. In addition, in physiological concentrations ANP may act preferentially in the distal nephron, causing natriuresis and potassium retention.

MANGANESE (Mn^{2+}) MIMICS SOME BUT NOT ALL RENAL ACTIONS OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN VIVO. H.M. Lafferty,* M. Gunning,* S. Anderson, J.L. Troy,* and B.M. Brenner. Brigham and Women's Hospital, Boston, MA.

In inner medullary collecting duct membranes, Mn^{2+} but not calcium (Ca^{2+}) increases basal guanylate cyclase (GC) activity and cGMP formation, and enhances GC sensitivity to ANP. To evaluate whether Mn^{2+} might exert in vivo effects similar to those of ANP, we administered $MnCl_2$ or $CaCl_2$ to rats by continuous intravenous infusion (0.65 μmol/min):

Group (n)	GFR	RPF	FE_{Na}	u cGMP
	ml/min		%	pm/min
$MnCl_2$ (7)				(n=4)
Pre	1.0±.1	3.3±.1	0.5±.1	3.7±1.2
30 mins.	1.5±.1*+	5.1±.2*+	1.5±.2*+	3.7±1.6
$CaCl_2$ (5)				(n=3)
Pre	1.1±.1	3.5±.1	0.4±.1	4.0±1.4
30 mins.	1.1±.1	3.5±.3	0.7±.2	4.4±1.3

(Means ± SEM; *p < .05 vs. Pre, +p < .05 vs. $CaCl_2$). Neither infusion changed blood pressure. The increases in glomerular filtration rate (GFR) and fractional excretion of sodium (FE_{Na}) with Mn^{2+} were similar to ANP effects, but the sustained increase in renal plasma flow (RPF) and lack of increase in urinary cGMP excretion (u cGMP) suggest that the actions of Mn^{2+} are independent of GC activation. Since Mn^{2+} also inhibits transcellular Ca^{2+} influx in muscle, the glomerular hemodynamic effects may be due to vasodilatation resulting from inhibition of renal vascular smooth muscle contraction. The natriuretic mechanism remains to be more fully clarified. Thus, Mn^{2+} has significant vasodilatory and natriuretic effects in vivo.

HETEROGENEOUS NATRIURETIC RESPONSES TO ATRIAL NATRIURETIC PEPTIDE (ANF) IN TWO MODELS OF CANINE ASCITES. E. Maher, P. Cernacek and M. Levy. Depts. of Physiology and Medicine, McGill University, Montreal, Quebec, Canada.

All of our normal dogs increase $U_{Na}V$ following i.v. ANF. When ANF at 175 ng/kg/min was given to chronic caval dogs (TIVC) or cirrhotic dogs (Cirrh.) while they were retaining sodium (45 mEq Na/day intake) and forming ascites, they divided 50:50 into those where $U_{Na}V$ showed no change or where $\Delta U_{Na}V$ increased markedly. Of 46 TIVC dogs, 22 responded to ANF with a $\Delta U_{Na}V$ of 185 ± 35 μEq/min, while in 24 dogs, $\Delta U_{Na}V$ was blunted (2 ± 1 μEq/min). In 19 cirrh. dogs, 9 were responders ($\Delta U_{Na}V$ = 60 ± 10 μEq/min), while 10 were non-responders ($\Delta U_{Na}V$ = 1.3 ± 0.6 μEq/min). $\Delta U_{Na}V$ could not be correlated to changes in GFR or C_{PAH}. Natriuretic responders or non-responders (NR) could not be differentiated in terms of atrial content of ANF, plasma levels or t½, systemic hemodynamics, plasma volume or plasma levels of renin (R) and aldosterone (A). They generated C.GMP equally. Renal denervation or vasodilatation did not increase $U_{Na}V$ in response to ANF in NR. Some TIVC dogs returned to sodium balance on 45 mEq/day Na intake, despite persistent ascites. Five NR dogs in balance now increased $U_{Na}V$ dramatically ($\Delta U_{Na}V$ = 90-340 μEq/min) following i.v. ANF. We conclude that blunting of $U_{Na}V$ in response to i.v. ANF is functional and reversible and occurs at a tubular level, probably in the medulla.

COMPARISON OF THE NATRIURETIC RESPONSES (UNaV) TO WATER IMMERSION (WI) AND ATRIAL NATRIURETIC PEPTIDE INFUSION (IANP) IN CIRRHOTIC PATIENTS (C). L. Legault, L. Warner*, W.M. Leung, L. Blendis*, K. Skorecki, A. Logan, Dept. Med., Univ. of Toronto, Canada

We have previously reported that C fall into two categories in terms of their UNaV to WI. 50% exhibit significant UNaV (R), while the others do not (NR), despite comparable and significant increases in plasma ANP (pANP). In order to determine whether UNaV resistance to WI in NR does indeed represent resistance to ANP, we compared pANP (pg/ml) and UNaV (mmol/hr) to IANP and WI in 8 C maintained on a 20 mmol Na diet:

	R(4)	NR(4)	normals(5)
baseline: UNaV	<0.1	<0.1	0.7±0.1
pANP	40±10	20±8	13±5
peak WI:UNaV	5.1±2.2*	.06±.02	2.8±.6*
pANP	118±42*	70±23*	19±6*
peak IANP:UNaV	6.2±1.8*	0.2±0.2	
pANP	236±7*	365±95*	

(p<.05 compared to baseline)

Those C who did not significantly increase UNaV with WI despite a significant rise in pANP (NR), also did not respond to IANP which achieved even higher pANP levels. We conclude that in NR, resistance to WI does indeed represent resistance to the natriuretic action of ANP; and that markedly raising ANP levels in such patients is not itself sufficient to induce a natriuresis.

DOSE RESPONSE OF ATRIAL NATRIURETIC FACTOR (ANF 102-126 OR WY 47663 IN PATIENTS WITH RENAL INSUFFICIENCY

J.F.E. Mann, Ch. Reisch, and E.Ritz (intr.by F.C.Luft) Dept. of Medicine, Univ. of Heidelberg, FRG

We investigated the effects of ANF on renal function and on blood pressure (BP) in 7 patients (pts) with renal impairment (serum creatinine 2.2-7 mg/dl), and in 4 normal volunteers. Methods: After an oral fluid load, fluid intake was matched with urine volume (UV); ANF was administered when urine output was stable. On separate days (randomized order) placebo, or 1,2,or 3 ug/kg ANF was given iv (bolus, single-blind). Results: Effects were dose-dependent. At 3 ug/kg ANF, maximal UV was 44±28 ml/min vs 256±32, UNa V 242±88 umol/min vs 814±56 in pts and volunteers respectively ($p < 0.05$). UCl^-V changed in parallel with UNa^+V while UK^+V, $UCa^{++}V$, and UPV exhibited no significant changes. Plasma aldosterone concentration fell in all volunteers after 3 ug/kg ANF but not in pts ($p < 0.01$ between groups). Endogenous creatinine clearance increased by > 20% in all volunteers but remained constant in pts ($p < 0.05$). Maximal systolic and diastolic BP decrease at 3 ug/kg ANF was 11±3 mmHg and 5±2 vs 8±2 and 5±1 in pts and volunteers respectively (not different between groups). Plasma concentration at baseline was higher in pts (42 ±10 fmol/ml vs 12±4, $p < 0.05$), and increased after 3 ug/kg ANF by >50 fold in all subjects (maximal concentration). Conclusion: The effects of ANF on renal excretory function, creatinine clearance and aldosterone secretion are blunted in pts with renal insufficiency while effects on BP appear to be unaltered.

ENHANCED EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN PATIENTS WITH CHRONIC RENAL FAILURE (CRF): POTENTIAL MECHANISM OF ACTION. H.Meyer-Lehnert, T.Bayer,* K.Glänzer,* H.J.Kramer. Med. Poliklinik, Univ. of Bonn, West Germany.

We examined the effect of 60 min α-hANP infusion (24 ng/min/kg) on GFR (C_{In}), RPF (C_{PAH}), cardiac index (CI, impedance cardiography) and blood pressure (BP) in 8 patients with CRF (51.9±3.4 yrs) and in 8 control (C) subjects (54.1±4.1 yrs) with normal renal function. During ANP, GFR increased in C by 16.2±1.4% and in CRF by 70.7±4.2% (p<.001 vs C) from 34.4±6.8 to 57.4± 9.9 ml/min (p<.001). RPF increased in CRF by 43.6± 6.4% and in C by 3.1±1.2% (p<.01). During ANP infusion, CI increased in CRF after 30 min from 2.91±0.08 to 3.12±0.09 l/min/m² (p<.001) and in C from 3.20 ± 0.11 to 3.39±0.13 l/min/m² (p<.05). Mean arterial BP was higher in CRF and decrease was greater than in C (9.1±1.0 vs 21.1±2.7%, p<.001). In CRF, GFR, RPF, and CI remained significantly elevated and BP was still significantly decreased 60 min after ANP infusion; in C, however, there was no difference compared to basal levels. Basal plasma levels of ANP and cGMP were elevated in CRF (ANP: 30.0±5.4 vs 76.0±14.0 pmol/l, p<.05; cGMP: 6.5±1.1 vs 14.3±2.9 pmol/ml, p<.05). During ANP infusion, peak levels of cGMP were higher in CRF (17.3±1.3 vs 27.5±3.2 pmol/ml, p<.05) and remained elevated 60 min after ANP (17.8±2.7 pmol/ml, p<.01 vs basal), while in C, cGMP had returned to basal levels (7.1±0.4 pmol/ ml, NS vs C basal; p<.01 vs CRF at 60 min). Thus, ANP has a greater and prolonged effect on systemic hemodynamics and renal function in CRF than in C. This effect may be due to higher levels of ANP and may be mediated by a sustained formation of the second messenger cGMP.

CLEARANCE (C) RECEPTORS MEDIATE INTERNALIZATION AND LYSOSOMAL HYDROLYSIS OF ANF IN FIBROBLASTS. D.R. Nussenzveig,* B.A. Fontoura,* R. Scarborough,* J. Lewicki,* and T. Maack. Dept. of Physiology, Cornell Univ. Med. Coll., N.Y., N.Y. and California Biotechnology, Mountain View, CA.

C-ANF receptors have as a major function the removal of ANF from the circulation. To study the mechanism of this function we determined C-ANF receptor-mediated internalization and lysosomal hydrolysis of ANF in cultured 3T3 Swiss albino mice fibroblasts (F). F contains a high density of C-ANF but not B-ANF receptors (Nussenzveig et al., Am. J. Hypertension 1:121A, 1988). ^{125}I-ANF_{1-28} was bound to F at 0°C and then the cells were washed and incubated at 37°C in presence or absence of the lysosomotropic agent NH_4Cl (10 mM). Cell surface and intracellular associated ^{125}I as well as TCA ppt and soluble ^{125}I released to the medium were determined at several time intervals. ^{125}I-ANF_{1-28} was rapidly internalized at an initial rate of ~10% of occupied receptors/min. Dissociation of intact ^{125}I-ANF_{1-28} from C-ANF receptors was negligible compared to its internalization. NH_4Cl markedly inhibited the appearance of TCA soluble radioactivity in medium from 74% to 28% of internalized radioactivity during 15 min of incubation. Correspondingly, NH_4Cl markedly increased intracellular radioactivity during this time. Blockage of C-ANF receptors by its specific ligand C-ANF_{4-23} practically abolished the internalization and hydrolysis of ^{125}I-ANF_{1-28}. Results demonstrate that binding of ANF to C-ANF receptors in fibroblasts leads to rapid internalization and subsequent lysosomal hydrolysis of the hormone. Thus, C-ANF receptor-mediated endocytosis is the basic mechanism of ANF removal from the circulation.

DOES ATRIAL NATRIURETIC PEPTIDE (ANP) CAUSE CHRONIC NATRIURESIS? H. L. Mizelle*, J. E. Hall, D. A. Hildebrandt*, and J.-P. Montani*. Department of Physiology & Biophysics, University of Mississippi Medical Center, Jackson, MS

Acute infusion of ANP causes marked natriuresis and diuresis. However, these effects are not sustained, possibly because of a decreased sensitivity of the kidney to ANP, neurohumoral changes, or because of a compensatory decrease in mean arterial pressure (MAP). The aim of this study was to determine whether physiological levels of ANP can cause chronic natriuresis if changes in MAP and neurohumoral influences are controlled. This was accomplished by quantitating the renal effects of chronic unilateral renal arterial infusion of ANP in conscious dogs on a normal sodium intake (~ 80 mEq/day) with the urinary bladder split to allow continuous measurement of renal function in the ANP infused and contralateral kidneys. Since both kidneys are exposed to the same MAP, neural activity, and circulating hormones, increases in sodium excretion (UNaV) in the ANP infused kidney, compared to the control kidney, should be sustained if ANP has a chronic natriuretic action. ANP was infused into one renal artery at two different rates, 2 ng/kg/min (n=4) and 4 ng/kg/min (n=3) for 7 days (d) each with 7 d of recovery between infusions, while the other renal artery was infused with vehicle. At the 2 ng dose, urine output (UO) and UNaV were elevated by 33.5 ± 1.0 % and 29.8 ± 5.5 % (7 day average), respectively, in the ANP infused compared to the control kidneys throughout the entire infusion period. ANP infusion at 4 ng/kg/min elevated UO 68.5 ± 1.8 % above the control kidneys (604 ± 13 vs 359 ± 6 ml/day) and UNaV 98.5 ± 3.6 % (50.2 ± 1.3 vs 25.4 ± 0.8 mEq/day). GFR was not different between the ANP infused and vehicle infused kidneys at either dose of ANP. These results demonstrate that ANP infusion at physiological levels causes sustained diuresis and natriuresis, with no change in GFR, when the influences of compensatory changes in MAP and neurohumoral factors are controlled.

SPECIFIC DOPAMINE$_1$(DA_1) RECEPTOR BLOCKADE INHIBITS THE NATRIURETIC AND PHOSPHATURIC EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP). F.V. Ortola,* I. Seri,* S.J. Downes,* B.M. Brenner, B.J. Ballermann. Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Blockade of endogenous ANP in rats with reduced renal mass (5/6 Nx) lowers fractional sodium (FE_{Na}), as well as fractional phosphate (FE_{Pi}) and calcium (FE_{Ca}) excretions. To test whether these ANP effects may be mediated by dopamine (DA), we infused 10 ng/kg/min ANP into normal rats followed by 4 μg/kg/min of the specific DA_1 receptor antagonist SCH 23390 (SCH) or vehicle. Urinary Na($U_{Na}V$) and Pi($U_{Pi}V$) excretion were determined during baseline, ANP and ANP + SCH infusions. (Mean ± SEM, n = 5, *p < 0.05 vs. ANP; +p < 0.05 vs. baseline).

	Baseline	ANP	ANP + SCH
$U_{Na}V$ (μEq/min)	1.3 ± 0.1	2.1 ± 0.9+	1.4 ± 0.3*
$U_{Pi}V$ (μEq/min)	0.5 ± 0.1	1.1 ± 0.2+	0.6 ± 0.1*

Thus, DA_1 receptor-blockade decreased the effect of ANP on $U_{Na}V$ and $U_{Pi}V$. GFR and BP remained stable and vehicle had no effect. We also infused SCH in rats with 5/6 Nx and measured FE_{Na} and FE_{Pi}. (n=5, *p 0.05 vs. baseline).

	Baseline	SCH
FE_{Na}(%)	0.36±0.05	0.19±0.03*
FE_{Pi}(%)	25.3±3.6	18.1±3.6*

Thus, DA_1 receptor-blockade also reduced FE_{Na} and FE_{Pi} in rats with 5/6 Nx. BP and GFR remained stable. Thus, the ANP-induced natriuresis and phosphaturia in normal and in 5/6 Nx rats may be mediated, in part, by DA, raising the possibility that ANP has an indirect DA-mediated action on renal solute transport, presumably at the level of the proximal tubule.

ANP SECRETORY AND NATRIURETIC RESPONSES TO VOLUME EXPANSION WITH EQUILIBRATED BLOOD IN 2-KIDNEY, 1-CLIP HYPERTENSION. Richard V. Paul and Andrzej Kalinsky*, Div. of Nephrology, Medical Univ. of South Carolina, Charleston, SC.

To investigate the mechanisms of exaggerated natriuresis in hypertension, we studied the plasma atrial natriuretic peptide (ANP) and renal responses to acute blood volume expansion in 2-kidney, 1-clip Goldblatt hypertensive rats. Acute studies were perfomed in anesthetized rats (n=6) 3 weeks after placing a 0.2 mm clip on the right (R) renal artery. After a 40-minute control period, volume expansion from a cross-circulated reservoir filled with donor whole blood was performed in two steps of 1 and 1.5% body weight, and urine collected separately from each kidney for 60 minutes after each step. Blood samples for plasma ANP measurement by radioimmunoassay were drawn during control periods and immediately after each volume expansion step. Results are shown with flow in µL/min and Na excretion in µEq/min:

	Control	1% exp	1.5%exp
MAP (mm Hg)	165±4	169±4	168±5
ANP (pg/ml)	107±26	168±31	427±154
L urine flow	5.5±0.8	27±7.1	42±13
L Na excretion	0.31±0.13	1.5±0.52	3.4±1.2
R urine flow	3.4±0.40*	7.1±2.4*	6.3±1.7*
R Na excretion	.043±.011*	.028±.007*	.092±.048*

(*p<0.05 vs. left)

Natriuretic and diuretic responses were markedly blunted on the clipped side, as compared with the non-clipped. Since both kidneys were exposed to the fourfold rise in plasma ANP, we conclude that intrarenal factors, possibly related to perfusion pressure, are the primary determinants of the natriuretic and diuretic response in this model.

ATRIOPEPTIN II (ANP) INDUCED INCREASE OF GLOMERULAR FILTRATION RATE(GFR) BUT NOT URINARY SODIUM EXCRETION (UNA) IS PROSTAGLANDIN (PG) DEPENDENT. A. pomerantz, M.Rathaus, E.Podjarny, L.Shilo, L.Shenkman, J.Bernheim (Intr. by J. Levi). Dpmts of Nephrology and Medicine C, Meir Hospital,Kfar-Saba, Israel.

ANP and PGE_2 may induce similar changes in GFR and UNa. The purpose of the present study was to ascertain whether the actions of ANP are mediated by stimulation of renal PG synthesis. Inuline clearance, UNa, mean arterial pressure (MAP) were measured in penthotal anesthesied rats before and after a continuous infusion of ANP (0.67 µg/Kg/min). Later on Lysin acetylsalycilate (ASA: 10mg/Kg was injected and 2 further clearance periods evaluated. Two series of experiments were performed to evaluate the effects of ANP on renal PG synthesis: glomerular, medullary and papillary PGE_2, PGI_2 and TXA_2 productions were measured (1) after in vivo injection of ANP 0.67 µg/min/20 min) (2) after in vitro incubation of ANP (10^{-7}M) for 30 min in a shaking bath. The results were: GFR increased from 0.93±0.28 to 4.06±1.68ml/min 100g ($p<.02$) after ANP. ASA prevented the ANP effect (GFR=1.54±0.45). UNa increased from 0.44±0.12 to 10.52±2.65 µmole/min ($p<.02$).ASA did not affect the ANP induced increase in UNa. In vivo infusion of ANP induced an increase in glomerular PGE_2 (8430±1539 vs 1858±237 pg/mg prot/30 min. $p<.01$) and PGI_2 (287±53 vs 123±25 $p<.01$) but did not change the medullary and papillary production. A similar pattern was observed in the in vitro experiments.It may be concluded that the ANP induced increase of GFR is prostaglandin dependent but not the natriuretic effect.

ABNORMAL RENAL RESPONSE TO ATRIAL NATRIURETIC PEPTIDE (ANP) IN EXPERIMENTAL NEPHROTIC SYNDROME (NS). Norberto Perico*, Massimo Cucchi*, Giuseppe Remuzzi*. Mario Negri Institute for Pharmacological Research, Bergamo, Italy. (intr. by R.J. Glassock).

To evaluate whether the reduced response to ANP in NS is due to an intrarenal defect, and the role of the renal sympathetic nerve, we used denervated isolated perfused kidney (IPK) from rats given adriamycin (ADR) or vehicle (C). After equilibration period, a basal 15 min clearance was followed by a constant infusion of rat ANP (1-28 Bachem; 450 ng/min) or saline (S) for 25 min. ANP infusion increased GFR, as creatinine clearance, in ADR but not in C kidneys (ADR, basal: 0.178±0.072; ANF: 0.348±0.081 ml/min.g K; $p<0.01$. C, basal: 0.521±0.110; ANF: 0.612±0.055 ml/min.g K). No GFR changes were found in ADR or C kidneys given saline. $U_{Na}V$ excretion and diuresis (V) were significantly enhanced by ANP infusion in C but not ADR kidneys.

	$U_{Na}V$ (µEq/min.g K)		V (µl/min.g K)	
	Basal	ANP inf.	Basal	ANP inf.
C+ANP	3.34±1.67	11.74±3.50*	37.5±12.1	114.9±25.0*
C+S	3.27±1.42	5.02±1.06	34.9±12.0	65.5±14.4**
ADR+ANP	0.45±0.24	0.84±0.57	7.8± 3.6	13.4± 3.4
ADR+S	0.40±0.27	0.79±0.59	6.7± 1.9	12.2± 5.1

Mean ± SD; n=8; ** $p<0.05$; * $p<0.01$ vs basal.

The percentage of $U_{Na}V$ during ANP infusion over basal was significantly higher ($p<0.01$) in C than in ADR. (C+ANF: 421%; C+S: 159%; ADR+ANF: 179%; ADR+S: 160%). Thus the blunted natriuresis to ANP is due to an intrarenal defect and is not related to sympathetic nerve activation.

RENAL AND SYSTEMIC EFFECTS OF INTRANASAL ADMINISTRATION OF HUMAN ATRIAL NATRIURETIC PEPTIDE (a-hANP). INTERACTION WITH 1-deamino-D-Arg-VASOPRESSIN (d-DAVP). Hans-Georg Predel*, Peter Kuklinski*, and Herbert J. Kramer. Med. Poliklinik, Univ. of Bonn, W. Germany

The diuretic, natriuretic and vasodilatory properties of i.v. infused a-hANP are well documented. In the present study we investigated the effects of an intranasal (i.n.) administration of 25 µg a-hANP on renal excretory function, blood pressure (BP), heart rate (HR) and its interaction with the renal effects of 20 µg d-DAVP in 10 healthy volunteers. The renal and hemodynamic effects occurred during the initial 30 min and returned to basal values at 60 min. Plasma cGMP significantly rose from 5.9±0.3 to 6.6±0.4 after 30 min and returned to 5.6±0.3 pmol/ml 60 min after a-hANP administration. Diuresis increased 4-5 fold, natriuresis and kaliuresis approx. 2-fold ($p<0.05$). Systolic and diastolic BP declined by 8% and 7%, resp., while HR remained unaltered. After 48 h the protocol was repeated with 20 µg d-DAVP administered i.n. 120 min before 25 µg a-hANP i.n. After 120 min d-DAVP alone had reduced diuresis by approx. 50%, while sodium and potassium excretion remained unaffected. Subsequent administration of a-hANP fully restored diuresis to control values. The data demonstrate that i.n. administration of a-hANP produces a significant diuresis, natriuresis and kaliuresis. The antidiuretic effect of d-DAVP is completely reversed by a-hANP which suggests that the diuretic effect of a-hANP is mainly related to an action of this peptide at the collecting duct level.

THE ROLES OF ATRIAL NATRIURETIC PEPTIDE (ANP) AND OF DOPAMINE (DA) RECEPTORS IN MEDIATING THE NATRIURESIS DURING HEAD-OUT WATER IMMERSION (HWI) IN HEALTHY SUBJECTS. H.G. Predel*, M. Erkeling*, H. Meyer-Lehnert, H.J. Kramer. Med. Poliklinik, Univ. of Bonn, W. Germany

Previous studies suggested that the natriuresis during HWI is related to increased ANP secretion. It was also claimed that the effects of ANP on renal hemodynamics and excretory function may be mediated at least in part by DA. In the present study we investigated the role of the renal dopaminergic system in the natriuresis during HWI in the absence and presence of the DA-receptor antagonist haloperidol (H). After a 1h pre-HWI period six healthy volunteers aged 26.3±1.6 yrs underwent 4h control (C) HWI followed by a 1h post-HWI period. After 48h the protocol was repeated after i.v. application of 15 μg/kg H. Plasma ANP of 8.4±1.6 (C) and 9.7±2.2 (H) rose during HWI to 32.9±3.0 (HWI) and 30.5±7.2 (HWI+H), and declined to 22.3±3.3 (C) and 23.5±2.4 (H) pmol/l, resp. Plasma cGMP rose from 16.6±1.1 (C) and 17.1±1.9 (H) before to 32.5±1.9 (C) and 29.6±3.1 pmol/ml (H) during HWI, resp. Urinary DA did not change during HWI without and with H. Within 2h of HWI urinary sodium excretion had increased from 12.9±3.1 and 15.1±3.1 to 18.0±3.3 and 35.5±8.0 mmol/h in the absence and presence of H, resp. In summary, although HWI was an adequate stimulus for ANP release into the circulation associated with a significant natriuresis, the present study failed to detect a mediating role of the renal dopaminergic system in the renal excretory response to endogenous ANP in man.

RELATIONSHIP BETWEEN RENAL INTERSTITIAL PRESSURE AND ATRIAL NATRIURETIC FACTOR IN THE ANTINATRIURESIS OF ACUTE CONGESTIVE HEART FAILURE. M. M. Redfield*, W. Miller*, J. Loftus*, J. C. Burnett Jr. Mayo Clinic, Rochester, MN

Increases in renal interstitial pressure (Pi) occur in response to exogenous atrial natriuretic factor (ANF) infusion or endogenous ANF release during volume expansion (VE) and decrease tubular reabsorption of sodium at or beyond the proximal tubule. Acute congestive heart failure (CHF) is characterized by elevated ANF without a natriuresis. As renal perfusion pressure (RPP) is decreased in acute CHF and is a modulator of Pi, we hypothesized that Pi is not increased in acute CHF despite the elevated ANF. To test the hypothesis, ten anesthetized dogs underwent VE and after recovery, acute CHF was produced by rapid ventricular pacing. +=p<.05 VE vs Baseline I, ++=p<.05 CHF vs Baseline II.

	Baseline-I	VE	Baseline-II	CHF
ANF,pg/ml	35±5	64±12+	130±35	500±89++
RPP,mmHg	109±3	119±3	123±3	96±2++
Pi,mmHg	7.4±2.0	14.9±3.0+	7.8±2.4	6.2±2.0
FENa,%	0.22±.11	6.18±.86+	5.12±.62	3.03±.50++
GFR,ml/min	34±2	45±4+	44±2	41±4

This study demonstrates for the first time Pi dynamics in acute CHF and reports an important dissociation between elevated ANF and Pi. This uncoupling of ANF and Pi may be mediated by the decrease in RPP and may contribute to the antinatriuresis of acute CHF.

ROLE OF ATRIAL PEPTIDE IN POST-OBSTRUCTIVE NATRIURESIS AND DIURESIS. ML Purkerson, EH Blaine, TJ Stokes and S Klahr, Washington University Medical School, St. Louis, MO.

In man and experimental animals, a marked natriuresis and diuresis are observed after relief of bilateral ureteral obstruction (BUO). The same phenomenon does not occur after relief of unilateral ureteral obstruction. The mechanisms underlying this postobstructive diuresis are not clear, although the role of several factors has been examined. While ECF volume expansion may occur during BUO and contribute to the natriuresis, studies in rats have shown that the natriuresis observed after release of obstruction is not merely due to volume expansion. Natriuretic factors accumulating during the period of obstruction may contribute to the natriuresis and diuresis seen after release of BUO in rats. The present studies examine the potential contribution of atrial peptide to the natriuresis and diuresis that occur after release of BUO. We found that plasma levels of atrial peptide are significantly elevated in rats with BUO as compared to normal rats or rats with unilateral ureteral obstruction. The contribution of endogenous atrial peptide to the natriuresis and diuresis which follows release of BUO of 24 hr duration was examined by the intravenous administration of heparin. Infusion of heparin, which binds atrial peptide and interferes with its biological effect, decreased the natriuresis and diuresis observed after release of BUO. Heparin administration also markedly blunted the natriuresis and diuresis observed after exogenous administration of atrial peptide. The finding of increased plasma levels of atrial peptide in rats with BUO together with the decrease in diuresis and natriuresis observed with the infusion of heparin after release of BUO in these animals indicates that endogenous levels of atrial peptide contribute to the natriuresis and diuresis that occur in this setting.

CAPTOPRIL (C) BLUNTS ATRIOPEPTIN II (ANP) EFFECT ON DIGOXINE LIKE IMMUNOREACTIVE FACTOR (DLI) AND NATRIURESIS (UNA) IN NA DEPLETED RATS. L.Shilo, A.Pomerantz, M.Rathaus, E.Podjarny, L.Shenkman, J.Bernheim. Dpmts of Nephrology and Medicine C, Meir Hospital, Kfar-Saba Israel. (Intr. by J. Levi).

DLI, a steroid like substance, has natriuretic effects. ANP stimulates DLI synthesis in normal and salt depleted rats (Life Sciences 42:1855,1988). Angiotensin II and ANP may present opposite effects. The purpose of the present study was to assess the interrelationships between ANP and DLI during converting enzyme inhibition. ANP was infused (0.67μg/kg/min) in Na depleted rats which received for 14d a low NaCl diet (0.01 mmole/100g) and 30mg/kg/d of C. GFR, blood and urinary DLI, UNa and mean afterial pressure (MAP) were measured before and after ANP. GFR increased from 320±50 to 700±130μl/min/100g ($p<.01$), a change similar to that found normally. Plasma and urinary DLI increased from 0.8±0.1 to 0.9±0.1 ng/ml and from 10.6±1.8 to 28±4.5 pg/min ($p<.05$) respectively. The basal values of urinary DLI were significantly elevated compared to control groups. The response to ANP was blunted. Non significant differences in urinary DLI, between control and treated groups, were found after ANP, demonstrating a decrease in the absolute excretion of DLI. UNa increased from 0.29±0.05 to 0.96±0.16 mole/min/100g BW ($p<.01$) but the magnitude of the natriuretic response was blunted compared to normals (4.48±1.11vs0.12±0.02). MAS remained unchanged in the treated group (83±7vs85±6 mmHg). In conclusion the response to ANP is differently affected by the absence of angiotensin II. While the effect on GFR is intact, the tubular response, the DLI production and the blood pressure changes are markedly blunted.

EFFECTS OF LUMINAL AMILORIDE OR ANF ON ^{22}Na EFFLUX FROM MEDULLARY COLLECTING DUCT IN VIVO. Harald Sonnenberg, Ursula Honrath,* and Douglas R. Wilson. Dept. of Physiol., Univ. of Toronto, Canada.

Recent in vitro studies have indicated that sodium transport inhibition in the medullary collecting duct (MCD) by atrial natriuretic factor (ANF) occurs via blockade of an amiloride-sensitive luminal sodium channel. We have shown, however, that ANF-induced reduction of MCD transport in vivo is much greater than that obtainable with amiloride. A newly-developed microperfusion technique allows us to study the effect of luminal administration of the two agonists on Na efflux in the rat MCD in vivo. A microcatheter is inserted retrogradely about 5mm into a duct, and artificial duct fluid containing ^{22}Na and ^{3}H-inulin is perfused. A decrease in the Na/inulin ratio in urine collected downstream represents unidirectional efflux of the ion. Setting the Na/In ratio in perfusate as 1, results were obtained as follows:

	Control	Amiloride ($2x10^{-4}M$)	ANF ($10^{-5}M$)
Ratio	0.49±.04	0.72±.05	0.72±.06
Depth (mm)	5.36±.06	5.44±.06	5.41±.06
No. of rats	12	9	8

Either amiloride or ANF in the perfusate caused significant increase ($p<0.01$) in the Na/In ratio, compared to control perfusate, indicating that each agonist reduced sodium efflux to a similar degree, when given from the luminal side. These results are compatible with the in vitro findings. However, since amiloride can not mimic the inhibition of MCD transport by systemic administration of ANF, they also indicate that a peritubular action at this nephron site is an important determinant of ANF natriuresis.

ATRIAL NATRIURETIC FACTOR (ANF) ENHANCES ERYTHROPOIETIN (Ep) PRODUCTION IN A RENAL CARCINOMA (RC) CELL LINE. Munehisa Ueno*, Jun Nakashima*, Jesse Brookins*, Barbara Beckman*, Isaac Rondon*, Frank Cole*, and James W. Fisher. Dept. of Pharmacology, Tulane Univ., and Ochsner Med. Inst., New Orleans, Louisiana.

ANF refers to peptide hormones released from cardiac atria in response to acute and chronic hypoxia which act on the kidney to produce natriuresis. Hypoxia is well-known to stimulate Ep production both in vivo and in vitro. However, the humoral factors involved in the mechanism of hypoxic stimulation of Ep production are still unclear. The present studies were carried out to determine the effects of human ANF on Ep production (RIA) in our Ep producing RC cell cultures. ANF produced a significant ($p<.05$) dose-related increase in Ep production (74.4±3.8 and 84.7±5.5 mU/ml at $10^{-7}M$ and $10^{-6}M$, respectively when compared with medium controls, 58.7±1.3, N=6). ANF also produced an increase in intracellular cyclic GMP (RIA) in concentrations of $\geq 10^{-8}M$ after 5 min incubation with the RC cells. Incubation of RC cells with 8 bromo cyclic GMP (10^{-7} to $10^{-5}M$) also caused a significant ($p<.05$) enhancement of Ep production (64.7±3.5 to 75.0±2.6 mU/ml) in RC cells when compared with controls (55.1±1.7 mU/ml, N=6). ^{125}I ANF binding to the particulate membranes of RC cells indicated the presence of specific high affinity (K_d=26nM, N=2) ANF binding sites suggesting that the increase in Ep production/secretion in response to ANF is coupled to an RC cell ANF receptor. In conclusion, ANF could be one of several factors released during hypoxia to increase Ep production/secretion.

LEUKOTRIENE D_4 (LTD_4) SELECTIVELY INHIBITS ATRIAL NATRIURETIC FACTOR (ANF)-DEPENDENT cGMP PRODUCTION IN PARTICULATE GUANYLATE CYCLASE OF RAT GLOMERULI. Kimio Tomita, Yoshio Terada*, Hiroshi Nonoguchi*, Yasuhiko Iino and Fumiaki Marumo. Second Dept. of Int. Med., Tokyo Med. & Dent. Univ., Yushima, Bunkyo-ku, Tokyo 113, Japan

Previously we suggested LTD_4 inhibits ANF(but not sodium nitroprusside;SNP)-dependent cGMP production in rat glomeruli (ISN, 1988, London). We investigated precise mechanisms of inhibitory effect of LTD_4 on ANF-dependent cGMP production by separating of guanylate cyclase of glomeruli into particulate and soluble fraction.

Glomeruli were isolated by a graded sieving technique with stainless steel mesh. Particulate fraction was obtained after centrifuged at 105,000g for 60min. Guanylate cyclase was assayed with 2mM isobutylmethylxanthine, 1mM GTP, Tris buffer. Cyclic-GMP formed (fmol/µg/3min) was assayed by RIA (Yamasa cGMP assay kit).

	Particulate	Soluble
Basal	2.52±0.04	1.08±0.27
ANF($10^{-6}M$)	9.06±1.79	1.22±0.43
ANF+LTD_4($10^{-7}M$)	4.70±0.70*	1.13±0.61
SNP($10^{-4}M$)	3.34±0.97	6.38±1.14
SNP+LTD4($10^{-7}M$)	2.58±0.62	5.87±0.84

* $p < 0.05$ vs ANF alone, n=6, m±SE

These data suggest that LTD_4 selectively inhibits ANF stimulated cGMP production in particulate guanylate cyclase in rat glomeruli. LTD_4 may counteract on ANF-induced mesangeal relaxation through this mechanism.

INTERACTION OF ATRIAL PEPTIDE (ANP) AND ANGIOTENSIN (AII) ON BLOOD PRESSURE AND VASCULAR PERMEABILITY. Jean Pierre Valentin,* Jean Ribstein* and Albert Mimran* (intr. by Lise Bankir). CHU Montpellier, France.

Atrial natriuretic peptide antagonizes the renin angiotensin system at different levels, and induces a fluid transfer from the intra- to the extravascular compartment. The influence of sodium intake (LS: low vs HS: high sodium for 4 weeks) and angiotensin-converting enzyme inhibition (LS-CEI: captopril given for 2 weeks to LS rats) on the effects of ANP infusion (0.1 µg/kg/min for 30 min) was assessed in anephric anesthetized rats by measuring changes in blood pressure (BP) and hematocrit (Hct). During ANP infusion, BP decreased by 5±1.6% in HS and 3.5±0.9 in LS rats; this response was similar in the LS-CEI group. The ANP-induced increase in Hct was 8.1±0.1% in HS and 5.8±0.3% in LS rats; this effect was abolished by CEI pretreatment. Infusion of angiotensin II at a subpressor dose (2.5 ng/kg/min) restored the response of Hct to ANP in LS-CEI rats. These results indicate that the effect of ANP on capillary permeability, and not that on arterial pressure, is modulated by A II, possibly because of opposing influences of ANP and AII on pre- and postcapillary resistances.

THE RELATION OF ATRIAL NATRIURETIC FACTOR (ANF) TO SODIUM INTAKE AND HEPATIC SINUSOIDAL PRESSURE IN CIRRHOTIC PATIENTS (C). L. Warner*, P. Campbell*, J. Miller*, A. Logan, K. Skorecki, and L. Blendis. Univ. of Toronto, Canada.

We compared the response of ANF to dietary sodium challenge in 6 C without (-) and 6 C with (+) ascites. UNaV (mmol/d) and plasma ANF (pg/ml) were measured after each of 7 days on 20 then 100 mmol sodium intake. Right atrial pressure (RAP) and corrected sinusoidal pressure (CSP) were determined at the end of the study:

	20mmol Na		100mmol Na	
	UNaV	pANF	UNaV	pANF
control	19±2	10±4	99±7	19±4
C(-)	17±3	24±10	80±5	34±10
C(+)	9±3*	35±10*	33±12**	106±31**

*p<.05; **p<.02; vs. control

C (-) patients achieved mean sodium balance on both diets, but only with a tendency to elevated pANF levels compared to normal control subjects. In contrast, C (+) remained in significant positive sodium balance on both diets despite marked elevation of pANF. There was a signficant correlation of pANF with CSP (r=0.8;p<.02), but not RAP.

We conclude that hepatic sinusoidal hypertension is associated with progressive resistance to ANF. In the absence of ascites elevated pANF levels are sufficient to maintain sodium balance, but with progression to ascites elevated pANF levels are not sufficient to overcome sodium retention.

CHRONIC REMOVAL OF CARDIAC ATRIAL NATRIURETIC PEPTIDE (ANP) CAUSES REVERSIBLE SYSTEMIC AND PULMONARY HYPERTENSION AND SALT AND WATER RETENTION MEDIATED BY RENIN, ALDOSTERONE AND VASOPRESSIN. C.Westenfelder, F. Birch, R. Baranowski, R. Brownley, * G. Burns,* D.B. Olsen,* C. Kablitz. Univ. of Utah and VA Med. Ctr., Div. of Nephrology & Artif. Org. Salt Lake City, UT

Whether ANP is essential for the chronic maintenance of normovolemia is unknown. We examined this issue in calves in which native hearts were replaced by artificial atria and ventricles. This caused a small but sustained decrease in extracted plasma ANP levels from 13.3±0.6 to 10.5±0.4 pg/ml (p<0.01). During 26 days of observation, all calves developed edema, a significant rise in mean aortic (from 112.3±7.2 to 119.7±1.6) and pulmonary artery pressure (from 21.6±2.0 to 31.3±0.9 mmHg). Postoperatively GFR and cardiac output were unchanged while serum Na fell significantly from 134.0±1.6 to 123.7±1.7 meq/L. Plasma renin activity rose postoperatively from 0.8±0.2 to 2.2± 0.3 ng/ml/hr, aldosterone from 23.3±4.0 to 47.4± 8.2 and vasopressin from 1.8±0.5 to 52.7±11.1 pg/ ml (all p<0.05). Infusion of autologous atrial extract reversed hemodynamic and hormonal manifestations. Conclusion: in the chronic maintenance of euvolemia ANP appears to function through its "tonic" suppression of volume-preserving hormones.

ATRIAL NATRIURETIC PEPTIDE (ANP) IN TWO TELEOST FISH, *GILA ATRARIA* (GA) AND *SALMO GAIRDNERI* (SG), IS SIMILAR TO HUMAN ANP AND IT MEDIATES SALT ADAPTATION. C. Westenfelder, R. L. Baranowski, D. K. Shiozawa,* R. Brownley,* and C. Kablitz. Univ. of Utah and VA Med. Ctr., Div. of Nephrology, Salt Lake City, and Brigham Young Univ., Dept. of Zoology, Provo, UT

Both GA and SG (rainbow trout) tolerate wide fluctuations in external salinity. The regulation of this adaptation is incompletely understood. We demonstrated previously that plasma ANP levels in GA correlated linearly with serum and external salt concentration, thus strongly suggesting that ANP may be an important mediator of salt adaptation in fish. In order to further characterize the ANP-like material detected in GA and in a salmonid, SG, we cultured atria and ventricles from both species. Culture supernatant and acetic acid (0.1 M) extract from atria and ventricles and plasma ANP-like material was examined by reversed phase HPLC and HP Gel Permeation Chromatography. Supernatants of atria and ventricles, cardiac extracts and plasma from both species of fish contained pico- to nano-molar concentrations of immunoreactive (ir) ANP (by RIA, using anti-hANP). Its molecular weight was 3 kD and co-chromatographed with hANP-(99-126). Plasma levels of ANP in both species were similar at comparable external and internal NaCl concentrations (fresh water adapted GA, 213±20 vs. SG, 184±13 pg/ml). Conclusion: 1) piscine ANP in two teleosts closely resembles hANP-(99-126); 2) ANP is a likely mediator of osmo- or volume control in salt tolerant fish.

EFFECT OF ATRIOPEPTIN III (AP-III) ON ISOLATED RAT AFFERENT (AA) AND EFFERENT ARTERIOLES (EA). B.H. Yuan*, J.B. Robinette*, J.D. Conger**, UCHSC and VAMC, Denver, CO.

Micropuncture studies in the rat indicate that the AP-III induced increase in glomerular filtration rate is due to a simultaneous decrease in AA resistance (R_A) and increase in EA resistance (R_E) resulting in increased glomerular capillary pressure. Qualitatively opposite changes in R_A and R_E require that AP-III induce a second *in vivo* vasoconstrictor messenger to increase EA tone (since AP-III directly dilates systemic arterial vessels) or AA and EA have intrinsically different receptor or post receptor mediated AP-III responses. To dissociate these possibilities, AA and EA were isolated from similarly hydrated, litter mate rat kidneys and perfused *in vitro* at 80 and 30 mmHg, respectively. Dose response curves to AP-III (10^{-12} to 10^{-5} M) were determined. In AII (10^{-9} M) or NE (10^{-7} M) preconstricted AA (each n=10), dilation occurred to AP-III (ED_{50} $4\pm9\times10^{-10}$ M and $1\pm8\times10^{-7}$ M, respectively). AP-III had no effect in AII (10^{-11} M) or NE (10^{-8} M) preconstricted EA; however, in untreated EA (n=12) AP-III caused vasoconstriction (EC_{50} $6\pm2\times10^{-9}$ M). The vasoconstrictor effect was reversed by histamine (10^{-8} M), but not by a thromboxane (TX) synthetase inhibitor OKY-046 (10^{-5} M) or enalapril (10^{-5} M). It is concluded that AA constrict and EA dilate to AP-III. The different responses are due to factors intrinsic to the arterioles. EA vasoconstriction is not mediated by AII or TX.

ENKEPHALINASE INHIBITORS INCREASE PLASMA ATRIAL NATRIURETIC PEPTIDE (ANP) LEVELS, GLOMERULAR FILTRATION RATE (GFR) AND FRACTIONAL EXCRETION OF SODIUM (FE_{Na}) IN RATS WITH REDUCED RENAL MASS. M.B. Zimmerman,* H.M. Lafferty,* M. Gunning,* P. Silva, L.E. Clarey,* B.M. Brenner, and S. Anderson. Brigham and Women's Hospital, Beth Israel Hospital, Boston, MA.

E.C. 24.11, an enkephalinase present in the kidney, degrades ANP in vitro. To study the effects of in vivo inhibition of this enzyme in rats with reduced renal mass, we infused the E.C. 24.11 inhibitor phosphoramidon (PHOS) or vehicle (VEH) into rats 4 weeks after 5/6 nephrectomy: (Means ± SEM; *p < .05 vs. Pre, +p < .05 vs. VEH)

Group (n)	GFR ml/min	FE_{Na} %	pANP pm/L	u cGMP pm/min
PHOS (6)				
Pre	.56±.07	0.7±0.3	56±3	6±1
During	.75±.07*+	3.4±1.2*+	124±26*+	31±6*+
VEH (6)				
Pre	.60±.07	0.3±0.1	54±3	6±2
During	.66±.08	0.5±0.1	56±6	8±2

Neither infusion changed blood pressure. PHOS augmented GFR and FE_{Na}, in association with increases in plasma ANP levels and urinary cGMP (u cGMP) excretion, while VEH was without effect. Similar functional effects were found with thiorphan, another E.C. 24.11 inhibitor. PHOS had no direct effect on the isolated perfused kidney, and did not potentiate the effect of added ANP. These results suggest that PHOS, acting at an extrarenal location, inhibits ANP degradation, thus increasing plasma ANP levels and u cGMP excretion, thereby enhancing the effects of endogenous ANP.

CHARACTERIZATION OF THE ARACHIDONIC ACID EPOXYGENASE REACTION PRESENT IN ISOLATED RAT KIDNEY PROXIMAL TUBULE CELLS (RKPTC). J.H. Capdevila, R. C Harris, A. Karara and E. Dishman. Division of Nephrology, Vanderbilt University. Nashville, TN.

The renal arachidonic acid epoxygenase catalyzes the NADPH and cytochrome P-450 dependent formation of four regioisomeric epoxyeicosatrienoic acids (EETs). The EETs have been documented as endogenous constituents of human urine, rat and rabbit kidney. The EETs posses potent vasoactive properties and alter renal ion fluxes and water reabsorption. As a continuation of the segmental analysis of the epoxygenase activity we have obtained freshly dispersed RKPTC and incubated them with [1-^{14}C] arachidonic acid (1-10 μM). Product extraction followed by HPLC resolution shows the time dependent epoxidation and W/W-1 oxidation of the exogenously added labelled fatty acid. Studies of the epoxidation of endogenous arachidonic acid were carried out by extracting RKPTC with $CHCl_3/CH_3OH$, followed by HPLC purification, catalytic hydrogenation, derivatization to pentafluorobenzyl esters and NICI/GC/MS analysis. Capillary GC resolution of the regioisomeric 5,6-, 8,9-, 11,12- and 14,15-EET shows the presence in cell extracts of 14,15- and 8,9-EET as major products. Utilizing synthetic [$^2H_8$]14,15-EET we have estimated the total cell EET content to be between 1-2 ng EET/mg cell protein. Preliminary results indicate that within 5 min of EGF (50 nM) addition there is a two fold increase in the epoxygenation of endogenous arachidonic acid.

In summary, these result show the presence of an active epoxygenase reaction in RKPTC. The enzyme system catalyzes the epoxidation of exogenous and, importantly, endogenous precursors. The stimulatory effect of EGF suggest a receptor mediated regulation of arachidonic acid epoxidation.

RABBIT PLACENTAE, RATHER THAN MATERNAL VASCULAR TISSUES, AS THE SOURCE OF ELEVATED SYSTEMIC EICOSANOID LEVELS OF PREGNANCY. G.P. Brown and R.C. Venuto. State University of NY at Buffalo, Schools of Nursing and Medicine, Buffalo, NY.

In pregnant (P) rabbits, elevated systemic levels of eicosanoids (eico.) may originate from the uteroplacental units and/or maternal vascular tissues (MVT). Eico. production (prod.) was determined in rabbit placental cotyledon and MVT (n=7 rabbits). Comparisons were made with vascular tissues from nonpregnant (NP) rabbits (N=7). Freshly isolated tissues were incubated at 37°C. Media aliquots were analyzed for eico. by RIA. Net prod. of eico. (pg/μg/ protein/15 min.) shown below was determined by subtraction of eico. present in media prior to incubation from that present at the end of incubation. Prod. was proportional to protein content and inhibitable with meclofenamate.

	PGE_2		6-Keto-$PGF_{1\alpha}$		TXB_2	
	NP	P	NP	P	NP	P
Mes. Art.	2 ± .4	3.0 ± .5	89 ± 8	73 ± 4	1.0 ± .2	1.0 ± .2
Renal Art.	1 ± .1	0.9 ± .1	52 ± 6	42 ± 4	0.6 ± .1	0.5 ± .1
Cotyledon	---	149 ± 41	---	12 ± 4	---	2.0 ± .6

(Mes. = mesenteric; TXB_2 - thromboxane B_2)

We conclude: 1) The pattern of eico. prod. in vascular tissues was 6-Keto-$PGF_{1\alpha}$ > PGE_2 > TXB_2; and in cotyledons it was PGE_2 > 6-Keto-$PGF_{1\alpha}$ > TXB_2. 2) Eico. prod. was similar in vascular tissues of P and NP rabbits. 3) In cotyledons PGE_2 and TXB_2 prod. was 50-166 and 2-4 times that in MVT, resp. Since placental weight was 1.8 ± 0.3 g with 6-12 placentae per rabbit, total prod. of PGI_2 in cotyledons may also exceed prod. in MVT. These data suggest that physiologically significant quantities of eico. may be secreted by uteroplacental tissue.

MODULATION OF RENIN SYNTHESIS BY LIPOXYGENASE PRODUCTS IN CULTURED HUMAN MESANGIAL CELLS. Dominique Chansel and Raymond Ardaillou (Intr. by K.F. Badr) INSERM 64, Hôpital Tenon, Paris, France.

We have shown that subcultured human mesangial cells synthesize and release renin mainly as inactive renin (Am.J.Physiol. 252 : F32, 1987). Further studies were designed to examine the regulation of this function by arachidonic acid metabolites. Total renin activity was measured after 24h incubation in the culture medium and the cellular extract of the cells (5.9 ±0.2 and 0.58 ± 0.09 ng/mg/h respectively). Both were stimulated at increasing concentrations (0.1-100 μM) of PGE_2 up to twice their basal values. There was in parallel a considerable increase of cyclic AMP (100 times its basal value) but no change of cyclic GMP. The stimulatory effect of PGE_2 on renin production was inhibited by 12-HETE from 0.1 nM and completely suppressed at 100 nM. Extracellular and intracellular renin were affected similarly. Neither basal and PGE_2-dependent cyclic AMP nor basal cyclic GMP productions were modified. 15-HETE, 12-HPETE and 15-HPETE had the same effects as 12-HETE. Intracellular calcium (Ca^{2+}_i) was unchanged in the presence of the lipoxygenase products. Oleyl-2-acetylglycerol, an analogue of diacylglycerol, and angiotensin II, whose cellular effects are mediated, in part, by protein kinase C (PKC), also inhibited PGE_2-stimulated renin production. These data suggest that the mode of action of the lipoxygenase products on renin production in human mesangial cells implies PKC stimulation.

LIPOXIN A4 (LXA4) ANTAGONIZES CELLULAR AND IN VIVO ACTIONS OF LEUKOTRIENE D4 (LTD4) IN RAT MESANGIAL CELLS (MC) BY COMPETING FOR A COMMON RECEPTOR. DK DeBoer*, M Schwartzberg*, CN Serhan*, and KF Badr. Vanderbilt University, Nashville, TN and Harvard University, Boston, MA.

LTD4 and LXA4 exert opposing (constrictor/dilator) effects on renal arterioles, but both reduce the glomerular ultrafiltration coefficient (Kf). Since the spatial orientation of the polar substituents (5S,6R) is identical in LTs and LXs and is a crucial requirement for biologic activity of LTD4, we investigated whether LXA4-induced falls in Kf were due to its activation of the LTD4 MC receptor.

Increasing concentrations of LXA4 competed potently for specific binding of 10 nM 3H-LTD4 to MC (n=3). Half maximal inhibition was obtained with 70 nM LXA4, compared to 10 nM for LTD4 itself. Ten nM (n=6) and 50 nM (n=3) LXA4 induced mild increases in MC inositol trisphosphate (IP3) generation: 48%* and 44*%, respectively (compared to 146%* and 106%* for equimolar LTD4) which were abrogated in the presence of 100-fold concentrations of the LTD4 receptor antagonist, SKF 104353 (n=2). Furthermore, LXA4-pretreatment (100 nM, n=3) prevented MC IP3 generation by 10 nM LTD4.

To test the in vivo relevance of these results, we established a dose-response curve for the GFR/RPF-reducing effects of intrarenal arterial (ia) LTD4 in anesthetized euvolemic rats [LTD4 doses: 0.5 (n=4), 7 (n=3) and 14 (n=3) μg/kg/min] in the absence or presence of ia LXA4 [1 μg/kg/min, n=3 at each LTD4 dose]. Mean % falls in GFR/RPF during LTD4 administration alone were 20*/22*, 27*/40*, and 70*/65* at the above doses, respectively (* $p<0.05$ vs pre-LTD4 baseline). In the presence of LXA4, these values were: 11/20*, 9/38*, and 42*/51*, rightwardly shifting the LTD4/GFR dose-response curve.

Thus, LXA4 competes for 3H-LTD4 binding to MC and abrogates LTD4-induced MC IP3 generation. Its own stimulation of MC IP3 is blocked by an LTD4 receptor antagonist. In vivo, LXA4 antagonizes LTD4-induced falls in GFR, but not RPF, implying selective prevention of LTD4-mediated reductions in Kf. These data suggest that LXA4 is an endogenous LTD4 MC receptor antagonist, and provide further evidence for counterregulatory interactions between arachidonate 5/15-lipoxygenase products during glomerular inflammatory injury.

DIRECT EVIDENCE THAT THROMBOXANE MIMETIC U44069 PREFERENTIALLY CONSTRICTS THE AFFERENT ARTERIOLE. Murray Epstein, Koichi Hayashi*, Rodger Loutzenhiser*, Nephrology Section, V. A. Med. Center, and Univ. of Miami, Miami, Florida.

Thromboxane (TX) has been implicated as a mediator of the deranged renal hemodynamics associated with a number of disorders. We have previously demonstrated that the TX-mimetic U44069 preferentially reduces GFR and filtration fraction (FF); suggesting a preferential constriction of pre-glomerular vessels (AJP 250:F619-F626,1986). To assess this possibility directly, we examined the effects of U44069 and diltiazem (DIL) on renal microvessels. Chronic unilateral hydronephrosis was induced in rats to facilitate direct visualization of the renal microcirculation. Hydronephrotic kidneys were excised and perfused on an inverted microscope. Afferent (AA) and efferent (EA) arteriolar diameters were measured in situ by image analysis as recently described (J.P.E.T. 246:522-528, 1988). The microvascular effects of U44069 (1.0 μM) and their reversal by DIL are presented below (n=6), along with the effects of each agent on GFR (ml/min/g) and FF (%) previously observed in normal isolated rat kidneys (n=6) perfused under identical in vitro conditions:

	basal	U44069	U44069+DIL		
[DIL]			0.1 μM	1.0 μM	10 μM
AA	18.4±0.6	13.2±0.7	15.1±0.5	16.5±0.5	17.9±0.5
EA	15.5±1.0	14.4±0.9	14.7±0.9	14.8±0.9	15.0±0.8
GFR	0.9±0.1	0.2±0.1	0.5±0.1	0.8±0.1	0.9±0.1
FF	2.3±0.2	0.4±0.1	0.9±0.1	2.0±0.2	2.1±0.2

(AA and EA diameters in μm, mean ± SE)

These findings constitute the first direct evidence that TX preferentially constricts the AA. Calcium antagonists completely reverse TX-induced AA vasoconstriction, suggesting a potential utility of these agents in ameliorating TX-induced renal hemodynamic abnormalities.

SULINDAC SULFIDE INHIBITS SYNTHESIS OF CYSTEINYL LEUKOTRIENES (CYLT) BY HUMAN KIDNEY TISSUE. Karl W. Dreyling[1], Hjalmar B. Steinhauer[1], Alexander Frankenschmidt[2], Marion Trautmann[3], Peter Schollmeyer[1] (intr. by Thomas Lüscher). [1]Dpt. of Medicine and [2]Dpt. of Urology, Univ. of Freiburg, FRG, and [3]Dpt. of Exp. Clin. Medicine, Univ. of Bochum, FRG

We investigated the effect of indomethacin (I), sulindac (SL), its active metabolite sulindac sulfide (SI) and the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) on the release of eicosanoids by human kidney.

Normal renal cortex from patients with hypernephroma (N=9) was incubated in Tyrode solution at 37°C for 20 min in the absence (A) and thereafter in the presence of 5μg/ml calcium ionophore A23187 (CAI). Release of CyLT (determined as immunoreactive LTC_4) and prostaglandin E_2 were measured radioimmunologically. Synthesis of CyLT (CAI) was confirmed by HPLC.

The release of CyLT (CAI) and PGE_2 (A) is shown (ng/g wet weight/20min).

		CyLT (CAI)	PGE_2 (A)
controls		1.6 ± 0.3 (9)	7.9 ± 2.3 (8)
I	(10μg/ml)	1.3 ± 0.3 (5)	0.6 ± 0.1*(5)
SL	(10μg/ml)	1.5 ± 0.2 (6)	6.9 ± 1.9 (5)
SI	(10μg/ml)	0.3 ± 0.06*(6)	1.9 ± 0.8 (5)
NDGA	(10μg/ml)	0.2 ± 0.03*(8)	7.5 ± 2.1 (6)

values represent the mean ± SEM of N experiments. *$p<0.01$ compared to controls.

The reduction of renal prostaglandin release by sulindac sulfide is less pronounced than by indomethacin. From these in vitro results the renal-sparing effect of sulindac sulfide might additionally be due to synthesis inhibition of vasoconstrictory CyLT.

RENAL AND VASCULAR PROSTAGLANDINS IN MINERALOCORTICOID HYPERTENSION IN RATS. Pierre Falardeau, Rutai Hui*, John Grose and Marcel Lebel. Clinical Research Institute of Montreal and Hôtel-Dieu de Québec, CANADA.

The goal of the study was to determine the role of prostaglandins in a new model of mineralocorticoid hypertension. The continuous s/c infusion of aldosterone over a period of four weeks was associated with a rise in blood pressure (147±3 vs 113±1 mmHg, mean ± SEM) and an increase in the renal production of PGE_2 (442±31 vs 103±12 ng/24 h), PGI_2 (16.4±3.0 vs 9.6±1.3 ng/24 h) and TxB_2 (4.8±0.4 vs 3.3±0.5 ng/24 h). The high salt diet had an inhibitory effect on the renal synthesis of PGE_2 but did not aggravate aldosterone- induced hypertension. Indomethacin (3.5 mg/kg/day s/c) did not modify the influence of aldosterone alone on the blood pressure, but worsened the hypertension of the rats receiving both aldosterone and a high salt diet (140±4 vs 157±5.3 mmHg). Indomethacin caused a 40-60% reduction in the renal and the systemic production of PGI_2 but did not inhibit at all the renal synthesis of PGE_2.

The results of this study indicate that: 1) aldosterone and high salt diet exert opposite effect on the renal synthesis of PGE_2; 2) renal and systemic prostaglandins do not play a role in aldosterone- induced hypertension; 3) vascular PGI_2 modulates the impact of dietary salt on blood pressure; 4) the vascular synthesis of PGI_2 is much more sensitive to the inhibitory effect of indomethacin than is the renal synthesis of PGE_2.

ALDOSE-REDUCTASE ACTIVITY MEDIATES RENAL PROSTAGLANDIN PRODUCTION IN STREPTOZOTOCIN DIABETIC RATS. J. Frey*, P. Zager, J. Jackson*, P. Eaton*, M. Scavini*. Univ. of New Mexico, VAMC, Albuquerque, NM, H San Raffaele, Milano.

Increased renal prostaglandin (PG) production may contribute to the hyperfiltration that accompanies the induction of streptozotocin (STZ) diabetes. We postulated that increased flux through the aldose-reductase pathway mediates the augmented renal PG production.

We studied the effects of an aldose-reductase inhibitor, sorbinil, on 24 h urinary PGE_2 excretion (UPGE2). Three groups of rats (n=24) were studied. Group I, maintained on normal chow, served as controls. Group II was rendered diabetic with STZ and fed normal chow. Group III also received STZ but was fed chow containing sorbinil (0.7%).

	UPGE2 (pmol/24 h)			
	Baseline	Day 1	Day 7	Day 14
I	80±13	59±4	115±25	73±9
II	43±5	128±114*	232±40#	202±50#
III	65±7	96±6	100±22	120±27

*p<0.05, #p<0.01 vs Baseline

Induction of diabetes increased UPGE2 in Group II (p<0.01) but did not in Group III (NS). On days 1, 7 and 14 UPGE2 were higher in Group II than either Groups I or III (p<.05). UPGE2 were similar in Groups I and III at 1, 7 and 14 days, respectively (NS).

Increased flux through the aldose-reductase pathway contributes to the augmented UPGE2 that accompanies the induction of STZ diabetes. Sorbinil administration prevents this phenomenon and may be useful in suppressing the glomerular hyperfiltration that accompanies early diabetes.

PURIFICATION OF PHOSPHOLIPASE A_2 FROM RAT KIDNEY. Joseph H. Gronich*, Joseph V. Bonventre, and Raphael A. Nemenoff*, Massachusetts General Hospital, Boston MA. 02114

Phospholipase A_2 (PLA_2) is the rate-limiting enzyme in the release of arachidonic acid from membrane phospholipids. PLA_2 activation in the mesangial cell is likely to play an important role in glomerular hemodynamics and inflammation. We have recently identified a soluble form of PLA_2 in rat renal mesangial cells, whose activity is stimulated by AVP and PMA. Stimulated activity is detected in the presence of saturating Ca^{2+} concentrations, suggesting the possibility of post-translational modifications of this enzyme. This enzyme has chromatographic properties identical to the major soluble form of PLA_2 in the whole rat kidney. The kidney enzyme has been purified several thousand fold by successive fractionation on DEAE-Sephacel, Phenyl-Sepharose, Sephacryl S-300 gel filtration and MONO-Q anion exchange chromatography. The enzyme appears to be a monomer of approximately 60,000 daltons, larger than other intracellular and secretory forms previously identified. The enzyme has an absolute requirement for Ca^{2+}, and is half-maximally activated at 500 nM Ca^{2+}. It hydrolyzes both phosphatidylcholine and phosphatidylethanolamine, but has low activity towards phosphatidylinositol. Using several mixed-micelle assays it also appears to have a greater affinity for phospholipids having unsaturated fatty acid side chains in the *sn-2* position. It is likely that this enzyme plays an important role in the hormonal regulation of prostaglandin production in the kidney, and is the target of regulation by a variety of hormones.

CULTURED SPRAGUE DAWLEY MESANGIAL CELLS (MC) PRODUCE A LIPOXIN (LX) LIKE SUBSTANCE. R. Garrick, H.L. Zuo*, S. Ogunc*, A. Goodman, P. Y-K Wong*. Depts of Med and Pharmacology, N.Y. Medical College, Valhalla, N.Y.

Lipoxygenase (LO) products of arachidonic acid, include leukotrienes (LT) and lipoxins (LX). Renal artery infusions of subclasses of LT and LX have differing effects on renal hemodynamics, and LT production is altered in some glomerular diseases. In this study normal rat first pass MC were incubated for 30 min at 37° with either NaOH-Methanol stabilized LTA_4 or vehicle alone (C). LTA_4-MC incubation yielded a LX-like product identified by its characteristic R.P. HPLC retention time, and UV absorbance at λ_{max} 302nm with shoulders at 289 and 317nm. No similar products occurred in C. Incubation of MC with 15-hydroperoxyeicosatetraenoic acid (15-HPETE) or its vehicle did not produce a LX-like product. The generation of a LX-like product from MC in the presence of LTA_4 suggests that there is active 15-LO in MC which inititates oxygenation at the 15 carbon to generate the "epoxytetraene" intermediate for subsequent transformation to LX. The absence of LX-like products following MC incubation with 15-HPETE suggests that 5-LO activity is not present under these conditions. The production of these LX-like mediators may be an important function of the MC cell in response to inflammation.

NOREPINEPHRINE (NE) STIMULATES PROSTANOID (PG) BIOSYNTHESIS IN CULTURED RABBIT RENAL MICROVASCULAR SMOOTH MUSCLE CELLS (SMC). S. Gupta*, A. Chaudhari, F. Farokhi*, M.A. Kirschenbaum. Nephrology Section, VA Med Center and UCI-Long Beach Med Prog, Long Beach, CA.

It has been shown that renal preglomerular microvessels (RMV) respond to a variety of vasoactive substances including NE, and it has been proposed that vasoconstrictive effects of NE can be antagonized by vasodilator PGs produced as a result of stimulation of RMV PG synthesis by NE. In the present study, we examined whether NE can stimulate PG synthesis in cultured SMC derived from rabbit RMV. We have recently developed methods to culture SMC from RMV and have characterized these cells by LM and EM and by the demonstration of the presence of CPK activity comparable to SMC derived from other vascular beds. Confluent cultured SMC grown in monolayers were used after 3-4 passages. Incubations were performed at 37° C for 15 min in HEPES buffer (lacking protein, Ca^{2+} or $NaHCO_3$), pH 7.4, without shaking. Both PGI_2 (measured as 6-keto-$PGF_{1\alpha}$) and PGE_2 released in the medium were determined by RIA. SMC produced 9-10X PGE_2 (10.7±0.5 ng/mg prot.) as compared to PGI_2 (1.3±0.3) under basal conditions. A dose-dependent (10^{-7}-10^{-4} M) increase in synthesis of both PGs was observed in the presence of NE and in the absence of added Ca^{2+}. A23187 (10 μM), in the absence of added Ca^{2+} or NE, resulted in 14-40X increase in SMC PG synthesis These data show (1) our ability to cultivate RMV SMC, (2) that SMC synthesize both PGE_2 and PGI_2, and (3) that NE produces a dose-dependent increase in SMC PG synthesis consistent with observations made in SMC derived from other blood vessels.

14,15-DIHYDROXYEICOSATRIENOIC ACID (DHET), A CYTOCHROME P450 ARACHIDONATE METABOLITE, INHIBITS VASOPRESSIN-(AVP) MEDIATED WATER TRANSPORT VIA A UNIQUE MECHANISM. D. Hirt*, M Breyer, J. Falck*, J. Capdevila* and H. Jacobson. Nephrology Div., Vanderbilt U.,Nashville,TN. and Mol. Genetics,UTSMC, Dallas, TX.

Arachidonic acid is metabolized in the cortical collecting tubule (CCT) via a cytochrome P450 NADPH-dependent epoxygenase to form a series of 4 regioisomeric epoxides: epoxyeicosatrienoic acids (EETs) and their respective hydrolysis products, diols: DHETs. All four regioisomeric DHET inhibit peak AVP-induced hydraulic conductivity (Lp 10^{-7}cm/atm/s±SE) in rabbit CCTs perfused *in vitro* at 37°C. 14,15-DHET causes potent inhibition of AVP-induced water flow at nanomolar concentrations. The effects of 10^{-7}M 14,15-DHET and 10^{-7}M PGE_2 on AVP- peak Lp were equipotent. Finally, 14-15-DHET reduced cyclic AMP-stimulated Lp,suggesting a post-cAMP inhibitory mechanism (ASN:1987,120A). Our present studies investigate the mechanism of DHET inhibition on AVP- induced water flow in CCT. Pretreatment (preTx) with 10^{-7}M 14,15-DHET significantly reduced AVP-peak Lp: 10μU/cc AVP, 228.9±9.4, n=21; DHET+AVP, 129.8±10.2,n=9 (p<.0001). PreTx with 5μM indomethacin, a cyclooxygenase inhibitor, failed to reverse DHET inhibition of AVP-peak Lp:130.6±12.2, n=5 (p<.0001). PreTx with 10^{-7}M staurosporine, an inhibitor of protein kinase C, also did not reverse the DHET suppression of AVP-peak Lp:114.4±14.9, n=5 (p<.0001). To examine the potential role of $[Ca^{++}]i$ in mediating DHET effects, we measured $[Ca^{++}]i$ in FURA-2 loaded CCTs. Bath 10^{-7}M 14,15-DHET had no effect on basal $[Ca^{++}]i$, n=3, despite the fact that these same CCTs demonstrated a brisk transient increase in $[Ca^{++}]i$ upon subsequent exposure to a pharmacologic dose (10mU / cc) of AVP.

We conclude: 1) 14,15-DHET is a potent inhibitor of AVP in CCTs; 2) the mechanism by which DHET mediates its effect is not via generation of cyclooxygenase products or through activation of protein kinase C; 3) additionally, DHET does not appear to induce a Ca^{++}transient which argues against a role for phosphoinositide turnover. These findings suggest that 14,15-DHET invokes a unique mechanism for its inhibitory effect on the activity of AVP in CCTs.

PLATELET-ACTIVATING FACTOR INHIBITS RECEPTOR-MEDIATED ADENYLATE CYCLASE ACTIVITY IN CULTURED RAT MESANGIAL CELLS. Mark Kester and Michael J. Dunn. Case Western Reserve Univ., Dept. of Medicine and University Hospitals, Cleveland,Ohio.

Platelet-activating factor (PAF) increases intracellular calcium concentration ($[Ca^{2+}]_i$) and stimulates arachidonate release leading to eicosanoid formation. Vasodilator eicosanoids stimulate mesangial cell (MC) adenylate cyclase (AC) activity. We investigated the potential interactions between PAF-stimulated $[Ca^{2+}]_i$ and cAMP in MC. PAF (10^{-6} M) decreased immunoreactive cAMP content, from control (0.2% BSA) values of .133 ± .007 to .098 ± .003 pmoles/ug prot/5 min in the presence of IBMX. In subsequent experiments, MC were pretreated for various times with 10^{-7} M PAF or control, followed by addition of iloprost (a PGI_2 mimetic), isoproterenol or forskolin and cAMP content was assessed at 5 min. Isoproterenol (10^{-6} M) or iloprost (10^{-6} M) elevated cAMP content from control values of .113 ± .028 to 1.54 ± .63 and 1.64 ± .27 pmoles/ug prot/5 min, respectively. Pretreatment of MC with PAF for 1 min reduced isoproterenol- and iloprost-stimulated cAMP content (52.2 ± 11.1% and 40.4 ± 12.6% of control values, respectively, $p < .01$, n=4) while failing to inhibit forskolin-stimulated cAMP content (116 ± 6%, n=2). This inhibitory effect of PAF could be mimicked with the calcium ionophore, A23187 (5×10^{-6} M). However, iloprost-stimulated cAMP content remained reduced in 5 and 15 min A23187 pretreated MC, at which times PAF no longer elevates $[Ca^{2+}]_i$ or significantly inhibits agonist-stimulated cAMP generation. Thus, the negative feedback regulation upon basal- and receptor mediated- AC activity induced by PAF may be mediated by a rise in $[Ca^{2+}]_i$ This "cross-talk" mechanism may augment the cellular actions of PAF by antagonizing eicosanoid stimulation of AC.

DIETARY FISH OIL DECREASES PROSTACYCLIN AND INCREASES RESISTANCE IN ISOLATED RAT KIDNEYS. B.L. Kasiske, M.P. O'Donnell, and W.F. Keane, Henn. Co. Med. Ctr., U. of Minn., Mpls, MN.

Mechanisms whereby diet-induced alterations in fatty acids may affect renal structure and function are unknown. Kidneys from rats fed chow supplemented with 18% coconut oil (CO, n=8), sunflower seed oil (SO, n=7), or fish oil (FO, n=8) were isolated from possible systemic influences of the diets and perfused with cell-free medium at constant pressure. Perfusate flow (F, ml/min/g), inulin clearance (C_{In}, ml/min/g), and urine prostaglandin excretion (pg/min/g) were measured. Results (mean ± SD, different superscripts indicate p<.05):

	F		C_{In}		6-keto-$PGF_{1\alpha}$	
	30min	100min	30min	100min	30min	100min
CO	54[a] ±7	58[a] ±8	.78[a] ±.23	.69[a] ±.09	141[a] ±112	347[a] ±166
SO	54[a] ±8	60[a] ±9	.91[a] ±.14	.53[a] ±.12	205[a] ±157	275[a] ±154
FO	42[b] ±6	44[b] ±8	.82[a] ±.26	.43[b] ±.28	82[b] ±33	161[b] ±103

TXB_2 and PGE_2 were not affected by diet. Morphologic injury was most extensive in the FO group, and its distribution suggested it was caused by ischemia. By regression analysis, the decline in C_{In} and the degree of injury were most closely linked to diminished prostacyclin (6-keto-$PGF_{1\alpha}$) production. Thus, diet FO-induced decreases in renal prostacyclin may cause increased vascular resistance and increased susceptibility to ischemic tubular cell injury.

PROSTACYCLIN (PGI_2) IS NOT A MAJOR PROSTANOID (PG) PRODUCED BY CULTURED RABBIT RENAL MICROVASCULAR (RMV) ENDOTHELIAL CELLS (EC). M.A. Kirschenbaum, V. Chopra*, A. Chaudhari. Nephrology Section, VA Medical Center and UCI-Long Beach Medical Program, Long Beach, CA.

The renal microvasculature is known to be a source of a number of PGs which have been proposed to influence vascular, tubular and glomerular function. We have recently developed methods to isolate RMV from the rabbit renal cortex and to culture EC from these RMV. RMV EC were grown in monolayers which demonstrated morphology consistent with that described for cultured EC derived from other blood vessels and demonstrated the presence of Wiebel-Palade bodies, Factor VIII antigen and angiotensin converting enzyme activity. The cultured EC synthesized a number of PGs under *in vitro* conditions in the following order: $PGE_2 > PGF_{2\alpha} > PGI_2 > TxA_2$ (3.0±0.5 > 1.6±0.4 > 0.6±0.1 > 0.2±0.1 ng/mg protein/15 min). RMV EC PGE_2 biosynthesis increased 9-12X in the presence of arachidonic acid (1 and 10 μM), 200X with A23187 (10 μM), 40X with thrombin (5 U/ml), and 30X with bradykinin (1 μM). PGI_2 biosynthesis increased, however, to a lesser extent. PG biosynthesis decreased 48-71% with mepacrine (10 μM) and 62-89% with indomethacin (100 μM) suggesting that these EC were metabolically responsive to a variety of PG stimulators and inhibitors. In summary, EC can be cultured from freshly isolated preglomerular RMV and have the ability to produce a number of vasoregulatory PGs under *in vitro* conditions. However, under the conditions employed in the present study and as compared to that seen in intact RMV, PGE_2 rather than PGI_2 appeared to be the primary PG produced by these cultured cells.

MODULATION OF GLOMERULAR PROSTAGLANDIN PRODUCTION BY EXTRACELLULAR MYO-INOSITOL. David K. Klassen, Robert C. Aiemian. Univ. of MD, School of Medicine, Balto., MD. Depletion of cellular myo-inositol (MI) has been postulated as a mechanism underlying progression of diabetic renal disease. We studied the effect of extracellular MI on the in vitro production of vasoactive prostaglandins by glomeruli isolated from diabetic and control rats, seven days after the induction of diabetes by injection of streptozotocin. Isolated glomeruli were incubated in Krebs-Ringer phosphate buffer and prostaglandins were assayed in the supernatant by RIA. Glomeruli from diabetic animals produced significantly more PGE$_2$ (2.17±0.19 vs. 1.41±0.14 ng/mg $p < 0.01$), 6-keto-PGF$_{1\alpha}$ (2.11±0.16 vs. 1.52±0.17 ng/mg $p < 0.05$), and thromboxane B$_2$ (1.82±0.14 vs. 1.17±0.15 ng/mg $p < 0.01$) than did controls. Incubation of diabetic glomeruli with 35 M and 350 M MI increased PGE$_2$ production in dose-response fashion by 16%±12% p 0.05 and 50%±15% p 0.001 (n=20), respectively compared to control. PGE$_2$ production in glomeruli from control animals increased less, 10%±16% NS and 18%±10% $p < 0.05$ (n=20) respectively. The inactive isomer scyllo-inositol was without effect in both diabetic and controls. Co-incubation with 20mM glucose, which may decrease MI uptake, tended to blunt the stimulatory effects of MI on glomerular prostaglandin production. Similar results were observed for both 6-keto-PGF$_{1\alpha}$ and thromboxane B$_2$. Angiotensin II stimulation of prostaglandin production was not synergistic with the effects of MI. We conclude that glomerular prostaglandin production is modulated by cellular MI levels. This may occur through effects on membrane phospholipid turnover and the availability of free arachidonic acid.

REGULATION OF EXTRACELLULAR MATRIX BY THROMBOXANE. P. Klotman, L. Bruggeman*, J. Hassell*, E. Horigan*, G. Martin*, and Y.Yamada*. Duke University, Durham, NC and NIDR, NIH Bethesda, MD

Increased renal thromboxane production is a key element in the pathogenesis of a number of renal diseases including subtotal nephrectomy, hydronephrosis, transplantation rejection and diabetes. The deleterious effects of thromboxane have been thought to occur as a result of local vasoconstriction or platelet aggregation. However, thromboxane, may have additional direct effects on the biosynthesis of extracellular matrix proteins and, as a result, may be linked directly to the development of glomerulosclerosis and interstitial fibrosis. To address this hypothesis, we measured the production of several components of extracellular matrix including heparan sulfate proteoglycan, and the A, B1 and B2 chains of laminin in differentiated teratocarcinoma (F9+) cells grown in culture. Cells were treated with increasing log doses of the thromboxane agonist U44619. Matrix proteins were separated by labelling F9+ cells with ^{35}S methionine followed by immunoprecipitation and resolution by electrophoresis on polyacrylamide gels. Intensity of the bands was quantitated by optical scanning of the autoradiographs. Thromboxane induced a dose-dependent increase in laminin A, B1 and B2 chains of approximately 50%. In contrast, heparan sulfate proteoglycan was reduced to non detectable levels. These effects were reversed by the thromboxane receptor antagonist GR32191B. The changes in basement membrane components induced by thromboxane agonist are similar to those observed in diabetic nephropathy and suggest that thromboxane may have a role in the abnormal production of basement membranes in this and in other renal diseases. Furthermore, these data suggest potential new strategies for therapy in the variety of renal diseases characterized by increased thromboxane production.

ROLE OF PROSTAGLANDINS IN RENORENAL REFLEXES. U.C. Kopp & L.A. Smith*. Dept. of Int. Med., Univ. of Iowa Col. of Med. & VA Med. Ctr., Iowa City, IA.

In rats stimulation of renal mechanoreceptors (MR) by increasing ureteral pressure 30 mmHg or renal chemoreceptors (CR) by renal pelvic perfusion with 0.9 M NaCl increases ipsilateral afferent renal nerve activity (ARNA), decreases contralateral efferent RNA and increases contralateral urine flow rate (V) and urinary sodium excretion (UNaV); a contralateral inhibitory renorenal reflex response. Since PGE2 injected into renal interstitium results in a contralateral diuresis and natriuresis that are abolished by ipsilateral renal denervation (Pawlowska et al Kid Int 33:280 1988), we examined if the renorenal reflex responses to renal MR or CR stimulation were affected by renal pelvic administration of indomethacin (indo) or meclophenamate (meclo) at 0.25 μg/min. Before indo or meclo, renal MR stimulation for 20 min increased ipsilateral ARNA (integrated voltage, expressed in percent of control) 65±11%, contralateral V from 5.9±0.7 to 8.4±1.2 μl/min/g and contralateral UNAV from 1.1±0.1 to 1.7±0.3 μmol/min/g (n=8, $p<0.01$). During renal pelvic perfusion with indo or meclo renal MR stimulation increased ipsilateral ARNA 19 ±5% ($p<0.01$) but did not affect contralateral V or UNaV; from 9.3±1.4 to 8.4±1.4 μl/min/g and from 1.7 ±0.3 to 1.5±0.3 μmol/min/g, a markedly impaired renorenal reflex response. Mean arterial pressure and ipsilateral renal blood flow were not affected by renal MR stimulation or renal pelvic administration of indo or meclo. Similarly, renal pelvic administration of indo blocked the renorenal reflex responses to renal CR stimulation. We conclude that renal prostaglandins are involved in the renorenal reflex responses to renal MR and CR stimulation.

BLEEDING TENDENCY IN CHRONIC RENAL FAILURE: EVIDENCE FOR AN INCREASED GENERATION OF PROSTACYCLIN IN THE MICROVASCULATURE AND A PLATELET STORAGE POOL DEFECT. P.A. Kyrle, F. Stockenhuber, G. Sunder-Plassmann, P. Balcke, K. Lechner. 1st Department of Medicine, University of Vienna, Austria.

An acquired platelet defect is suggested to be the underlying cause for the bleeding tendency in patients with chronic renal failure. We sudied the formation of 6-keto-prostaglandin F$_{1\alpha}$ (6-k-PGF$_{1\alpha}$) and thromboxane B$_2$ (TxB$_2$) and the release of beta-thromboglobulin (beta-TG) at the site of platelet-vessel wall interaction in blood emerging from a standardized injury of the cutaneous microvasculature in 8 uremic patients on regular hemodialysis treatment and in 19 normal subjects. In the uremic patients the mean level of 6-k-PGF$_{1\alpha}$ was 1.7 fold higher than in the control subjects indicating an increased PGI$_2$ formation in chronic uremia. Formation of TxB$_2$ was similar in the uremic subjects and in the normals excluding a major defect of the platelet prostaglandin metabolism in chronic renal failure. Significant smaller amounts of beta-TG were found in patients with chronic renal failure indicating the presence of a storage pool defect in chronic uremia.

	controls (n=19)	uremic patients (n=8)
6-k-PGF$_{1\alpha}$	265 ± 45	450 ± 49 pg/ml P < .005
TxB$_2$	6386 ± 2622	6290 ± 2312 pg/ml n.s.
beta-TG	4726 ± 763	2955 ± 479 ng/ml P < .001

We therefore conclude that the hemorrhagic diathesis commonly seen in patients with chronic renal failure is - at least partially - due to a platelet storage pool defect and an increased generation of PGI$_2$ in the microvasculature.

LEUKOTRIENE (LT) SYNTHESIS IN EXPERIMENTAL MEMBRANOUS NEPHROPATHY: POTENTIAL ROLE OF THE GLOMERULAR MACROPHAGE. Elias A. Lianos, Dept. of Med., Nephrology Division, Medical College of Wisconsin, Milwaukee, WI.

Enhanced glomerular synthesis of hydroxyeicosatetraenoic acids (HETE) and LT has been described in experimental glomerulonephritis (J.C.I. 76:1355, 1985; J.C.I. 82, 1988). The role of glomerular macrophages (Mϕ) as a potential source of glomerular HETE and LT was assessed in passive Heymann nephritis (PHN), a non-infiltrative form of membranous nephropathy. Female Lewis rats received a single IV proteinuric dose of sheep serum raised against rat antigenic fraction Fx1A. Glomeruli were isolated at 5 h, 18 h and 5 days and incubated with the ionophore A23187 at 37°C for 45 min. 12-HETE and LTB_4 were extracted, separated by HPLC and quantified by RIA. Synthetic rates at 5 h were compared to those in rats depleted of glomerular Mϕ using whole body x-irradiation (x-irr.) prior to induction of PHN. Controls received non-immune sheep sera (PHN-C). Irradiated controls received either non-immune sera (PHN-C/x-irr) or x-irr. alone. Mϕ depletion was assessed by staining for presence of Iα antigen bearing cells in isolated glomeruli. Results (ng of LTB_4 or 12-HETE/mg glom. protein, mean ± SE) were as follows:

Groups	12-HETE	LTB_4
PHN - C (n=13)	36.05 ± 5.8	2.26 ± 0.25
PHN - C/x-irr. (n=10)		1.29 ± 0.25†
x-irr. alone (n=7)		1.00 ± 0.13†
PHN - 5h (n=17)	81.80 ± 15.3†	4.57 ± 0.75†
PHN - 5h (x-irr.) (n=8)	29.07 ± 3.45	1.57 ± 0.35††
PHN - 18h (n=4)		7.62 ± 2.88†
PHN - 5 days (n=4)		8.45 ± 3.20†

(†), $p<0.05$, compared to control; (††), $p<0.05$ compared to PHN - 5h.

In all x-irr. groups there were significant decrements in glomerular LTB_4 levels and a 3-5 fold decrement in glomerular Iα bearing cells. X-irr. also induced a 4 to 8 fold decrement in circulating leukocytes which did not correlate with changes in LTB_4. As no arachidonate lipoxygenase activity has been demonstrated in cultured glomerular cell populations, it is concluded that the resident Mϕ is a potential source of glomerular LTB_4. In PHN the enhanced glomerular eicosanoid synthesis commences early and could be related to an altered state of activation of this cell type in response to immune injury.

EICOSANOID PRODUCTION BY THE KIDNEY OF THE GOLDFISH (CARASSIUS AURATUS). J. Lowenstein, Renal Section, Dept of Med. NYU School of Medicine, New York, NY

Carroll et al (J Hyper. 1986, S33-S42) reported that rabbit renomedullary cells metabolize arachidonic acid (AA) via the cytochrome P450 system to yield oxygenated products which are vasoconstrictor and inhibit Na+-K+ATPase. We examined the metabolism of AA by the kidney of the goldfish, a fresh water teleost in which sea water adaptation results in marked reduction in GFR and fractional reabsorption of sodium.

Goldfish were maintained in fresh water or 1/3 sea water. Whole renal homogenates were incubated with tritiated AA, with or without indomethacin (INDO) or nordihydroguaiaretic acid (NDGA), an inhibitor of lipoxygenase. Renal microsomes were incubated with or without added NADPH (1mM). The major metabolites, averaging 47.7% of recovered radioactivity, comigrated with 12-or 15-hydroxyeicosatetraenoic acid (HETE) while prostaglandins accounted for only 1.6%. INDO had little effect but NDGA reduced HETE production by 86%. Microsomes from fresh water fish synthesized a NADPH-dependent prostanoid not identified in sea-water adapted fish.

The findings demonstrate that the predominant eicosanoids of the goldfish kidney are products of either lipoxygenase or cytochrome P450 oxidation. These products may mediate the changes in renal hemodynamics and sodium reabsorption seen following sea water adaptation.

SERUM RENOTROPIC FACTOR (RF) STIMULATES PROSTAGLANDIN (PG) PRODUCTION BY PRIMARY CULTURES OF RABBIT KIDNEY CELLS. Joy L. Logan* and Bryant Benson* (intr. by Ulrich F. Michael). V.A. Med. Centr. and Depts. of Int. Med. and Anat., Univ. of Arizona, Tucson, Arizona.

Compensatory renal growth (CRG) may be regulated by a serum RF. Our previous work has suggested that renal PGs may act as mediators of CRG in response to the RF. This hypothesis was further tested by examining the effects of serum from uninephrectomized (NX) rabbits on PGE_2 production by primary cultures of rabbit kidney cells. The activity of the serum RF was assessed by the cellular uptake of ^{3}H-thymidine (^{3}H-T) [dpm x 10^{-3}/plate]. PGE_2 levels [pg x 10^{-2}/ml] were measured in the media of cells incubating with rabbit serum obtained pre-NX or 7, 9 and 14 days after NX. Data are means + SEM for 6-9 plates.

	pre-NX	7-NX	9-NX	14-NX
^{3}H-T:	10.7±0.3	13.3±0.4[a]	17.3±1.2[b]	11.6±0.4
PGE_2:	6.4±0.3	9.1±0.9	14.3±1.8[b]	11.0±0.9[a]

[a] $p<0.05$ and [b] $p<0.01$ compared to pre-NX

The differences in media PGE_2 were not due to levels of PGE_2 in added serum. These data provide further confirmation of the existence of a RF, and new support for the hypothesis that PGs participate in the cellular events which lead to CRG.

THROMBOXANE A_2 (TxA_2) ACTIVATES HUMAN MESANGIAL CELL ADENYLATE CYCLASE AND INHIBITS SERUM- AND GROWTH FACTOR-STIMULATED PROLIFERATION. Paolo Mene',* Hanna E. Abboud, and Michael J. Dunn. Dept. of Medicine, Case Western Reserve University, University Hospitals and VA Medical Center, Cleveland, Ohio.

The platelet and leukocyte product TxA_2 stimulates glomerular mesangial cell contraction through a signal transduction mechanism initiated by a rapid rise of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) and sustained increases of intracellular pH. Contrary to most stimuli of phospholipase C, TxA_2 has only weak mitogenic activity. We therefore investigated whether TxA_2, similar to other eicosanoids such as PGE_2 and PGI_2, also stimulates intracellular cyclic AMP (cAMP) accumulation, a growth inhibitory pathway in human mesangial cells. The TxA_2 mimetic U-46619 dose-dependently increased immunoreactive cAMP in the presence of 0.1 mM IBMX, from a basal of 162±4 to 1200±49 pmol/mg protein/3 min at 10 uM. Consistent with cAMP stimulation, addition of U-46619 10 min prior to exposure of quiescent cells to 1-17% fetal bovine serum (FBS) for 24 hrs potently and dose-dependently inhibited [^{3}H] thymidine ([^{3}H]TdR) incorporation (10% FBS 620±47% over basal, +10 uM U-46619 317±56%, $p<0.01$), and blocked FBS-stimulated increases in cell number (basal 51±2, FBS 76±4, U-46 56±3x10^3 cells/cm^2, NS vs basal). Similarly, 10 uM U-46619 inhibited the effects of 10 ng/ml platelet-derived growth factor, epidermal growth factor or basic fibroblast growth factor on [^{3}H]TdR incorporation, by 55, 79 and 88% respectively. A selective stimulus of mesangial $[Ca^{2+}]_i$, angiotensin II, did not affect FBS-stimulated proliferation, indicating cAMP as the potential mediator of growth inhibition by TxA_2. The dual signaling mechanism for TxA_2 points to multiple functional interactions between eicosanoids and growth factors in glomerular inflammation.

RENAL HEMODYNAMIC RESPONSES TO 5,6-EPOXYEICOSATETRAENOIC ACID (5,6 EET) IN THE RAT: INTERACTIONS WITH THE CYCLOOXYGENASE (CO) PATHWAY. KA Munger*, K Takahashi*, J Ebert*, and KF Badr. Vanderbilt Univ. Nashville, TN.

We have recently reported the induction of renal cytochrome P450-linked arachidonic acid monoxygenase and epoxygenase enzyme systems following reduction in renal mass, during untreated insulin-dependent diabetes, and in mid-term pregnancy. The present studies examined the renal actions of 5,6-EET, a major product of the epoxygenase pathway, reported as being a potent vasodilator in isolated extrarenal vascular beds.

Intrarenal arterial administration of 5,6-EET [1 (n=3) and 2 (n=5) µg/kg/min] to anesthetized euvolemic male Munich-Wistar rats had no effect on systemic arterial pressure, but resulted in dose-dependent reductions in RPF and GFR which, at the higher dose, decreased, on average, from 4.20 to 3.33* and from 1.15 to 0.91* ml/min, respectively. [* : $p<0.025$ vs baseline]. The FF (0.27) was unchanged. Since 5,6-EET may act as a substrate for CO, we examined the role of this pathway in mediating its renal effects. In a separate group of rats (n=5) pre-treated with the CO inhibitor ibuprofen (20 mg/kg), 5,6-EET (2 µg/kg/min) resulted in significant renal vasodilatation (RPF increased from 4.38 to 4.81 ml/min*) associated with a rise in GFR (1.09 to 1.24 ml/min). FF was again unchanged (0.25 to 0.26).

Thus, intrarenal administration of 5,6-EET in vivo evoked vasoconstrictor, and not vasorelaxant, responses. CO inhibition, however, resulted in 5,6-EET-induced renal vasodilatation, suggesting that its renal vascular actions arise either from its own bioconversion to, and/or its stimulation of the secondary release of, a vasoconstrictor CO product. These observations provide evidence for potentially relevant biologic interactions between products of the cytochrome P450 and CO pathways of arachidonic acid metabolism in the regulation of renal vascular tone.

SELECTIVE INHIBITION OF GLOMERULAR THROMBOXANE-SYNTHASE IN RAT MODELS OF PROGRESSIVE GLOMERULOSCLEROSIS. P. Salvati*, F. Pugliese, C. Ferti*, L. Pierucci*, R. Ferrario* and C. Patrono*. Farmitalia Carlo Erba Research Labs, Milan and Dept of Pharmacology, Catholic Univ, Rome, Italy.

A lower sensitivity of renal cortical vs platelet thromboxane(TX)-synthase to several imidazole-analogue inhibitors (TXSI) has been suggested both in animals and man. We compared the effects of FCE 22178 [5,6-dihydro-7-(1H-imidazol-1-yl)-2-naphtalene-carboxylic acid] on platelet and glomerular TXB_2 production in normal rats and in two models of glomerular disease, i.e. subtotal renal ablation (SRA) and age-related glomerulosclerosis in the Milan normotensive strain (MNS). Incubations of whole blood and isolated glomeruli were performed as previously described (JPET 228:472,1984). Basal glomerular production of TXB_2 was (mean±SD n=6): 8.7±1.0 in SRA, 2.5±0.4 in MNS, and 1.9±0.6 ng/mg protein/h in controls. A significantly ($p<0.01$) lower IC_{50} for inhibition of glomerular vs platelet TXB_2 was observed in both normal and diseased rats. Thus, IC_{50} values averaged 3.2×10^{-8} vs 1.7×10^{-6}, 1.9×10^{-8} vs 1.8×10^{-6} and 7.0×10^{-8} vs 1.0×10^{-6} M in glomeruli and whole blood of SRA, MNS and control rats, respectively. In MNS glomeruli, inhibition of TXB_2 synthesis was associated with a dose-dependent increase in PGE_2 synthesis up to 70% at 10^{-7}. These results suggest that glomerular TX-synthase might be more susceptible to inhibition by FCE 22178 than the platelet enzyme. We conclude that a tissue-selective TXSI may represent a valuable pharmacological tool in exploring the functional significance of PG-endoperoxide metabolism in chronic glomerular disease.

STIMULATION OF GLOMERULAR PROSTAGLANDINS (GP) SYNTHESIS BY POTASSIUM LOAD PREVENTS RENAL FAILURE IN NA DEPLETED RATS TREATED WITH CAPTOPRIL (LNA-C). M.Ratheus, E.Podjarny, A.Pomerantz, J.Bernheim. Dpmt. of Nephrology, Meir Hospital, Kfar-Saba, Israel. (Intr. by J. Levi).

In Na depleted rats, treatment with captopril induce renal failure, associated with low synthesis of glomerular prostanoids (Am.J.Physiol, 254:F358-F363, 1988). Potassium (K) stimulate GP in normal rats (N). The effect of K (80 mM KCL in the drinking water) on inulin (GFR) and PAH (RPF) clearances (ml/min/100gBW) was therefore measured in KCl loaded LNa-C rats (n=9) as compared with N (n = 19).To separate the effect of K or Cl, 2 groups of LNa-C received either K or Cl. (80 mM, pH 7.0) as drinking solutions.

Mean BP was low in all LNa-C groups.Plasma $NaHCO_3$ and pH were normal in all. The effects of the same loads on GP (pg/mg prot/30 min) PGI_2 (I) and TXA_2(T) was studied in 5 additional groups (n=,each).

The results:

	GFR	RPF	I	T
N	0.94±.07	2.23±.2	34±11	34±4
LNa-C	0.27±.03*	0.76±.2*	20±3	85±4 *
LNa-C/KCl	0.67±.16°	1.90±.3°	466±62*	847±192*
LNa-C/K	0.66±.23°	1.72±.5°	671±53*	967±208*
LNa-C/Cl	0.29±.08	0.80±.2	228±95*	332±142*

*=$p<0.01$ vs N: °=$p<0.01$ vs LNa-C

The I/T ratio was decreased in LNa-C rats (.0.27 vs 0.86 in N).After K stimulation, I/T ratio returned to normal values: 0.64 (LNa-C/KCl) and 0.77 (LNa-C/K), $p=0.01$ vs LNa-C for both. After Cl alone the ratio remained low (0.42). In conclusion, K but not Cl, prevents the development of renal failure in LNa-C rats. The increased synthesis of vasodilatory GP may play a pivotal role in this effect.

ACTIVATION OF POLYOL PATHWAY STIMULATES RENAL PRODUCTION OF VASODILATORY PROSTAGLANDINS. M. Scavini*, P. Zager, J. Frey*, R. Eaton*, J. Jackson*. Univ. of New Mexico, Albuquerque, NM, and H San Raffaele, Milano, Italy.

Increased renal production of vasodilatory prostaglandins (PG) may contribute to the hyperfiltration that accompanies early experimental diabetes (Metab 36:95, 1987). We postulated that activation of the polyol pathway increases renal production of vasodilatory PG, and that this phenomenon can be prevented by aldose-reductase (AR) inhibition. We studied the effects of polyol accumulation on the urinary excretion rates (UER) of PG. We used the classic model for polyol accumulation, the galactose-fed rat, to avoid the metabolic complications of diabetes. Three groups (n=18) of rats were studied. Group I was fed normal chow. Group II was fed normal chow supplemented with galactose (30%). Group III received chow containing galactose (30%) and an AR inhibitor (sorbinil 0.7%). UER of PG (pmol/24 h) were measured in each group after 151-240 days on the respective diets. UER of PGE_2 and 6-keto-$PGF_{1\alpha}$ increased in response to galactose feeding. Administration of sorbinil prevented these changes.

	Group I	Group II	Group III
PGE_2	30±7	106±18*	40±7
6ketoPGF$_{1\alpha}$	127±1	201±19*	136±12

*p<.01 II vs I and III

UER of 11-dehydro-TxB_2, however, were similar in groups I and II (10±3 vs 15±3 pmol/24 h, NS).

Activation of polyol pathway increases UER of vasodilatory PG. AR inhibition prevents this phenomenon and may be useful in suppressing hyperfiltration of early diabetes.

IDENTIFICATION OF GLOMERULAR THROMBOXANE (TX) RECEPTORS: ROLE IN MODULATION OF GLOMERULAR HEMODYNAMICS. Judith Solomon*, Barry Wilkes, Peter Mento*, Mary Maita* and Carolyn Macica*. Division of Nephrology & Hypertension, Dept. of Medicine, North Shore Univ. Hospital and Cornell University Medical Center, Manhasset, NY 11030

TX has important actions on renal glomeruli and may contribute to the progression of glomerular disease. The aim of this study was to identify and characterize glomerular TX receptor sites. Glomeruli (>95% pure) were isolated by graded sieving and centrifugation from normal male S-D rats. Binding studies were performed using the stable TX receptor antagonist, ^{3}H-SQ29548 (SQ). Specific binding was saturable, reversible, and varied with glomerular protein. Scatchard plots of equilibrium binding revealed one class of high affinity receptor sites (K_D = 14.3±2.4 nM, Bmax = 361.1±21.9 fmol/mg, N = 5). Specific binding was inhibited by TX agonists (U46619, U44069) and antagonists, but not by PGE_2, PGF_{2a}, angiotensin II or bradykinin. The effects of receptor occupancy and blockade on renal function were studied in vivo:

	MAP (Torr)	GFR (ml/min/100gBW)	RBF	FF
No Drug	108±6	0.49±.05	1.27±.10	0.39±.03
U46619	112±3	0.19±.04*	0.47±.15*	0.49±.04*
SQ+U46619	108±2	0.56±.09	1.70±.15	0.33±.02

*P < 0.01 vs no drug or SQ+U46619

Stimulation of TX receptors resulted in glomerular vasoconstriction and a rise in FF which were blocked by SQ. These studies demonstrate by ligand techniques the presence of specific functional receptor sites for TX on renal glomeruli.

PHOSPHOLIPASE A_2 (PLA_2) IS COUPLED TO A PERTUSSIS TOXIN (PT)-INHIBITABLE G PROTEIN IN CULTURED RAT INNER MEDULLARY COLLECTING TUBULE (RIMCT) CELLS. I. Teitelbaum, and A. Strasheim,*. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

Studies were performed to examine the role of guanine nucleotide-binding regulatory proteins in the stimulation of PLA_2 in RIMCT cells. Incubation with PT (100 ng/ml, 16 hr) decreases basal PGE_2 production from 26.29±4.89 to 8.72±1.85 pg/µg (n=6, p<.01). PGE_2 production in response to the non-specific PLA_2 agonist, ionomycin (0.1 µM), is not affected by PT, 149.04±25.36 vs 128.86±12.04 (n=6, NS). To assess the effect of PT on receptor-mediated PLA_2 activition, we employed epidermal growth factor (EGF, 1 µM). EGF increases PGE_2 from 12.37±1.23 to 48.44±6.35 (n=5, p<.02); this increase is eliminated by PT as PGE_2 production in PT-treated cells is only 17.15±4.29 (NS vs basal). To ensure that inhibition by PT is due to ADP-ribosylation of a G protein rather than the lectin-like effects of PT, we examined the effects of the non-hydrolyzable compound, GTPγs (10 µM).

C	E	γs	E+γs	E+γs+PT
20.4±4.4	31.1±4.5	15.8±3.0	130.0±35.1	9.2±2.5

In saponin-permeabilized cells, EGF alone has only a modest effect on PGE_2 production. While GTPγs alone is without effect it markedly potentiates the effect of EGF (n=3, p<.05) and this, too, is inhibited by PT. The effect of PT is not due to enhanced cAMP content (AJP 251:F671,1986) as PT has no effect on cAMP in the absence, 19.23±5.81 vs 19.45±2.29 (n=7, NS), or presence, 39.82±4.72 vs 39.80±4.65 (n=4, NS), of EGF. We conclude that a PT-inhibitable G protein transduces receptor-stimulated but not ionomycin-stimulated PLA_2 activity.

DISSIMILAR RESPONSES TO INSULIN AND PROSTANOID (PG) INHIBITION IN DIABETIC (D) AND NONDIABETIC (ND) PERFUSED KIDNEYS (IPK). JS Stoff, DM McCarthy,* and AJ Cohen. Univ of Massachusetts Medical Center, Worcester, MA.

Previous studies in the IPK have shown that insulin causes vasodilation in ND but paradoxical vasoconstriction in D. To examine whether these dissimilarities were due to differences in the biosynthesis of vasoconstrictive and vasodilatory PGs, we perfused D (2 to 4 weeks after STZ) and ND (STZ-vehicle) with the cyclooxygenase inhibitor, indomethacin (I), and the thromboxane synthetase inhibitor, UK38485 (UK) in control perfusions (C) and in the presence of insulin (INS, 100uU/ml). All kidneys were perfused at constant arterial pressure (100-110 mmHg) and preconstricted with angiotensin (AII, 100 ng/min).

Renovascular Resistance (RVR, ml/min/g)

	C	INS	C+I	INS+I	C+UK	INS+UK
D	9.3±.7	11.9±.8*	10.0±.6	9.1±.6	6.1±.4*	7.6±1.0*
ND	11.9±.8	7.4±.6*	8.9±.4*	9.4±.6*	9.8±1.4	6.7±.6*
pvsD	<.02	<.005	NS	NS	<.05	NS

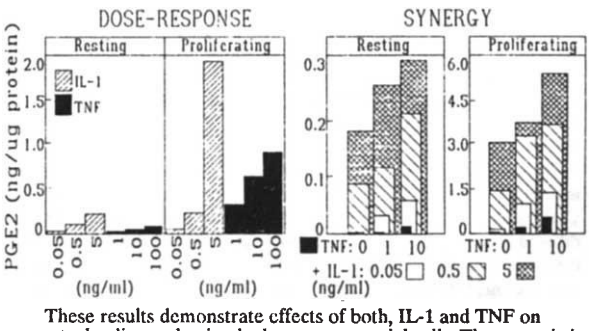
* p <.05 vs C

INS induced vasoconstriction in D but vasodilated ND. I alone (C+I) had no effect but prevented INS-induced vasoconstriction in D and INS-induced vasodilation in ND. UK dilated D (C+UK) and prevented INS-induced vasoconstriction in D (INS+UK), while permitting INS-induced vasodilation in ND. INS vasoactivity is mediated by both vasoconstrictor and vasodilator PGs and blocked by I in D and ND. In D, however, the predominant hemodynamic effect of INS is thromboxane-mediated and blocked by UK.

INTERLEUKIN-1ß (IL-1) AND TUMOR-NECROSIS FACTOR α (TNF) SYNERGISTICALLY INDUCE PGE_2 RELEASE IN HUMAN MESANGIAL CELLS

N.Topley*, J.Floege*, V.Kaever*, K.Resch* (intr.by C.Olbricht). Dept. Molecular Pharmacology, University Hospital, Hannover, FRG

Increasing evidence supports a role for the glomerular mesangial cell in both, homeostatic and proinflammatory reactions within the glomerulus. However, to date most studies have utilized proliferating mesangial cells to study cell stimulation. We therefore studied both, resting cells (viable, but non-proliferating; following 48 hrs culture in serum free medium) and cells proliferating in medium with 10% fetal calf serum. Human mesangial cells stimulated for 24 hrs with rIL-1 and rTNF produced PGE_2 (measured by RIA) in a dose- and time-dependent manner (reaching a plateau by 18 hrs). The production (expressed as pg/µg protein) by proliferating cells however was in all cases markedly higher than in resting cells (see figure). After the stimulation period of 24 hrs both, resting and proliferating cells were viable as assessed by Acridine Orange staining and resting cells could be reinduced to proliferate maximally. When IL-1 and TNF were added simultaneously at varying doses to both, resting and proliferating cells, the resulting 24 hr PGE_2 production showed a marked synergistic increase. Results (means of 3 separate exp.) are given in the figure.



These results demonstrate effects of both, IL-1 and TNF on prostaglandin production by human mesangial cells. The synergistic action of these cytokines suggests a potentially potent effect on the course of inflammation in the kidney.

CLASSIFICATION OF PROSTAGLANDIN (PG) RECEPTORS (R) BASED ON COUPLING TO PHOSPHOLIPASE C (PLC) OR ADENYLYL CYCLASE (AC) in UMR-106 CELLS. D. Yamaguchi, B. Merritt, J. Green, C. Kleeman, and S. Muallem. VAMC West Los Angeles, Cedars-Sinai Med. Ctr., and UCLA School of Medicine, Los Angeles, CA.

PGs bind to plasma membrane Rs to evoke their actions in different tissues. We utilized PG second messenger generation to logically classify PG Rs. PGs can stimulate both an increase in cAMP and free cytosolic Ca2+ ((Ca2+)i). The increase in (Ca2+)i is independent of ambient Ca2+ and is correlated with stimulation of the phosphoinositide cycle. The potency rank for PG-stimulated rise in (Ca2+)i was F2α>D2$\gg$E2>E1>I2>A2. Stimulation with an F2α dose that gave maximal (Ca2+)i increase followed by stimulation with maximal doses of F2α or D2 did notfurther increase (Ca2+)i. Maximal doses of E2,E1, A2, or I2 following F2α or D2 stimulation did elicit a second transient rise in (Ca2+)i. Similarly, initial stimulation with maximal doses of E2, E1, or A2 followed by stimulation with E2, E1, or A2 did not result in a second Ca2+ transient. When F2α or D2 followed E2, E1, or A2 stimulation, a second transient Ca2+ rise occurred. The potency rank for PG-stimulated cAMP production was E2>E1>A2>I2$\gg$F2α>D2. Simultaneous stimulation with E2 and E1 did not result in additivity of cAMP generation. Pretreatment with E2 desensitized cells to respond to E1, A2, or I2 by cAMP generation but had no effect on the increase in (Ca2+)i. Conclusions: 1) PLC and AC signal transduction systems are coupled to different PG Rs; 2) there are three subclasses of PG Rs--two linked to PLC and one linked to AC; 3) F2α and D2 bind to a common PLC-associated R while E2, E1, A2, and I2 bind to a second common PLC-associated R.

VASORELAXANT AND NATRIURETIC RESPONSES TO LEUKOTRIENE B4 (LTB4) IN THE RAT KIDNEY: FUNCTIONAL IMPORTANCE OF THE 12(R) STEREOCHEMISTRY. A Yared, K Takahashi*, A Brash*, J Capdevila*, and KF Badr. Vanderbilt University, Nashville, TN.

LTB4 is an established mediator in renal inflammatory injury. To investigate its intrinsic actions which are independent of its effects upon leukocyte (PMN) activation, we infused LTB4 (10 nM) into the renal artery of 7 Munich-Wistar rats. At this concentration, LTB4 does not attract or activate rat PMNs, and PMN infiltration was not observed in LTB4-infused kidneys. Despite constant arterial pressure and GFR, LTB4 increased RPF (3.5±0.4 to 4.0±0.3 ml/min*) and dramatically augmented urinary sodium, but not potassium, excretion rate (UNaV) from 0.61 to 2.71 µEq/min*. Inhibition of cyclooxygenase, lipoxygenase, and cytochrome-P450 (cP450) arachidonic acid (AA) metabolism by 5,8,11,14-eicosatetraynoic acid (ETYA, n=5) did not abolish LTB4-induced changes in RPF and UNaV. * p<0.05 vs control.

These renal responses to LTB4 were identical to those of the AA-cP450 metabolite 12(R)-hydroxyeicosatetraenoic acid [12(R)-HETE). Since the two eicosanoids share the (R) configuration at the C12 chiral center and may occupy a common receptor on human PMNs, we examined their potentially related structure-function activities in the kidney. Synthetically prepared C12-(S) stereoisomers of LTB4 [12(S)-LTB4] (n=3) and of 12(R)-HETE [12(S)-HETE] (n=3) were without effect on RPF (4.1±0.5 to 4.2±0.6and 3.1±0.1 to 3.2±0.1 ml/min) or UNaV (0.99±0.15 to 1.13±0.04 and 1.43±0.69 to 1.59±0.66 µEq/min).

Thus, we report novel renal actions for LTB4 (vasorelaxation and natriuresis) independent of PMN activation or other AA metabolites. These actions are shared by 12(R)-HETE. Since the olefin geometry is different between LTB4 and 12(R)-HETE , but is identical between these eicosanoids and their respective biologically inactive stereoisomers 12(S)-LTB4 and 12(S)-HETE, it is therefore the common R configuration at the C12 alcohol which constitutes an essential prerequisite for optimal recognition and biologic activity at a proposed LTB4/12(R)-HETE renal receptor site.

MULTIPLE FORMS OF ANGIOTENSIN-CONVERTING ENZYME (ACE) cDNA. Kenneth E. Bernstein* and Brian M. Martin*, Dept. of Pathology, Emory Univ., Atlanta, Ga., NIMH, Bethesda, Md. (intr. by Wallace G. Campbell, Jr.)

ACE plays a major role in blood pressure regulation by converting angiotensin I to angiotensin II. We have isolated two classes of mouse kidney ACE cDNA based on patterns of hybridization to 5 ACE specific oligonucleotide probes. One such cDNA, ACE.11, is a 4691 bp class I cDNA that encodes 991 amino acids. The protein sequence of ACE.11 appears to be composed of three domain-like regions, each of approximately 300 amino acids. While the first and third protein regions are highly homologous, the second region is similar to the protein sequence of the 1033 bp class II ACE cDNA, ACE.31. The proteins encoded by ACE.11 and ACE.31 are more homologous than their DNA sequences. Both classes of ACE cDNA identify mRNA of 4900 and 4150 nucleotides in mouse kidney and lung RNA. In contrast only ACE.11 hybridizes to testis mRNA, detecting a single strongly hybridizing band of 2500 bases. Southern analysis of mouse genomic DNA with clone specific restriction fragments demonstrates that ACE.11 and ACE.31 are encoded on separate genomic segments. These data strongly suggest that within mouse kidney and lung mRNA, there are multiple messages encoding ACE-like sequence. ACE may be a family of similar proteins.

RENAL SENSITIVITY TO ANGIOTENSIN II IN HUMAN DIABETES MELLITUS IS NOT DECREASED. Staffan Björck*, Mattias Aurell, Lars-Einar Bresäter*, Hans Herlitz*, Lennart Welin*, John Wikstrand*,. University of Göteborg, Sahlgrenska hospital, Dept. of Nephrol. & Clin. Physiol., Göteborg, Sweden.

Reduced angiotensin II (AII) sensitivity has been proposed to be responsible for the increased GFR and RPF in diabetic animals. To test this hypothesis in man, we investigated the renal effects of low dose angiotensin II infusion in 9 patients with insulin dependent diabetes mellitus, 25±3 years old, without complications and in 7 healthy age-matched controls. The GFR (^{51}Cr-EDTA-clearance) and RPF (PAH-clearance) were measured before and during infusion of 0.5 and 1.5 ng/kg/min. Basal levels of plasma renin activity (PRA) and AII were measured.

	basal	0.5ng/kg/min	1.5ng/kg/min
GFR (ml/min/1.73m^2)			
diabetics	125±13*	117±7*	109±15#
controls	109±10	100±11	97±13
RPF (ml/min/1.73m^2)			
diabetics	524±131	415±71#	373±79#
controls	534±107	438±88	389±123#
Filtr fraction			
diabetics	0.26±0.06	0.29±0.05*	0.30±0.04#
controls	0.21±0.03	0.24±0.04#	0.27±0.07#

(means±SD). *=p<0.05 diabetic vs controls. #=p<0.03 vs basal. The small change in blood pressure was similar in both groups and increased from 115±10/81±11 mm Hg to 123±11/84±11 mm Hg in the diabetics and from 118±8/76±8 to 125±5/87±6 in the controls. The basal PRA and AII levels, that did not differ between groups, were 1.1±0.4 ng/ml/hr and 5.0±1.4 pg/ml in the diabetics and 0.9±0.8 and 5.1±3.9 in the controls. It is concluded that the glomerular hyperfiltration in uncomplicated diabetes mellitus in man is not due to a reduced renal sensitivity to AII.

NaCl CONCENTRATION DEPENDENCE OF MACULA DENSA MEDIATED RENIN SECRETION. Josephine P. Briggs, Ole Skott*, Ama Ofosu-Appiah*, and Ann Smart*. Dept. of Int.Med., University of Michigan, Ann Arbor,MI.

Studies were performed to define the relationship between macula densa (MD) tubular fluid NaCl concentration and renin secretion, using a newly developed technique which permits control of luminal composition at the MD in isolated superfused JGA's. The MD-containing tubule segment was dissected together with adherent glomerulus from rabbit kidneys, and perfused from either the TAL or the DCT using isolated perfused tubule techniques. The complex was superfused continuously, and fluid collected at ten min intervals. Renin was assayed in presence of excess substrate using antibody trapping followed by RIA of angiotensin I. In time control studies, single JGA renin secretion rate (RSR) averaged 10.9±4.8 nGU/min and was unaffected by a mock perfusate change. In 8 JGA's, RSR was measured with 4 perfusates of varying [NaCl], presented in a random order with a Latin squares design:

[Na] mM	140	80	45	25
[Cl] mM	122	60	25	7
RSR (nGU/min)	13.2±4.2	15.7±4.7	27.9±6.6	38.6±6.2
	n.s.	p<.05	p<.05	

Previous studies in rat have shown a similar pattern of luminal NaCl concentration dependence of tubuloglomerular feedback (TGF). TGF responses are half maximum at a luminal Cl of 35, and increases above 65 mM do not result in augmentation of responses. The results suggest that the TGF mechanism and MD-mediated renin secretion may share a common signal mechanism, and that increasing extracellular formation of angiotensin is unlikely to account for TGF-mediated vasoconstriction.

RESPONSE OF INTRARENAL RENIN (IR) TO NEONATAL UNILATERAL URETERAL OBSTRUCTION (UUO) AND UNINEPHRECTOMY (UNX). S. El-Dahr*, R.L. Chevalier, R.A. Gomez, M.J. Peach*, and R.M. Carey*. University of Virginia Medical Center, Departments of Pediatrics, Internal Medicine and Pharmacology, Charlottesville, Virginia.

Chronic UUO results in renal vasoconstriction which is selectively attenuated by angiotensin converting enzyme inhibition. In contrast, the intact kidney following contralateral UUO or UNX undergoes hypertrophy and vasodilatation. To evaluate the role of IR in these responses, Sprague-Dawley rats were subjected to sham operation (S, n=10), left ureteral ligation (UUO, n=12), or right nephrectomy (UNX, n=7) within the first 2 days of life. At 4 weeks of age, kidney sections were immunostained for renin using a specific polyclonal antirat renin antibody. The kidney weight (KW, mg), % stained juxtaglomerular apparatuses (%JGA), % length of staining of the afferent arteriole (%LAA) and renal renin content (RRC; ngAI/mg protein/hr) were determined for the left (LK) and right (RK) kidneys. Results (mean±SE; * p < 0.01 vs sham; # p < 0.01 vs UNX; + p < 0.05 vs left kidney):

	S		UUO		UNX
	LK	RK	LK	RK	LK
KW	680±20	680±10	450±30*	800±60*+	960±60*
%JGA	55±2	54±1	81±1*#	74±2*+	48±3
%LAA	32±2	32±3	70±4*#	66±6*	33±2
RRC	22±2	17±2+	42±6*	16±2+	36±3*

Plasma renin activity and mean arterial pressure were not significantly different among the 3 groups. Changes in IR suggest that renal vasoconstriction in the chronically obstructed kidney may be mediated by recruitment of renin-containing microvascular cells. However, the response of IR in the intact kidney varies with the stimulus to hypertrophy: after UUO, %JGA and %LAA were increased and RRC was decreased; while after UNX, renin distribution was not affected and RRC was increased. This adaptive response may be mediated by a neural or humoral signal.

METABOLISM OF ALDOSTERONE IN THE ISOLATED PERFUSED KIDNEY AND LIVER.
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The interaction between kidney and liver on the metabolism of aldosterone was studied in the isolated perfused kidney and isolated perfused liver and in the combined isolated perfused kidney and liver of male Wistar rats using recirculation with a hemoglobinfree medium. The renal arterial pressure and the hepatic perfusate flow were kept constant. 4-14C-aldosterone was added at an initial conc. of 1800 pg/ml. Perfusate aldosterone was measured by a RIA and 14C radioactivity was determined in the samples.

Equal diureses, GFR, fractional sodium and potassium reabsorption were observed in the isolated perfused kidney and the combined model. Similarly equal values of portal vein pressure, bileflow and oxygen consumption were found in the combined model and the isolated liver. After 90 minutes of perfusion aldosterone conc. declined with 34% in the isolated kidney and with more than 90% in the isolated liver as well as in the combined model. The corresponding perfusate levels of 14C radiometabolites was 23%, 43% and 48% of the initial 14C added, respectively. The total biliary excretion of 14C was 26% and 16% in the isolated liver and the combined model; while the total urinary excretion of 14C was 1.7% and 2.6% in the isolated kidney and the combined model. The average clearance rate of aldosterone in the isolated kidney was 0.93 ml/min compared to a GFR of 0.72 ml/min. In non-filtering kidneys the clearance rate of aldosterone was 0.29 ml/min. In the isolated perfused liver and in the combined model the metabolic clearance rate of aldosterone was 13.1 and 10.9 ml/min, respectively.

In conclusion, hepatic metabolism was the major eliminating pathway of aldosterone, and biliary excretion was predominant. Renal elimination amounted to less than 10 % pr. kidney of the total clearance rate of aldosterone. It consisted mainly of glomerular filtration followed by tubular reabsorption; while 25% was eliminated by peritubular degradation.

EARLY EFFECTS OF ALDOSTERONE ON THE Na:K PUMP IN ISOLATED CORTICAL COLLECTING TUBULES (CCT). Yoshiaki Fujii* and Adrian I. Katz. Univ. of Chicago, Dept. of Medicine, Chicago, Illinois.

Sustained exposure to aldosterone [A] increases the abundance and activity of the Na:K pump in its main renal target site, the CCT. However, the occurrence and mechanism of the early interactions of [A] (i.e., within the time span of its effects on electrolyte transport) with the CCT pump, especially in adrenal-intact animals, remain disputed. In this study we evaluated the short-term in vitro effects of [A] on Na-K-ATPase hydrolytic activity and on ouabain-sensitive ^{86}Rb uptake ($^{86}Rb_u$), a measure of the transporting rate of the pump, in microdissected CCT from adrenal-intact rats. Na-K-ATPase activity was unchanged by 2 h incubation with [A], 10^{-8} M (904 + 84 SE vs. 984 ± 72 pmol/mm/h, NS). In contrast, [A] produced a striking increase in the 1 min (initial velocity) $^{86}Rb_u$, from 19.5 ± 1.6 to 41.7 ± 4.0 pmol/mm/min, $P < 0.01$. This effect was time-dependent (30 min lag) and dose-related (detected at 10^{-10} M, maximal at $\geq 10^{-8}$ M). Additional studies were performed to assess whether the pump stimulation was secondary to increased Na entry. Incubation with [A] in the presence of 10^{-4} M amiloride (tubules with patent lumen), or in a Na-free medium (choline Cl) did not alter the enhanced $^{86}Rb_u$ observed with [A] alone, and this effect was still seen upon addition of nystatin, i.e., when cell Na concentration was no longer rate-limiting. In contrast, the [A]-induced enhancement of $^{86}Rb_u$ was blunted by concurrent incubation with cycloheximide.

The results indicate that the early effect of [A] on the Na:K pump in CCT represents enhanced turnover rate of existing pumps that precedes (and may promote) the later increase in the biosynthesis of new pump units. This effect is probably not mediated by increased Na entry, but appears to require new protein synthesis.

RENIN GENE EXPRESSION DURING SALT DEPLETION. R.A. Gomez, R.L. Chevalier, A.D. Everett*, M.J. Peach*, R.M. Carey*. University of Virginia School of Medicine, Charlottesville, Virginia.

To define whether intrarenal renin synthesis and distribution are affected by salt depletion, a control group of adult, male Wistar-Kyoto rats (C, n=7) was compared to a group of rats receiving a low sodium diet containing trace amounts of sodium chloride (0.01 - 0.02%) for 7 days (SD, n=10). Kidney total RNA was extracted and dot and Northern blots prepared and hybridized to a full length ^{32}P-renin cDNA. Kidney renin mRNA levels were higher in the SD than in the C group. The intrarenal distribution of renin was assessed by immunocytochemistry with a polyclonal antirat renin antibody (gift of Dr. T. Inagami) applied to formalin-fixed tissue. The number of immunostained arterial vessels (AV) and the length of afferent arteriolar immunostaining (LAA) was determined, expressed as a percentage (%) of the total and compared between groups. Both the % AV and % LAA were higher in SD (80±10 and 80±15%) than in C (43±9 and 33±15%) rats ($p < 0.05$). In addition, 27±18% of glomeruli in SD rats contained renin in their glomerular tufts. We conclude that SD enhances intrarenal renin synthesis and redistributes intrarenal renin within the preglomerular and glomerular microvasculature. Consequently, SD may induce a recruitment of cells that in the basal state were not expressing the renin gene.

PARATHYROID HORMONE (PTH) MODULATES AII-STIMULATED ALDOSTERONE SECRETION. C.M. Isales*, P. Q. Barrett, M. L. Brines*, W. B.Bollag*, H. Rasmussen*. Yale Sch. Med., Depts. Medicine & Physiology, New Haven, CT.

Although Ca^{++} regulating hormones have been implicated in the etiology of hypertension, their exact role remains controversial. In vivo studies have yielded conflicting data concerning the modulation of the renin-angiotensin-aldosterone axis by PTH Using bovine adrenal preparations, we evaluated PTH as a potential modulator of glomerulosa cell function, and measured the intraadrenal distribution, the affinity and the steroidogenic activity of the PTH receptor.

In vitro autoradiography of adrenal tissue incubated with ^{125}I-PTH revealed binding which was limited to the outer zone (glomerulosa layer) of the cortex and was 40% specific. No binding was observed in other regions of the adrenal. Using purified glomerulosa cell membranes, receptor affinity was determined and by Scatchard analysis a single class of high affinity receptors (Kd 0.4 nM) was demonstrated.

In the absence of other secretagogues, PTH (0.01-1.0 nM) modestly stimulated aldosterone secretion from glomerulosa cells from a control value of 15 pg/min/10^6 cells to a maximum of 30 pg/min/10^6 cells at 1 nM PTH, $p<0.01$. In contrast, a dose of PTH (0.1 nM) which by itself minimally increased secretion (+10 pg/min/10^6 cells) greatly enhanced the secretory rate when added with AII (0.1-10.0 nM). The magnitude and the AII-dose dependence of this potentiation by PTH varied according to the time over which secretion was measured. During the first 15 min of incubation, PTH shifted the AII-aldosterone dose response curve (Ka 1.0 to 0.3 nM) without significantly increasing the maximal secretory response (46.4 pg/min/10^6 cells vs 57.8 pg/min/10^6 cells,$p>.05$). In contrast during a sustained response (30-45 min), PTH greatly enhanced the maximal secretory response (AII:109.4pg/min/10^6cells; AII+PTH:219.2 pg/min/10^6 cells, $p< 0.005$) without shifting the Ka for AII activation.

These data indicate that high affinity PTH receptors are present in the zona glomerulosa and that at physiological concentrations of PTH and AII a synergism occurs resulting in greatly enhanced aldosterone secretion.

PHYSIOLOGICAL DOSES OF ANF PARTIALLY INHIBITS SODIUM RETAINING ACTIONS OF AII IN DOGS. J.P. Granger, M.J. Solhaug*and J.W. Scott*. Eastern Virginia Med. Sch., Dept. of Physiol., Norfolk, VA

Previous microperfusion studies in rats have indicated that high concentrations of atrial natriuretic factor (ANF) inhibits AII-stimulated proximal Na reabsorption. The purpose of this study was to determine whether physiological doses of ANF has an important antagonizing effect on the Na retaining actions of AII in anesthetized dogs with the endogenous renin angiotensin system blocked by converting enzyme inhibition. The effects of an intrarenal infusion of AII at rates of .5, 1.0, and 1.5 ng/kg/min on renal handling of Na were determined in the presence (N=5) and absence (N=3) of physiological increases in circulating levels of ANF (5 ng/kg/min, I.V.). Intrarenal infusion of AII at rates of .5, 1.0, and 1.5 ng/kg/min into normal dogs decreased urinary excretion of Na ($U_{Na}V$) to 43, 37, and 34% of control. Fractional excretion of lithium (FE_{Li}, an indicator of Na delivery from the proximal tubule) also decreased to 70, 57, and 60% of control. AII infusion had no significant effect on GFR. Pretreatment of dogs with ANF significantly blunted the effects of AII on $U_{Na}V$ and FE_{Li}. In the presence of ANF, intrarenal infusion of AII at rates of .5, 1.0 and 1.5 ng/kg/min decreased $U_{Na}V$ to only 72, 84, and 97% of control, respectively. FE_{Li} decreased to 92, 71, and 76% of control in response to AII. These data suggest that physiological increments in plasma ANF may partially inhibit the proximal tubule effects of AII, thereby affecting Na excretion. This affect of ANF could be an important mechanism whereby ANF affects proximal tubule function.

MODULATION OF PLASMA ALDOSTERONE CONCENTRATION BY CHANGES IN $[H^+]$. G.V. Jones,* B.M. Wall, H.H. Williams, D.G. Sapir and C.R. Cooke. VAMC and Univ. of TN, Memphis, TN and The Johns Hopkins Univ. School of Med., Baltimore, MD.

To assess the effect of changes in extracellular $[H^+]$ on plasma aldosterone (PA) concentration while controlling other factors influencing aldosterone secretion, studies were performed on 3 anephric and 9 non-nephrectomized patients undergoing hemodialysis. All subjects were studied twice, once with high (35meq/l) and once with low (15-17.5 meq/l) dialysate HCO_3 concentrations. Body weight and plasma K^+ were maintained at constant levels. Blood samples were collected by constant withdrawal into iced syringes during 30 minute intervals prior to and following dialysis for determination of integrated PA, cortisol (PC), Na^+ and K^+ concentrations. Plasma renin activity (PRA) and $[H^+]$ were determined in samples drawn separately. Pre and post dialysis values (mean ± SE) were:

	High HCO_3			Low HCO_3		
	Pre		Post	Pre		Post
PA	8.5±1.5	p<0.005	5.8±1.5	7.2±1.7	p<0.04	9.3±2.3
PRA	0.4±0.2	NS	0.5±0.3	0.3±0.1	NS	0.2±0.1
PC	11.9±2.6	NS	9.2±0.9	11.6±2.2	NS	10.2±1.4
Na^+	139±0.6	NS	139±0.9	140±0.6	p<0.003	135±1.3
K^+	4.7±0.2	NS	4.6±0.2	4.8±0.1	NS	4.7±0.1
H^+	44.5±2.0	p<0.002	36.7±1.2	43.1±1.0	p<0.005	53.3±2.4

PA (ng/dl), PRA (ng/ml/hr), PC (µg/dl), Na^+ and K^+ (meq/l), H^+ (neq/l). ΔPA in high HCO_3 studies (-2.7±0.7) was significantly different ($p<0.005$) from ΔPA in low HCO_3 studies (2.1±0.9). Regression analysis of data from both low and high HCO_3 studies showed a highly significant correlation between ΔPA and ΔH^+ ($r=0.74$, $p<0.005$) but no correlation between ΔPA and ΔPRA, ΔPC, ΔNa^+ or ΔK^+. These studies show a relationship between PA and extracellular $[H^+]$ that strongly suggests that H^+ may play a role in the regulation of aldosterone secretion.

CIRCULATING ACTIVE RENIN HETEROGENEITY CHANGES AFTER STIMULATION OF RENIN SECRETION. SA Katz*, JA Opsahl, and PA Abraham. Hennepin County Medical Center, and Univ. of Minnesota, Minneapolis, MN

Human active renin is composed of multiple forms with variable isoelectric points between 5.46 and 4.84. The relative proportions of five major renin forms were compared in human essential hypertensive peripheral venous plasma before and 2 hrs after stimulation of renin release with converting enzyme inhibition (Quinapril) and upright posture. Five major active renin forms were separated by shallow gradient isoelectric focusing and quantitated by radioimmunoassay of generated Angiotensin I.

Plasma renin activity increased significantly from 1.2 to 5.1 after renin stimulation (N=5).

Isoelectric point (IP) and average proportions (%) of circulating active renin forms before (B) and after stimulation (S) of renin secretion (N=5) (Mean±SEM): *p < .01; B vs S

IP	5.46	5.22	5.05	4.88	4.84
B	13.8±2.1	19.9±1.9	22.8±4	21.1±2.3	22.4±5.4
S	22±2.3	24±1.7	22.2±2.6	19.3±1.1	12.5±2.7
%Δ	*+59.4%	*+20.6%	-2.6%	-8.5%	*-44.2

Stimulation of renin secretion was accompanied by a significant increase in the proportions of the two most basic renin forms and a significant decrease in the proportion of the most acidic form. These data are consistent with the hypotheses that stimulation of renin secretion results in preferential secretion of the more basic forms, and/or that the liver preferentially degrades the more basic forms.

EFFECT OF ENALAPRILIC ACID ON THE SINGLE NEPHRON HEMODYNAMIC RESPONSES TO PERITUBULAR CAPILLARY INFUSION OF ANGIOTENSIN I. Kenneth D. Mitchell* and L. Gabriel Navar. University of Alabama at Birmingham, Birmingham, AL 35294.

The present study was performed to determine the effect of acute administration of the angiotensin-converting enzyme (ACE) inhibitor, enalaprilic acid (MK 422), on the single nephron hemodynamic responses to peritubular capillary infusion of angiotensin I (ANG I). Stop-flow pressure (SFP) measurements were obtained in pentobarbital-anesthetized rats during control conditions and during successive peritubular capillary infusions (each at a rate of 20 nl/min) of 10^{-3}M MK 422 and 10^{-5}M ANG I + 10^{-3}M MK 422. SFP was not altered during infusion (4.3±0.3 min) of 10^{-3}M MK 422 (40.6 ± 0.6 vs 39.7 ± 0.5 mmHg, n=27). Subsequent infusion of 10^{-5}M ANG I + 10^{-3}M MK422 into the same vascular sites reduced SFP to 21.5 ± 3.2 mmHg (P<0.01, n=27); responses similar to those obtained with 10^{-5}M ANG I alone. Similarly, in 2 rats receiving a systemic infusion of MK 422 (1-3 mg/kg/hr), peritubular infusion of 10^{-5}M ANG I alone or 10^{-5}M ANG I + 10^{-3}M MK 422 decreased SFP from 38.1 ± 1.3 to 14.9 ± 5.7 mmHg (P<0.01, n=7 tubules). In contrast, the effects of peritubular ANG I infusion on SFP in normal rats were blocked by the angiotensin II (ANG II) receptor antagonist, saralasin (10^{-5}M). These findings indicate that intrarenal conversion of ANG I to ANG II occurs, at least in part, at a site which is inaccessible to acutely administered MK 422, or, that there is an alternative pathway for the intrarenal conversion of ANG I to ANG II.

ENALAPRIL (E) SUPPRESSES THE EFFECT OF HIGH PROTEIN DIETS ON ALBUMINURIA BUT NOT ON GLOMERULAR FILTRATION RATE IN NEPHROTIC RATS V. Martin*, F.N. Hutchison, G.A. Kaysen. VAMC Martinez CA. University of CA Davis, CA.

Dietary protein (DP) was increased from 8.5% (LP) to 40% (HP) in 24 rats with passive Heymann nephritis (HN), 12 of which received E (HPE), to evaluate the relationship between changes in GFR (ml/m) and albuminuria (UalbV, mg/d), and to define how GFR and UalbV were affected by E.

	HP		HPE	
Day	GFR	UalbV	GFR	UalbV
0	0.94+0.9	189+20	1.01+.11	181+11
1	1.28+.11*	309+45*	1.32+.13*	218+22
2	1.33+.07*	457+36*	1.21+.09*	287+31*#
4	0.92+.04	570+70*	0.94+.04	373+29*#
14	0.58+.10	552+81*	0.86+.14	65+2*#

# P<0.05 vs HP, * P<0.05 vs day 0

GFR increased at 1 and 2 days, but decreased by day 4, unaffected by E. UalbV increased on day 1 in HP, and continued to increase despite the fall in GFR after day 2. UalbV initially increased in HPE at day 2, but decreased after day 4 in a time dependent fashion. Similar studies were performed in 23 HN rats fed LP, of which 10 received E (LPE). UalbV fell with time in LP, but decreased more in LPE. The % decrease in UalbV by day 14 was the same in LPE (73%) and HPE (82%). E modifies the increase in UalbV caused by HP without affecting the protein induced changes in GFR, suggesting that these events are not the result of the same process, and that angiotensin II does not play an important role in the changes in GFR. The proportional reduction in UalbV in both LPE and HPE suggest that E exerts an effect that is independent of DP.

ANGIOTENSIN II (AII) AND VASOPRESSIN (VP) INCREASE INTRACELLULAR CALCIUM ACTIVITY (Ca_i) IN SINGLE JUXTAGLOMERULAR CELLS (JGC) IN PRIMARY CULTURE. Orson Moe*, Alberto Tejedor*, Robert J. Alpern, William Henrich. University of Texas Southwestern Medical Center and VAMC, Dallas, TX.

The inhibitory effects of AII and VP on renin release may be mediated by an increase in systemic pressure and/or by a direct effect on the JGC. Since Ca_i may modulate renin release, we examined the effect of AII and VP on Ca_i in individual JGCs. JGCs were isolated from rat kidney cortex by density gradient centrifugation, and grown in primary culture for 2 days. 80% of these JGCs stained positively witn anti-rat renin antibody and isoproterenol (10^{-5} M) increased renin secretion from 20±8 to 46±17 ng/ml/h (n=6, p<0.05). Ca_i was measured microfluorometrically on single JGCs loaded with FURA 2 and perfused at 37°C. Ca_i was calculated from the ratio of fluorescence intensities at excitation wavelengths of 350 and 380 nm with an emission wavelength of 510 nm. Each cell was calibrated using ionomycin. Resting Ca_i in JGCs was 148±24 nM (n=23). AII (10^{-6} M) increased Ca_i from 125±23 to 209±40 nM (n=17, p<0.05). VP (10^{-6} M) increased Ca_i from 215±56 nM to 373±106 nM (n=6, p<0.05). In most cells Ca_i peaked at 150-250 sec and then returned toward control values.

In conclusion, both AII and VP transiently increase Ca_i in cultured JGCs. This effect on Ca_i may mediate a direct inhibitory action of both hormones on renin secretion.

ANGIOTENSIN II (AII) DIRECTLY INCREASES SODIUM TRANSPORT BY RENAL BRUSH BORDER MEMBRANE (BBM). ROLE OF PHOSPHOLIPASE A_2 (PLA). G. Morduchowicz* and N. Yanagawa, Nephrology Div., Sepulveda VAMC, UCLA Sch. of Med., Los Angeles, CA.

Previous studies demonstrated the presence of AII receptors in BBM. We have recently reported that AII directly causes a dose-dependent increase in BBM Na^+/H^+ exchange (Clin. Res. 36:523, 1988). Since PLA may serve as the AII signal transducer in renal tubular cells, we have tested the role of PLA in AII effect on BBM Na^+/H^+ exchange. BBM vesicles were isolated from rabbit kidneys and amiloride sensitive Na^+ uptake was measured under an outward pH-gradient. Addition of PLA inhibitor, mepacrine (10^{-4} M), abolished the effect of AII (10^{-9} M) (n=4, *p<Ø.Ø5 vs. control).

	Control	AII	MP	AII+MP
Na^+ uptake (nm/mg/5sec)	2.11 ±Ø.17	3.18* ±Ø.33	2.54 ±Ø.4Ø	2.34 ±Ø.12

Direct addition of PLA activator, melittin (5ØØng/ml) also increased amiloride-sensitive BBM Na^+ uptake (2.87± Ø.48 vs. 3.81 ± Ø.35 pm/mg/5sec, n=4, p<Ø.Ø5). The effect of AII was abolished by pretreatment of BBM with pertussis toxin (5ØØng/ml), suggesting the involvement of G-protein. These results suggest that AII increases BBM Na^+/H^+ exchange possibly through activation of PLA via G-protein.

EFFECT OF DIETARY PROTEIN (DP) ON REGIONAL BLOOD FLOW AND VASCULAR REACTIVITY TO ANGIOTENSIN II (AII). Brian M. Murray, SUNY at Buffalo, Department of Medicine, Buffalo, New York.

Rats given high DP exhibit increased plasma renin activity, resistance to the systemic pressor effect of AII, and a fall in renal but not systemic vascular resistance. This study was designed to compare the effect of DP on blood flow and vascular reactivity in the renal and mesenteric vascular beds. Male SD rats (200-250g) were fed isocaloric diets for two weeks containing either 6% (LP) or 50% (HP) protein and studied under inactin anesthesia. Renal vascular resistance (RVR) was significantly lower on HP than LP diet (HP 17.2, LP 24.9 mmHg ml^{-1} min, P <.05), whereas mesenteric vascular resistance (MVR) was not significantly affected (HP 6.8, LP 7.6 mmHg ml^{-1} min, P=NS). Rats fed HP exhibited decreased vasoconstrictor responses to infused AII in both the mesenteric (%ΔMVR) and renal (%ΔRVR) vascular beds (See Table).

Vascular Response to AII (250 ng Kg^{-1} m^{-1}) (*p <.5)

	Untreated		Post-Captopril		Post-Meclofenamate	
	%ΔMRV	%ΔRVR	%ΔMVR	%ΔRVR	%ΔMVR	%ΔRVR
LP	12	97	56	180	NA	156
HP	3*	31*	48	72*	NA	107

While captopril (10mg/Kg iv) restored the response to AII in the mesenteric bed, the renal response remained blunted. Meclofenamate (5mg/Kg iv) restored the renal response to AII in the HP-fed rat. Thus, the vasodilatory effect of HP appears to be selective for the kidney. Also, the mechanisms of resistance to AII appear to differ in the two vascular beds. Enhanced prostaglandin production is implicated in the renal bed, whereas in the mesenteric, elevated circulating levels of AII appear to be responsible.

EFFECT OF SARALASIN VS CAPTOPRIL RENOGRAPHY IN TWO-KIDNEY, ONE-CLIP (2K,1C) HYPERTENSION. Joseph V. Nally, Erdal Erturk,* Luis A. Bedoya,* Harry S. Clarke,* Nicholas T. Stowe. Cleveland Clinic Foundation, Cleveland, OH.

Angiotensin-converting enzyme (ACE) inhibition enhances diagnostic changes in the renogram of subjects with renovascular hypertension. In our canine model of 2K,1C hypertension, we compared the effects of the ACE inhibitor captopril (C) (1.5 mg/kg plus 1.5 mg/kg/min), the A-II competitive antagonist saralasin (SAR) (25 µg/kg plus 2.5 µg/kg/min) and atrial natriuretic factor (ANF) (Auriculin A 1.0 µg/kg plus 0.1 µg/kg/min) upon BP, renal function (C_{IN} and C_{PAH}), and the Tc-99m-DTPA and I-131 hippuran (HIP) renograms.

C (N=9) lowered BP (139±6 vs 106±4 mmHg, p<.02), decreased C_{IN} (16.0±3 vs 11.0±.2 ml/min, p<.03) of the stenotic kidney (SK), and dramatically altered the time activity curves of SK (Hypertension 8:685, 1986). ANF (N=9) also lowered BP (150±4 vs 123±6 mmHg, p<.01) but did not adversely affect C_{IN} nor the renograms of SK. SAR (N=10) transiently increased then significantly decreased BP. SAR resulted in cortical retention/delayed excretion of both the Hip and DTPA studies, yet C_{IN} was not reduced.

Conclusion: 1) Alterations in DTPA and Hip renograms are related to A II-dependent, intrarenal events rather than simply the reduction in systemic blood pressure. 2) Changes were more dramatic with the ACE inhibitor C. 3) Transient increase in BP and less dramatic changes in renography after SAR may relate to its partial A-II agonistic activity.

SODIUM BUT NOT ITS ANION REGULATES RENAL ANGIOTENSINOGEN mRNA. Mitchell Pivor, Victor J. Dzau, Julie R. Ingelfinger. Brigham and Women's Hosp, Children's Hosp & Harvard Med School, Boston, MA

High salt diet decreases renal angiotensinogen (ang-n) mRNA expression and plasma renin activity. Not only Na^+ but also Cl^- is important in salt regulation of plasma renin, yet the role of Na^+ vs. its anion at the molecular level is unknown. To investigate the role of cation vs. anion in ang-n mRNA regulation, we placed 3 month old Sprague Dawley rats in metabolic cages and fed them 1.5 days of a low NaCl diet (.02%), followed by a high NaCl vs. high NaHCO3 intake for 3 days (n=5/gp). Controls were fed either low NaCl diet for 1.5 days (n=5) or for the entire 4.5 day study period (n=5). Mean Na^+ intake was not significantly different in the high NaCl gp compared to the high NaHCO3 gp. Mixed blood pH was 7.49±.01 in NaHCO3 gp cf. 7.42±.01 in controls (p<.05), while remaining the same in the NaCl gp (7.44±.01). Plasma renin concentration was suppressed more in high NaCl (1.86±.47) vs. high NaHCO3 (4.07±.67), (p<.05), and both high salt groups were decreased cf. to the control 4.5d low NaCl (7.26±79), (p<.05). Renal renin content was equal in all gps. Renal ang-n mRNA decreased 1.5x in both the high NaCl and high NaHCO3 gps. cf. both low NaCl controls (p<.05). Thus, Na^+ alone is important in the decrease of renal ang-n mRNA levels, while both Na^+ & Cl^- ions regulate plasma renin levels. These findings may have important implications in understanding the regulation and function of the intrarenal renin-angiotensin system.

REGULATION OF RENAL ANGIOTENSINOGEN IN NORMOTENSIVE AND GENETIC HYPERTENSIVE RATS. Richard E. Pratt,* Wen Min Zuo,* Mariano Ubeda,* Allen J. Naftilan,* Victor J. Dzau,* Brigham and Women's Hosp., Harvard Med. School, Boston, MA. introduced by Julie R. Ingelfinger.

Angiotensinogen (ang-n) mRNA has been found in the kidney. We have shown that renal ang-n mRNA is regulated in WKY by sodium (Na). However, the response in SHR is unknown. To examine this, adult male WKY and SHR, were fed 0.02% or a 1.6% Na diet. On both diets renal ang-n mRNA levels in the SHR were significantly lower than in the WKY. Renal ang-n mRNA level increased in the WKY with Na restriction. In contrast, there was no Na regulation at ang-n mRNA levels in the SHR. To determine if this was a specific defect in Na responsiveness two other protocols were tested. WKY and SHR were fed a 5 day 4% or 0.03% potassium (K) diet. A second group were injected with dexamethasone (dex) and sacrificed in 8 hours. In both protocols, renal ang-n mRNA were higher in WKY than SHR. However, unlike Na restriction, K loading or dex resulted in increases in renal ang-n mRNA in both strains. These results indicate that, compared to WKY, SHR contains lower levels of Ao mRNA which fail to respond to Na restriction. This is a specific defect in Na responsiveness, since both rats respond similarly to K loading and dex. These results may have implications in the renal physiology of these animals.

STIMULATION OF Na^+/H^+ EXCHANGE BY ANGIOTENSIN II IN RENAL PROXIMAL TUBULE CELLS. Gaetano Saccomani, Kenneth D. Mitchell and L. Gabriel Navar. University of Alabama at Birmingham, Birmingham, AL 35294.

Angiotensin II (ANG II) stimulates $^{22}Na^+$ uptake by ouabain-treated rabbit proximal tubule cell suspensions in the presence of an outwardly directed proton gradient. This stimulatory effect was abolished by amiloride, suggesting that ANG II may affect Na^+ transport via an Na^+/H^+ antiport. In the present study, we examined the effect of ANG II on the rate of proton efflux from acid-loaded proximal tubule cells using the pH-sensitive fluorescent dye 2',7'bis (carboxyethyl)-5(6)-carboxyfluorescein (BCECF). Cells were acid-loaded in a Na^+-free buffer (pH 7.4) isosmotically balanced with TMA-Cl containing 20mM NH_4Cl and 4μM BCECF at 37°c for 30 min. After removal of external NH_4Cl, which results in a rapid acidification of the cells, the Na^+-dependent rate of intracellular alkalinization was determined in the absence and presence of ANG II. 10^{-10}M ANG II increased the rate of alkalinization by 56±4% over the control (n=5); addition of 1.0mM amiloride abolished the effect of ANG II. The ANG II antagonist, saralasin (10^{-8}M), alone enhanced the rate of proton efflux by the proximal tubule cells but abolished the stimulatory effect of ANG II. The present findings, together with the previous results on Na^+ uptake, indicate that ANG II stimulates the amiloride-sensitive Na^+/H^+ exchange system in isolated proximal tubule cells.

INTRARENAL PRODUCTION AND METABOLISM OF ANGIOTENSIN II. Garry Reams, Daniel Villarreal*, Halina Krol*, and John H. Bauer. Truman VA Hospital and Univ. of Missouri School of Medicine, Columbia, Missouri.

Our ability to separate (by HPLC) and to measure (by RIA) the angiotensin octapeptide (A_8) has allowed us to investigate the origin of urinary angiotensin. Studies were performed in anesthetized, uninephrectomized dogs. Tritiated angiotensin II (H_3A_{II}) was infused into the aorta above the renal artery to assess the recovery of the angiotensin octapeptide in renal tissue, renal venous plasma and urine.

Data, H^3A_8 by scintillation counting (cpm/ml) and A_8 by RIA (pg/ml), are presented below.

	Baseline	0.5*	2.0*	2.5*
cpm/ml (N=4)				
aorta H^3A_8	--	131	647	1017
renal vein H^3A_8	--	42	179	249
urine H^3A_8	--	0	0	0
tissue (cpm/g)	--	--	--	0
pg/ml (N=3)				
aorta A_8	13.5	13.6	17.1	19.2
renal vein A_8	5.7	7.2	8.2	8.6
urine A_8	0.8	2.0	1.5	1.4
tissue (pg/g)	--	--	--	33.2

*Rate of infusion of H^3A_{II} (ng/kg/min)

H_3A_{II} had little or no effect on MAP, GFR, RBF or urinary flow rate.

The fractional removal rate of systemic A_8 was 74 to 77%; essentially all systemically delivered A_8, filtered by the kidney, was catabolized (probably at the level of the proximal tubule). Urinary A_8 was not derived from the glomerular filtrate. Renal tissue A_8 was not derived from the systemic circulation. Urinary A_8 may be a marker for the intrarenal generation of A_8.

IMMUNOHISTOCHEMICAL LOCALIZATION OF RENIN IN TUBULES IN DIPHENYLTHIAZOLE (DPT) RENAL CYSTIC DISEASE (RCD). V.E. Torres, M.J. Moore,* K.P. Offord,* T. Inagami,* K.E. Holley. Mayo Clinic, Rochester, MN; Vanderbilt Univ., Nashville, TN.

In a study on the enhancement of DPT-RCD in high renin states (KI 33:1130, 1988), immunoreactive renin in tubular cells was occasionally noted. To characterize this tubular renin, 4 μ kidney sections from 10 rats on high (HS) or low (LS) sodium diets and 1% DPT for two months, stained for renin by the indirect immunoperoxidase technique were used for morphometric analysis. All cortical (CT) and medullary (MT) tubules with renin and at least 500 CT and 300 MT without renin randomly selected using a plot sampling technique were measured and means obtained for each animal. JGA and arterioles with renin were counted.

	External tubular diameter (μm)					
	Cortex			Medulla		
Renin in tubules	HS	LS	Both	HS	LS	Both
+	56±12†	61±17	58±14†	78±26	108±27	93±30†
-	41±2	59±13*	48±12	56±8	76±20	66±28

Mean±SD; *from HS, p=0.008; †from renin(-), p<0.05

Most tubules did not contain renin. The diameter of the tubules without renin was larger in LS rats than in HS. The diameter of the tubules with renin was larger than that of those without. JGA and arteriolar renin were markedly reduced in HS rats, but a similar number of tubules contained renin in the HS and LS groups. The preferential localization of renin in dilated tubules and the dissociation of tubular and vascular renin suggest that tubular renin in DPT-RCD may be related to cystogenesis. Alternatively, this localization may be due to increased uptake of filtered renin caused by tubular obstruction.

RENAL HEMODYNAMIC AND EXCRETORY EFFECTS OF THE RENIN INHIBITOR A-64662 IN SODIUM-DEPLETE ANESTHETIZED MONKEYS. K.M. Verburg, J.R.C. Kadam, M.A. Chekal, and H.D. Kleinert: (Intr. T.J. Opgenorth) Abbott Laboratories, Abbott Park, IL.

The purpose of this study was to investigate the renal hemodynamic and excretory responses elicited by intravenous administration of the renin inhibitor A-64662 in sodium-deplete anesthetized monkeys. The experiments were performed after uninephrectomy and consisted of nine 15-min renal clearance periods equally divided into control, experimental, and recovery observations. Each monkey received either vehicle (V; n=6) or A-64662 (A; n=6) as a 10 µg/kg bolus followed by a 1.0 µg/kg/min infusion.

		CONTROL	EXPERIMENTAL	RECOVERY
MAP	V	83±4	83±4	81±3
(mmHg)	A	82±4	*70±4	82±3
RBF	V	47±4	44±3	43±3
(ml/min)	A	47±5	*58±7	48±6
PRA	V	5.8±1.9	5.3±1.8	4.4±1.5
(ng/ml/h)	A	5.4±1.1	*0.7±0.3	2.6±0.7

$\bar{X}$±SE, avg of 3 periods; *p<0.05 vs control and V; MAP=mean arterial pressure, RBF=renal blood flow, PRA=plasma renin activity

Glomerular filtration rate was unaffected by A-64662 (6.7±0.6 vs 6.5±0.6 ml/min) or vehicle (6.6±1.0 vs 6.9±0.7 ml/min). Sodium excretion tended to fall (2.1±1.0 vs 0.8±0.5 µEq/min) during A-64662 infusion. The results indicate that renal vasodilation occurs during systemic administration of A-64662 in sodium-deplete anesthetized monkeys.

PREDISPOSITION TO NON-PROGRESSIVE DIABETIC NEPHROPATHY - ROLE OF ANGIOTENSIN II (AII). C. Whiteside, J. Thompson. Membrane Biology Group, Department of Medicine, University of Toronto, Toronto, Canada.

Microalbuminuria and renal hypertrophy in the presence of Captopril was examined in pancreatectomized mongrel dogs (N=4) receiving 10-20 U NPH and 10-20 U Regular insulin daily, with blood glucose 300 ± 75 mg/dl $\bar{X}$ ± SD. Increased microalbuminuria measured by radioimmunoassay did not develop up to 22 months: controls (C), 2.6 ± 1.0µg/min/kg; diabetics (D), 2.8 ± 1.6 µg/min/kg, $\bar{X}$ ± SD. Serial fractional anionic dextran renal clearances remained unchanged over 18-22 months. Another series (N=5) following unilateral nephrectomy (NX) developed mesangial expansion visible on light microscopy at 12 months, twice the rate of non-nephrectomized dogs. Renal hypertrophy was unchanged with Captopril (5mg/kg) over 8-12 months Δ renal Wt: D + NX, 1.22 ± .98 g kidney/kg body wt; D+NX + Captopril, 1.35 ± .29 g/kg. ^{125}I-AII binding to isolated glomeruli (N=3) revealed a K_d:C, 3.43± 2.7 nM; D, 1.51±.18 nM; D+NX, 9.93±5.47 nM; D+NX+Captopril, 4.12±1.20 nM. This represents less affinity than observed in normal (K_d .68± .07 nM) or Streptozotocin (K_d .55 ± .07 nM) rat glomeruli. Receptor sites increased with NX: C, 839±95fmol/mg protein; D, 847 ± 306fmol/mg; D+NX, 1324±155 fmol/mg; D+NX+Captopril, 1520±47 fmol/mg. The pancreatectomized dog is a model of non-progressive diabetic nephropathy. Glomerular permeability, AII receptors and renal growth are not modulated by AII.

EFFECT OF ENALAPRIL ON RENAL HYPERTROPHY AND GLOMERULAR ANGIOTENSIN II (AII) RECEPTORS IN DIABETIC AND REMNANT RAT KIDNEYS. C. Whiteside, J. Thompson. Membrane Biology Group, Department of Medicine, University of Toronto, Toronto, Canada.

Enalapril (E) inhibits progressive glomerulosclerosis in diabetic and remnant rat kidneys. Effect of E on renal hypertrophy and glomerular AII receptors in diabetic and unilaterally nephrectomized rats was examined. Normal and Streptozotocin-treated male Sprague-Dawley rats (N=40) had unilateral nephrectomy. Half of controls (C), diabetics (D), nephrectomized controls and nephrectomized diabetics (DN) received E 50 mg/L in drinking water. Diabetic rats received daily subcutaneous 4-10 U NPH insulin. At 3 months, the diabetic rats had elevated albuminuria (all C, .09 ± .06 µg/min; all D, .57±.39 µg/min, $\bar{X}$± SD); inhibited with E (D+E, .26 ± .02 µg/min). Renal hypertrophy was additive in the combined model (C, 3.3 ± 0.3 g kidney/kg body wt; D, 5.3± 0.6 g/kg; DN, *8.4 ± 2.1g/kg) which was partially inhibited with E (DN+E, *6.0±1.0g/kg, *p<.05). ^{125}I-AII binding to isolated glomeruli revealed diabetics had increased receptor affinity K_d (all C, .70±.11 nM; all D, .54± .14 nM; all D+E, .51±.10 nM), but receptor number (all C, 737±197fmol/mg protein; all D, 746 ± 152fmol/mg; all D+E, 638 ± 125fmol/mg) remained unchanged. Renal hypertrophy in diabetes is exacerbated by nephrectomy and partially inhibited by E. Diabetes increases glomerular AII receptor affinity at 3 months, but receptor number fails to up-regulate in the presence of E.

GLOMERULAR ANGIOTENSINASE A IN THE RAT: INCREASE OF ENZYME ACTIVITY FOLLOWING FUROSEMIDE TREATMENT AND 1 1/3 NEPHRECTOMY. Gunter Wolf*, Friedrich Thaiss*, Jürgen E.Scherberich*, Wilhelm Schoeppe* and Rolf A.K.Stahl, Dept. Internal Medicine,Div. Nephrology, University Hospital, Frankfurt/M F.R.G.

Angiotensinase A (ATA), an enzyme located in rat glomeruli, degrades angiotensin II (A II). In order to study the activity of ATA in conditions with an activated renin-angiotensin system (RAS), male Wistar-rats (n=4) were treated with furosemide (20 mg/kg body weight, i.p.) every 8 hours for 4 days or were reduced in renal mass by 1 1/3 nephrectomy. Glomeruli were isolated by sieving techniques and ATA activity was evaluated in the supernatant of perfused glomeruli. ATA in furosemide treated rats was 5.5 ±2.6 mU/mg glomerular protein (mean±SD; p<0.05 vs control: 1.5 ± 1.1 mU/mg, n=6). In vitro superfusion of isolated glomeruli with furosemide (2 mg/ml buffer) had no effect on ATA activity. In addition, ATA activity was significant higher (p<0.05) in rats with 1 1/3 nephrectomy (n=8) five weeks after surgery (5.8 ± 1.9 mU/mg) when compared with controls. The increase in ATA following renal ablation was almost completely abolished by enalapril pretreatment (50 mg/l in the drinking water for 5 weeks). The data demonstrate that glomerular ATA activity is increased in conditions with elevated A II. Activation of this enzyme might be of significance in the regulation of glomerular function when the RAS is stimulated.

PHOSPHATIDIC ACID (PA) INHIBITS VASOPRESSIN (AVP)-INDUCED WATER TRANSPORT IN CORTICAL COLLECTING DUCTS (CCD) THROUGH PROTEIN KINASE C (PKC) ACTIVATION. Y Ando*, RC Harris, HR Jacobson, and MD Breyer. Div. Nephrol., Vanderbilt Univ., and V.A. Med. Ctr., Nashville, TN.

We have previously shown that pretreatment (preTx) of isolated perfused rabbit CCD with 25μg/ml PA (made from egg yolk lecithin by phospholipase D hydrolysis), inhibited 10μU/ml AVP-induced increase in hydraulic conductivity (Lp) (JCI 80:590). This suggests that PA is not merely an intermediate of phosphoinositide (PI) turnover but may play an important role in signal transduction. The present study was undertaken to characterize futher this PA action (See table). PreTx with 5μM indomethacin (IND), which attenuates the Ca^{++} ionophore-induced suppression of AVP action (JCI 81:1578), had no effect on PA suppression of AVP-induced peak Lp. PreTx with 100nM staurosporine (SSP), a PKC inhibitor, totally abolished the inhibitory action of PA. An arachidonic acid (AA)-free synthetic PA, 25 μg/ml dipalmitoyl PA (dpPA, C16), reproduced this SSP- reversible inhibition by PA. A shorter carbon chain PA, 25μg/ml dilauroyl PA (dlPA, C12), had no significant effect.

AVP-induced peak Lp ($x10^{-7}$ cm/atm/s) [mean±SE (no. of exp.)]

	cont	PA	dpPA	dlPA
cont	166.7±4.9(25)	91.7±8.9(11)	28.6±4.9(5)	161.5±11.4(4)
IND	166.4±20.5(4)	96.4±7.4(5)	not tested	not tested
SSP	172.1±11.6(4)	184.5±11.4(4)	178.2±14.0(4)	not tested

To test whether PA stimulates PI turnover in the collecting duct, as has been reported in other cells, inositol monophosphate (IP_1) accumulation in the presence of LiCl was examined in primary cultured rat papillary collecting duct cells. DpPA, but not dlPA, significantly stimulated IP_1 production (142.7±15.8 and 129.6±19.7% of control, respectively. n=10).

We conclude: 1)PA inhibition of AVP action is apparently mediated by PKC but not by AA or its cyclooxygenase metabolites. 2)The fatty acid composition is critical for this PA action. 3)The mechanism for PA activation of PKC remains undefined but may involve phospholipase C activation.

AGGREPHORES HAVE LITTLE, IF ANY ROLE IN WATER CHANNEL DELIVERY IN RAT COLLECTING DUCT. G.Ding* B.McGovern,* P.Singhal, N.Franki,* and R.M. Hays. Albert Einstein College of Medicine and Long Island Jewish Hospital, New York, NY.

In toad bladder, aggrephore fusion is the principal mode of vasopressin-stimulated water channel delivery to the luminal cell membrane. Thin section electron microscopy, the best means of distinguishing between true aggrephores and small vesicles, showed 6.0 ± 2.1 (SE) fused aggrephores/100 sq.microns in this tissue. However, the toad bladder delivery system is not a universal one; in frog bladder, only 0.1 fused aggrephores/100 sq. microns were seen. Here, channel delivery by vesicles, rather than aggrephores, appears to be the dominant mechanism, with aggrephores acting as channel storage reservoirs.

We now report the frequency of fused aggrephores by thin and serial sectioning in the collecting ducts of 2 Sprague-Dawley rats, 30 min after vasopressin infusion (mean urine osmol 1047). Results for cortical, outer and inner medullary collecting duct were:

	CCD	OMCD	IMCD
Area examined (sq. microns)	561	775	260
Fused aggreph./100 sq.microns	0.2	0.6	0.4

In the Brattleboro rat, freeze-fracture of the IMCD showed shallow pits containing particles as early as 10 minutes after vasopressin, consistent with vesicular delivery.

Thus, fused aggrephores are rare in the rat collecting duct, averaging 1 or less per cell, and, as in frog bladder, aggrephores play little or no role in water channel delivery.

IMPAIRMENT OF THE HYDROOSMOTIC ACTION OF VASOPRESSIN IN ISOLATED CORTICAL COLLECTING TUBULES OF RABBITS CHRONICALLY TREATED WITH LITHIUM. Elie Cogan*, Joëlle Nortier* and Maurice Abramow* (intr. by J.J.Grantham). Dept. of Physiol. and Pathophysiol. Free Univ. of Brussels (U.L.B.), Brussels, Belgium.

Chronic lithium administration is known to induce an impairment of the renal water conservation processes. We have previously shown that, when present in the luminal fluid, lithium inhibits the hydroosmotic action of vasopressin (AVP) in isolated perfused rabbit cortical collecting tubules (CCT). The aims of the present experiments were to study the effects of chronic lithium administration to the rabbit both on the water metabolism in vivo and on the hydraulic conductivity (Lp) of CCT in vitro.

The hydroosmotic action of AVP (25 μU/ml) and of 8-Bromo-cAMP (10^{-4} M) was studied in CCT isolated from rabbits fed with lithium chloride during 3 weeks (Li) and in CCT from control rabbits (C). Lp ($nl.cm^2.s^{-1}.atm^{-1}$) was measured at 37°C in the presence of a transepithelial osmotic gradient (lumen:125 mosm/kg; bath: 290 mosm/kg) with ^{125}I-iodothalamate as a volume marker. AVP-stimulated Lp was inhibited by 65% in Li rabbits (Lp= 7.65±1.66; n=5 p<0.001) as compared to C rabbits (Lp=21.39± 1.51; n=5). In contrast, the hydroosmotic effect of 8-Bromo-cAMP was only slightly altered by previous Li treatment (Li:14.31±1.27; n=5; C: 19.03±1.47; n=7; p<0.05). Water drinking, diuresis, and urinary osmolality were not affected by lithium administration. The maximal urinary concentrating ability was not modified by lithium treatment (pre Li: 1485±87 mosm/kg; post Li: 1567±135 mosm/kg; n=7; NS). A mathematical model simulating water reabsorption along the CCT predicts that a reduction of Lp as observed in the Li tubules, is not enough to prevent a complete osmotic equilibration at the end of the CCT in vivo .

We conclude that: 1) In the rabbit, lithium administration induces an impairment of the hydroosmotic action of AVP in the CCT which is mainly due to an inhibition of the adenylate cyclase 2) The inhibition of AVP action can be demonstrated in vitro before any detectable impairment of the water conservation process.

VASOPRESSIN (AVP) DEPENDENT PHOSPHORYLATION OF A 38 Kd PROTEIN IN HUMAN PLATELETS. Y. Granot, V. Van Putten,* and R.W. Schrier. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

This communication provides evidence that AVP directly induces the specific tyrosine phosphorylation of a 38 Kd protein in human platelets. This phosphorylation was observed using a specific anti-phosphotyrosine antibody (PT-ab) that has previously been shown to immunoprecipitate the tyrosine phosphorylated insulin receptor. "Western" immunoblotting studies revealed four major tyrosine phosphorylated proteins with apparent M.W. of 58, 54, 50 and 38 Kd. Two of these proteins (58 and 50 Kd) comigrate with proteins identified by a specific monoclonal antibody raised against the protooncogene protein product $pp60^{c\text{-}src}$. The $pp60^{c\text{-}src}$ has been found in relatively high concentrations in human platelets and possesses tyrosine phosphorylated residues. The AVP dependent tyrosine phosphorylation of the 38 Kd protein occurs at hormone concentrations between 10^{-10} and 10^{-6} M. The time course of this protein phosphorylation is biphasic, mimicking the changes in Ca_i due to AVP stimulation of the V_1 receptor. Maximal AVP stimulation (up to 10-fold) is observed within 1 min of exposure to the hormone. Forskolin (10^{-5} M), an activator of the catalytic subunit of adenylate cyclase, as well as phorbol ester (10^{-6} M), an activator of protein kinase C, do not produce a similar 38 Kd protein tyrosine phosphorylation. These observations indicate for the first time that AVP signal transduction in human platelets may be associated with direct or indirect biochemical activity that regulates the specific tyrosine phosphorylation of a 38 Kd protein.

Cl-HCO_3 EXCHANGE IN SARCOLEMMAL VESICLES FROM VASCULAR SMOOTH MUSCLE (VSM). Rakha D. Halligan*, and Andrew M. Kahn. University of Texas Medical School, Houston, Texas.

Intracellular pH is an important modulator of VSM tone, but the mechanisms which control pH_{in} in this tissue are not well understood. We wished to demonstrate the presence of and characterize Cl-HCO_3 exchange in VSM. ^{36}Cl uptake was studied in a sarcolemmal-enriched vesicle preparation obtained via Mg aggregation and differential centrifugation of homogenized bovine superior mesenteric artery (SMA). In the presence of an outwardly directed HCO_3 gradient (HCO_{3in} = 25 mM, HCO_{3out} = 5 mM), 30 second Cl uptake was stimulated over 4 fold relative to the absence of intravesicular HCO_3. 30 second Cl efflux was stimulated by an inwardly directed HCO_3 gradient. Cl uptake in the absence of intravesicular HCO_3 was insensitive to DIDS and furosemide and was stimulated by an inside positive membrane potential. The HCO_3 gradient-stimulated component of Cl uptake was inhibited 55% and 45% by 1 mM DIDS and furosemide, respectively and was insensitive to a change in membrane potential. HCO_3 gradient-stimulated Cl uptake was not increased by an outwardly directed Na gradient. These data demonstrate the presence of an Na-independent, electroneutral, DIDS and furosemide sensitive Cl-HCO_3 exchanger in sarcolemmal vesicle from bovine SMA. This transport system, in conjunction with the sarcolemmal Na-H exchanger, may play an important role in acid-base homeostasis in VSM.

A NEW FUNCTION FOR THE MAIN RENAL ARTERY (M-RA): A MAJOR SOURCE OF RENAL ENDOTHELIUM DERIVED RELAXATION FACTOR (EDRF). L Ichikawa, M.L. Hughes*, R.C. Harris & V. Kon. Vanderbilt University Med. Ctr., Nashville, TN.

Since M-RA offers little resistance to renal blood flow, it is largely regarded as a conduit connecting the systemic circulation and renal microvasculature. However, it has frequently been observed that after manipulation of M-RA, the kidney loses its responsiveness to vasodilators. One hour after arterial endothelial denudation by rubbing the left (L) M-RA, we assessed renal plasma flow rate (RPF) and GFR in L and right (R) kidneys in 8 rats. RPF and GFR were measured before (CONT) and during i.v. infusion of acetylcholine (ACH, 150 μg//kg/min), atrial natriuretic peptide (ANP, 70 ng/kg/min) or nitroprusside (NPR, 3-5 μg/min). Results (mean, § $p<0.05$ vs R) include:

	RPF				GFR			
	CONT (ml/min)	ACH (Δ%)	ANP (Δ%)	NPR (Δ%)	CONT (ml/min)	ACH (Δ%)	ANP (Δ%)	NPR (Δ%)
R	2.88	+22	+19	+37	0.86	+ 9	+13	- 22
L	2.44	- 10§	+35	+57§	0.75	- 23§	+29	+20

In R, RPF and/or GFR increased in response to both EDRF-dependent (ACH) and EDRF-independent vasodilators (ANP and NPR). By contrast, in L, RPF and/or GFR failed to increase following ACH and rose only with ANP and NPR. Micropuncture study of the renal microcirculatory dynamics during i.v. ACH infusion revealed that afferent and efferent arteriolar resistances were significantly higher, and ultrafiltration coefficient significantly lower in denudated than non-denudated kidneys, on average by 6%, 26% and 74%, respectively. In a separate study to further evaluate M-RA's contribution to renal vasodilation, ACH (85 μg/kg/min, n=6) was infused through a micropipette into either the proximal or distal portion of endothelium-intact M-RA (left diagram). Although proximal infusion caused significant increases in RPF and GFR, on average, by 8% and 10%, bypassing the M-RA endothelium by distal infusion failed to increase RPF (-24%) and GFR (-22%).

These data suggest that EDRF derived from M-RA has a major role in determining renal hemodynamics by acting on regulatory hemodynamic effectors within the downstream microcirculation.

ACTIVITIES OF CYCLIC-3',5'-NUCLEOTIDE PHOSPHODIESTERASES (PDE) IN CELLS OF INNER MEDULLARY COLLECTING DUCTS (IMCD). S. Homma, S. Takeda, P. Morgano, and T.P. Dousa, Nephrol. Res. Unit, Mayo Clinic, Rochester, MN.

cAMP and cGMP serve as 2nd messengers in actions of AVP and ANP on epithelium of IMCD. PDE plays a key role in determining cellular content of cAMP and cGMP in response to these peptides. We studied activities of cAMP-PDE and cGMP-PDE in cytosol and membrane fractions of IMCD cells from normal mouse. IMCD cells were prepared from slices of inner medullary tissue by the method of Granier, et.al. (Am J Phys: 241, F94, 1984). Homogenized IMCD cells were separated cytosolic and membrane fractions by ultracentrifugation at 100,000 x g. The membranes in pellet were extracted prior to assay by buffer with Triton-X-100, and PDE were assayed using 1 μM cAMP or cGMP as substrates. The specific activity of cAMP-PDE was similar in cytosol and membrane fractions, whereas cGMP-PDE was slightly higher in membranes. Both rolipram (RP) and cilostamide (CS) markedly inhibited cAMP-PDE. cAMP-PDE was inhibited to a higher ($p < 0.02$) degree by RP than by CS, namely in cytosol. In summary, in mouse IMCD cells cAMP-PDE and cGMP-PDE are distributed equally between cytosol and membrane fractions. The inhibitory responses of cAMP-PDE to RP and CS suggests the presence of a high activity of cAMP-PDE isozymes type-III in cells of IMCD.

Na-H EXCHANGE IS DOWN REGULATED IN CONFLUENT VASCULAR SMOOTH MUSCLE CELLS (VSMC'S) DUE TO LOW AFFINITY FOR H_{in}. Andrew M. Kahn, Julius C. Allen*, Steven S. Navran*, Charles L. Seidel*, Harnath Shelat*, and Ruth Zimmer*. University of Texas Medical School - Houston and Baylor College of Medicine, Houston, Texas.

The proliferation of primary cultures of VSMC's from canine femoral artery in the presence of serum is markedly inhibited as cultures approach confluence. Since mitogen-stimulated cell proliferation has been linked to stimulation of Na-H exchange activity, we wished to examine the relationship between the activity of Na-H exchange and serum treatment in confluent VSMC's. In the presence of 10% fetal calf serum (FCS), pH_{in} was 7.15 (measured with BCECF fluorescence). ^{22}Na uptake was measured as a function of pH_{in} which was preset by the K-nigericin method. In the presence of 10% FCS, lower pH_{in} resulted in higher amiloride-sensitive Na uptake. pH_{in} at half maximal Na uptake ($pK_{0.5}$) was 6.7. Amiloride-sensitive Na uptake was increased at each pH_{in} (7.5 - 5.5) by 18 hour serum deprivation, but $pK_{0.5}$ was unchanged. Addition of 10% FCS for 10 minutes to serum deprived cells further increased Na uptake, and shifted $pK_{0.5}$ to 7.1. We conclude that Na-H exchange is down regulated in serum treated confluent VSMC's due to low affinity for H_{in}. Na-H exchange is stimulated by serum deprivation by an unknown mechanism, and further stimulated by readdition of serum due to increased affinity for H_{in}. Low pH_{in} caused by Na-H exchange down regulation may contribute to inhibition of proliferation as VSMC's approach confluence.

ARGININE VASOPRESSIN (AVP) DESENSITIZATION (DS) IN VASCULAR SMOOTH MUSCLE CELLS(VSMC). R.W. Schrier, K. Okada*, P. Tsai* and C. Caramelo*. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

To further understand the mechanisms of VSMC DS to pressor hormones, we examined variations in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) and ^{45}Ca efflux in the presence of AVP and angiotensin II (AII). Pretreatment of the cells with 10^{-9}, 10^{-8} and 10^{-7}M AVP reduced the response to a second dose of AVP(10^{-7} M) by 41.1%,79.7% and 100% in less than 30 sec. At these doses, the DS was homologous, since pretreatment of the VSMC with AII(10^{-7}M) had no effect on the Ca^{2+} mobilization induced by AVP(10^{-7}M). However, AVP or AII doses of 10^{-6}M or greater induced cross DS involving competition for a common ionomycin-sensitive Ca^{2+} pool. Receptor occupancy was only partially responsible for the DS phenomenon, since washing off of the AVP with glycine buffer (pH 4.7, 2 min, + physiological saline, pH 7.4) restored 37.5% of the Ca^{2+} response only after 5 min. This procedure effectively removed 92.5% of the specifically-bound hormone and neither the first dose of AVP nor glycine washing interfered with the binding of the second dose of AVP, as assessed by ^{3}H-AVP. The AVP-DS was partially reversed (29.8% of response reappearance at 2 min) by protein kinase C (PKC) inhibition (24 h VSMC preincubation with 10^{-6}M PMA), suggesting a significant, although not exclusive, role of PKC in the VSMC-DS. Also, the absence of an effect of pertussis toxin (.1 to 1 ug/ml, 24 h) on AVP-DS suggests that a G protein of the G_i type is not involved in the AVP-DS phenomenon. AVP-DS in VSMC therefore involves PKC activation, receptor occupancy and Ca2+ availability from intracellular stores.

ROLE OF EXTRACELLULAR pH IN ARGININE VASOPRESSIN (AVP) BINDING AND ACTION IN RAT VASCULAR SMOOTH MUSCLE CELLS (VSMC) IN CULTURE. P. Tsai*, K. Okada*, C. Caramelo*, and R.W. Schrier. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

Extracellular acidosis and alkalosis induce vasodilation and vasoconstriction, respectively. This study was undertaken to examine the effect of extracellular pH (pH_e) on V_1 receptor (V_1R) binding, intracellular pH (pH_i), cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) and VSMC contraction. Incubation of VSMC at varying pH_e for 60 min at 4°C affected total binding to V_1R (pH, %bound: 6.6, 25; 7.0, 66; 7.4, 100; 7.8, 133; 8.2, 139; n=3). $^{45}Ca^{2+}$ uptake also changed with different pH_e (pH, ^{45}Ca uptake, counts/mg prot: 6.6, 9199; 7.0, 10507; 7.4, 13504; 7.8, 12261; 8.2, 10833; n=8). Basal and maximal dose AVP-induced $[Ca^{2+}]_i$ were both decreased at pH_e 7.0 as compared to 7.4 after 60 min (basal: 44 vs 64nM; 10^{-6} M AVP: 297 vs 431nM, both $p<.01$, n=6) but were not affected by pH_e 7.8. pH_i also changed at different pH_e as compared with pH_e 7.4 (7.0, -0.25 pH units; 7.8, +0.15 pH units; $p<.01$, n=7). The % VSMC contraction in response to maximal dose AVP (10^{-6}M) was less at pH_e 7.0 than at pH_e 7.4 (32.2 vs 15.3%, $p<.01$, n=5) and enhanced at pH_e 7.8 (32.2 vs 41.2%, $p<.05$, n=5). The effect of extracellular acidification to decrease the VSMC response to AVP is therefore associated with reduced V_1R binding, pH_i, basal $[Ca^{2+}]_i$ and mobilization of $[Ca^{2+}]_i$; in contrast, the enhanced AVP response of VSMC induced by extracellular alkalinization is associated with only increased V_1R binding and pH_i. Thus, factors in addition to intracellular Ca^{2+} mobilization may mediate the effect of acid-base states on AVP-mediated VSMC contraction.

VASOACTIVE PEPTIDES (VPs) PROMOTE GROWTH OF CULTURED MESANGIAL CELLS (MCs). R. Sterzel, M. Ganz, M. Perfetto* and R. Unwin*. Yale Univ. Med. Sch., New Haven, CT.

We examined if VPs with known effects on the renal microcirculation stimulate growth of vascular smooth muscle-like MCs. Early subcultures (#2-5) of rat MCs in microtiter plates, made quiescent in 0.5% FCS, were incubated with a VP for 1, 2 or 3 days and their replication rate assayed by measuring ^{3}H-thymidine uptake. Effects of arginine vasopressin (AVP), oxytocin (OT), bradykinin (BK), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) and substance P (SP) were tested at concentrations of 10^{-6} to 10^{-10} M. They were studied in the absence or presence of AVP (10^{-7} M), which is mitogenic for MCs through Ca^{2+}-dependent activation of protein kinase C (KI 33: 156, 1988). Results (means of 3-5 replicate assays), expressed as maximal % change of DNA synthesis over baseline (without or with AVP), during the 3-day study were (*P<0.05):

	AVP	OT	NPY	VIP	BK	SP
Control	680*	591*	14	360*	516*	8
AVP	-	-15	121*	15	21	151*

No mitogenic effects (without or with AVP) of angiotensin-II, cholecystokinin-8, neurotensin, beta-endorphin, enkephalin or somatostatin were seen. The data indicate that VPs, alone or in combination, and seemingly unrelated to their vascular effects, can induce growth of MCs. OT, VIP and BK directly stimulate and do not add to the AVP effect, while NPY and SP potentiate AVP-induced MC replication. These findings suggest that OT, VIP and BK activate the mitogenic mechanism(s) used by AVP, whereas NPY and SP synergize with AVP by a different action.

ORTHOSTATIC STIMULATION OF VASOPRESSIN(AVP)IN HYPONATREMIC CIRRHOSIS(HYPO-NA). R.Wehrle*, P.Gross*, A.Schömig*, W.Rascher*[1]. Univ.of Heidelberg, Dept.of Medicine, Heidelberg and Univ.of Essen, Dept.of Pediatrics [1], Essen, FRG. (Intr.by R.J.Anderson)

The stimuli of nonosmotic AVP in HYPO-NA have remained debated. To test a role of circulatory stimuli of AVP in HYPO-NA we measured AVP after 40 min of supine rest(C)and again after 20 min of subsequent standing upright(TILT;tilt table). Epinephrine(E)and N-epinephrine(NE)were also measured. Studies were done in HYPO-NA(P_{Na}=129+1 mM/l;n=15; stage CHILD B)and in age and sex matched normonatremic cirrhosis(NORMO-NA;n=10;CHILD B)and healthy volunteers(n=10). - We observed: In all groups of patients mean arterial blood pressure(RR)fell comparably at the begin of orthostasis and returned to baseline later. In healthy volunteers RR was 92+2 mmHg, AVP, E and NE were normal and failed to change in response to tilt. In NORMO-NA RR was comparable to that of healthy volunteers(C:91+3 mmHg) although orthostasis caused a rise of E(C:0.3+.05; TILT:0.5+.08; p<.05)but not of NE or AVP. In contrast in HYPO-NA RR was lower(83+2.3 mmHg; p<.05) than in NORMO-NA; AVP(C:4.1+.9 pg/ml; TILT:9.5+3.3; p<.05)and NE(C:2.5+.5 nM/l; TILT:5.3+1.1; p<.05)were both increased during orthostasis; E was not. - The data shows a specific increase of AVP reaching near-pressor concentrations during tilt table orthostasis in hyponatremic cirrhosis. The simultaneously observed increase of N-epinephrine suggests an involvement of arterial baroreceptors in this response. In conclusion nonosmotic AVP in hyponatremic cirrhosis is under control by circulatory stimuli, at least during orthostasis.

FUNCTIONAL HETEROGENEITY AMONG ANATOMICALLY DISTINCT FIBROBLASTS: IMPLICATIONS FOR IMMUNE REGULATION OF FUNCTION. R.J. Alvarez*, T. Haverty and E.G. Neilson, Renal Sect., U. of Pa., Phil., PA.

Fibroblasts might be heterogeneous in their function and response to modulation depending on their anatomic location. We wanted to examine the hypothesis that mouse tubulointerstitial fibroblasts (SJL/K) might be functionally distinct from syngeneic dermal fibroblasts (SJL/S). We, therefore, contrasted the growth and biosynthesis of matrix proteins in response to various immune relevant molecules.

RIA Procollagen Secretion (% Δ from control)

Factor	Type I	III	IV	V
SJL/K				
IFN (10^3 μ/ml)	80	50	-100	90
EGF (10ng/ml)	100	NC	100	NC
TGF_β (1ng/ml)	50	50	NC	100
IL-1 (100μ/ml)	100	100	150	100
SJL/S				
IFN (10^3 μ/ml)	60	700	20	500
EGF (10ng/ml)	-70	-70	-80	-90
TGF_β (1ng/μl)	-99	-90	-90	-90
IL-1 (100 μ/ml)	40	20	35	—

^{3}HTdR Incorporation (Stimulation Indices)

	TGF_β	EGF	IL-1	IFNγ
SJL/K	3.4	2.3	1.3	0.6
SJL/S	15.0	14.4	1.2	0

Dot blot hybridization to mRNA from γIFN-treated SJL/K with α_1(I) cDNA shows that relative mRNA levels increase 5-fold in parallel with that observed at the protein level. Our results indicate that immune modulation of fibroblast function is heterogeneous, bidirectional, and in some cases, regulated by transcriptional events.

ROLE OF DOPAMINE (DA) IN RENAL RESPONSE TO AMINO ACID INFUSION. Richard Amerling,* Alan G. Wasserstein. Renal Section, U of Pa., Phila., Pa.

The role of dopamine in the renal response to protein feeding is controversial. We studied 4 healthy male volunteers during water diuresis under 4 experimental conditions in random order at one week intervals: (1) IV amino acid infusion, 8 mg/kg BW/min x45 min (AA); (2) AA plus the DOPA-decarboxylase inhibitor, carbidopa (AA+CD); (3) CD only; (4) time control (D5W, 250 ml x45 min). Urine DA and plasma DOPA were determined by HPLC. Statistical comparison was by ANOVA for repeated measures. AA increased urine DA; the response was abolished by CD (AAxCD interaction, $p<0.05$). CD increased plasma DOPA ($p<0.02$), and AA may enhance this response. AA increased clearance of PAH (CPAH) and creatinine (CCr); these responses were blunted by CD (AAxCD interaction, $p<0.005$ for CPAH and $p<0.05$ for CCr). Iothalamate clearance was not altered by AA or CD. AA increased fractional excretion (FE) of Ca ($p<0.01$), PO_4 ($p<0.05$), Na ($p<0.01$), and K ($p<0.001$); CD may blunt natriuresis and kaliuresis (AAxCD interaction, $p=0.14$ and $p=0.12$, respectively). AA accentuated an inital phosphaturia and subsequent antiphosphaturia seen in time control ($p<0.05$). AA increased plasma glucagon in DA-independent fashion ($p<0.002$). Peak urine DA, CPAH, FENa and FECa occurred 1 hr after AA, peak FEK 2 hrs after AA. In conclusion: (1) AA increases urine DA, probably by increasing DOPA delivery to the kidneys; (2) AA-induced increases in CPAH and CCr are dopamine-dependent; (3) AA-induced calciuria and phosphaturia are not dopamine-dependent, but a role of DA in AA-induced Na and K excretion is not excluded.

INSULIN-LIKE GROWTH FACTOR I SYNTHESIS BY HUMAN GLOMERULAR MESANGIAL CELLS. David C. Aron*, Wahoub M. Hout*, and Hanna E. Abboud. VA Medical Center, Case Western Reserve University, Department of Medicine, Cleveland, Ohio.

Insulin-like growth factor I (IGF I) has been found in the kidney, but its precise cellular localization is not clear. Since IGF I is an autocrine factor in many tissues and since mesangial cells (MC) have been shown to possess IGF I receptors, we have examined if MC produce IGF I. Concentrated MC conditioned medium was applied to a Sephadex G100 column equilibrated in 6 M guanidine-HCl. Two major species were identified by IGF I RIA - apparent MWs of 7.5k and 25k daltons. To determine whether the high MW species possessed IGF I binding activity, appropriate fractions were desalted, incubated with ^{125}I-IGF I (thr 59) for 2 h at 30°C and applied to a Sephadex G100 column equilibrated in a non-dissociating phosphate buffer. The major peak of radioactivity was confined to a high molecular weight region; there was no radioactivity in the fractions corresponding to 7.5k daltons. Further characterization of 7.5k-dalton IGF I immunoreactive species by reverse phase HPLC showed that it co-eluted with synthetic human IGF I. Isoelectric focusing revealed it to have a pI between 8.1 and 8.5, corresponding to the known pI of human IGF I of 8.25. Northern analyses of poly(A)$^+$RNA from MCs and human liver using a cDNA probe for human IGF I showed that a 2.0 kb transcript predominates in MC unlike the 1.1 kb species found in liver. We conclude that (1) MC secrete IGF I and IGF I binding activity; and (2) IGF I gene expression in MC differs from that in liver. Since MC possess IGF I receptors, these data suggest that IGF I may be an autocrine factor that regulates MC functions.

MESANGIAL CELL (MC), GLOMERULAR, AND RENAL VASCULAR RESPONSES TO ENDOTHELIN (EN) IN THE RAT. KF Badr, MD Breyer, K Takahashi*, JJ Murray*, M Schwartzberg*, J Ebert*, T Inagami* and RC Harris. Vanderbilt Univ. Nashville, TN.

In view of its potential physiologic/pathophysiologic significance, we investigated the actions of EN in cultured MCs and anesthetized rats. Addition of 1 nM EN to MCs plated on a silicone rubber substrate (n=5) increased the intensity and number of tension-generated wrinkles, and caused their reappearance in forskolin pre-relaxed cells. Ten sec following exposure of fura-2 loaded MCs to 10 nM EN (n=8), single cell intracellular calcium concentration ([Ca]i), measured as fluorescence emission from rapid alternating excitation at 340/380 nm, increased from a mean baseline value of 66±11 (SE) to a peak of 684±250 nM ($p<0.05$) followed by a sustained elevation at 145±42 nM. In separate experiments (n=3) anion exchange HPLC revealed the appearance of polyphosphoinositide peaks (IP1, IP2, IP3) in the first minute following exposure of ^{3}H-myoinositol pre-loaded MC to 10 nM EN. No such peaks were observed in vehicle-stimulated controls.

Sustained I.V. infusion of EN (10 nM) in 6 Munich-Wistar rats decreased RBF (flow probe) from 8.4±0.6 to 4.7±1.0 ml/min* at 20 min, with no change in arterial pressure (AP). At 20 min, AP began to increase gradually from 124±3 to 133±4 mmHg* over 60 min. Glomerular micropuncture during the non-hypertensive period revealed increases in afferent and efferent arteriolar resistances [from 2.3±0.2 to 3.8±0.5* and from 1.1±0.1 to 2.0±0.3* resistance units] leading to a fall in nephron plasma flow rate (137±11 to 90±14 nl/min*). No significant change was noted in transcapillary hydraulic pressure difference (33±2 to 37±2 mmHg), but the glomerular ultrafiltration coefficient (Kf) fell from 0.097±0.035 to 0.031±0.011 nl/(s.mmHg)* as did and SNGFR (41±3 to 19±3 nl/min*). * $p<0.05$ vs control.

Thus, EN exerts potent constrictor effects on afferent and efferent arterioles, the onset of which precedes its sytemic hypertensive action. It contracts MCs in vitro and lowers Kf through stimulation of MC IP3 generation and elevation of [Ca]i. These findings suggest the presence of EN-specific receptors on rat MC and propose a role for this novel vasoactive peptide in the control of MC function, glomerular filtration, and renal vascular tone.

CARDIOVASCULAR AND RENAL EFFECTS OF ENDOTHELIN (EN) IN THE DOG AND IN THE RAT. Robert O. Banks. Dept. Physiology & Biophysics, Univ. of Cincinnati, Cincinnati, OH.

We have evaluated the effect on renal function of a recently isolated, vasoconstrictor peptide, EN, synthesized by endothelial cells in culture (Yanagisawa et al. Nature 332:411, 1988). EN (Peninsula Labs) was infused into the left renal artery of 6 pentobarbital (PB) anesthetized dogs at 1 $\eta g\ min^{-1}\ kg^{-1}$ for 20 min. Renal blood flow (RBF, flow probe) slowly decreased ($p<.05$) from 3.79±.25 (SE) to 3.22±.14 ml $min^{-1}gm^{-1}$ at 20 min and remained lowered for at least 30 min after EN. The glomerular filtration rate (GFR) increased ($p<.05$) from 39±2 to 47±4 ml/min during the first 10 min of EN infusion but then returned to baseline; Na excretion tended to increase during EN infusion but decreased significantly after EN. There were no systemic effects of EN at this dose. In the rat, EN was infused (iv) into PB anesthetized females for 30 min at 0.12 $\mu g\ min^{-1}kg^{-1}$ (longer infusions or higher doses were fatal) without (n=5) or with (n=5) the angiotensin (ANG) II antagonist, Sar^1-Thr^8 ANG II, infused during the entire experiment at 0.1 $\mu g\ min^{-1}kg^{-1}$. EN alone caused mean arterial blood pressure (MAP) to slowly increase from 107±3 to 136±4 mmHg ($p<.05$) at 30 min but then decreased to 83±6 mmHg 60 min after EN infusion. The GFR decreased from 2.7±.2 to 0.7±.3 ml/min at 30 min ($p<.05$) and then increased to only 1.3±.2 ml/min 1 hr following EN. The competitive ANG II antagonist completely blocked the EN-induced changes in blood pressure and renal function (MAP was 104±3 mmHg and GFR was 2.4±.4 ml/min before and during EN plus the ANG II antagonist infusion). These data indicate that EN activates ANG II receptors via a slowly developing, long-lasting process and that the kidney is a major site of action of the vasoconstrictor.

THE EFFECTS OF ADENOSINE A1 AGONIST ON TRANSEPITHELIAL POTENTIAL DIFFERENCE AND CYTOSOLIC CALCIUM CONCENTRATION IN THE CORTICAL THICK ASCENDING LIMB. P. Darwin Bell, Martha Franco, and Michael Higdon. Univ. of Alabama at Birmingham, Birmingham, Al 35294.

Adenosine has been shown to influence transport in several epithelia. The purpose of this study was to determine if activation of adenosine A1 receptors influence transepithelial transport in the cortical thick ascending limb (cTAL) and whether this effect may occur through a change in cytosolic calcium concentration ($[Ca^{2+}]$). Isolated cTAL's were incubated in 5uM Fura 2AM for 1 hr at 21°C. The tubule was cannulated and warmed to 37°C. Transepithelial potential difference (V_T) was measured using standard techniques. The A1 agonist, N^0-cyclopentyladenosine (CPA), was used to activate adenosine A1 receptors. Control V_T was +3.2±0.47mV (n=9) in isotonic symmetrical solutions. Addition of CPA inhibited V_T by 25±3% (720nM CPA) and by 40±6% (1080nM CPA). Fura 2 fluorescence was measured at excitation wavelengths of 340nm and 380nm and an emission wavelength of 510nm. Control $[Ca^{2+}]$ was 89±11nM (n=14). CPA increased cTAL $[Ca^{2+}]$ by 50±15% (360nM CPA), 126±17% (720nM CPA), and 198±38% (1080nM CPA). This increase in cTAL $[Ca^{2+}]$ was completely inhibited by the concomitant addition of 8-cyclopentyl-1,3-dipropylxanthine a specific A1 receptor antagonist. Interestingly, we found that CPA only increased cTAL $[Ca^{2+}]$ when added to the luminal perfusate; no effect was seen when CPA was added to the bathing solution. Accordingly, these studies indicate that adenosine A1 receptors may exist on the luminal membranes of the cTAL and suggest that inhibition of transport by activation of these receptors may occur through an increase in $[Ca^{2+}]$.

PRODUCTION OF TUMOR NECROSIS FACTOR (TNF) BY RAT MESANGIAL CELLS (MC) IN RESPONSE TO BACTERIAL LIPOPOLYSACCHARIDE (LPS). L. Baud, J-P. Oudinet, M.N. Peraldi, E. Rondeau, and R. Ardaillou. (Intr. by K.F. Badr). INSERM 64, Hôpital Tenon, Paris, France.

In immune-mediated glomerulonephritis, infiltration of glomeruli by macrophages is associated with TNF production. The present study was designed to determine whether MC could contribute to glomerular TNF synthesis. TNF activity determined using L-929 lytic assay was detectable in the culture medium of MC only when exposed to LPS (0.1-10 µg/ml) for periods longer than 1h. It reached 415± 87 U/ml 14h after addition of 1 µg/ml LPS. This cytotoxic factor was identified as TNF since : 1) its activity was suppressed by an anti-mouse TNF polyclonal antibody. 2) its molecular weight (100-140 Kd) corresponded to that of murine TNF under non denaturing conditions ; 3) mRNA encoding TNF was expressed by MC 2h after addition of LPS as determined by Northern blot analysis. TNF synthesis by MC was regulated by their production of prostaglandin E_2 (PGE_2) and cyclic AMP (cAMP). Indeed, indomethacin (1 µM) both suppressed PGE_2 production and increased TNF synthesis from 286 ± 66 to 696 ± 177 U/ml. Conversely, exogenous PGE_2 (0.1-300 nM) reduced TNF synthesis. 8 bromo-cAMP (0.1-100 µM) and histamine (10 µM-1mM) which enhance the cAMP content of MC promoted similar dose-dependent inhibitions. It is concluded that TNF is released by MC upon stimulation by LPS and that its synthesis is regulated by both PGE_2 and cAMP. This production could participate in the mechanism of endotoxin-induced glomerular injury.

CHARACTERIZATION OF A MEMBRANE BOUND FORM OF PREPROEGF FROM KIDNEY. Julia A. Breyer, Matthew D. Breyer, Stanley Cohen, Harry R. Jacobson, Vanderbilt Univ., Div. of Nephrology, Nashville, Tennessee. High levels of EGF are found in urine and high levels of the MRNA for the EGF precursor preproEGF are found in the kidney. PreproEGF has yet to be purified from any tissue and nothing is known about its potential processing. We demonstrated by Western blotting that antibodies to murine (m) EGF recognize a high molecular weight protein in both the soluble and membrane fractions of homogenates of mouse kidneys. The membrane protein can be released by incubation of membranes at 37° or by solubilization with 1% triton. The triton solubilized protein is glycosolated as evidenced by its binding to wheat germ lectin (WGL), con A, and lentil lectin columns. Elution from WGL yields significant purification. The WGL purified protein is a substrate for the EGF binding protein arginine esterase generating a smaller molecular weight fragment. The WGL protein is also a substrate for pepsin which generates a fragment that reacts with the anti-EGF antibody and runs at the same molecular weight as EGF on a gel. WGL eluate contains a protein that competes for binding with EGF to EGF receptors. This binding is enhanced by pretreatment with arginine esterase and inhibited by preincubating with anti(m)EGF antibody. Thus, membranes from mouse kidney contain a high molecular weight immunoreactive protein, presumably prepro EGF which can be processed by the EGF binding protein.

REGULATION OF HORMONAL RESPONSES IN MEDULLARY THICK ASCENDING LOOP (MTAL) BY ADENOSINE. Maria A. Burnatowska-Hledin* and William S. Spielman. Michigan State University, East Lansing, Michigan.

The present study was undertaken to determine the involvement of the inhibitory (A_1) adenosine receptor coupled to adenylate cyclase in the regulation of cAMP production induced by hormones known to affect transport in mTAL. Rabbit mTAL cells were isolated by solid phase immunoadsorption, grown until confluent, and the effects of the inhibitory analog of adenosine, N^6-cyclohexyladenosine (CHA, 50 nM), on vasopressin (VP), isoproterenol (IP), calcitonin (CT), and prostaglandin E_2 (PGE_2) (1 uM) induced changes in cAMP production were examined. CHA inhibited VP, IP, CT and PGE_2 induced increase in cAMP by ~50%. This inhibitory effect of CHA could be abolished by pretreating cells with pertussis toxin (PT) for 12-20 hr. A specific A_1 inhibitor, 8-cyclopentyl-1,3-dipropylxanthine, (CPX) (1 uM), also abolished the inhibitory effect of CHA. CHA induced increase in cytosolic free calcium ($[Ca^{2+}]_f$) and inositol tris-phosphate production was also abolished with PT and CPX. To test whether the rise in $[Ca^{2+}]_f$ was involved in CHA induced inhibition of hormone stimulated cAMP production, cAMP was measured in cells loaded with 65 uM Quin2-AM for 20 min. Buffering of $[Ca^{2+}]_f$ had no effect on the inhibitory action of CHA, suggesting that the rise in $[Ca^{2+}]_f$ was not needed for CHA induced inhibition of hormone stimulated cAMP production. These results indicate that adenosine regulates hormone induced changes in cAMP production via an inhibitory A_1 receptor. The role of the $[Ca^{2+}]_f$-linked receptor remains to be determined.

ABNORMAL GROWTH FACTOR RESPONSE BY HUMAN ADULT POLYCYSTIC KIDNEY DISEASE (APKD) EPITHELIA IN VITRO. K. Das, P.A. Gabow, P.D. Wilson, Dept. Physiol UMDNJ-RWJ Med. Sch., Piscataway, NJ; Denver General Hospital, Co.

Our previous studies have shown that individually microdissected normal rabbit and human renal tubules grown in serum free media exhibit maximal mitogenic response to: dexamethasone (D) alone in collecting tubules (CT); D+insulin (I) in proximal convoluted (PCT) and straight tubules (PST); and to D+I+T_3 in thick ascending limb (TAL) cultures. APKD epithelia, which show accelerated growth kinetics also showed an abnormally enhanced response to D+I and D+I+T_3. The mitogenic response to peptide growth factors was determined by rendering confluent cultures quiescent by removal of growth factors from the media for 48 hrs and subsequent reincubation in the presence of growth factor(s), for 48 hrs and [H^3]-thymidine (1μCi/ml) for 16 hrs. Epidermal growth factor (EGF); transforming growth factor-β (TGFβ); insulin like growth factor I(IGF-I) and arginine vasopressin (AVP) were tested. %[^{3}H]-thymidine labelled nuclei was:

	D+I	D+IGF-I	EGF	EGF+D+I	TGFβ+D	AVP
APKD	52	12	40	68	15	8
PST	35	14	0	25	0	10

Thus EGF is an abnormally potent mitogen for APKD epithelia and TGFβ is less growth inhibitory than in normal epithelia. Abnormal responses to EGF and TGFβ may contribute to the accelerated proliferation of APKD epithelia during cyst formation.

SERINE PROTEINASE INHIBITORS BLOCK THE SYNTHESIS OF PLATELET-ACTIVATING FACTOR (PAF) BY RAT CULTURED MESANGIAL CELLS (MC) AND PERITONEAL MACROPHAGES (PM). Camussi G, Tetta C, Bussolino F, Montrucchio G, Baglioni C.Cattedra di Nefrologia Sperimentale,Univ.Napoli,Lab.Immunop.Univ.Torino,Dept Biol.Sci.,SUNY at Albany.

MC and PM produce PAF when stimulated with C3b opsonized baker's yeast particles(BY)(MC:2.5+0.9; PM:11.9+3.8 ng/ml),tumor necrosis factor(TNF 10ng/ml)(MC:2.1+0.9;PM:12.5+3.2) or Calcium Ionophore A23187 (1 ug/ml)(MC:2.4+0.6;PM:20.6+4.2)in serum-free medium. It is not known whether serum proteinase inhibitors interfere with PAF production as described for serine proteinase inhibitor TPCK (J.Exp.Med.166:1390,1987).By gel filtration chromatography, plasma fractions containing α1-proteinase inhibitor (PI) and α1-antichymotrypsin (AC) inhibited PAF synthesis. PI and AC (10 ug/ml) inhibited BY- and TNF- induced PAF synthesis in MC (PI+BY:0.9+0.3; AC+BY:1.1+0.2; PI+TNF:0.2+0.1;AC+TNF:0.9+0.2 ng/ml) and in PM (PI+BY:4.8+1.2; AC+BY:6.7+3.4;PI+TNF:0.8+0.4;AC+TNF:2.8+0.9 ng/ml) In contrast, PAF production induced by Calcium Ionophore A23187 was unaffected by both PI and AC. The finding that serum antiproteinases are inhibitory at concentrations 100-fold lower than those present in plasma rises questions as to the ability of phagocytosis and TNF to stimulate PAF production in vivo by MC and PM, when exposed to the vast excess of antiproteinases present in serum. Synthesis of PAF may require inactivation of these proteinase inhibitors or it may be limited to zones of close contact between cells where antiproteinases are excluded

EFFECT OF DIETARY PROTEIN ON RENAL INSULIN-LIKE GROWTH FACTOR I (IGF-I) FOLLOWING UNI-NEPHRECTOMY IN RATS. A M El Nahas, J E Le Carpentier, D J Hill. Clinical Sciences Research Centre, Northern General Hospital, Sheffield, UK.

We undertook this study to explore the effect of dietary protein manipulations on the IGF-I content of remaining kidneys following UNx in rats. Groups of adult male Wistar rats were fed either a low (8% casein) protein diet (LPD), MPD (18%) or HPD (78%). Thirty days later, they underwent a right UNx (12 rats) or a sham operation (8). Four days later the left kidney was removed. At the time of UNx and sacrifice, the kidneys were weighed and their protein, DNA and IGF-I content measured by RIA after acid extraction. At the time of sacrifice, kidney weight (KW) varied proportionally to the dietary protein intake in both sham and UNx rats. Following UNx the percentage increase in KW was LPD: 11%, MPD: 18%, HPD: 37%. The difference between the 3 groups was even more significant ($p<0.001$) when CRG was calculated by comparing kidney/body weight of UNx and sham animals. In UNx rats, renal IGF-I varied proportionally to dietary protein intake (DPI) and KW; $r = 0.95$, $p<0.01$; in the remaining kidney, IGF-I was LPD: 299+77ng/gm KW (M+SD), MPD: 507+102ng/gm KW and HPD: 526+60ng/gm KW, LPD vs MPD and vs HPD Mann-Whitney, $p<0.01$. This observation suggests IGF-I might, through an autocrine or a paracrine mechanism, be involved in the pathogenesis of CRG. It also shows that a LPD blunts CRG and prevents the rise in IGF-I following UNx. This might suggest an effect of dietary protein on intra-renal growth factors.

SHORTTERM REGULATION OF NaKATPase IN RAT MEDULLARY THICK ASCENDING LIMB (MTAL). Effect of AVP. J Fryckstedt*. Dept of Pediatrics, Karolinska Institute, Stockholm, Sweden.

NaKATPase is the major energy source for salt transport in MTAL. To study whether there is a short-term regulation of NaKATPase in MTAL, MTAL segments were dissected from rat kidneys, permeabilized and preincubated with nucleotides or hormone. NaK-ATPase was determined as ouabain sensitive ^{32}P-ATP hydrolysis at Vmax for K and ATP, while Na in medium (Na_m) varied. Intracellular Na (Na_i) was approximately the same as Na_m. In control MTAL NaKATPase (pmol Pi/mm/h) was 2438±367 at Na 20mM and reached Vmax (3640±339) at Na_m 50. (Values are mean ±SD. n=3-6 rats, in each rat 12-16 tubules were analyzed.) An inhibitor of GTP dependent activation of G-protein, GDP S (400/uM) significantly decreased NaKATPase activity to 2375±290 at Na_m70 but had no effect on NaKATPase activity at Na_m20. A nonhydrolyzable GTP analogue GppNHp (100 /uM) significantly increased NaKATPase activity to 3382±69 at Na_m20, but had no effect at Na_m70. GDPβS and GppNHp had no effect on NaKATPase purified from renal medulla. AVP will in MTAL stimulate transcellular Na transport (AJP 241: F432, -81) and at the same time increase cell volume (KI 33:427, -88). At Na_m20, AVP decreased NaKATPase activity in a dose dependent manner. Inhibition was first observed at 10^{-11}M and maximal at 10^{-9}M (1438±224 vs 2437±367 in controls). At Na_m70, AVP had no effect on NaKATPase activity. dBcAMP 10^{-3}M also inhibited at Na_m20. Conclusion: NaKATPase activity is modulated by more than one G-protein. The effect is Na dependent. At normal Na_i AVP inhibits MTAL NaKATPase which might explain AVP induced cell volume increase.

REDUCED ONTOGENIC EXPRESSION OF EPIDERMAL GROWTH FACTOR (EGF) IN A MURINE MODEL OF RENAL CYSTIC DISEASE. Vincent H. Gattone II, Niu Fu-wen*, Glen K. Andrews*, Robert M. Klein* and James P. Calvet*. Univ. of Kansas Med. Ctr., Depts. of Anatomy & Cell Biology and Biochem. & Molecular Biology, Kansas City, Kansas.

The kidney synthesizes EGF, however the function of renal EGF is unknown. Renal cystic disease is associated with hyperplasia of renal tubular epithelium. This hyperplasia may be regulated by endogenous growth factors. Ontogeny of EGF in kidneys of a genetic murine model of cystic disease (C57BL/6J-cpk) was examined using immunohistochemical and nucleic acid hybridization techniques. Immunohisto-chemically, renal EGF was initially detected in normal siblings at 1 week of age and increased through 3 weeks. While there was some evidence of EGF at the same time points in cystic kidneys, the amount of EGF immunoreactivity appeared reduced. Using a nucleic acid probe for preproEGF, the expression of mRNA was examined at similar time points. Expression of the EGF gene in phenotypically normal siblings increased from 1 through 3 weeks of age. However, there was no significant EGF expression in cystic kidneys.

In conclusion, EGF expression in a murine model of renal cystic disease is dramatically reduced. This suggests that abnormal EGF expression may contribute to the abnormal hyperplastic growth of tubular epithelia by altering normal cell growth regulatory processes.

α- AND ß-ADRENERGIC BINDING SITES IN HUMAN KIDNEY DETECTED BY AUTORADIOGRAPHY. E Fuchs*, P Neumann*, B Salzbrunn*, G Flügge*, H-J Gröne* (intr. by CJ Olbricht). German Primate Center and Dept. of Pathology, University of Göttingen, FRG.

In order to gain insight into the hemodynamic and tubular effects of catecholamines in the kidney it is necessary to know the distribution and properties of their binding sites. In contrast to the rat, human renal α- and ß-adrenergic binding sites (BS) are poorly characterized. We therefore investigated the distribution of ^{3}H-prazosin (α_1), ^{3}H-rauwolscine (α_2), and ^{125}I-cyanopindolol (ß) BS in tumor free parts of human kidneys. BS were visualized by autoradiography and quantified by densitometry.

Like in other species, the α_1 ligand bound to cortical tubules (K_D 1.02 nM; B_{Max} 378 fmol/mg tissue equivalent) revealing no labeling of glomeruli. In contrast to rat and dog, α_2-BS were only detected on collecting ducts of the human renal medulla (K_D 1.78 nM; B_{Max} 140 fmol/mg tissue equivalent). Furthermore, whereas in rat, dog, and cow ß-adrenergic BS have been found before on glomeruli, in the human kidney, ^{125}I-cyanopindolol only binds to the interlobular arteries and to collecting ducts.

These results indicate that the renal sites of α- and ß-adrenergic substances in man differ from those of non-human mammals.

HUMAN CALCITONIN GENE RELATED PEPTIDE (CGRP) AND CALCITONIN (CT) EXERT DIFFERENT HEMODYNAMIC AND RENAL EFFECTS. Markus P. Gnädinger*, Dominik E. Uehlinger*, Peter Weidmann, Sidney G. Shaw*, Roman Muff*, Wolfgang Rascher*, and Jan A. Fischer*. Med. Poliklinik, Univ. of Berne, and Klinik Balgrist, Univ. of Zurich, Switzerland.

CGRP has recently been discovered and found to possess vasodilating properties. We investigated whether CGRP may also modify renal function. Ten healthy young men were evaluated under steady-state clearance conditions. Following 1 hr of equilibration and 2 20 min-baseline clearance periods, human CGRP was infused iv. at rates of .3 and 1.0 µg/kg/hr for 1 hr each. Six men received on separate days also infusions of isomolar CT and of carrier only (.9% NaCl, .5 ml/min). Fluid balance was kept stable. CGRP lowered diastolic blood pressure (BP) by 25%, increased heart rate, plasma catecholamines and renin activity, and doubled the fractional excretions (FE) of sodium (Na^+) and chloride (Cl^-) (P<.01-.001) in the presence of an unchanged glomerular filtration rate and reduced effective renal plasma flow (P<.05); FE of calcium (Ca^{++}) and phosphate (P_i) were constant. In contrast, CT slightly increased systolic BP, did not affect diastolic BP, increased $FENa^+$ and $FECl^-$ 4-fold and $FECa^{++}$, FEP_i and diuresis 3-fold (P<.01-.001). Plasma aldosterone, arginine vasopressin and atrial natriuretic factor were unaffected by CGRP or CT. Carrier infusion did not modify measured variables. Conclusions: CGRP can in humans acutely lower BP and reduce tubular Na^+ reabsorption. CGRP differs from CT by marked hemodynamic, weaker natriuretic and lacking calciuric and phosphaturic properties.

INVOLVEMENT OF CYCLOOXYGENASE (CO) AND CYTOCHROME P450 ARACHIDONATE METABOLITES (P450/AA) IN RAT RENAL RESPONSES TO EPIDERMAL GROWTH FACTOR (EGF). RC Harris, K Takahashi*, J Capdevila* and KF Badr. Vanderbilt Univ., Nashville, TN.

EGF contracts cultured rat mesangial cells and its intrarenal infusion decreases RPF and GFR. We examined the role of AA metabolites in mediating these responses. Intrarenal EGF infusion increased urinary iPGF2α by 300%* and 30 nM EGF stimulated iPGF2α, but not iTxB2, production in isolated glomeruli by 58%*. The TxA2 receptor antagonist SQ29548 did not block EGF's vasoconstrictive effects. During selective CO inhibition (ibuprofen), intrarenal EGF infusion (n=5) led to a fall in mean BP from 117 to 98 mmHg* accompanied by increases in RPF: 3.8 to 5.6* and GFR: 0.90 to 1.10* ml/min. When total, or selective P450-mediated, AA metabolism were inhibited by the additional administration of 5,8,11,14-eicosatetraynoic acid (ETYA, n=5) or ketoconazole (n=2), the EGF-induced vasodilation observed during CO inhibition alone was abolished. In fact, in these rats, EGF evoked vasoconstrictor responses (RPF 4.3 vs 3.3* and GFR 1.05 vs 0.73 ml/min*) (not inhibited by saralasin).

To ascertain the source of EGF-stimulated P450/AA metabolites, suspensions of rat glomeruli or proximal tubules were exposed to 30 nM EGF and P450-catalyzed epoxidation measured by GC/MS. One min. post-exposure to EGF, proximal tubule, but not glomerular, epoxidation products were increased by 50-100%* vs control. [* : $p < 0.05$ vs control].

Thus, EGF stimulates the renal generation of PGF2α and P450/AA metabolites. PGF2α augments intrinsic EGF-induced vasoconstriction. CO inhibition unmasks a potent renal and systemic vasodilator action of EGF, due to its stimulation of the renal biosynthesis and systemic release of vasodepressor P450/AA metabolites, particularly epoxidation products.

L-AROMATIC AMINO ACID DECARBOXYLASE (LAADC) ACTIVITY ALONG THE RAT NEPHRON AND THE EFFECT OF Na INTAKE. M. Hayashi, Y. Yamaji, W. Kitajima, and T. Saruta, (intr. by A.I. Katz), Saitama Central Hospital, Saitama, and Univ. of Keio, Tokyo, Japan.

Extraneural dopamine (DA) is thought to be synthesized by LAADC in tubular cells and to modulate Na excretion during high Na intake, but previous histochemical studies of LAADC localization in the nephron were not consistent. To determine the location of LAADC, and whether changes in Na intake regulate this enzyme, LAADC activity was measured in microdissected nephron segments from rat kidneys. DA formed by isolated tubules incubated with 250 μM L-dopa was quantitated by HPLC or with the more sensitive radioenzymeassay (REA). LAADC activity was present only in proximal convoluted tubules (PCT, 208 ± 19 ng/cm/h) and proximal straight tubules (PST, 81 ± 9 ng/cm/h), whereas no significant activity was detected in other nephron segments (cortical and medullary thick ascending limbs, distal convoluted tubules, and cortical and medullary collecting ducts) by either HPLC or REA. The K_m for L-dopa was 113 μM, and DA production was completely inhibited by carbidopa, a specific inhibitor of LAADC. Enzyme activity in PCT from surgically denervated kidneys (282 ± 27 ng/cm/h) was not significantly different from that in contralateral kidneys (247 ± 30 ng/cm/h), indicating that LAADC activity of the isolated tubules was of extraneural origin. LAADC activity (217 ± 26 vs 189 ± 23 ng/cm/h) and apparent K_m (98 vs 99 μM) were similar in PCT of rats kept for 7-10 days on a low- or high salt diet. These results show that LAADC is present only in the PCT and PST of the rat nephron, and suggest that the increased urinary DA excretion during high salt intake is not modulated by changes in tubular L-aromatic amino acid decarboxylase activity.

CORTICOSTERONE METABOLISM IN TOAD BLADDER IN VITRO. A. Hartsell*, K. Hierholzer*, N.A. Kurtzman, and S. Sabatini, Depts of Internal Medicine and Physiology, Texas Tech Univ HSC, Lubbock, TX and Free Univ of Berlin, Germany.

Recent studies have demonstrated that the mammalian kidney metabolizes steroid hormones. Corticosterone (B) receptors have been found in cortical collecting tubule. At least 4 metabolites of B have been identified in rat renal tissue and urine. It is not known whether these metabolites have any biologic function. We studied the effects of the parent compound B and 3 of its metabolites on water flow and sodium transport in toad bladder. Using HPLC and GC-MS, we also analyzed the oxidoreductase pathways for B metabolism in toad bladder. AVP- and cyclic AMP-stimulated water flow was unaffected by B, 11-dehydrocorticosterone-B (IV), and 11-dehydro-20-dihydrocorticosterone-B (I). Water flow was significantly inhibited by 20-dihydrocorticosterone-B (II). Sodium transport was unaffected by B, but was stimulated following a 5-18 hr incubation with II and IV. Analysis of the oxidoreductase pathways in toad bladder revealed that the parent compound B was irreversibly oxidized to IV. Metabolite IV was then converted at a high rate to I and to lesser amounts of II. We conclude that the renal metabolites of corticosterone are biologically active and may serve an important modulating role on both sodium and water transport.

EVIDENCE THAT IGF-I INCREASES RPF AND GFR IN FASTED RATS. R. Hirschberg and J.D. Kopple, Division of Nephrology and Hypertension, Harbor-UCLA Medical Center, Los Angeles, CA.

Growth hormone (GH) injections increase RPF and GFR in man but only after many hrs. Thus, other agents may mediate GH effects on renal function. We tested whether IGF-I may cause the same renal effects as GH. Rats fasted for 60-72 hrs were anesthetized and then underwent serial PAH and inulin clearances of 20 min each in 2 studies. In Study 1, after equilibration and baseline measurements, rats were injected with rIGF-I, 25 ug/kg, followed by an infusion of 25 ug/kg over 20 min, and 10 more clearances were performed. In Study 2, rats received infusions of indomethacin (IND, 2.5 mg/kg/hr) or somatostatin (SRIF, 2.5 ug/hr) continuously throughout the study. rIGF-I injections and infusions were given as in Study 1; 4 clearance periods were performed after the rIGF-I or vehicle infusion. In both studies, controls received vehicle instead of rIGF-I. In Study 1, rIGF-I induced a rapid, sustained rise in RPF and GFR and fall in renal vascular resistance (RVR); the maximum changes were +29±3SEM%, +25±3% and -26±4%, respectively ($p<0.05$). Total plasma IGF-I rose from 0.5±0.1 before to 1.0±0.2 U/ml at the end of the rIGF-I infusion ($p = 0.069$). In Study 2, IND but not SRIF blocked the rIGF-I effect on renal hemodynamics. In both studies, FF and MABP did not change. We conclude that IGF-I increases RPF and GFR and lowers RVR in fasted rats. These effects of IGF-I may require eicosanoids but may not require peptide hormones suppressed by SRIF, IGF-I may mediate the actions of GH on renal function.

EFFECT OF DIETARY SODIUM ON DIABETIC GLOMERULOPATHY HarEr Huang, Praveen Chander, Renee Garrick, Alvin Goodman and Leonard G. Meggs. New York Medical College, Department of Medicine, Valhalla, New York

Several lines of evidence suggest a role for dietary NaCl in the progression of diabetic nephropathy; restriction of NaCl attenuates renal hyperfiltration and glomerular injury in streptozotocin (STZ) diabetic rats; NaCl modulates angiotensin II (AII) and atrial natriuretic peptide (ANP), two vasoactive hormones with important effects on the renal microcirculation and NaCl has been implicated in systemic hypertension, a factor associated with accelerated progression of diabetic nephropathy. In this study we describe the long term (8 months) effects of increased dietary NaCl in STZ diabetic rats.

	LOW	REGULAR	HIGH
BG(mg/dl)	420±6.3	412±5.4	408±5.3
BP(mmHg)	153±3.8	158±4.6	184±6.7*
Upro(mg/24h)	114.3±26.4	129.9±28.6	314.2±76.5*
GFR(ml/min/kg)	4.9±0.7	6.9±1.0	9.4±0.9**

*P $\lesssim$.01 **P$\lesssim$.05

No evidence of hypertensive vascular change was observed in the HIGH Na group, although systolic BP was elevated by month 2. Advanced glomerular changes were present in the HIGH Na Group, consisting of mesangial expansion, segmental and global glomerulosclerosis with hyalinosis. Urinary PG excretion (PGE_2, TxB_2, PGF_2, 6 Keto PGF) was increased (P$\lesssim$.01) and plasma AII suppressed in HIGH Na group (P$\lesssim$.05). Plasma ANP did not differ among the 3 groups. Our findings indicate, chronic ingestion of HIGH NaCl diet altered the balance of renal vasoactive hormones, increases BP and GFR and renal injury in STZ diabetic rats.

SPECIFIC INHIBITION OF NEUTRAL ENDOPEPTIDASE 24.11 (NEP) REDUCES PROTEINURIA IN NEPHROTIC RATS. FN Hutchison and V Martin*. VAMC, Martinez, CA and University of CA, Davis, CA

Converting enzyme inhibitors (CEI) reduce albuminuria (UAE, mg/d) in rats with passive Heymann nephritis (PHN) and in other models of proteinuric renal disease. CEIs inhibit degradation of kinins, as well as generation of angiotensin II, thus the antiproteinuric action may result from either or both of these effects. Phosphoramidon (P) selectively inhibits NEP, the enzyme that degrades kinin, but has little effect on CE. Rats with PHN received either P, 10mg/kg/d (n=9), enalapril, 40mg/kg/d (E:n=10), or no treatment (C:n=9). GFR, UAE, and fractional clearance of albumin (FC) were measured daily for 3 days before and 3 days after treatment began.

	P	E	C
UAE_{1-3}	334±47	313±47	354±45
UAE_{days}	228±23[ab]	188±35[ab]	316±45
FC_{1-3}	.0323±.005	.0282±.006	.0317±.004
FC_{days}	.0174±.004[ab]	.0154±.007[ab]	.0330±.008

(a:p<0.05 vs day 6, b:p<0.05 vs C) UAE and FC decreased significantly in both P and E compared to the pretreatment period. UAE and FC were not different in P and E at day 6, but were significantly less than in C. Blood pressure decreased significantly in E, but not P or C. GFR was constant in all 3 groups and UAE and FC did not change over time in C. Specific inhibition of NEP with an agent that does not significantly modify converting enzyme activity reduces UAE in nephrotic rats. The decrease in UAE in response to CEI may be due to potentiation of kinin activity rather than to modulation of the renin angiotensin system.

RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEPO)-INDUCED HYPERTENSION (HPT): DEVELOPMENT OF AN EXPERIMENTAL MODEL. N. Jamgotchian*, M.S. Hu*, P. Abdella*, R. Jackson*, M. Berger*, M. Golub*, N. Yanagawa, and D.B.N. Lee. VA Med. Ctr., Sepulveda and UCLA Sch. of Med., Los Angeles, California.

Blood pressure (BP) elevation occurs with rHuEPO therapy, particularly in previously hypertensive patients. The aim of this study was to develop an experimental model for studying this form of HPT. Ten male spontaneously hypertensive rats (SHR, 10-wk-old) were given subcutaneous rHuEPO (150 U/kg) in albumin every other day for 2 wks (EXP). Ten control SHR (C) received albumin only. Pretreatment and final weights were not different between EXP and C. Hematocrit (Hct) in EXP was higher than C after the 3rd injection (52.8±1.4 vs 46.8±1.5, P<0.02) and remained stable to the end of the study. Initial indirect tailcuff systolic BP (mm Hg) was not different between EXP and C (148±3 vs 150±2). BP rose with time in both groups, but was consistently higher in EXP than in C after the 6th day: day 6 (170±2 vs 163±2, P<0.05); day 10 (175±1 vs 163±2, P<0.001) and day 14 (179±2 vs 167±2, P<0.001). No correlation was observed between BP and Hct. Contractile response of tail-artery segments to transmural nerve stimulation (TNS, 1-40 Hz) and exogenous norepinephrine (NE, $1x10^{-9}$- $6.7x10^{-5}$M) were measured in vitro. No difference was observed between EXP and C. In vitro incubation of arterial segments from untreated SHR with rHuEPO (up to 75U/ml for 1 hr) also caused no change in contractile response to TNS. Conclusions: 1. RHuEPO in SHR causes rise in Hct and induces rapid and sustained exacerbation in BP. 2. No correlation is seen between the BP elevation and the rise in Hct. 3. RHuEPO does not increase vascular reactivity to TNS and NE. 4. SHR is a promising model for investigating the mechanism of rHuEPO-induced HPT.

ENDOTHELIN DECREASES URINE VOLUME, GFR, AND RPF BY ITS DIRECT ACTION ON THE KIDNEY, BUT DOES NOT AFFECT MESANGIAL CELL FUNCTION. T Katoh*, H Chang*, T Okuda*, S Uchida* and K Kurokawa. IVth Dept Med, Univ Tokyo Faculty Med, Tokyo.

Endothelin (ET) is a potent vasoconstrictive peptide derived from vascular endothelium. In the present study, we tested direct effects of synthetic porcine ET on rat kidney in vivo and on cultured rat mesangial cells. ET was infused into left renal artery of anesthetized rats at a rate of 20 or 40 pmol/hr. At this dose, ET's effects were seen only in left kidney (LK) and no effect attributable to ET was seen in right kidney. ET at 20 pmol/hr did not change urine volume (V), but decreased renal plasma flow (RPF) and glomerular filtration rate (GFR) of LK by 27.6% and 30.8%, respectively. At 40 pmol/hr, ET reduced V, RPF, and GFR of LK by 72%, 77.5%, and 78.8%, respectively. Filtration fraction (FF) and FENa in LK remained unchanged. When Ca channel blocker, nicardipine, was infused iv at a rate of 25 µg/hr, V, GFR, and RPF of LK did not change with 20 pmol/hr ET: with 40 pmol/hr ET, V did not change and RPF and GFR fell by 65.1% and 32.0%, respectively, decrements less than those without nicardipine. Cultured rat mesangial cells showed no contraction and cell [Ca] measured by fura-2 did not change in response to 10^{-9} to 10^{-6}M ET while they contracted and cell [Ca] rose in response to 10^{-7}M angiotensin II. These findings document direct renal action of ET and suggest that mesangial cells may not participate in the renal action of ET. Data also suggest that ET, as an endogenous vasoconstrictor, may play some role in the regulation of renal hemodynamics in physiological and pathophysiological conditions.

SYSTEMIC AND RENAL VASOCONSTRICTIVE EFFECTS OF ENDOTHELIN. A.J. King,* S. Anderson, and B.M. Brenner. Brigham & Women's Hosp., Boston, MA.

Endothelin, an endothelial cell-derived peptide, is a potent vasoconstrictor in vitro (Nature 332:411). To examine the in vivo hemodynamic effects, single bolus doses of synthetic endothelin were infused intravenously into anesthetized euvolemic Munich-Wistar rats. The maximal absolute change from baseline in mean arterial pressure (MAP), glomerular filtration rate (GFR), renal plasma flow rate (RPF), and filtration fraction (FF) occurred within 15-30 minutes: (Means ± SEM, n = 4-5 per dose; *p < 0.05 vs. baseline; †p < 0.05 vs. vehicle, VEH)

Dose	MAP	GFR	RPF	FF
pm	mmHg	-------ml/min-------		
VEH	+ 1±1	+0.1±0.1	+0.2±0.4	+0.01±0.01
25	+ 5±1*	-0.1±0.1	-0.6±0.1*	+0.03±0.01
75	+17±3*†	0.0±0.1	-0.8±0.3*†	+0.08±0.02*†
150	+24±2*†	-0.3±0.1*	-1.6±0.1*†	+0.11±0.01*†
300	+42±2*†	-0.8±0.1*†	-3.2±0.3*†	+0.06±0.03*†
400	+54±4*†	----------unmeasurable----------		

Endothelin induced a dose-dependent rise in MAP; LD_{50} was 450 pm. RPF fell disproportionately to GFR; thus FF increased, implying predominant efferent arteriolar vasoconstriction. Hematocrit rose markedly with the highest doses, suggesting a reduction in plasma volume. Endothelin was modestly natriuretic at doses (75 and 150 pm) which raised MAP and did not severely impair GFR. When the rise in renal perfusion pressure was prevented with an aortic snare, 75 pm induced a fall in GFR and RPF, and was antinatriuretic. This potent renal and systemic vasoconstrictor may be important in the pathophysiology of clinical microvasculopathies.

ENDOTHELIN: AN ENDOGENOUS SODIUM TRANSPORT INHIBITOR ? Herbert J. Kramer, Harald Meyer-Lehnert, Helgard Stelkens*, Angela Bäcker* and Christine Wanning*. Med. Poliklinik, Univ. of Bonn, W. Germany

We have previously shown that the putative natriuretic hormone, an endogenous sodium transport inhibitor, inhibits Na-K-ATPase activity, displaces ^{3}H-ouabain, and immunoreacts with digoxin antibodies. Its vasoconstrictor activity appears to be mediated by a rise in intracellular Ca^{++}, a property which may be shared by endothelin, a newly discovered vasoactive peptide isolated from porcine aortic endothelium. In the present study we therefore investigated the in vitro-effects of endothelin at concentrations of 10^{-9} to 10^{-5}M on Na-K-ATPase derived from hog cerebral cortex, on intracellular Ca^{++} in human thrombocytes by the fura-2 fluorescence technique and its immunoreaction with a digoxin antibody (NEN). Endothelin at conc. of 10^{-9}, 10^{-7} and 10^{-5}M inhibited Na-K-ATPase by 6.1%, 17.6% and 30.5%, resp., when preincubated with the enzyme for 60 min as compared to 50% inhibition by 1.5 x 10^{-6}M ouabain. No interaction with the digoxin antibody was observed (less than 8% displacement at a conc. of 4 x 10^{-5}M). Endothelin, at a conc. of 2 x 10^{-6}M, increased intracellular Ca^{++} in platelets from a basal level of 70±8 nM by 174% as compared to 136% in the presence of 10^{-5}M ouabain after 60 min preincubation. Thus, endothelin exerts its vasoconstrictor activity presumably by stimulating transmembrane Ca^{++} influx but may also act as an endogenous sodium transport inhibitor by directly affecting the Na-K-ATPase enzyme.

CONTRACTION OF RAT MESANGIAL CELL (MC) INDUCED BY ADENOSINE (ADO): ROLE OF THE A_1 RECEPTOR TYPE. S. Lamas*, A. Olivera*, D. Rodriguez-Puyol*, J.M. Lopez-Novoa*. (Intr. by P. Vinay). Fundacion Jimenez Díaz- CSIC, Madrid, Spain.

Ado is a nucleoside with known vasodilator properties in multiple organs. By contrast, in the kidney, it increases afferent arteriole resistance and decreases GFR. We tested the hypothesis of MC contraction and subsequent Kf decrement as a contributing mechanism to ADO-induced GFR reduction and tried to define possible receptors in a functional manner. We studied the effects of ADO and selective A_1 and A_2 agonists and antagonists on primary cultures of rat MC. MC were incubated at room temperature with ADO, cyclohexylADO (CHA) (A_1), s-phenyl isopropylADO (S-PIA) (A_1) or n-ethylcarboxamideADO (NECA) (A_2) all at 10^{-3}-10^{-7}M. In experiments with antagonists a 10 min preincubation was performed with PD 116,948 (A_1) 10^{-5}-10^{-7}M or PD 115,199 (A_2) 10^{-5}-10^{-7}M. Microphotographs were taken at 0 and 40 min and planar cell surface area (PCSA) measured by a computerized planimeter. ADO, CHA and S-PIA reduced PCSA in a dose dependent fashion respect to control cells ($p<0.01$) whereas no modification was observed with NECA. Furthermore, PD 116,948 completely blocked ADO effect in contrast with PD 115,199 which did not change ADO-induced PCSA reduction. Inasmuch as MC contraction represents real decrement of ultrafiltration surface, it may play a role in ADO-induced GFR reduction. This effect is mediated through an A_1-type receptor which is present in MC.

THE RESPONSE OF PROXIMAL TUBULAR CELLS (PTC) TO GROWTH FACTORS CHANGES DURING TERMINAL DIFFERENTIATION

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To study how the growth potential changes during differentiation of PTC, primary cultures of PTC were prepared from infant(I,10 d), weanling(W,20d) and adolescent(A,40d) rats as described previously (AJP,251,C455). The mitotic rate of the cells was determined with 3H-Thymidine autoradiography (6 or 24h) and measured as labelling index (Li) in both high (HD) and low density (LD) cells in colonies.

After 48h in culture in DME+10%FBS Li was larger in W/LD than in I/LD and A/LD cells (54±4 vs 32±2 and 30±2% respectively ($p<0.05$,n=4-6)). After 24h serum deprivation Li of I/LD cells was lower than that of W/LD and A/LD (4±1 vs 10±1 and 10±2 ($p<0.05$,n=3-4)). In contact inhibited HD cells absolute values of Li were lower but the relations between I,W and A similar to that of LD.

The mitotic response of the serum deprived cells was studied following addition of FBS. Li increased in I/LD cells from 1±0 to 6±3%. The increase was much larger in W/LD (2±0 to 21±7) and A/LD (2±1 to 27±4%) ($p<0.05$,n=3-4). FBS addition caused a significant 11 fold increase in Li of both W and A HD cells. Addition of EGF (10nM) or IGF-1 (300ng/ml) did not stimulate Li of I and W cells, whereas Li of A/LD cells increased to 9±1 and 16±3% respectively ($p<0.05$,n=4). In A/HD cells no effect was seen by EGF whereas IGF-1 increased Li from 1±0 to 10±3 ($p<0.05$,n=4)

Single cell nuclear c-fos was measured by immunofluorescence and quantitative image analysis in I and A cells. FBS addition to deprived cells significantly increased nuclear c-fos 2-3 fold in A HD and LD cells with a peak at 60 min. In I cells this increase was not seen.

Conclusion;The mitogenic response to growth factors changes during terminal differentiation in both HD and LD cells .

ROLE OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I) IN COMPENSATORY RENAL GROWTH (CRG) IN DWARF RATS. J E Le Carpentier, D J Hill*, A M El Nahas. Departments of Nephrology and Paediatrics*, Northern General Hospital, Sheffield, UK.

The relative contribution of systemic hormones such as growth hormone (GH) and renal autocoid growth factors such as IGF-I, to the pathogenesis of CRG remains unknown. We studied CRG in a new mutant strain of dwarf rats known to be selectively deficient in GH and displaying low circulating levels of IGF-I. Six adult male dwarf rats underwent a right uni-nephrectomy (UNx) whilst 4 had a sham operation. Seven days later, UNx rats were sacrificed and their left kidney removed. Groups of adult male Wistar rats were similarly studied. At the time of UNx and sacrifice, their kidneys were weighed and their IGF-I content measured by an RIA after acid extraction. Serum IGF-I was also measured pre and post-UNx. CRG was estimated by comparing right and left kidney weights as well as kidney/body weight ratios. In spite of significantly lower levels of GH and IGF-I in dwarf rats, CRG was comparable in dwarf (14±5%, M±SD) and Wistar (18±3%) rats. This could be explained by a comparable rise in intra-renal levels of IGF-I following UNx in both experimental groups; Wistar - sham: 308±15ng/gm KW (M±SD), Wistar - UNx: 417±75 ($p<0.025$), dwarfs - sham: 301±53, dwarfs - UNx: 447±60 ($p<0.005$). This occurred in the absence of parallel changes in circulating IGF-I. These data show that CRG takes place in growth hormone deficient rats and suggests a possible autocrine or paracrine role for IGF-I in CRG following UNx in normal and growth hormone deficient rats.

ENDOTHELIN INDUCES A PREDOMINANTLY AFFERENT ARTERIOLAR VASOCONSTRICTION THAT IS REVERSED BY NIFEDIPINE. Rodger Loutzenhiser*, Murray Epstein, Koichi Hayashi*. Nephrology Section, V. A. Med. Center, and Univ. of Miami, Miami Florida.

Endothelin, a peptide recently isolated from endothelial cells, is the most potent known mammalian vasoconstrictor (Nature 332:411-415, 1988). It is postulated to directly activate potential-dependent Ca channels (PDC's) in vascular smooth muscle. Suggestions that endothelin mediates vasoconstriction in pathophysiologic states prompted us to examine its actions on the renal microcirculation. Chronic hydronephrosis was induced in rats to facilitate visualization of the renal microvessels. Hydronephrotic kidneys were excised and perfused in vitro on an inverted microscope. Afferent (AA) and efferent (EA) arteriolar diameters were measured in situ, using videomicroscopy as described previously (J.P.E.T. 246:522-528, 1988). At 0.3 nM, endothelin elicited preferential vasoconstriction of the AA; reducing AA diameter by 46±7% ($p<0.025$), and EA diameter by only 12±7% ($p>0.2$). The AA-vasoconstriction elicited by endothelin was reversed by NIF:

	basal	Endothelin	Endothelin + NIF	
[NIF]		0.1 μM	1.0 μM	10 μM
AA	18.4±0.6	9.9±1.3	12.5±1.1	15.5±1.3	17.5±1.4
EA	17.3±1.6	15.1±1.4	15.1±1.2	15.1±1.4	16.1±1.4

(mean ± SE, n=3, vessel diameters in μm)

Our findings that endothelin preferentially constricts the AA, and that this action is reversed by NIF are consistent with previous observations suggesting a predominant role of PDC's in the activation of this vessel. Thus, calcium antagonists constitute an appropriate pharmacologic approach to reverse endothelin-induced renal hemodynamic abnormalities.

AVP REGULATION OF ARACHIDONIC ACID (AA) METABOLISM IN RENAL EPITHELIAL CELL LINE (LLC-PK1): ACTIVATION OF TRIGLYCERIDE METABOLISM. Diane Leone*, Jeffrey Stoff, Thom. Honeyman*. UMAMedCtr, DeptMed&Physiol. Worcester, MA

Free arachidonic acid (AA) conc is limiting for cellular eicosanoid synthesis and is regulated by phospholipases which hydrolyze membrane lipids, lipases which cleave triglycerides (TG) and reacylation enzymes which incorporate AA into membrane phospholipids (PL).

We studied vasopressin (AVP) regulation of AA metabolism in confluent LLC-PK$_{1-6}$ (p210-220), pulse labelled with ^{3}H-AA (1-5uCi, 10^{-8}M x 120 min). Neutral lipids were extracted and separated by TLC. ^{3}H-AA was incorporated into: PL(87.9%), TG(12.2%), AA(<.1%), cholesterol esters (<.01%). The effect of increased [AA] substrate and AVP (1mU/ml x 15min) were studied:

CONDITION	PL		TG	
	amount	%	amount	%
10-6M AA	103±3.8	87.9±1.4	14.4±2.3	12.2±1.3
10-4M AA	1210±240	17.5±0.8	5525±1365	79.0±0.7
10-6M+AVP	116±4.45	87.7±1.9	18.5±0.1	13.8±0.4
10-4M+AVP	2280±140	19.9±1.2	10410±530	86.3±0.6

Data are Mean±SEM and amount in picomoles.

AA is principally incorporated into PL at low substrate conc while at high substrate conc there is reversal with incorporation into TG. TLC separation of PL indicated incorporation of ^{3}H-AA into phosphatidyl-choline and phosphatidyl ethanolamine (2.5:1). AVP increased incorporation of AA into TG both at low ($p<.1$) and at high ($p<0.05$) substrate conc.

These data indicate that conversion of AA into TG is an important metabolic pathway in LLC-PK1 and that AVP may exert control on TG metabolism. Since AVP activates adenylate cyclase in LLC-PK1 these data are consistent with the presence of a hormone sensitive (cyclic AMP-dependent) lipase analogous to the adipocyte. Thus, AVP may activate AA metabolism through multiple pathways.

THE RENAL VASODILATORY ACTION OF CALCITONIN (CT) AND ITS EFFECT ON EXPERIMENTAL ACUTE RENAL FAILURE (ARF) INDUCED BY ISCHAEMIA. S.F.Lui*, Z.Varghese*, D.Nair*, P.Sweny*, J.F. Moorhead. Royal Free Hospital, London, England.

Renal vasoconstriction is an aetiological factor of the pathogenesis of ARF. There is some evidence to suggest that CT is a potent renal vasodilator. We have studied the dose response of CT infusion on renal haemodynamics in Sprague Dawley (SD) rats, and its effect on experimental ischaemic ARF.

(1) GFR and ERPF (inulin and PAH clearance) were measured in 3 groups of 6 SD rats before and during the infusion of either 0.04, 0.1 and 0.2 iu/kg/hr of CT. ERPF and GFR were maximally increased with 0.1 iu/kg/hr of CT infusion (62% and 67% respectively, $p< 0.04$).

(2) Ischaemic ARF was induced in 2 groups of 12 SD rats by clamping the right renal artery for 40 minutes and left nephrectomy. The CT group was given an infusion of CT (0.1 iu/kg/hr in albumin solution), started 1 hr before and continued for 24 hr after clamping. The control group was given the same volume of albumin solution. The mortality rate was lower but did not reach statistical significance in the CT group (17% vs 42%). Renal function was monitored for 7 days, and was significantly better at day 4 post-operation in the CT group. Serum Cr was lower (219 umol/l vs 598 umol/l, $p < 0.01$), and CrCl higher (289 ul/min vs 123 ul/min, $p< 0.05$). It is concluded that Calcitonin is a potent renal vasodilator and if given prophylactically, can ameliorate ischaemic renal damage.

EFFECTS OF AN INCREASE IN PLASMA DOPA LEVELS ON URINARY CATECHOLAMINE EXCRETION. Eric S. Marks, Robin W. Stull,* Harry R. Keiser,* and David S. Goldstein.* Dept. of Med., Uniformed Services Univ of Health Sciences & NIH, Bethesda, Maryland

Urinary dopamine (DA) is believed to be primarily derived from intratubular decarboxylation of plasma delivered DOPA (D). Plasma D originates from cells of neural crest origin possessing tyrosine hydroxylase activity. This study describes the effect on urinary D and DA excretion produced by increases in plasma D. Urine and plasma was collected from conscious, unstressed, semi-restrained female Wistar-Kyoto rats (n=6-8) that received either L-DOPA (0.5µg/kg/min IV), tyrosine (600 mg/kg/hr IV) or the peripheral decarboxylase inhibitor alpha-difluoromethyl dopa (DFMD) (50mg/kg IP). Urine levels are based on one-hour collections. L-DOPA, tyrosine, and DFMD increased plasma dopa concentration (pg/ml) 27-, 11-, and 33-fold respectively. DOPA is not normally detectable in rat urine. L-DOPA, tyrosine and DFMD produced excretion of 29.7±25.8, 59.2±16.1 and 382.2±81.3 ng/60min of D. L-DOPA and tyrosine increased urinary dopamine excretion 41- and 20-fold, while DFMD decreased DA from 92.7±15.6 to 71.8±15.4 These results demonstrate that L-DOPA and tyrosine infusion increase urinary D and DA excretion by increasing plasma D. Tubular D transport capacity is limited as demonstrated by the appearance of urinary D. Decarboxylase inhibition markedly increases plasma D but fails to completely block intrarenal DA production. The limiting step in urinary DA production appears to be substrate delivery rather than a lack of amino acid decarboxylase capacity.

PATHOPHYSIOLOGY OF HYPERTENSION IN DIALYSIS PATIENTS TREATED WITH ERYTHROPOIETIN. G. Mayer, E. M. Cada, U. Watzinger, G. Ludvik, U. Barnas, H. Graf. 2nd Dept. of Medicine, University of Vienna, Austria.

Hypertension is one of the major side effects of erythropoietin (EPO) treatment in dialysis patients. In order to investigate the underlying pathogenetic mechanisms we determined resting hemodynamic parameters (cardiac output and total peripheral resistance) before (hemoglobin = 6.6 g/dl) and after (hemoglobin = 11.5 g/dl) partial correction of anemia in 18 patients. To avoid antihypertensive drug interference 9 patients, in whom therapy was not changed throughout the study period were evaluated seperately. Cardiac output was measured non invasively using the indirect Fick's principle (CO_2 rebreathing maneuvre). Hemodynamic changes were identical in both groups and consisted of a significant decrease of cardiac index ($p<0.005$), an increase of total peripheral resistance ($p<0.0005$) and an increase of diastolic blood pressure ($p<0.05$).

We conclude from our studies that the increase of blood pressure in patients treated with EPO reflects the reversal of hemodynamic adaptations to anemia. The anemic patient on dialysis appears to have a decreased peripheral resistance due to hypoxic vasodilation. After correction of anemia most probably his "true" peripheral resistance reappears representing renal hypertension.

IDENTIFICATION OF SPECIFIC, HIGH AFFINITY ENDOTHELIN (EN) BINDING SITES IN RAT RENAL PAPILLARY (P) AND GLOMERULAR (G) MEMBRANES. E.R. Martin, B.M. Brenner and B.J. Ballermann. Brigham and Women's Hospital, Boston, MA.

EN is a newly described, potent 21-amino acid vasoconstrictor peptide produced by vascular endothelial cells, (Nature 332:411 '88), which increases blood pressure and glomerular filtration fraction, and reduces GFR when infused in rats (King et al, ASN '88). EN furthermore inhibits Na^+ transport in rabbit inner medullary collecting duct cells (Zeidel et al, ASN '88). We therefore set out to identify specific EN binding sites in rat P and G membranes. At 25 °C specific ^{125}I-EN binding to P and G membranes was time-dependent, reached equilibrium at 4 h and remained stable for a further 2 h. Specific binding was saturable as determined by incubating P and G membranes with increasing concentrations of ^{125}I-EN. Scatchard plots derived from saturation studies were linear, suggesting binding sites with a uniform affinity. The equilibrium dissociation constant (K_D) averaged 662±151 and 1309±123 pM, and the binding site density (R_O) 7666±920 and 5831±348 fmol/mg protein for P and G, respectively. Unlabeled EN inhibited ^{125}I-EN binding in P and G in a concentration-dependent fashion, with half-maximal inhibition at 108±13 and 140±22 pM, respectively, (n=3). Angiotensin II, atrial natriuretic peptide, and EN-related peptide did not compete for binding, indicating specificity of the sites for EN. We postulate that these high affinity binding sites in G and P membranes represent EN receptors which mediate the renal actions of this novel peptide.

STIMULATION OF PHOSPHOINOSITIDE (PI) HYDROLYSIS BY ADENOSINE IN RABBIT KIDNEY. Shari McArdle*, Lal C. Garg and Fulton T. Crews*. Univ. of Florida, Gainesville, FL.

Adenosine is a metabolite of renal tubular cells and is known to influence the renal function. In order to investigate the second messenger system for the renal actions of adenosine, we determined the effects of adenosine on PI hydrolysis in the tissue slices from cortex (CTX), outer medulla (OM) and inner medulla (IM) of the rabbit kidney. The method involved the incubation of the slices with [^{3}H]-inositol for its incorporation into PI and the measurement of the release of [^{3}H]-inositol phosphates (IP) in the presence of lithium which prevents dephosphorylation of IP. The results of [^{3}H]-IP formation are expressed as % of total [^{3}H]-inositol incorporation into the membranes.

Tissue	N	Control	Adenosine (100 µM)
CTX	3	1.9 ± 0.4	-0.2 ± 1.9
OM	8	0.9 ± 1.9	15.6 ± 7.1*
IM	7	7.1 ± 1.8	6.2 ± 3.8

Values are mean ± SE of N animals. * $P < 0.05$ *vs* Control.

No significant effect of adenosine was found on the release of IP in the CTX or IM. Adenosine produced a 15-fold increase in IP release in the OM. Our results suggest that adenosine produces its renal effects in OM through activation of PI messenger system.

ENDOTHELIN CONSTRICTS THE RENAL CIRCULATION AND STIMULATES RENIN IN VIVO. W. L. Miller*, M. Redfield*. J. C. Burnett, Jr., Mayo Clinic, Rochester, MN

Endothelin, an endothelium-derived peptide, demonstrates calcium-dependent contractile activity in vitro and may serve a counter-regulatory role to the vasodilatory and renin inhibitory actions of endothelium-derived relaxing factor(s). The integrated biologic actions of endothelin, however, have not been evaluated. This study was, therefore, designed to test the hypothesis in anesthetized dogs that endothelin decreases renal blood flow (RBF) and glomerular filtration rate (GFR), stimulates renin (PRA), and elevates systemic blood pressure (MAP) in vivo. †p<.05;x±SEM;n=6

	Control	Endothelin (50ng/kg/min,iv)	Washout#
MAP,mmHg	98±7	123±7†	101±5†
GFR,ml/min	38±4	0.7±0.3†	48±2†
RBF,ml/min	267±33	63±20†	173±28†
UNaV,μEq/min	48±14	1.2±0.3†	13±3.6†
PRA,ng/ml	3.7±1.0	6.9±1.7†	5.6±1.6
ANF,pg/ml	39±6	180±53†	74±10†

#1 hour post-endothelin infusion

We conclude that endothelin is a potent systemic and renal vasoconstrictor, which decreases GFR and RBF in asssociation with a protracted anti-natriuresis. Further, these studies demonstrate that, despite increases in ANF, endothelin stimulates renin release.

MOUSE RENAL CORTICAL BLOOD FLOW (RCBF) AND EGF CONTENT DO NOT INCREASE ACUTELY AFTER UNINEPHRECTOMY (NX). D.W.Moskowitz, S.L.Bonar*, and H.M.Druce*,VA Medical Center and St. Louis University School of Medicine, St. Louis, MO.

Increased renal arterial blood flow has been reported to occur within 5 min after Nx in the dog (Krohn, J.Urol. 103:564, 1970), as has increased renal EGF content 24 hr after Nx in the rat (Anderson, Fed.Proc. 46:1331, 1987). We studied RCBF by laser-Doppler velocimetry in adult (3-6 month old, 30-45 gm) and weanling (4-5 wk old, 15-20 gm)C57B10 mice of both sexes. RCBF in the left kidney was monitored before and for up to 4.5 min after ligation of the right renal artery. RCBF did not increase in either adult (n=8) or weanling (n=7) mice. Using similar operating conditions, we measured whole kidney EGF content. One kidney was removed and quickly placed in a dry ice-acetone bath, followed by removal of the second kidney at various times (0 sec-24 hr) after the first. Each kidney was extracted in 5% acetic acid and EGF was quantitated by competitive radioreceptor assay, using A431 cell membranes. No significant change in whole kidney EGF content was seen at any time after Nx in either adult or weanling C57B10 mice of either sex. In addition, there was no significant difference in EGF content of the first (control) kidney between adult (1.73 ± 0.343 ng EGF/mg protein, mean ± SEM, n=157) and weanling (2.08 ± 0.381, n=27) mice; nor between male (1.35 ± 0.267, n=100) and female (1.65± 0.223, n=28) adult mice, nor between male (1.98 ± 0.313, n=13) and female (2.18 ± 0.690, n=14)weanling mice.

We conclude that, in the mouse, renal EGF content is not affected by age or sex, and that no detectable change in RCBF or renal EGF content accompanies the onset of compensatory renal growth.

DOPAMINE BLOCKADE (DAB) BLUNTS NATRIURESIS AND LITHIURESIS INDUCED BY LOW DOSES OF NICARDIPINE (NIC): EVIDENCE FOR A DOPAMINE (DA) RECEPTOR-RELATED TUBULAR EFFECT OF CALCIUM CHANNEL BLOCKING AGENTS (CCBA). A.Montanari, D.Vallisa, G.Ragni, M.Serventi, A.Novarini and P.Coruzzi (intr. by J.P.Knochel) - Istituto di Semeiotica Medica,Università di Parma - Italy

In order to study possible direct renal tubular effects of CCBA independent of renal hemodynamic changes and their relationship to the DA-ergic system, we performed studies in hypertensives (EH) using NIC at the dose of 0.01 mg/kg BW, thus minimizing its renal vasodilatory effect. In a group of 5 EH (I), mean arterial pressure (MAP, mmHg), ERPF and GFR (infusion of inulin and PAH), urinary excretion of Na and Lithium (Li, single oral dose, used as a marker of proximal tubular reabsorption) were measured during 1 hour before (C1) and after acute i.v. NIC. Four-six days later the experiment was repeated with NIC + metoclopramide (MCP) 10 mg as DA-blocking agent. In experiments II (n=5), after 1 control hour (C), MCP alone was given at 2nd hour and MCP + NIC were administered at 3rd hour. In the results, derived values of renal blood flow (RBF, ml/min), precapillary renal vascular resistance (PVR, dyne.min.cm^{-2}), excreted fraction of filtered Na (FENa%) and fractional reabsorption of Li (1-FELi)% are included.

		MAP	GFR	RBF	PVR	(1-FELi)%	FENa%
EXP I (n=5)	C1	124±25	121±19	949±111	9.0±2.3	81.0±5.5	1.01±0.60
	NIC	120±24	119±30	960±141	8.8±2.4	73.9±8.6*	1.67±0.59*
	C2	124±22	124±29	940±106	8.9±1.7	79.8±4.2	1.09±0.54
	NIC + MCP	123±21	118±30	930±172	9.1±1.7	79.1±5.2	1.19±0.41
EXP II (n=5)	C	120±24	132±21	1131±401	8.2±4.5	78.0±5.3	0.91±0.11
	MCP	117±25	129±28	1097±391	7.9±4.7	78.8±4.3	0.93±0.23
	MCP + NIC	117±25	132±29	1139±405	7.9±4.1	77.5±4.7	1.04±0.19

* Significant difference versus C1

The present data show that NIC increases FENa% and even more markedly decreases (1-FELi)% in the absence of any change in GFR, RBF and PVR. Furthermore MCP alone has no effects on renal function while it abolishes almost completely NIC-induced changes in FENa% and (1-FELi)%. Thus, NIC may suppress tubular transport, mainly in the proximal tubule, through a direct tubular mechanism. This latter seems to be correlated with the modulation of tubular transport by tubular DA receptors.

CHARACTERIZATION OF ALPHA - 2 RECEPTORS IN AMBYSTOMA PROXIMAL TUBULES. Renu Nigam* and Nikolas S. Morgunov* (intro. by D. Hirsch). Dept. Physiol. & Biophys., Dalhousie Univ., Halifax, N.S., Canada.

Alpha-2 (clonidine) response was investigated in isolated perfused salamander proximal tubules. In control HCO_3 Ringer's, basolateral cell membrane (V_{bl}) and transepithelial (V_{te}) potentials averaged -56.6 ± 7.5 mV (n=58) and -3.6 ± 0.6mV (n=53), respectively. Within 2.9 ± 0.2 minutes of clonidine (10^{-6} M) addition to the bath superfusate, V_{bl} depolarized by 5.1 ± 0.5 mV (p<0.001:n=35). However, the response was transient in nature and V_{bl} returned to control values within 12.0 ± 0.8 min. Removal of clonidine from the bath superfusate produced a transient hyperpolarization of V_{bl}, qualitatively similar in magnitude and duration to that of the depolarizing response. Bilateral Na replacement with N-methyl-D-glucamine (n=9) or the addition of 0.5mM SITS to the bath superfusate (n=8), completely blocked the clonidine response. In contrast, bilateral replacement of bicarbonate (HEPES substitution) or organic substrates (10 mM mannitol substitution) reduced the clonidine response by 85% (p<0.01;(n=3) and 51% (p<0.01:n=4), respectively. In conclusion, the data suggest that alpha-2 receptor stimulation increases the activity of a SITS inhibited, sodium dependent basolateral transporter(s). Substitution studies implicate Na/HCO_3 and Na/organic substrate cotransport systems. The nature of the organic substrate is under investigation.

TUMOR NECROSIS FACTOR (TNF) STIMULATES PROSTAGLANDIN (PG), CYCLIC AMP (cAMP), and DNA SYNTHESIS BY CULTURED RAT MESANGIAL CELLS (CMC). J. Perez, L. Baud and R. Ardaillou. (Intr. by K.F. Badr). INSERM 64, Hôpital Tenon, Paris, France.

Infiltration of glomeruli by macrophages is associated with local production of TNF in nephrotoxic serum nephritis. We investigated the possibility that TNF interacts with mesangial cells to stimulate PG, cAMP, and DNA synthesis as observed in this model. TNF increased the production of PGE_2, $PGF_{2\alpha}$, and 6 keto $PGF_{1\alpha}$ by cycling CMC exposed to 10 % serum (8-, 6.5- and 3.5-fold control values, respectively). This response was detectable from 6h and maximum after 48h of incubation with TNF (0.1-100 ng/ml). TNF stimulated PG synthesis by cycling CMC exposed to serum free medium and by non-cycling CMC as well. Actinomycin D and cycloheximide inhibited TNF-induced PG synthesis, suggesting that the induction requires RNA and protein synthesis. TNF also stimulated cAMP production (2-fold control values). [^{3}H] thymidine uptake was not modified by TNF in CMC exposed to 10% serum, but was enhanced in CMC with serum-free medium. Pretreatment by indomethacin suppressed the effect of TNF on PGs but only reduced that on cAMP indicating that PGs mediate just partly the increase in cAMP. Under these conditions, TNF stimulated [^{3}H] thymidine uptake by CMC exposed to 10 % serum, suggesting that TNF-induced PG synthesis antagonized this growth effect. It is concluded that TNF released from macrophages infiltrating the glomeruli modulates mesangial cell functions during glomerulonephritis.

BOMBESIN (BBS) STIMULATES PROLIFERATION OF RAT MESANGIAL CELLS (RMC) IN CULTURE. Francesco Pugliese, Maria C. Anania*, Giulio A. Cinotti*, and Gianfranco Delle Fave*. Dept. of Medicine, Univ. of Rome " La Sapienza", Rome, Italy.

BBS and BBS-like peptides have been shown to be mitogens for a variety of cell types. We have investigated whether BBS could promote glomerular mesangial cell proliferation often associated with glomerular diseases. Synthetic BBS dose-dependently stimulated DNA synthesis measured as ^{3}H-thymidine (TdR) incorporation into quiescent, confluent RMC monolayers incubated for 48 hrs in the absence of serum (FBS). 29±24 %, 126±76 %, and 231±119 % (mean ± SD) elevation in TdR uptake above basal occurred at BBS 10^{-10} M, 10^{-8} M and 10^{-6} M, respectively. Corresponding increments in cell count by 43.6 ±18 %, 101±39 % and 191±34 % respectively, were observed. Addition of the specific BBS-receptor antagonist D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}-substance P (10^{-6} M), which by itself did not affect RMC proliferation, eliminated the BBS stimulated TdR incorporation by RMC at BBS 10^{-10} M, and blunted the stimulation by 57 % at BBS 10^{-8} M and by 47 % at BBS 10^{-6} M. Corresponding decrements in cell count were also observed.

These data suggest that BBS/BBS-like peptides have functional receptors in the glomerular mesangium mediating proliferation, with possible implications in glomerular diseases.

PROLONGED RBC SURVIVAL AND HEMATOPOIETIC RESPONSE TO RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEPO) IN CHRONIC RENAL FAILURE (CRF) John E. Prior,* Alan Terzian,* Allan B. Schwartz, Kwan E. Kim, Gary S. Mintz,* S. Benham Kahn,*. Hahnemann University, Department of Medicine, Philadelphia, PA

rHuEPO was administered 3x/week to 8 pts with CRF in a double blind placebo study. Creatinine clearance (GFR) avg 14.4 (8-28) ml/min at Hct 29.8% (29-31). Baseline RBC survival by ^{51}Cr T1/2 was highly correlated with GFR (r=.871, p<.01). Repeat ^{51}Cr T1/2 at Hct 40% at 8 wks was prolonged by 4 days.

Hct ↑ from 29.8 to 40% at 43 days. s Iron ↓ from 69 to 38mg%. s Ferritin ↓ from 217 to 75 mg%. Retics ↑ from 1.4 to 7.4%. Doses were witheld in all pts to avoid polycythemia, then titrated to maintain Hct 38-42%. No untoward effects noted. Closely monitored BP ↑ from 138/79 (100 MAP) to 149/87 (108 MAP) correlated with ↑ Hct. One pt required titration of BP meds.

Conclusion: rHuEPO is an effective stimulator of erythropoiesis in pts with CRF. Early retic release is noted. Orderly sequential ↓ s ^{51}Iron; ↓ s Ferritin precedes ↑ Hct. RBC ^{51}Cr T1/2 was highly correlated with GFR. A prolongation of RBC survival noted at 8 wks of rHuEPO correlated with early release of younger RBCs.

PROXIMAL TUBULAR FLUID FROM SALINE VOLUME EXPANDED DONOR RATS INHIBITS VOLUME FLUX IN PROXIMAL TUBULES OF NON EXPANDED RECIPIENT RATS. Sri Reddy, Cheryl C. Cochineas, and Akos Z. Györy. Dept. of Med. Univ. of Sydney & Renal Med. at The Royal North Shore Hosp. Sydney, Australia, 2065.

Volume expansion is known to inhibit proximal tubular Na transport. We have previously shown that, during both mannitol saline (Györy and Willis, Pflügers Arch. 396: 110-116,1983) and saline volume expansion (SVE) (Györy, Chan and Reddy, Pflügers Arch. 404:S136-S142, 1985) inhibition of both volume flux (J_{vl}) and Na transport (transepithelial Na concentration difference) was dependent on the type of fluid used: whether artificial (AS, 0-20%) or harvested autologous fluid (HTF, 40-50% inhibition).

Using the shrinking drop technique (evaluator blinded, precision of duplicate measurements 18%) we measured J_{vl} (nl/mm/min) in non expanded (recipient) rats with AS alternating with either HTF or with harvested proximal tubular fluid from donor rats (HTF_D) which had undergone SVE ($U_{Na}V$ control = 0.33±0.15, exp. = 27.2±1.96 µEq/min, mean±SE). With AS, J_{vl} was 1.89±0.14, with HTF 1.95±0.31 whereas with HTF_D it was 1.10±0.36, a highly significant reduction with respect to both AS and HTF ($p<0.001$ and 0.01, n=11-18). Subsequent SVE of the recipient rats resulted in a J_{vl} with HTF of 0.71±0.13, not significantly different from HTF_D ($p>0.4$).

These results support our previous observations that following volume expansion a Na transport inhibiting factor appears in HTF not present in this fluid when rats are not volume expanded. Importantly however, these data now demonstrate that this factor is also transferable resulting in proximal tubular transport inhibition in non expanded rats.

MAPPING OF 125-I EPIDERMAL GROWTH FACTOR (I-EGF) BINDING SITES ALONG THE RABBIT NEPHRON. R. Redha*, C. Lopez*, J.A. Breyer*, H.R. Jacobson, and M.D.Breyer. Div. of Nephrol., Vanderbilt Univ. and V.A.M.C., Nashville, Tn.

EGF is a 53 amino acid polypetide with potent mitogenic effects on cultured cells. Recently the thick ascending limb has been identified as a major site of synthesis for preproEGF. Also a specific 170 kD EGF receptor has been demonstrated in whole kidney homogenates. However both the localization and the functional role of renal EGF receptors remain undefined. We therefore examined the distribution of I-EGF binding to microdissected rabbit nephron segements.

We have previously reported the presence of saturable and specific binding of I-EGF to microdissected pars rectae (PR) (Clin. Res, 36: 515A,1988). We now further characterize this binding and map these binding sites in dissected nephron segments. I-EGF binding to PR at 1°C was reversible: 50%off-rate of 35 min. I-EGF binding to PRs was not decreased by a 500 fold excess of by either PTH or insulin, peptide hormones of comparable size, with well documented proximal tubule receptors. In contrast TGF- alpha a functional and structural homolog of EGF singnificantly reduced I-EGF binding to PR by 57±12.2% ($p<0.025$). In separate studies performed in collagenase digested kidneys, using a more stringent wash, the segmental distribution of specific I-EGF binding was determined. Under these conditions (1°C, 2 h) binding of I-EGF to collagenase and non-collagnease treated PR's was not different. Significant specific binding (amoles/cm) was greatest in PR 90±8.4, followed by proximal convoluted tubules 75.7±18.03, , cortical collecting tubules 65±20.3, and outer medullary collecting duct 28.2± 11.5. Specific binding to glomeruli was also identified (5.25±1.17amol/glom.). In contrast no specific binding to cortical or medullary thick ascending limb of Henle was observed.

We conclude: 1) specific I-EGF binding sites in glomeruli, proximal tubules and CCTs correspond with previously demonstrated sites of EGF action in the kidney; 2) The absence of specific EGF binding in the thick limb is of interest since this segment is a major site of prepro-EGF mRNA synthesis 3) The lack of uniform EGF binding along the nephron argues for selective renal sites of EGF acttion.

EGF BINDING IS DECREASED IN COMPENSATORY RENAL HYPERTROPHY. **E. Sack*, J.A.L. Arruda, and Z. Talor, Dept. of Medicine, U of IL, WSVAMC and Cook County Hospital, Chicago, IL.**

We have recently demonstrated the existence of specific high affinity EGF binding sites in basolateral membranes from rabbit kidney. Following unilateral nephrectomy, there is compensatory hypertrophy of the remaining kidney with an increase in the Na-H antiporter activity. We therefore postulated that EGF may play a role in these processes. To investigate this hypothesis we measured the Na-H antiporter in brush border membranes and EGF binding in basolateral membranes prepared three weeks after unilateral nephrectomy (UNX). Following UNX, BUN and creatinine were unchanged but the kidney weight increased significantly from 8.7 ± 0.4 to 11.5 ± 0.8 g ($p < 0.01$). UNX was associated with a significant increase in the Vmax of the Na-H antiporter (measured by acridine orange quenching) from 251 ± 36 to 397 ± 35 FU/300 µg protein/min ($p < 0.005$), without a change in Km. Scatchard analysis of EGF binding to basolateral membranes showed that the Bmax of the high affinity binding sites decreased following UNX from 0.45 ± 0.07 to 0.29 ± 0.04 pmol/mg protein ($p < 0.025$), without a change in Kd. Thus, in compensatory renal hypertrophy there is a significant increase in the Vmax of the Na-H antiporter and a significant decrease in the Bmax for the high affinity EGF binding sites. We conclude that compensatory renal hypertrophy is associated with a decrease in the maximal number of binding sites for EGF in renal cortical basolateral membranes. These findings may suggest EGF receptor down regulation in compensatory renal hypertrophy.

REACTIVE OXYGEN SPECIES (ROS) CONTRACT ISOLATED GLOMERULI AND CULTURED RAT MESANGIAL CELLS. D. Rodríguez-Puyol, I. Duque, L. Obregón, M. A. Moliz, L. Hernando (Intr. by R. R. Robinson). Department of Nephrology, Fundación Jiménez Díaz, Madrid, Spain.

ROS could play some role in the reduction of GFR observed in some pathological conditions, such as glomerulonephritis or drug nephrotoxicity. To test this hypothesis and to analyze if these effects could be mediated by changes in the K_f, we measured glomerular cross-sectional area (GCSA) of isolated glomeruli and planar cell surface area (PCSA) of cultured mesangial cells incubated with Xanthine (X)/Xanthine-oxidase (XO) (X: 0.2 mM. XO: 2 mU/ml). Neither buffer nor X or XO alone modified GCSA or PCSA. However, XXO induced reductions of GCSA (21 ± 3 %) and PCSA (11 ± 2 %) which were statistically significant ($p<0.01$). Preincubation with superoxide dismutase (SOD: 5 µg/ml) did not modify the effect of XXO, but catalase (C: 20 µg/ml) completely abolished the glomerular or cellular response to XXO. Direct incubation with H_2O_2 induced dose-dependent reductions in GCSA and PCSA which were statistically significant from 10^{-6} M and 10^{-5} M respectively, and which were inhibited by C. BN 52021 10^{-5} M, a PAF antagonist, completely inhibited the action of H_2O_2 on isolated glomeruli or cultured mesangial cells. These results suggest that ROS, by decreasing the K_f, could mediate the reduction of GFR observed in some kidney diseases. The dependence of these effects on other cellular mediators must be carefully assessed, but it seems that PAF could be involved.

IN SITU HYBRIDIZATION OF EPIDERMAL GROWTH FACTOR mRNA IN THE MOUSE KIDNEY. Eduardo C. Salido,* Pauline H. Yen,* Larry J. Shapiro,* Delbert A. Fisher,* and Luciano Barajas. Dept. of Pathol., Pediat., and Biol., Chem. UCLA School of Med. and Howard Hughes Med. Inst., UCLA School of Med., Harbor-UCLA Medical Center, Torrance, CA.

The expression of epidermal growth factor (EGF) in the adult mouse kidney was studied by both in situ hybridization and immunocytochemical techniques. Kidneys were perfused in vivo with 4% buffered paraformaldehyde and frozen sections were cut. The presence of prepro-EGF mRNA was studied in sections of mouse kidney by in situ hybridization using a ^{3}H-labeled prepro-EGF cDNA probe and a ^{35}S-labeled antisense RNA probe; a ^{35}S-labeled sense RNA probe was used as control. In addition, rabbit anti-EGF serum was utilized to immunolocalize the peptide by the avidin-biotin complex immunoperoxidase method. Both EGF immunoreactivity and prepro-EGF mRNA hybridization were localized to the thick ascending limb of Henle (TALH) and the distal convoluted tubule (DCT), while the macula densa was negative. The glomerulus, the proximal portion of the nephron and collecting system were negative. We conclude that the TALH and DCT cells are responsible for the synthesis of EGF in the mouse kidney.

THE EFFECT OF PLATELET ACTIVATING FACTOR (PAF)-RECEPTOR ANTAGONIST ON PROTEINURIA AND GLOMERULAR EICOSANOID SYNTHESIS IN RAT IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN). D. Sauter*, S. Iskandar, and M. Rahman. Hines VA Hospital and Loyola University, Hines, IL., and Bowman Gray School of Medicine, Winston-Salem, NC.

PAF is a lipid mediator of inflammation released by a variety of cell types including mesangial cells. We examined the pathogenetic role of PAF released from endogenous glomerular cells in cationic bovine gamma globulin (CBGG)-induced ICGN. This is a complement- and leukocyte-independent model, devoid of cellular infiltration and characterized by enhanced glomerular eicosanoid production. Rats were preimmunized with CBGG and divided into experimental and control groups. Each group was challenged with daily i.v. injections of CBGG. The experimental group received the PAF-receptor antagonist L-652,731 (1.6 mg/kg) i.v. one hour prior the CBGG injections, followed six hours later by an oral dose of the antagonist (20 mg/kg). The control group received the vehicle.

Proteinuria was significantly reduced in the antagonist-treated group, 77.4 ± 13.2 mg/24 hour, n=9, versus 127 ± 5.6mg/24 hour, n=10 for control; $p<0.02$. Administration of the antagonist did not attenuate the increment in glomerular thromboxane synthesis seen in this model. There were no clear differences between the groups in the intensity and the distribution of immunofluorescence staining for BGG or rat IgG.

We conclude that PAF released by endogenous glomerular cells could have a role in the development of proteinuria in non-infiltrative models of glomerulonephritis.

UPTAKE OF MACROMOLECULES (MM) BY CULTURED MESANGIAL CELLS (MC) IS ENHANCED BY ANGIOTENSIN II (AII). P. Singhal, A. Santiago*, R. Hays, D. Schlondorff, Long Island Jewish Med. Ctr. and Albert Einstein College of Medicine, New York.

MC endocytose MM, which may be important in immune injury. We have previously reported that endocytosis of MM by MC is receptor mediated (AJP 252, F627, 1987) and that MC express specific IgG Fc receptors (JCI 1988). We examined the effect of AII on the uptake of IgG coated particles by MC to determine whether AII directly stimulated MM endocytosis.

Colloidal gold particles (10nm) were prepared and coated with radioiodinated IgG. Mc (3rd passage) on 24 well plates were treated with either vehicle alone (control) or AII (10..-6..M) for 10 min. and incubated for 30 min. with 5x10..5..CPM of IgG particles, washed extensively and the radioactivity was measured. Four sets of experiments (each in quadruplet) were carried out and the results were as follows:

	Control	AII	P
Mean±SEM (CPM/well)	3519±305	4628±258	<0.05

The AII-induced increase in uptake of MM may represent mere surface binding or relate to later events of endocytosis. To differentiate this, MC were pre-treated with Cytochalasin-B (10..-5..M) prior to the addition of AII. Uptake of MM by MC, treated with AII and Cyto-B was significantly ($P<0.02$) decreased to 2378±172 CPM/well when compared to AII treated cells only.

Our results suggest that AII enhances the uptake of MM by MC independent of hemodynamic factors and that this may occur as a result of increased intracellular traffic.

INHIBITION OF Na-K-ATPase (Na-K) IN RAT RENAL CORTICAL TUBULE CELLS (RCTC) BY LOCALLY FORMED DOPAMINE (DA) INVOLVES DA_1, BUT NOT DA_2, RECEPTORS. I. Seri, B.C. Kone, S.R. Gullans, B.M. Brenner, B.J. Ballermann. Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

By measuring Na-K-dependent O_2 consumption (Na-K-d-QO_2) and ^{86}Rb uptake we have shown that DA, formed locally from L-dopa (L-D), inhibits Na-K activity in rat RCTC. This finding has now been confirmed by quantification of K^+ release from RCTC with an extracellular K^+ electrode. L-D (100 µM) induced a K^+ efflux of 33±6 nmol/mg prot./min (29±6% of ouabain-sensitive K^+ release), which started 30 sec after L-D addition, and was inhibited by carbidopa.

Inhibition of Na-K by locally formed DA has also been demonstrated in permeabilized rat proximal tubules (PT). Surprisingly, the effect appeared to be DA receptor (R)-independent. We therefore studied the involvement of DA Rs in this process in intact RCTC. With combined DA_1 and DA_2 R blockade (10 µM SCH 23390 + 10 µM S-sulpiride), 100 µM L-D inhibited Na-K-d-QO_2 by 7±3% (n=3), compared to a 16±3% inhibition by L-D alone ($p < 0.05$, n=3). DA R blockade alone did not influence QO_2. Like L-D, the selective DA_1 R agonist SKF 82526 (SKF) (10 µM) inhibited Na-K-d-QO_2 by 21±3% ($p < 0.05$, n=3), whereas the selective DA_2 R agonist LY-171555 (10 µM) had no effect.

Attenuation of L-D-induced Na-K inhibition by DA R blockade suggests involvement of DA Rs. Since the DA_1, but not the DA_2, R agonist mimics the effect of L-D on Na-K-d-QO_2, it is concluded that DA_1 Rs mediate, at least in part, the effect of locally formed DA on Na-K in intact rat RCTC.

CYCLOOXYGENASE INHIBITION ENHANCES RAT INTERLEUKIN 1-ß (Il 1-ß)-INDUCED PROLIFERATION OF RAT MESANGIAL CELLS (MC) IN CULTURE.
Rolf A.K.Stahl, Friedrich Thaiss, Sabine Kahf, Alan Shaw*, Wilhelm Schoeppe, Dept. of Medicine, Division of Nephrol., University of Frankfurt and *Glaxo-IBM Geneva, Switzerland.

The cytokine interleukin 1 (Il 1) stimulates proliferation of MC and prostaglandin (PG) formation. A possible role of cyclooxygenase products on Il 1 mediated growth of MC is, however, unknown. Therefore, we evaluated the effect of cyclooxygenase inhibition on DNA recombinant rat Il 1-ß induced proliferation of rat MC in culture. MC were studied after the second subculture. Proliferation was estimated by 3H-thymidine incorporation and counting of total cell number. Rat Il 1-ß in concentrations between 1 to 100 ng/ml resulted in a stimulation of proliferation of MC between 30 and 80%. Il 1-ß (10ng/ml) increased MC PGE_2 formation within 24 h significantly (control: 268± 24pg/10ug protein; Il 1-ß: 465± 37 pg/10ug).Coincubation of MC with Il-1-ß (10ng/ml) and indomethacin (INDO) (1 ug/ml) increased 3H-thymidin incorporation in MC by 227% at 24 h and 367% at 48h. The increase in 3H-thymidine incorporation in MC was paralleled by an increase of 48% in total cell number. INDO alone stimulated MC proliferation by 138%. The Il 1-ß and INDO induced stimulation of cell proliferation is inhibited in a dose dependent matter (between 20 and 90%) by PGE2 (10^{-6} to 10^{-9} M). These data demonstrate that cyclooxygenase inhibition enhances Il 1-ß induced proliferation of rat MC. The antiproliferative effect of PGE2 might play a role in glomerular diseases where Ill 1-ß mediates MC proliferation.

RECOMBINANT HUMAN ERYTHROPOIETIN (r-HuEPO) ACTIVATES A BROAD SPECTRUM OF HEMATOPOIETIC STEM CELLS. F. Stockenhuber, K. Geissler, G. Sunder-Plassmann, R.W. Kurz‡ Ch. Jahn, W. Hinterberger, P. Balcke. 1st Department of Medicine, Univ. of Vienna, ‡KFJ Hospital, Austria.

The numbers of circulating hematopoietic progenitor cells CFU-GM, BFU-E and CFU-GEMM were assayed weekly by means of a commonly applied in vitro clonal assay in 20 uremic subjects on regular hemodialysis before and during treatment with r-HuEPO. 11 out of these patients were treated with 80 U (group 1), the others (group 2) with 50 U r-HuEPO per kg body weight 3 times a week. Circulating BFU-E, CFU-GEMM and CFU-GM increased significantly within 1 week of treatment in both groups.

	before	after 3 weeks of r-HuEPO therapy	
	n=20	group1 n=11	group2 n=9
CFU-GM	88±54	162±58+	150±47 cells/ml+
BFU-E	112±70	465±147+++	373±125 cells/ml++
CFU-GEMM	4±4	20±12+	15±11 cells/ml+

+ $P<0.05$ ++ $P<0.01$ +++ $P<0.005$

These findings demonstrate that chronic administration of even 50 U r-HuEPO per kg 3 times a week results in a significant increase not only of the erythroid progenitor cell BFU-E but also of the pluripotent stem cell CFU-GEMM and the non erythroid stem cell CFU-GM. The mechanism of r-HuEPO induced increase of non-erythroid progenitors remains unknown. The increase of BFU-E was followed by a rise of hematocrit with a delay of 3 weeks in group 1 and of 4 weeks in group 2. Monitoring of circulating BFU-E provides early information regarding red blood cell production, which allows to predict whether the dose of r-HuEPO is sufficient to achieve an increase of hematocrit.

COMPARISON BETWEEN CALCITONIN (CT) AND DOPAMINE (DP) AS A RENAL VASODILATOR. Z.Varghese*, S.F. Lui*, A.Ali*, D.Nair*, P.Sweny*, J.F. Moorhead. Royal Free Hospital, London, England.

There is some evidence to suggest that CT is a potent renal vasodilator. We have studied the dose response of CT infusion (5 and 10 iu/hr) on renal haemodynamics in 8 normal voluteers, and compared the renal haemodynamic effects of CT (5 iu/hr) with DP infusion (2.5 ug/kg/min) in 11 normal voluteers. ERPF and GFR were measured by inulin and p-amino-hipurran clearance under steady state conditions, before (baseline value: BL) and during the infusion of CT or DP (Inf).

Infusion	GFR (ml/min) BL	Inf	% change	ERPF (ml/min) BL	Inf	% change
CT 5u/hr	111	128*	+16%	564	684*	+21%
CT 10u/hr	112	129*	+15%	591	751*	+25%
CT 5u/hr	109	123*	+13%	557	647*	+16%
DP	96	107**	+11%	577	869*	+50%

(* $p<0.01$, ** $p<0.05$ from baseline value)

5 u/hr and 10 u/hr of CT infusion induced similar increase in ERPF and GFR. Although the increases in ERPF were significantly greater with DP infusion, the increases in GFR were similar. This suggests that the site of action of CT is different from that of DP. CT may exert its renal vasodilatory effects mainly on the preglomerular vasculature. It is concluded that CT is as at least as effective as DP in increasing GFR, and may be considered as an alternative renal vasodilator.

IMMUNOREACTIVE ENDOTHELIN (IR-ET) IN PLASMA OF HEMODIALYSIS PATIENTS K. Totsune‡, T. Mouri‡, K. Takahashi‡, M. Ohneda‡, M. Sone‡, T. Furuta‡, T. Saito* and K. Yoshinaga‡ (intr. by K. Kurokawa) 2nd Dept Int Med, Tohoku Univ Sch Med, Sendai, Japan.

In order to study the physiological roles of endothelin (ET), a vasoconstrictor peptide recently found by Yanagisawa et al., we measured plasma ET levels in 10 normal subjects, 10 hypertensive patients (pts) and 16 hemodialysis (HD) pts. Two types of RIA methods were tested. One was an extraction method in which 3 ml of plasma was applied on a SEP-PAK C_{18} cartridge, and another was a direct method using 100 µl of plasma per tube. Synthetic ET and ET antisera were supplied from PEPTIDE INSTITUTE INC. ET was labelled with ^{125}I by a modified chrolamine T method. By the extraction method, recovery of ET was 50-70 % and the lower limit of sensitivity was 5 pg/tube. Plasma IR-ET was not detectable in normal subjects and hypertensive pts (lower than 7 pg/ml). In HD pts, plasma IR-ET levels were elevated in 12 of 16 pts (10.9±2.0 pg/ml, Mean±SD), and did not significantly change during HD (before: 11.2±2.0, after: 12.5±1.6 pg/ml, n=10). By the direct method, ET was again undetectable in normal and hypertensive subjects except one patient respectively. In HD pts, plasma IR-ET levels were 190±95 pg/ml before HD and 220±135 pg/ml after HD (n=16). These data suggest that ET probably has some pathophysiological roles as a circulating hormone in HD pts. The reason of a large discrepancy between direct and extracted results in HD pts was not clear. It requires further investigations to confirm whether or not the discrepancy is due to IR-ET like substances with a large molecular weight existing in HD patients' plasma.

ENDOTHELIN (E) INHIBITS Na/K-ATPase IN INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. M. L. Zeidel, B. Kone, H. Brady, S. Gullans, B. M. Brenner. Harvard Medical School Boston, Mass.

E, a potent vasoconstrictor released by vascular endothelial cells, can induce natriuresis in vivo. The present studies examined the regulation of Na transport by E in suspensions of rabbit proximal tubule (PT) and IMCD cells. E reduced oxygen consumption (QO_2) by 18 ± 1% in IMCD cells, but did not alter QO_2 in PT cells. E inhibited QO_2 half maximally at 5×10^{-11} M, suggestive of a physiologic effect. Several lines of evidence indicate that E reduces QO_2 by inhibiting the Na/K-ATPase: 1) E gave nonadditive inhibition with ouabain and blocked the stimulatory effect of amphotericin B on QO_2 (+29 ± 4% in the absence of E, 0 ± 5% in the presence of E; n=6, $p<0.001$); 2) E inhibited ouabain-sensitive ^{86}Rb uptake by 46.6 ± 8.6% at 10 sec, and by 35.4 ± 5.3% at 30 sec, without altering uptake at 60 min; 3) Addition of E to IMCD cells induced an initial net K efflux rate of 32.2 ± 4.8 nmol/min/mg prot, vs 6.3 ± 4.0 for veh, consistent with inhibition of the Na/K-ATPase. Because PGE_2 inhibits Na/K-ATPase in IMCD cells (Kidney Int. 33:268, 1988), we examined the effect of the cyclooxygenase inhibitor, ibuprofen (I) on the QO_2 response to E. In the presence of veh, E reduced QO_2 by 21 ± 2%; in the presence of I, E reduced QO_2 by 8 ± 3% (n=8, $p<.001$).

In conclusion, E inhibited QO_2 in IMCD but not PT cells. E inhibited Na/K-ATPase as shown by QO_2, ^{86}Rb uptake and K^+ electrode studies. E may act on Na/K-ATPase via cyclooxygenase products such as PGE_2, the latter also known to inhibit Na/K-ATPase in these cells.

GLUCOSE AND MYO-INOSITOL (MI) MODULATE COLLAGEN BIOSYNTHESIS BY PROXIMAL TUBULAR CELLS IN CULTURE. F.N. Ziyadeh, S. Goldfarb, M. Watanabe*, and T.P. Haverty, Dept. of Med., U. of Penna., Phila., PA

Increased accumulation of extracellular matrix (ECM) is a hallmark of diabetic nephropathy (DN). In advanced DN, interstitial fibrosis is prominent. We studied the role of hyperglycemia and associated cellular inositol depletion in the pathogenesis of increased ECM in a murine proximal tubular cell line (MCT) by measuring Type IV and Type I collagen biosynthesis.

Following 48-hour synchronization in serum free media at 100 mg/dl glucose (G-100) and 40 μM MI, cells were grown for 72 hours in varying concentrations of G and MI and spent media were assayed for Type I and Type IV collagens by RIA. In 450 mg/dl G (G-450), vs G-100, Type IV collagen secretion was increased by 5-fold (218 ng/10^5 cpm to 1,105) and Type I by 3.8-fold (from 1.62 to 6.23 ng/10^5 cpm). Addition of MI produced a dose-dependent correction of the excess collagen formation. At 800 μM MI in G-450 cells, collagen secretion was normal.

To test for altered collagen gene transcription, we assayed for relative collagen mRNA using dot blots of cytosolic mRNA hybridized with type I and IV ^{32}P-labelled cDNA probes. G-450 medium produced a 2.6-fold increase in Type IV mRNA and a 1.6-fold increase in Type I mRNA.

These data suggest that hyperglycemia, presumably through reduced cellular MI levels, stimulates MCT cells to synthesize increased collagen matrix through increased transcription. Tubular cell collagen secretion induced by cell MI depletion may contribute to the increased fibrogenesis which characterizes advanced DN.

CHRONIC EFFECTS OF NADOLOL (N) VS. ATENOLOL (A) ON RENAL FUNCTION IN MALE HYPERTENSIVES. S. Bagby, D. Palac*, C. Gray*, M. Eldred*, T. Krishnamurthy*. Depts. Med., Nuclear Med., Portland VAMC, Portland, Oregon

Beta blockers may differ in effects on glomerular filtration rate (GFR) and renal plasma flow (ERPF), an issue relevant to long-term preservation of renal function. 19 mild-moderately hypertensive males aged 35-65 yrs. completed 3 sequential 8-wk study periods with active drug (A or N, titrated to diastolic BP = 90 mmHg) in Periods 1 and 3, Placebo washout in Period 2. Drug order was fixed and single-blinded in 8 pts (A -- N), randomized and double-blinded in 11 (4/11 A -- N). GFR and ERPF were simultaneously measured by plasma disappearance curves of ^{99}Tc-DTPA and ^{125}I-orthohippurate and calculated using 1- and 2-compartment models respectively. Filtration fraction (FF) was calculated as GFR ÷ ERPF. Results in pts with data from all 3 Periods (mean ± SD) are:

	n	Nadolol	Atenolol	Placebo
GFR	15	134 ± 35	138 ± 32	140 ± 26
ERPF	13	476 ± 95	449 ± 114	460 ± 113
FF	12	.28 ± .08	.30 ± .05	.31 ± .06

There was no significant drug effect by ANOVA, although N showed a trend toward lower FF (in 10 of 12 pts. cf. Placebo). Thus, on the short term, long-acting beta blockers N and A did not adversely affect renal function or intrarenal hemodynamic profiles.

MATERNAL ENVIRONMENT DURING SUCKLING ALTER PROTEINURIA AND GLOMERULOSCLEROSIS IN MATURE SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE (WKY) RATS. SH Azar, V Kabat*, and C Bingham*. Dept. of Med. and School of Stats., Univ. of Minnesota, Minneapolis, MN.

Milk factor(s) induced by dietary salt affect blood pressure (BP) and glomerular filtration variability in mature SHR and WKY offspring. The kidney is immature at birth. We wanted to know if this milk factor (s) acting during the early postnatal development of the kidney changed its susceptibility to hypertensive injury later in life. Females were randomly assigned to a high, 4% (H), or to a low, 0.3% (L), NaCl diet to be given during lactation (n=7-8/strain/diet). At birth litter size was reduced to 8 pups then crossover of SHR and WKY pups was done. Control pups remained with their mothers. At 19 days pups received L or H diets (16 groups, 9-10 pups/group) for 10 months. Then we assessed 24 h protein excretion (Prot) and renal morphology. We analyzed the data with multifactorial ANOVA. The results showed that SHR ($F=100$, $p<.0000$) and H ($F=13.21$, $p<.0006$) increased Prot. Pup H diet increased Prot in SHR but not in WKY, the effect being greater for SHR pups with WKY-H nurses, $F=10$, $p<.002$. For WKY-H pups Prot was positively associated with H nurse diet but not with nurse strain. Glomerulosclerosis was present mainly in SHR-H and was more severe when the nurses were on H. The results suggest that renal susceptibility to injury induced by salt and hypertension in the SHR pup was modulated by nurse dietary salt. However, the interactive effects of pup high salt diet with the salt induced milk factor(s) upon renal injury also involve other mechanisms, WKY-H pups with normal BP excreted more protein when they were nursed by WKY-H. Perhaps prevention of renal diseases should start with maternal nutrition.

MEASUREMENT OF ABNORMAL LITHIUM TRANSPORT IN RED CELLS OF HYPERTENSIVES BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY, Vinod K. Bansal, Duarte Mota de Freitas*, and Ravichandran Ramasamy*, Department of Medicine (Renal and Hypertension Section), Loyola University Stritch School of Medicine, Maywood, IL 60153 and Department of Chemistry, Loyola University of Chicago, Chicago, IL 60626.

The rates of lithium (Li^+) transport in red blood cells (RBC) of hypertensive patients (HT) are reported to be significantly higher than those of normotensives. However, these measurements were made by flame photometry, a technique which involves physical separation of the intra- and extracellular compartments prior to analysis. In our study, we developed a nuclear magnetic resonance (NMR) method, based on the ^{7}Li nucleus, which discriminates between the two pools of Li^+ in RBCs without cell lysis. The non-invasive NMR approach takes advantage of the different spin-lattice relaxation rates of the two Li^+ pools and employs a modified inversion recovery (MIR) pulse sequence. Using the MIR approach, we found that the rates of Na^+-Li^+ exchange were higher in hypertensives (0.51 ± 0.04 mmole of Li^+/L of RBC x h, n = 10) as compared to normotensives (0.21 ± 0.02 mmole of Li^+/L of RBC x h, n = 10). These correlated closely to the Na^+-Li^+ transport rates measured by atomic absorption (HT 0.53 ± 0.02, n = 10; Normotensive 0.20 ± 0.03, n = 10). Our study confirms abnormal Li^+ transport in RBC in HT by a different technique, which may be superior because of its non-invasive nature.

INTRACELLULAR pH_i (ipH) IN LYMPHOCYTES FROM THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). D.C. Batlle A. Saleh*, and G. Rombola*. Northw. Univ. and Lakeside VA, Chicago, IL.

Various defects in ion transport have been described in leukocytes from the SHR model of genetic hypertension. Increased activity of the Na+/H+ exchanger of SHR as compared to WKY cells has been recently found. This could result in an increase in ipH of SHR cells. Alternatively, a reduction in ipH could be a signaling mechanism for the activation of the Na+/H+ antiporter. We measured ipH in thymus-derived lymphocytes loaded with BCECF-AM, a pH sensitive dye, at 37°C for 30 min. Studies were performed in young SHR (4 weeks of age) and age-matched WKY rats with similar blood pressure (tail cuff pressure 87±3.6 and 85±2.6 mmHg, respectively) and in older rats (16-20 wks of age) in which blood pressure was markedly different (170±4.5 and 106±4.8 mmHg, respectively, $p<0.001$). Steady-state ipH was measured in a HCO_3-free medium (HEPES, pH 7.4) to maximize the activity of the Na+/H+ exchanger. Participation of this exchanger in the regulation of steady-state ipH was inferred from a fall in pH_i (≈ 0.10 pH units) upon exposure to a specific inhibitor (EIPA, 200 μM). Steady-state pH was found lower in SHR as compared to WKY lymphocytes both at 4 weeks of age (7.12±0.004 and 7.33±0.004, $p<0.05$) and at 16-20 weeks of age (7.06±0.03 and 7.24±0.02, $p<0.001$). The data demonstrate a previously unrecognized alteration, reduced ipH, in the SHR model of hypertension which appears genetically determined insofar as it is evident before and after the development of hypertension. Reduced pH_i, in turn, could result in enhanced activity of the Na+/H+ antiporter in SHR cells.

ALTERATIONS IN RENAL Na,K-ATPase ACTIVITY AND AFFINITY INDUCED BY HIGH SALT DIET IN THE DAHL SALT SENSITIVE (SS) RATS. A Bertorello*, M-L Melzi*, U Ekblad*, and A Aperia. Dept of Pediatrics, Karolinska Institute, Stockholm, Sweden.

We have demonstrated that changes in proximal tubule (PT) Na,K-ATPase activity are important for adaptation to high salt diet (HS) in Sprague Dawley (SD) rats (AJP 254: F795, 88). We have now examined renal Na,K-ATPase activity in PT dissected from Dahl salt resistant (SR) and SS during normal (NS) and HS diet. HS was accomplished by replacing drinking water with 0.9% saline for ten days. Mean arterial blood pressure (mmHg) was in SR/NS 103±13, SS/NS 113±9, SR/HS 113±7 and SS/HS 150±7. Na,K-ATPase activity was determined as ouabain sensitive ^{32}P-ATP hydrolysis in single permeabilized PT segments under Vmax conditions for Na,K, and ATP and expressed in pmol Pi/mm tubule/h. In SR, HS induced a significant decrease in enzyme activity compared to the NS group (1276±160, n=4 NS vs 841±83, n=5 HS, $p<0.05$). (n, rats studied. 15-20 tubules analysed in each rat). Findings are similar to those reported in SD rats. In the SS, HS induced a significant increase in enzyme activity (1104±152, n=7 NS vs 1521±91, n=7 HS, $p<0.05$). In the next protocol the activity of Na,K-ATPase purified from the outer renal cortex (containing ≥89% PT cells, AJP 249:F891) was determined as ^{32}P-ATP hydrolysis at Na concentration from 6 to 140 mM. During NS the $K_{0.5}$ for Na was the same for Na,K-ATPase from SS and SR (35 and 35 mM Na respectively). During HS the $K_{0.5}$ for Na was lower in SS than in SR (22 and 33 mM Na respectively). Conclusion: In Dahl SS rats HS diet induces a paradoxical increase in renal PT Na,K-ATPase activity and an increase of its Na affinity.

SUPPRESSION AND MASKING OF RENAL VEIN RENIN PROFILES INDUCED BY MANNITOL PROPHYLAXIS. Robert L Benz, Brendan P. Teehan, Charles R. Schleifer, and Miles H. Sigler, Lankenau Hospital, Phila., Pa.

Renal vein renin (RVR) profiling is the physiologic and diagnostic standard for evaluating significant renal artery stenosis. False negative RVR profiles with failure of lateralization in anatomically apparent high grade stenosis complicates the dx of renovascular hypertension (RVH). Mannitol prophylaxis (MP) is a regimen utilized for preventing contrast dye nephropathy.

Five pts. with RVH were studied prospectively to determine whether MP induces false negative RVR profiles. RVR samples were obtained pre and post infusion of 12.5 gms MP. RVR sampling preceded angiography. Pts. received oral ACE inhibitor stimulation prior to testing. In each study renin secretion from the ischemic kidney was suppressed. Mean ischemic side renin values fell from 21.9 to 11.1 ng/ml/hr ($p=0.01$). Lateralization was lost in 3 of 5 pts. Mean suppression of the non-involved side and IVC renin was not significant ($p=0.55$ and 0.21, respectively). To evaluate MP induced volume expansion or dilutional effects pre and post Hct, serum Na^+ and osmolality were obtained. No significant changes were found ($p = 0.73$, 1.00 and 0.76). Mean $U_{Na}V$ increased from 0.04 pre MP to 0.15 meq/min. post MP.

In conclusion, MP results in significant suppression of renin release by the ischemic kidney in RVH despite ACE inhibitor stimulation. This suppression can alter the RVR profile producing a false negative ratio. The mechanism of MP suppression may relate to increased distal sodium delivery or improved renal blood flow at the arteriolar level.

PRE-EXISTENT HYPERTENSION ALTERS THE SITE OF MICROVASCULAR INJURY IN REMNANT KIDNEYS. A. Bidani, M.Schwartz, K.Griffin*, R.Loutzenhiser*, K.Hayashi*, M.Epstein, E.Lewis. Rush Medical College, Chicago and Univ. of Miami, Florida.

Acute ablation of renal mass in normotensive rats leads to systemic hypertension (HTN), proteinuria, and progressive glomerular injury in remnant kidneys (RK). The effect of pre-existent HTN at the time of 5/6 ablation on the course and pattern of morphologic injury was examined in standard protein (22%) diet-fed male SHR rats (b.w.200-300g). Systolic BP (mmHg), Scr (mg/dl), protein excretion (mg/24°) and renal autoregulatory ability were measured before and at sacrifice 3-6 weeks after ablation. Results mean ± SEM.

	PREABLATION			POSTABLATION		
	BP	Scr	Upro	BP	Scr	Upro
SHAM(n=12)	161±6	.5±.04	15±1	170±4	0.6±.03	17±1
RK (n=19)	157±4	.5±.03	16±1	171±5	1.4±.1	43±3

Renal autoregulation was impaired in remnant kidneys and was associated with severe microvascular injury. In contrast to remnant kidneys of other strains, however, the injury was mostly confined to midcortical and juxtamedullary regions. In complementary studies, similar autoregulatory behavior of afferent arterioles (AA) and interlobular artery (ILA) segments was observed by videomicroscopy in isolated perfused hydronephrotic, non-ablated SHR kidneys. These data imply that both ILA and AA contribute to normal autoregulatory protection in SHR kidneys, which is impaired by 5/6 ablation. Acute transmission of pre-existent HTN to a "non-adapted" microvasculature transforms "benign" HTN to "malignant" HTN in remnant kidneys of SHR and shifts the site of injury to more proximal segments.

CALCIUM METABOLISM OF RESISTANCE ARTERIES (RA) AND MESENTERIC ARTERY MYOCYTES (MAM) IN PRIMARY AND LONG-TERM CULTURE: ALTERATIONS IN HYPERTENSION. Richard D. Bukoski*, Peggy DeWan*, and David A. McCarron. Oregon Hlth Sci Univ, Div of Neph, Portland, Oregon.

It has been proposed that elevated free intracellular Ca^{2+} $[Ca^{2+}]_i$ may be responsible for enhanced vascular reactivity and hyperplastic growth described in RA of spontaneously hypertensive rats (SHR) compared with normotensive Wistar Kyoto rats (WKY). Studies examining $[Ca^{2+}]_i$ in subpassaged, cultured MAM have yielded variable results. We examined basal levels of $[Ca^{2+}]_i$ and norepinephrine (NE)-induced mobilization of $[Ca^{2+}]_i$ in isolated RA of SHR and WKY, and contrasted the results with those obtained using MAM derived from superior mesenteric arteries. All tissue was isolated from 12-wk old male rats; $[Ca^{2+}]_i$ was estimated using Fura-2. Between strains, basal $[Ca^{2+}]_i$ levels were not different in either isolated RA, or MAM in primary culture or 1^{st} passage. However, $[Ca^{2+}]_i$ was elevated significantly in MAM of SHR at passages 3, 5, and 7 ($p < 0.05$). This elevation was temporally correlated with enhanced proliferation of SHR myocytes. Also, while NE induced an increase in $[Ca^{2+}]_i$ in RA and primary MAM of both strains, subpassaged MAM were not affected. In summary, intrinsic differences in $[Ca^{2+}]_i$ metabolism are not expressed in RA or primary MAM of SHR and WKY, although elevated $[Ca^{2+}]_i$ and enhanced proliferation rates are observed in those cells after subpassaging. These data indicate that subculture induces phenotypic expression of intrinsic genetic differences in myocytes of SHR and WKY or selects for a sub-population of cells with said differences.

ROLE OF PROTEIN KINASE C (PKC)-MEDIATED MECHANISMS IN VASCULAR SMOOTH MUSCLE CELL (VSMC) CONTRACTION. C. Caramelo*, K. Okada*, P. Tsai* and R.W. Schrier. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

The PKC stimulator, PMA, and the pressor hormone, arginine vasopressin (AVP), both contract VSMC in culture. We therefore studied the relative contribution of PKC to the AVP-mediated response by: 1) inhibition of PKC with H7($5x10^{-5}$M) and 2) PKC desensitization (PKC-DS) by preincubation with 10^{-6}M PMA for 24 h. These procedures reduced the AVP(10^{-7}M)-induced VSMC contraction by 32.2% and 43.2%, respectively. These same procedures reduced PMA(10^{-7}M) contraction by 67.1% and 79.2%. The AVP-induced cytosolic Ca^{2+} peak (424±42 nM) was not affected by H7 (411±51 nM) or PKC-DS (406±33nM), suggesting that PKC does not exert an acute regulatory effect on the AVP-mediated Ca^{2+} transient. The effect of PKC-induced intracellular alkalinization on the VSMC response was also examined. VSMC were preincubated for 24 h with or without 10% fetal bovine serum (FCS). In the presence of PMA(10^{-7}M), the FCS cells developed no changes in intracellular pH (pH_i), whereas the pHi of the cells without FCS increased by .18 +.03 units at 10 min. However, the contractile response was similar in both groups (42.3 ±4.6 and 41.6 ±3.4% of the total cells, p=NS). In addition, the Na^+ transport inhibitor, amiloride ($5x10^{-5}$M), inhibited contraction in both groups to a similar degree. The dissociation between the pH and contractile responses to PMA suggests that alkalinization is not mandatory for PKC-mediated VSMC contraction to occur, and that the inhibitory effect of amiloride may be mediated by mechanisms additional to Na^+/H^+ antiporter blockade.

SODIUM INDUCED INCREASE AND POTASSIUM INDUCED FALL IN BOTH PLASMA RENIN ACTIVITY (PRA) AND STROKE IN STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RATS (SHRsp). Maria Jose F. Camargo,* Massimo Volpe,* Mark S. Pecker,* Jean E. Sealey, and John H. Laragh. Cardiovascular Center, Cornell University Medical College, New York, NY

The incidence of strokes and death in SHRsp is modulated by a high K diet. To examine the relationship of these changes to PRA, SHRsp were studied for 12 weeks on 3 different dietary regimens, starting at 6 wks of age.

In Group A, (regular chow, n=26), PRA did not change with time (5.3±.35, 5.2±1.6, 5.0±1, 7.8±1.8 ng/ml/hr); in Group B (high Na/high K, n=36), PRA increased at 12 weeks (5.3±.35, 4.1±0.9, 5±1 to 11.1±2.9); and in Group C (high Na/low K, n=36), it increased more and earlier, from 5.3±.35, 3.4±0.8 to 13.7±3.9 and 22.3±3.6 at 0,4,8 and 12 weeks, respectively. Blood pressure and growth were similar in the 3 groups. The incidence of stroke and/or death during the 12-wk period was 8.6%, 19.5% and 50% in Groups A,B, and C, respectively.

The results show that 1) the incidence of stroke and death in SHRsp is independent of BP levels; 2) high Na intake in SHRsp causes a paradoxical rise in PRA which is attenuated by high K intake. These studies suggest that high Na intake in SHRsp leads to disruption of the physiological regulation of renin secretion. Excessive renin secretion may be a factor in the development of strokes since renin was highest in Group C, which had the most strokes, and since both renin and strokes were markedly reduced in high salt rats fed a high potassium diet (Group B).

INHIBITION OF CATION TRANSPORT PATHWAYS ALTERS CYTOSOLIC CALCIUM (Cai) IN VASCULAR SMOOTH MUSCLE CELLS (VSMC). D.B. Corry, N. Yanagawa, M.L. Tuck*. Olive View and Sepulveda VAMC, UCLA, Los Angeles, CA.

Changes in cation transport could lead to increased Cai and enhanced arterial smooth muscle tone in hypertension. Using the Fura 2/AM method we studied the interaction between cation transport systems and Cai in rat cultured VSMC. Addition of ouabain (10^{-3}M) to inhibit Na,K-pump in VSMC from Wistar rats resulted in small increments in Cai (140 ± 23 to 200 ± 16 nM, n=4). In contrast, addition of Ethyl-isopropylamiloride (EIPA, 10^{-5}M) to inhibit Na,H-antiport in VSMC led to a decrease in Cai (118 ± 23 to 77 ± 26 nM, n=5). Addition of bumetanide (10^{-5}M) to inhibit Na,K,Cl-cotransport (CoT) in VSMC produced a marked rise in Cai (126 ± 5 to 1121 ± 295 nM, n=5). This effect of bumetanide on Cai was attenuated by preaddition of EIPA. The effect of bumetanide on Cai was also compared in VSMC from Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Addition of bumetanide (10^{-5} M) resulted in a greater increase in Cai in SHR as compared to WKY. Thus, 1) in VSMC inhibition of CoT increases Cai to a greater extent than inhibition of Na,K-pump; 2) Modulation of Cai by CoT is amplified in SHR vs. WKY. We conclude that alterations in the CoT pathway could contribute to VSMC contractility in hypertensive disorders through changes in Cai.

DIFFERENCES IN INTRACELLULAR IONIC REGULATION OF PROXIMAL TUBULAR CELLS CULTURED FROM NORMOTENSIVE (MNS) AND HYPERTENSIVE (MHS) MILAN-STRAIN RATS. M. Crabos*, H. F. Cantiello*, G. Bianchi#* and C. Lechene. Boston, Mass. and Milano, Italy#.

Cells from primary cultures of renal proximal tubules isolated from MNS and MHS rats were grown in vitro for three days as a first step to assess possible markers involved in genetic hypertension. Leak rates were derived from time courses of changes in intracellular Na and K contents after addition of 5 mM ouabain, measured by electron probe analysis. Na,K pump activity was also studied as ouabain-sensitive K uptake and Na efflux at different intracellular Na concentrations. Initial rates for Na and K fluxes (mmole/mole P/min) were similar in MHS vs MNS for Na 35.9± 6.9 (n=3 rats) vs 29.4± 2.4 (3). and for K 36.6 ± 2.9 (3) vs 36.7 ± 3.9 (3). In contrast, intracellular ionic contents were different in MHS vs MNS. Intracellular Na was 0.122± 0.004 (n=627 cells) vs 0.175± 0.005 (543), intracellular K was 1.17 ± 0.008 vs 1.22 ± 0.009 and Cl was 0.353 0.006 vs 0.438± 0.009 mmoles /mmole P, all data, $p < .0002$ by unpaired t test. Intracellular volume calculated as the sum of intracellular ionic contents was higher in MHS cells by 12 % as compared to MNS, $p< 0.0002$. The data on Na,K pump activity indicate similar apparent Vmax. for both cell types 78.6 vs. 82.3 mmoles K /mole P/min. In contrast, the apparent Km was 28% higher in MHS vs MNS 0.15± 0.011 vs 0.117 ± 0.008 mmoles Na/mole P. In conclusion, 1) leak rates are similar, 2) intracellular ionic contents are highly statistically different, and 3) a higher apparent Km for Na in MHS. Thus, MHS cells may have a different activation of Na,K pump by intracellular Na than MNS cells.

RENAL HYPERTENSION FOLLOWING AORTIC CONSTRICTION IS ABOLISHED BY CONVERTING ENZYME INHIBITION BUT NOT BY LOW SALT DIET. A-C Eklöf* and B Sahlgren*. Dept of Pediatrics, Karolinska Institute, Stockholm, Sweden.

Constriction of the aorta proximal to the renal artery (PAC) in 40-day-old Sprague-Dawley rats results in hypertension both proximal and distal to the constriction. Three weeks after constriction mean arterial blood pressure (MAP, mmHg) was in carotid artery (CA) 228±29 and in femoral artery (FA) 163±15 vs 136±10 in sham operated control rats (C). GFR (ml/min) was significantly reduced (2.09±0.35 vs 2.97±0.57 in C). Filtration fraction (%) was significantly increased (42.1±4.8 vs 28.5±5.0 in C). Plasma angiotension II (AII) levels (pg/ml) were 54±47 in PAC and 30±15 in C (NS). Glomerular angiotensin receptor density (pmol/mg protein) were 933±273 in PAC and 1034±217 in C (NS). Kd was also the same in PAC and C. In PAC rats LS diet did not significantly alter MAP in CA and FA, GFR and filtration fraction. Plasma AII was increased significantly and to the same extent in C and PAC during low salt (LS) diet. In C rats LS and HS diet caused a down and up regulation of AII receptors respectively. This was not seen in PAC rats (HS diet accomplished by replacing drinking water with normal saline for 10 days). In PAC rats orally treated with converting enzyme inhibitor (CEI) from the time of constriction the MAP rise was significantly attenuated (153±26 in CA and 134±20 in FA). PAC rats treated with CEI had the same GFR and filtration fraction as C rats. The results suggest that renal hypertension following aortic constriction is due to an increase in renal vascular resistance beyond the afferent arteriole which is dependent on local AII production and independent of Na balance.

IMPAIRED ENDOTHELIUM-DEPENDENT RESPONSES IN RESISTANCE ARTERIES OF HYPERTENSIVE RATS. Dennis Diederich, Zhihong Yang*, Fritz R. Bühler* and Thomas F. Lüscher. Dept. of Research and Medicine, University Hospital, Basel/Switzerland

Endothelial cells can modulate vascular tone by releasing endothelium-derived relaxing (EDRF) and contracting (EDCF) factors. Alterations in EDRF and/or EDCF in hypertension could contribute to increased vascular resistance. Mesenteric resistance arteries (200 μ in diameter) of Wistar Kyoto (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) were suspended in a myograph filled with physiological salt solution (37°C) and aerated with 95% O_2 and 5% CO_2. In rings contracted with norepinephrine, acetylcholine (ACh; 10^{-9}-10^{-4}M) evoked endothelium-dependent relaxations (84± 4%, IC_{50} 7.3± 0.1; n=31). Hemoglobin (10^{-5}M) but not meclofenamate (10^{-5}M) reversed the relaxation induced by ACh delineating EDRF as the mediator. Nitric oxide (NO; 5x 10^{-9}-10^{-5}M) induced comparable relaxations as ACh (88±3%). In SHRSP, endothelium-dependent relaxations to ACh were impaired (54± 4%, n=24; $p<0.05$). Relaxations to NO were comparable in WKY and SHRSP. Meclofenamate but not the thromboxane synthetase inhibitor CGS-13080 normalized endothelium-dependent relaxations to ACh in SHRSP. Thus: (1) Endothelium-dependent relaxations are mediated by EDRF (i.e. nitric oxide) in resistance arteries; (2) the relaxations are blunted in SHRSP; (3) the blunted relaxations in SHRSP are not due to a decreased release of EDRF, but rather to the concomitant release of a prostanoid-like EDCF.

RENAL FUNCTION AND SYSTEMIC BLOOD PRESSURE ALTERATIONS AFTER EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY IN A CANINE MODEL. Erdal Erturk,* Nicholas T. Stowe, Stevan B. Streem,* Joseph V. Nally, Gordon N. Gephardt,* Ronald Lorig,* Andrew C. Novick. Cleveland Clinic Foundation, Cleveland, OH.

Although extracorporeal shock wave lithotripsy (ESWL) is being advocated for treatment of upper urinary tract stones, some concern exists regarding its long term effects on kidney function and elevation of blood pressure. In this study, kidneys of anesthetized, uninephrectomized dogs (N=6) were subjected to 3000 shocks at 18KV of power with the Dornier HM3 unit. Systemic blood pressure, plasma renin activities, and inulin clearance were measured before, and at days 2 and 30 after ESWL. In 4 of 6 experiments, mean systemic blood pressure increased by an average of 17±3 mmHg ($P<.05$) at 48 hours and decreased in 2. Inulin clearance decreased in the first 48 hrs following ESWL from a control value of 23.9±5.0 ml/min to 10.9±4.5 ml/min ($p<.05$) with a return to control values, 25.7±4.7 ml/min at 30 days (n=4). In three experiments, plasma renin activities were 1.2±0.1 ng/ml/hr before ESWL, 3.2±0.4 ng/ml/hr at day 2, and 1.7±0.2 ng/ml/hr at 30 days. MRI of the kidneys (n=3) was normal in one, with subcapsular fluid collection in another, and intraparenchymal bleeding in the third.

In conclusion, transient decreases in glomerular filtration rate with elevations of plasma renin activity and systemic blood pressure were observed following ESWL in this model.

DISSOCIATION OF HYPOGLYCEMIC AND ANTINATRIURETIC RESPONSES TO INSULIN (INS) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). C.D. Finch*, G.M. Davis, J.D. Bower, and K.A. Kirchner, Univ. of Mississippi Med. Ctr., Jackson, MS.

SHR have reduced peripheral INS sensitivity. To determine whether SHR also demonstrate reduced response to INS antinatriuretic effect, urinary sodium excretion was determined during sustained (3% BW) volume expansion in SHR and Wistar-Kyoto (WKY) rats (N=8/group) before and during euglycemic INS administration (85 mU/kg load and 8 mU/kg/min). SHR and WKY time controls received the vehicle for INS administration. Mean arterial pressure (MAP) was greater (160±6 vs 112±4 mm Hg; P<.001) and inulin clearance (C_{IN}) less (897±66 vs 1291±91 l/min/gKW; P<.01) in SHR than WKY prior to INS infusion. Initial fractional sodium excretion (FeNa) was not different between groups. INS reduced (P<0.02) FeNa from 0.59±0.17 to 0.15±0.06% in SHR and reduced (P<.005) FeNa from 0.86±0.17 to 0.32±0.08 in WKY rats. FeNa was unchanged in SHR and WKY time controls. The percent reduction in sodium excretion produced by INS was similar in both rat (SHR: 76±8% vs WKY: 53±11%; P-NS). MAP and C_{IN} during INS infusion were not different from respective baseline values in either group. Glucose requirement to maintain euglycemia was greater (P<.02) in WKY than SHR (15.4±2.5 vs 4.1±2.6 mg/min/kg BW). We conclude (1) while SHR have reduced sensitivity to the hypoglycemic effects of INS, the antinatriuretic response of SHR to INS is not different from WKY's; (2) acute INS infusion does not increase MAP in either SHR or WKY rats.

ARTERIAL PRESSURE AND RENAL FUNCTION DURING CHRONIC HYPERINSULINEMIA. John E. Hall, Thomas G. Coleman*, and H. Leland Mizelle*. Dept. Physiology & Biophysics, Univ.Miss.Med.Ctr., Jackson, MS

Although hyperinsulinemia has been postulated to cause obesity-associated hypertension, the chronic effects of increased insulin (INS) levels on mean arterial pressure (MAP) and renal function have not been reported. The aims of this study were to determine whether hyperinsulinemia, comparable to that found in obesity hypertension, elevates MAP or potentiates the hypertensive effects of angiotensin II (ANGII). Studies were conducted in conscious dogs with kidney mass reduced by 2/3rds in order to increase their susceptibility to hypertensive stimuli. In 7 dogs maintained on a Na intake of 319 mEq/day, iv INS (1.0 μU/kg/min) for 28 days (d), with plasma glucose held constant by iv glucose infusion, raised fasting INS from 9.9±1.5 to 50-79 μU/ml (x̄, 28 d) but did not increase MAP which averaged 106±2 and 102±2 mmHg during control and 28 d of INS infusion, respectively. Urinary K excretion decreased from 75.5±2.3 to 31-42 mEq/day during the first 7 d of INS infusion and then returned toward control. INS also caused transient Na retention followed by renal "escape" that was associated with increased GFR (13-27%). PRA and plasma aldosterone were not altered by INS. In 2 dogs infused with ANGII (2.0 ng/kg/min) to cause mild hypertension, INS infusion (1.0 μU/kg/min) for 28 d did not increase MAP further. Thus, chronic hyperinsulinemia did not elevate MAP, even when kidney mass was reduced, and did not potentiate the hypertensive effects of ANGII. Apparently, additional factors besides hyperinsulinemia are responsible for obesity-associated hypertension.

RENAL ARTERY STENOSIS AND ERYTHROPOIETIN P. Grutzmacher, H. W. Radtke, R.A.K. Stahl, K. Rauber*, W. Schoeppe.Depts.Nephrol.,Radiol.* Univ. Hosp., Frankfurt, FRG

Renal artery stenosis (St) has been claimed to induce polyglobulia by enhanced secretion of erythropoietin (EPO).

To evaluate its clinical relevance. we studied peripheral and renal venous EPO (fetal mouse liver cell assay) and renin levels (RIA) in 37 consecutive pts out of a series of 66 hypertensive pts with St. Pts with unilat. St were subdivided according to the severity of St (gr. I: St < 50%, gr. II: St > 50% with a renal vein renin ratio < 1,5, gr. III: St > 50% with a renin ratio > 1,5). Compared to 36 healthy controls (C), periph. EPO levels were moderately elevated (St: 50 ± 38 vs C 25±16 U/l, p < 0,001).EPO levels in both renal veins were equal in gr.I (St 41±29 vs contralat. kidney 44±26 U/l), whereas in gr.II and III EPO levels were increased in the stenotic side (gr.II: 60±28 U/l; gr. III: 111±67 U/l; p < 0,03). Peripheral EPO level increased correspondingly (gr.I:35±23 U/l; gr.II:45±28 U/l; gr.III:76±52 U/l, p=0,001).

Borderline polyglobulia was observed in 6/66 pts. However mean Hct, Hb and RBC did neither differ among the subgroups nor from C.(Hct: m: St 44±6 vs C 45±2%, f: St 41 ±5 vs C 43±2%).

Data indicate, that renal artery stenosis increases the secretion of EPO. This is demonstrable only in stenoses exceeding 50%, but already in the absence of renin hypersecretion. Unilateral EPO hypersecretion results only in a moderate increase of peripheral EPO level,which is probably too moderate to induce true renal polyglobulia.

EFFECTS OF ANTIHYPERTENSIVE AGENTS ON VASCULAR RESPONSES. L. Hartich*, P. Shultz, J. Tolins, K. Coffee*, L. Raij. VAMC and U. of Minnesota, Minneapolis, MN.

We have shown that in hypertension (HPN) both endothelium dependent (EN-D) as well as independent (EN-I) responses to vascular relaxants are depressed, but improve after anti-HPN therapy. In these studies we investigated whether anti-HPN agents themselves affect EN-D and/or EN-I responses to agonists of vascular relaxation. Normotensive Sprague- Dawley rats were given for 2 weeks either tap water (W) or W containing captopril (CAP) enalapril (EPL) or hydralazine (HYZ) in concentrations shown to be equihypotensive in hypertensive rats; these concentrations did not significantly lower BP of normotensive rats. Aortic rings (with and without EN) were suspended in organ chambers for isometric tension recording. The % relaxation induced by the EN-I agent Na nitroprusside (10^{-8}M) was significantly but similarly enhanced in rats given CAP, EPL and HYZ compared with rats given W (75 ±8%, 81±6% and 80±6% vs 50±5%, respectively, p < 0.05). In all rats acetylcholine (Ach), 10^{-9}-10^{-4} M, caused relaxations in rings with, but not in those without, EN. EN-D relaxations were significantly enhanced in CAP rats but not in W, EPL or HYZ rats: the concentration of Ach (-log M) required to evoke 50% relaxation (IC50) was reduced in rats given CAP (7.9±0.1) vs W (6.2±0.2), HYZ (7.1±0.2) or EPL (6.8±0.2, p<0.05). Indomethacin 10^{-5}M did not prevent the effect of Ach while both pyrogallol 10^{-4} M and Hb 10^{-5} M, inhibitors of the endothelium derived relaxing factor NO, inhibited Ach induced relaxations. These studies suggest that anti-HPN agents may differ in their ability to affect vascular relaxations in response to EN-D and EN-I agonists. This may influence the overall beneficial effect of anti-HPN agents in cardiovascular pathology.

COMPARISON BETWEEN EFFECTS OF CALORIE RESTRICTION AND CAPTOPRIL IN NZB x W MICE. Hans Herlitz*, Christian Svalander*, Andrej Tarkowski* and Gunnar Westberg, Univ. of Göteborg, dept. of Nephrology and Pathology, Sahlgren's Hospital, Göteborg, Sweden.

Both calorie restriction and treatment with the ACE-inhibitor Captopril have been shown to elicit beneficial effects in mouse strains that develop a disease similar to systemic lupus erythematosus (SLE). To compare the two treatments in NZB x W mice with respect to survival, BP, weight development, proteinuria, hematuria and renal histopathology, 3 groups of animals were studied: Control (group A, n=15); Captopril 30 mg/kg (group B, n=10); and calorie restriction with alternate day feeding (group C, n=15). All treatments were started at the age of 2 months. Tail systolic BP was measured with a strain gauge technique. The survival at 24 months in groups A-C was 15, 40 and 52% respectively. Systolic BP after 12 months treatment was 143±2, 128±2 and 141±2 mmHg (mean±SE). Body weights after 2, 9 and 18 months were 23,1±0,5, 34,2±0,5 and 40,1±1,8 g (A), 23,9±0,7, 30,1±0,9 and 32,8±1,3 g (B) and 22,7±0,5, 31,4±0,6 and 34,5±1,1 g (C). Proteinuria $\geq$ 2+ and hematuria $\geq$ 2+ occurred in 71 and 27% in group A, 0 and 0% in group B, and 37 and 30% in group C. General proliferative glomerular changes were found in 69, 50 and 54% in groups A-C.

It is concluded that calorie restriction and Captopril treatment both tend to improve survival and renal histopathology in NZB x W mice. Captopril alone decreased BP and the incidence of proteinuria.

RENAL FUNCTION CURVE(RFC) AND INTRARENAL HEMODYNAMICS IN ESSENTIAL HYPERTENSION(EHT). M Imanishi*, G Kimura, T Sanai*, Y Kawano*, S Kojima*, K Yoshida*, H Abe*, T Ashida*, H Yoshimi*, M Kawamura*, M Kuramochi* and T Omae*. Dept. of Medicine, Natl. Cardiovascular Ctr., Osaka, Japan

Interrelationships between RFC(pressure-natriuresis relationship) and intrarenal hemodynamics were investigated to clarify the entity of RFC.

Two week studies were performed in 33 patients with EHT who were given a regular sodium diet in the 1st week and a sodium-restricted diet in the 2nd week. Intrarenal hemodynamics were estimated using clinical data such as renal clearances by Gomez's equations. RFC was drawn by plotting $U_{Na}V$ on the ordinate as a function of MAP on the x-axis. The extrapolated x-intercept (A: 109±2 mmHg) of RFC was strongly correlated in a 1:1 fashion with the sum of the arterial pressure drop from the aorta to glomerulus plus the opposing pressures against glomerular filtration($r=0.7$, $p<0.001$), suggesting the difference between MAP and A represents the effective net filtration pressure. The gross filtration coefficient(k_F) of glomerular capillaries was estimated as 0.154±0.018 [ml/s]/mmHg, very close to the normal value, suggesting k_F is not affected in EHT, and was strongly correlated($r=0.9$, $p<0.001$) with the slope of RFC(19.2±2.8 [mEq/day]/mmHg), the reciprocal of which is the sodium sensitivity index.

Major characteristics of RFC, the x-intercept and slope, could be explained mainly by the intrarenal hemodynamic parameters, the afferent arteriolar resistance and k_F, respectively, in EHT.

RENAL DENERVATION PREVENTS NATRIURESIS AFTER ACUTE UNILATERAL NEPHRECTOMY (AUN) BY REDUCING BASAL AND STIMULATED PLASMA IMMUNOREACTIVE (IR) γ-MSH CONCENTRATION. M.H. Humphreys, S-Y. Lin,* and E. Wiedemann,* Divisions of Nephrology and Endocrinology, San Francisco General Hospital and UCSF, San Francisco, CA.

AUN initiates a neurocirculatory reflex which results in natriuresis from the remaining kidney through an increase in the plasma concentration of the natriuretic peptide γ-MSH. Denervation (DNX) either of the kidney to be removed (ipsi) or of the remaining kidney (contra) prior to AUN prevents the postnephrectomy natriuresis. In 8 rats undergoing sham AUN, sodium excretion ($U_{Na}V$) did not change and plasma IR-γ-MSH concentration was 74±21 (SD) fmol/ml. In 20 rats subjected to AUN, $U_{Na}V$ increased from 895±451 to 2334±1005 neq/min ($p<.001$) and IR-γ-MSH was 104±31 fmol/ml ($p<.05$ vs sham). In rats with unilateral renal DNX, sham AUN had no effect on $U_{Na}V$ from either kidney, but IR-γ-MSH activity was reduced to 36±23 fmol/ml ($p<.002$ vs. sham AUN in innervated rats). After ipsi DNX, AUN caused no increase in contra $U_{Na}V$ (929±340 to 952±386 neq/min) and IR-γ-MSH was again reduced to 27±24 fmol/ml ($p<.001$ vs AUN in innervated rats). After contra DNX, AUN also had no effect on $U_{Na}V$, 2327±860 to 2499±888 neq/min, and IR-γ-MSH likewise was reduced to 19±25 fmol/ml ($p<.001$).

These results indicate that unilateral DNX blocks postnephrectomy natriuresis by preventing the increase in plasma IR-γ-MSH caused by AUN. DNX also lowers basal IR-γ-MSH levels. These observations consequently suggest that renal afferent nerve input is necessary for both basal and stimulated plasma IR-γ-MSH concentration.

MECHANISM OF ACE-INHIBITION INDUCED CHANGES ON HIPPURATE RENOGRAPHY IN THE TWO-KIDNEY ONE CLIP (2K-1C) HYPERTENSIVE DOG. GJ Jonker*, D de Zeeuw*, RM Huisman*, GK van der Hem* (Intr. by LW Statius van Eps). Univ. Hosp. Groningen, The Netherlands.

ACE-inhibition (ACEi) improves the sensitivity of I-123 hippurate renography for the detection of a renal artery stenosis (RAS) by delaying the excretion of the tracer on the stenotic side. To test the mechanism of this, we studied (apart from renography) functions of both kidneys separately in 5 conscious chronically-instrumented 2K-1C dogs before and after ACEi. Urine of both kidneys was collected via an externally controllable ureter device (deviating only during the experiments). Experiments (n=9) were performed 2 weeks after induction of a mild RAS. After 2-h equilibration, three 20-min control periods were followed by three 20-min periods after 10 mg enalaprilic acid i.v., measuring urine flow (UV), renal blood flow by flow probe (RBF), GFR and ERPF by constantly infused radiolabeled iothalamate and hippurate.

	Stenotic side		Non-stenotic side	
	control	ACEi	control	ACEi
GFR (ml/min)	39±5	7±3*	49±5	53±4
ERPF(ml/min)	111±13	34±17*	128±15	176±14*
UV (ml/min)	19±4	1±.4*	28±7	41±9
RBF (ml/min)	191±19	233±30	-	-

Thus, UV and GFR fell dramatically on the stenotic side. RBF remained stable, whereas ERPF fell. Repeating the experiments during isotonic mannitol infusion prevented the ACEi-induced fall in UV. ERPF now rose in parallel with RBF, and GFR only fell slightly (35%). These results suggest that ACEi-induced alterations of the hippurate renogram in RAS are due to the nearly halting of UV, and may not reflect true changes in RBF and/or GFR.

ENDOGENOUS DOPAMINE (DA) REGULATES RENAL SODIUM TRANSPORT IN NORMOTENSIVE BUT NOT HYPERTENSIVE RAT. Pedro A. Jose, Peter Cody*, and Gilbert M. Eisner. Georgetown Univ. Med. Ctr., Dept. Peds. & Physiol. & Biophysics, Washington, DC

The DA-1 agonist SKF 38393 (SKF) induces a natriuresis in normotensive, Wistar Kyoto (WKY) but not in hypertensive, Aoki-Okamoto rats (SHR) (Kidney Int 33:306,1988). We studied the role of endogenous renal DA on sodium excretion by infusing a selective DA-1 antagonist, SCH 23390 (SCH), into the renal artery of inactin anesthetized WKY (n=6) or SHR (n=6). In WKY, SCH blocked the natriuretic effects of SKF on a mol/mol basis. SCH (1.2 ng/g kidney/min) infused by itself into the renal artery did not affect mean arterial pressure (MAP), glomerular filtration rate (GFR), or absolute ($U_{Na}V$) or fractional (FeNa) sodium excretion in WKY. The infusion of SCH at 120 ng/g kidney/min was chosen since this should result in the occupation of 96% of renal DA-1 receptors (radioligand binding studies). The results (M±SEM) are:

Rat	MAP ul/min/g	GFR ul/min	$U_{Na}V$ uEq/min	FeNa %
WKY(control)	119±5	420±20	3.0±.2	5.5±.2
(SCH)	119±5	411±24	2.6±.1*	4.9±.1*
(Recovery)	114±6*#	416±24	2.3±.2*#	4.3±.3*#
SHR(control)	161±3	496±21	2.8±.3	4.0±.4
(SCH)	161±3	459±22*	2.9±.3	4.5±.3*
(Recovery)	153±3*#	414±48	2.4±.4	4.0±.3#

*=p <.05 vs control, #=p <.05 vs SCH 23390, ANOVA with replications, Scheffe's test or paired t test with Bonferroni correction. Each period is 40 min. The effects of SCH persisted for at least 40 min after the drug was discontinued. **Conclusion**: Endogenous DA regulates renal sodium transport in normotensive but not hypertensive rats. These results taken together with our previous report that exogenous DA-1 agonist increased sodium excretion in WKY but not in SHR in the face of similar DA-1 receptor density and affinity suggest a defective post DA-1 receptor transduction in SHR.

EFFECTS OF DIETARY FAT ON RED CELL MEMBRANE FLUIDITY (r), ATPase ACTIVITY, AND BLOOD PRESSURE (BP) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Njeri Karanja*, Anna W. Sasaki*, Henry R. Thompson*, David A. McCarron. Oregon Hlth. Sci. Univ. Portland, Oregon.

Dietary fats may modulate BP by influencing cell membrane lipids and bound-enzymes that regulate transcellular ion distribution. The relationship between BP, r, and the activities of the Mg^{2+}, Na^+/K^+, and both basal and calmodulin-stimulated Ca^{2+}-ATPases were examined in 31 SHR fed 20% menhaden oil (MO), butterfat (BF) or corn oil (CO) from 5-24 wk of age. Mean ATPase activities (nmol Pi/mg protein/min) and r were:

	Mg^{2+}	Na^+/K^+	Basal Ca^{2+}	Activ. Ca^{2+}	r
MO	37.1±2.6	38.7±4.7	17.0±3.0	75.9±3.7	0.110±0.02
BF	21.0±2.7	33.3±2.8	10.9±1.5	53.3±4.8	0.070±0.01
CO	30.0+4.5	39.9+3.6	12.5+2.8	72.4+2.1	0.106+0.01

Mg-ATPase was higher in MO relative to BF ($p<.04$) and CO ($p<.018$). Activated Ca-ATPase was high in MO relative to BF ($p<0.001$) and in CO relative to BF ($p<0.007$) but did not differ between CO and MO. Basal Ca and Na/K activities did not differ among diets. Fluidity was highest in BF compared to CO and MO diets. Compared to the CO-fed animals, who had a continuous rise in BP, ($p<.001$), the MO and BF animals exhibited BP attenuation. MO animals had the lowest BP. In contrast, the effects on membrane ion transport and fluidity were virtually identical for MO and CO, and divergent for BF. These results suggest that the type of dietary fat will effect BP; however, the concurrent influences on membrane ion properties do not necessarily parallel the BP responses.

RENAL RESPONSES TO INTRAVENOUS INFUSION OF KAPPA OPIOID AGONIST, KETOCYCLAZOCINE, IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS (SHR). D.R. Kapusta, S. Y. Jones* and G.F. DiBona. Dept. Int. Med., U. Ia. Col. Med. & VAMC, Iowa City, IA 52242

Changes in renal function were examined during intravenous (i.v.) infusion of the kappa opioid agonist, ketocyclazocine (KC), in conscious SHR (n = 8). Renal sympathetic nerve activity (RSNA) was measured as integrated voltage and expressed as percent of control. Urine was collected during i.v. infusion of isotonic saline control (20 min.) and the kappa agonist KC (20 μg/kg/min) consecutive 10 min. samples) for glomerular filtration rate (GFR), renal plasma flow (RPF), urinary flow rate (V) and urinary sodium excretion ($U_{Na}V$). Results: KC significantly increased mean arterial pressure (MAP) (166±5→182±8 mmHg) and heart rate (408±7→ 467±8 bpm) for 30 min. Values are means ± S.E. for data collected during control, 40, 70 and 100 min. after start of KC infusion. ° per gram kidney weight; * p < 0.05 vs control.

	GFR° ml/min	RPF° ml/min	V° μl/min	$U_{Na}V$° μeq/min	RSNA %
control	1.39±.09	4.90±.29	17±3	2.38±.45	100
40 min	0.99±.04	4.36±.42	43±4*	0.39±.10*	182±12*
70 min	0.87±.05	4.57±.21	43±5*	0.18±.04*	176±6*

Naloxone (50 μg/kg i.v.) restored $U_{Na}V$ and RSNA but not V to control level. Significant increases in V and decreases in UNaV were obtained in rats with bilaterally denervated kidneys. Conclusion: The kappa opioid agonist KC produces a diuresis and antinatriuresis independent of changes in renal hemodynamics; naloxone blocks the antinatriuresis but not the diuresis. The effects on renal sodium handling appear independent of changes in RSNA.

A CALCIUM ENTRY BLOCKER, NICARDIPINE(N), DILATES AFFERENT ARTERIOLE IN ESSENTIAL HYPERTENSION(EHT) G Kimura, F Deguchi*, S Kojima*, T Ashida*, H Yoshimi*, H Abe*, Y Kawano*, K Yoshida*, M Kawamura*, M Imanishi*, M Kuramochi* and T Omae*. Dept. of Medicine, Natl. Cardiovascular Ctr., Osaka, Japan

Effects of Ca-entry blocker, N, on intrarenal hemodynamics were studied in EHT.

Four-week studies were performed in 8 patients with EHT who were given a regular sodium diet in the 1st & 3rd weeks, and a sodium restricted diet in the 2nd & 4th weeks. 60 mg/day of N was administered in the 3rd & 4th weeks. Afferent (R_A) and efferent(R_E) arteriole resistances, and glomerular pressure(P_G) were calculated using Gomez's equations. $U_{Na}V$ was plotted on the ordinate as a function of MAP before and after administration of N. Assuming the difference between MAP and the x-intercept of this renal function curve represents the effective filtration pressure, R_A, P_G and gross filtration coefficient(k_F) were also calculated.

Although MAP on a regular salt diet was reduced from 125±3 to 109±2 mmHg by N, RBF (670±40 ml/min) and GFR(79±2 ml/min) were not altered. R_A was estimated to be reduced from 9300±900 to 7300±700 dyns.sec.cm^{-5}, while no changes in R_E(4900±400 dyns.sec.cm^{-5}), P_G(50±1 mmHg) and k_F(0.180±0.041 [ml/sec]/mmHg). Estimations by analyzing the renal function curve were consistent with those by Gomez's equations.

EHT has been characterized by a prominent increase in R_A, resulting in P_G kept normal. Ca-entry blocker restores intrarenal hemodynamics in EHT by dilating afferent arteriole alone. The reason why RBF and GFR were not increased by N seemed due to a parallel reduction in MAP.

DEFECTIVE DOPAMINE-1 (DA-1) RECEPTOR-ADENYLATE CYCLASE (AC) COUPLING IN MICRODISSECTED PROXIMAL CONVOLUTED TUBULE (PCT) FROM SPONTANEOUSLY HYPERTENSIVE RAT (SHR). Shohei Kinoshita* and Robin A. Felder* (intr. by Pedro A. Jose), University of Virginia Medical Center, Charlottesville, VA

SHR retains sodium despite high renal concentrations of DA. DA-1 agonists decrease sodium transport in PCT of normotensive Wistar Kyoto rats (WKY). The intrarenal arterial infusion of DA-1 agonist induces a natriuresis in WKY but not in SHR. To determine a mechanism for the apparent lack of natriuretic effect of DA-1 agonist in SHR, we studied the DA-1 receptor in the microdissected PCT from SHR and WKY by autoradiographic methods and by measuring AC activity. Specific binding of the DA-1 antagonist ^{125}I-SCH 23982 (defined by 10 uM SCH 23390, a DA-1 antagonist) to PCT was concentration dependent and saturable in SHR and WKY. Saturation isotherm analysis yielded similar dissociation constant (K_d, 70.7 ± 5.1 and 55.3 ± 4.6 nM) and maximum receptor density (B_{max}, 17.5 ± 3.3 and 15.7 ± 3.6 fmol/mg protein) in 24 wk old SHR (n=5) and WKY (n=5) respectively. DA-1 receptor K_d and B_{max} were also similar in 18 wk old SHR and WKY. Basal PCT AC activity was similar in WKY (58.7 ± 21.2 fmol/3 mm PCT/20 min, n=5) and SHR (64.2 ± 10.9, n=5). In WKY, the novel DA-1 agonist SND-919 (1 uM) increased PCT AC activity by 40%. Fenoldopam (F) another DA-1 agonist increased PCT AC activity in a dose related fashion with significant increases noted at 100 nM F in WKY (n=5). In contrast, in SHR, F had no effect on AC activity at 100 nM but did increase AC activity at 10 uM (n=5). The stimulatory effect of F (10 uM) on PCT AC activity (M ± SE) was less in SHR (5.4 ± 1.5 fmol/3 mm PCT/20 min, n=5) than in WKY (14.6 ± 3.1 fmol/3 mm PCT/20 min, n=5) ($p < .05$). In WKY but not in SHR, F-stimulated AC activity in PCT was blocked by the DA-1 antagonist, SCH 23390 (n=4), but not by the beta adrenergic antagonist, (-)-propranolol (n=4) demonstrating specificity.

Conclusion: These data suggest a DA-1 second messenger coupling defect in the PCT of the SHR. This may explain in part the lack of natriuresis following intrarenal DA-1 agonist infusion in SHR.

Correlates of "Prehypertension". JM Kotchen*, G Guthrie Jr*, P Taylor*, M Berk*, M McFadden*, C Ott, T Kotchen. WVU Medical School, Morgantown, WV

Hemodynamic and hormonal correlates of "prehypertension" were studied in white men, age 31± 0.4SE years, who have participated in a blood pressure (BP) study since adolescence in 1973. From 1140 subjects, 9 males with BP consistently in the upper quartile for BP (HBP) and 10 from the lower half the BP distribution (LBP) were admitted for study. Subjects consumed a high salt diet (250 meq Na+) for 3 days followed by 80 mg furosemide and a 10 meq Na+ diet. Body weights of the two groups were similar. With salt depletion, BP decreased ($p<0.05$) comparably in HBP (130±4/91±3 mmHg to 124±3/89±4) and LBP (115±3/76±2 to 110±2/80±2). Group negative Na+ balance and extracellular volume (inulin space) reduction were the same. Cardiac output (doppler) did not differ in HBP (5.8±0.6 l/min) and LBP (5.5±0.6) and was not affected by salt depletion. Plasma norepinephrine (NE) was similar in HBP (1.36±0.18 nM/l) and LBP (0.92±0.18) and increased ($p<0.05$) comparably in response to stimulation by nitroprusside and lower body negative pressure. On high salt, PRA in HBP (0.8±0.1 ng/ml/hr) was lower ($p<0.05$) than in LBP (1.5±0.3); plasma aldosterone (aldo) was also lower ($p<0.01$) in HBP (3.2±0.6 ng/dl) than in LBP (8.4±0.8). Aldo responses to graded infusions of angiotensin II (A-II) were less ($p<0.01$) in HBP, although pressor responses did not differ. We conclude that salt sensitivity, cardiac output, and neural activity (stimulated NE) are similar in young adults with sustained HBP and LBP. HBP is associated with decreased PRA, aldo, and aldo responses to A-II. Low PRA and aldo may be a consequence of sustained HBP since childhood.

SIMPLIFIED SCINTIGRAPHIC DETECTION OF RENOVASCULAR HYPERTENSION (RVH). RT. Kopecky, FD. Thomas,* JG. McAfee,* D. Patchin,* B. Hellwig,* M. Roskopf.* SUNY Health Science Center, Depts. of Medicine and Radiology, Syracuse, NY.

Captopril (C) enhanced Tc-DTPA renography can detect RVH, but 2 scans (pre & post-C) and several hrs are needed. We evaluated alternate protocols using iv enalaprilat (E) in RVH rats (sys BP>150) 3-6 wks after left renal artery clipping.

Protocol 1: Sequential renograms using 150 and 1500 μCi Tc-DTPA were done in 75 min. E (30 μg/kg iv) or C (3 mg/kg ip) was given prior to second scan. Stenotic kidney (SK) & normal kidney (NK) DTPA clearance (C_{DTPA}) was derived from regression equations relating plasma clearance to renal uptake from .5-1.5 min, in ml/min/100 g, ±SEM,n=16. C reduced SK C_{DTPA} (.32±.02 to .20±.03, p<.001) and SK/NK ratio but not NK C_{DTPA} (.40±.03 to .41±.03, NS). E reduced SK C_{DTPA} (.31±.04 to .19±.03, p<.005) but NK C_{DTPA} also fell slightly (.43±.03 to .35±.04, NS) thus SK/NK ratio did not fall significantly. E also reduced BP transiently while C did not (Hyperten 10:181,1987).

Protocol 2: E (30 μg/kg) was given to furosemide pretreated RVH rats 5 min after 200 μCi Tc-DMSA. Rate of DMSA uptake (% dose/min) into SK & NK was determined by linear regression and expressed as SK/NK ratio for period before (0-5 min) and after (5-10 min) E. Without E (± furosemide) SK/NK ratios were similar before and after 5 min. With E, SK/NK ratio fell from .75±.08 at 0-5 min to .51±.09 at 5-10 min (p<.02,n=15). In RVH rats: 1) sequential Tc-DTPA renograms done in only 75 min can detect RVH. C is superior to E, since systemic BP effects of E impair NK as well as Sk GFR 2) a single Tc-DMSA renogram can detect RVH by comparing SK to NK DMSA uptake before and after E.

REGULATION OF INTRACELLULAR pH (pH_i) BY AORTIC VASCULAR SMOOTH MUSCLE CELLS (VSMC) DURING RECOVERY FROM AN ACID LOAD. M. LaPointe* and D.C. Batlle, Northw. Univ. and Lakeside VA, Chicago, IL.

pH_i may be involved in VSMC contractility. We sought to examine the acid extruding mechanism(s) that participate in pH_i regulation by rat aortic VSMC during an acid load. Serum starved VSMC's in subculture (2-7th passage) were loaded with BCECF and then acid-loaded with nigericin to a pH_i of ≈ 6.50 in the presence of external HCO_3 or in its absence (HEPES). pH_i recovery was continuously monitored over a 10 min. interval by measuring BCECF fluorescence at two excitation wavelengths (500/440 nM) as to calculate the increase of pH_i towards normal (ΔpH) (Table). *p<0.05 as compared to HCO_3, otherwise NS.

	Controls		Na Free		EIPA	
	1 min	10 min	1 min	10 min	1 min	10 min
HCO_3	0.34±0.05	0.78±0.04	-0.02±0.03	0.12±0.06	0.08±0.02	0.55±0.04
HEPES	0.29±0.07	0.76±0.13	-0.05±0.03	0.17±0.06	-0.02±0.06	0.28±0.10*

The initial rate of pH_i recovery (1 min) was comparable in the presence and in the absence of HCO_3, was completely Na-dependent and EIPA sensitive, and accounted for about 40% of the complete recovery seen after 10 min. About 80% of the complete pH_i recovery was Na-dependent both in the presence and absence of HCO_3 but was only partially obliterated by Na+/H+ inhibition by EIPA (to 70 and 37% of control in HCO_3 and HEPES buffer, respectively). We conclude that 1) Na+/H+ exchange can fully account for the initial rapid phase of pH_i recovery from an acid load; 2) complete pH_i recovery, in the presence of external HCO_3, is largely mediated by a Na-dependent transporter(s) other than the Na+/H+ exchanger.

INFLUENCE OF LOW DIETARY K ON KIDNEY FUNCTION AND BLOOD PRESSURE IN BORDERLINE HYPERTENSIVE (BHT) AND NORMOTENSIVES (NT). WJ Lawton, AE Fitz, CA Sinkey,* RA Coleman*. Int. Med., VA Med. Ctr. and U of Iowa, Iowa City, IA.

We postulated that Lo K/Hi NaCl diet would enhance our previously shown NaCl-induced increase in diastolic blood pressure (DBP) in BHT during upright posture. NT and BHT white men (18-35 yrs) received a 30mEq K/400mEq Na (Lo K) and 100mEq K/400mEq Na (Hi K) diet. On Day 5 of diet, U calcium (Ca) (mg/24h) in NT=320±62 (Lo K) and 197±50 (Hi K),p<.05. U Ca in BHT=367±19 (Lo K) and 285±20 (Hi K),p<.01. DBP, renovascular resistance (RVR) and UNa (μEq/min) were measured supine (SUP) and ½h standing (UP). $\bar{x}$±SE; *=p<.05, #=p<.01 (Hi vs Lo K)

	NT (n=8)		BHT (n=8)	
	Lo K	Hi K	Lo K	Hi K
Weight	80±2.9	78.3±2.5	86.4±3.9	84.7±4.1*
Hematocrit	40.1±1.0	42.7±1.2*	40.8±1.0	41.8±1.9
DBP-SUP	81±1	79±1	82±2	82±2
DBP-UP	81±3	71±3#	89±3	84±3*
RVR-SUP	0.20±.01	0.20±.01	0.20±.01	0.18±.02
RVR-UP	0.26±.02	0.22±.01*	0.24±.02	0.24±.02
UNa-SUP	391±38	267±33*	364±26	328±23*
UNa-UP	302±88	186±19*	295±18	193±19#

Plasma renin activity (PRA), aldo, and norepinephrine(PNE) were not different between the 2 groups. Lo K vs Hi K diet augments the abnormal orthostatic increase in DBP in BHT and increases standing RVR in NT. Lo K is associated with weight gain, lowered hematocrit, increased 24h U Ca, and increased SUP UNa. Lo K effects are not related to PRA or PNE. The effects of Lo K diet appear to be mediated by volume expansion. Dietary K must be considered in treatment programs for hypertensives.

OSMOTIC ACTIVATION OF THE Na^+/H^+ EXCHANGER IN VASCULAR SMOOTH MUSCLE CELLS. John Lowe* and Harlan E. Ives. Div. of Nephrology, UCSF.

In many systems, osmotically-induced cell shrinkage leads to the activation of ion transport systems which cause cell uptake of ions and water with correction of cell volume. Na^+/H^+ exchange (amiloride-inhibitable ΔpH_i/min x 10^{-4}) was measured in BCECF-loaded, cultured vascular smooth muscle cells. Osmolarity of the medium was increased by 25 to 200 mOsm with various salts and non-electrolytes.

	25	50	100	200 mOsm
Sucrose	54	134	348	1434
NaCl	52	149	268	1180
Urea	51	121	201	590
Acetamide	27	51	161	228

Thus, activation of the Na^+/H^+ exchanger depended strongly on the permeability of the agent used and non-linearly on the tonicity of the medium. Responses to sucrose, urea, and acetamide were blocked by Na^+ removal. Our cultured vascular smooth muscle cells contain a Cl^-/HCO_3^- exchanger, but this transporter was not primarily activated by cell shrinkage, as sucrose did not induce acidification in Na^+-free, HCO_3^- containing solutions. Neither colchicine (1-10 μM), cytochalasin B (1-20 μM), nor lowering and buffering of cytoplasmic calcium with MAPTAM (40 μM) affected osmotic activation of Na^+/H^+ exchange. We conclude that vascular smooth muscle cells exhibit a mechanism for activation of Na^+/H^+ exchange that appears to depend on cell volume per se and not on cytoplasmic tonicity, cell calcium, microfilaments, or microtubule integrity.

MAGNETIC RESONANCE STUDY OF TWO-KIDNEY, ONE CLIP GOLDBLATT MODEL OF HYPERTENSION. JoAnn Lindenfeld*, Joseph I. Shapiro, Laurence Chan. Univ. of Colorado HSC, Denver, Colorado.

Magnetic resonance imaging (MRI) and spectroscopy (MRS) at 1.89 Tesla were used to evaluate the degree of hypertrophy and atrophy in two groups of rats made hypertensive by placing a silver clip on the left renal artery. At two weeks following placement of the clip all rats were hypertensive as measured by tail cuff (avg systolic pressure 194+16mm Hg). In the first group of rats (n=9) P-31 MRS at 32.6 MHz was used to determine changes in adenine nucleotides and intracellular pH (pHi). No changes in the steady-state ATP concentrations were observed, however pHi in the clipped kidney was 7.63+0.26 vs 7.30+0.34 in the unclipped hypertrophied kidneys (p<0.01). A second group of rats (n=6) were used for proton MRI to determine the degree of atrophy in the clipped kidney and hypertrophy in the contralateral kidney. There were significant differences in the areas of the clipped and unclipped kidneys - 0.94+0.35 vs 2.05+0.46 cm. (p<0.01). We conclude that significant differences in the clipped and unclipped kidneys can be detected in this model of renovascular hypertension using both MRS and MRI which suggests exciting possibilities in the evaluation of human renovascular hypertension.

ENDOTHELIN OVERRIDES ENDOTHELIUM-DERIVED RELAXING FACTOR IN HYPERTENSIVE RESISTANCE ARTERIES. Thomas F. Lüscher, Dennis Diederich, Zhihong Yang*, and Fritz R. Bühler* . Dept. of Research and Medicine Research, University Hospital, Basel/Switzerland

Endothelin is a new peptide. In large arteries, it is a potent vasoconstrictor. An altered response to endothelin in hypertension might contribute to increased peripheral vascular resistance. Mesenteric resistance arteries (200 μ) of Wistar Kyoto (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) were suspended in a myograph filled with physiological salt solution (37°C; 95% O_2/5% CO_2). In rings with and without endothelium, norepinephrine (NE), serotonin (5-HT) and potassium chloride induced similar maximal contractions. The ED_{50} value of endothelin (7.3±0.1) was 85-fold lower than that of NE (5.4±0.1) and 20-fold lower than that of 5-HT (5.9±0.1,n=7-11;p<0.005). Contractions to endothelin and NE were similar in WKY and SHRSP, while ED_{50} of 5-HT was 2-fold lower in SHRSP than in WKY. Acetylcholine (ACh;10^{-9}-10^{-5}M) induced endothelium-dependent relaxations which were inhibited by hemoglobin (10^{-5}M) but not meclofenamate (10^{-5}M) delineating endothelium-derived relaxing factor (EDRF) as the mediator. In normal rats, EDRF completely reversed contractions induced by endothelin (94±3%), while in SHRSP relaxations were blunted (42±8%;p<0.05). Thus: (1) endothelin is a potent vasoconstrictor of resistance arteries; (2) the contractions are unaltered in SHRSP, but (3) their reversal by EDRF is severely impaired indicating an imbalance between endothelium-derived constricting and relaxing factors in hypertension.

THE USE OF CAPTOPRIL (CP) IN THE SCREENING AND EVALUATION OF PATIENTS WITH RENOVASCULAR HYPERTENSION (RVH). Robert Merrill,* Peter Smolens. Dept of Medicine, UTHSCSA, San Antonio, Tx

The peripheral vein plasma renin activity (PRA) response to CP has been proposed as a useful test to discriminate RVH from other forms of hypertension. In addition, CP has been reported to be useful in amplifying the renal vein renin ratio (RVRR) and thereby help predict success of revascularization in patients (PTS) with unilateral renal artery stenosis(U-RAS). This maneuver is less useful in PTS with bilateral(B)RAS. We studied 17 patients with moderate to severe hypertension to determine the efficacy of the CP screening test to predict the presence of RVH. In addition we have evaluated the use of the combination of salt depletion and CP administration to see if this improves the ability of the renal vein renin ratio (RVRR) to predict the success of revascularization. Four PTS had U-RAS , 5 PTS had B-RAS and 8 PTS had no significant renal artery lesions(NRAS). Systolic(S) and diastolic (D)BP changes and PRA in salt replete PTS(U_{Na}> 40mEq/L)before and 1 hour after CP(50mg) were (±1SD):

	%ΔSBP	%ΔDBP	PRA (pre/post)
NRAS	11±4	6±6	2.81±3.70/9.01±8.38
RAS	15±8	18±7	11.1±15.7/35.5±39.3

Thus while ΔBP and PRAs were greater in the RAS group, the degree of scatter prevented the differences between the RAS and NRAS groups from being significantly different. Using the criteria of Muller et al (AJM 80:641,1986), 2/8 NRAS PTS had a + test and 2 others were borderline +. However, 8 of the 9 with RAS(including 2/3 with serum creatinine >1.5) were correctly identified. RVRR pre and 45 min post 25mg of CP in salt depleted (SD) PTS with (U) or (B) RAS is shown below along with BP response to revascularization (improved (I) or cured (C)):

RAS type	U	U	U	U	B	B	B	B	B
RVRR pre	1.2	1.8	1.6	1.5	17.4	1.1	2.7	2.0	2.3
RVRR post	1.1	1.0	2.0	1.4	12.9	2.2	2.0	2.3	1.5
BP resp		I	I	C		I	I		

We conclude that the PRA response to CP can be useful in identifying PTS with RAS but its ability to discriminate these PTS from those without RAS may be more limited than previously reported. RVRR lateralization in SD PTS is not improved with CP.

HEMODYNAMIC CONSEQUENCES OF DIURETIC THERAPY IN PRIMARY ALDOSTERONISM. SK Mujais, F Fouad and E Bravo. Northwestern U, Chicago IL and Research Div, Clev Clin Found, Cleveland OH.

Reduction of blood volume by diuretics is a basic component of blood pressure control in primary aldosteronism (1° aldo). We examined the hemodynamic effects of this reduction in 8 patients with 1° aldo treated for 2 to 7 months. Diuretics significantly reduced mean arterial pressure (MAP 138 ± 3.9 to 107 ± 4.7 mmHg, $p<0.01$), cardiac output (CO 5.3 ± 0.2 to 4.4 ± 0.2 L/min, $p<0.01$), and blood volume (BV 97 ± 2.7 to 85.7 ± 1.2 % of normal, $p<0.01$), while total peripheral resistance remained unchanged (TPR 49.4 ± 3.1 to 46 ± 4.9 U). The change in MAP correlated with that in CO ($r=0.6$, $p<0.05$) and TBV ($r=0.64$, $p<0.05$). The reduction of MAP by diuretics in 1° aldo involves multifactorial interaction of chronically reduced BV and CO, and failure of TPR to increase in response to these reductions, a pattern distinct from that encountered in essential hypertension. Failure of diuretics to normalize the circulatory system and the persistent reduction in cardiac output would, in our opinion, favor surgical therapy for aldosterone secreting adenomas.

NOVEL VASOCONSTRICTOR RAISES CELL CALCIUM IN VASCULAR SMOOTH MUSCLE CELLS. Takako Mitsuhashi*, R. Curtis Morris, and Harlan E. Ives. Nephrol. Div. and CVRI, Univ. of Calif., San Francisco.

Endothelin is a potent vasoconstrictor peptide produced by vascular endothelial cells (Nature 332:411, 1988). This peptide exhibits homology to α-scorpion toxin and has been proposed to activate dihydropyridine-sensitive Ca^{2+} channels in vascular smooth muscle (VSM). To study the response of cultured VSM cells to endothelin, intracellular Ca^{2+} was measured in cells loaded with fura 2. KCl-induced depolarization and the dihydropyridine Ca^{2+} channel agonist BAY K-8644 (10 μM) both raised cell Ca^{2+} more than 3 fold from a resting level of 140 ± 14 nM. The effect of KCl was completely blocked by the inhibitory enantiomer of BAY K-8644 (40 μM). Thus, our cultured cell system exhibits Ca^{2+} channel activity. Synthetic endothelin (4×10^{-8} M) raised calcium in VSM cells from 140 ± 20 to 910 ± 143 nM within 15 sec. Calcium subsequently fell to basal levels within 30 min. Thrombin (1 U/ml) gave qualitatively similar results. Neither the endothelin nor the thrombin-induced Ca^{2+} response was blocked by verapamil (10 μM) nor by the inhibitory enantiomer of BAY K8644 (40 μM). Furthermore, when cells were incubated in solution containing 0 Ca^{2+} and 4 mM EGTA, the Ca^{2+} response to both endothelin and thrombin remained intact. Thus endothelin, like thrombin, appears to raise cell Ca^{2+} in vascular smooth muscle cells by mobilization of intracellular stores. While endothelin may also activate Ca^{2+} channels, this is clearly not its only mode of action.

EFFECT OF SANDIMMUNE® ON ISOLATED BLOOD VESSELS. E. Müller-Schweinitzer*, J. Mason* (introduced by L.C. Moore). Preclinical Research, Sandoz, Basel, Switzerland

Renal dysfunction and hypertension, two frequent side-effects of Sandimmune (SIM), are believed to be caused by increased vascular reactivity. To examine the mechanism of SIM-induced vascular changes, isometric force generation to various agonists was studied in vitro. Arterial rings were suspended in a Krebs-Henseleit solution (mmol/l:NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, EDTA 0.03) at 37°C, gassed with 5% CO_2 in O_2, at a resting tension of 500 mg.

Aorta, femoral, mesenteric or renal arteries from normotensive and spontaneously hypertensive male, Wistar rats did not contract in response to SIM (3 μmol/l) within 2 hr of incubation, and the contraction to noradrenaline and relaxation to acetylcholine were both unchanged.

In contrast, aortic rings from normotensive female, Wistar rats, treated for 1 week with SIM (10 mg/kg/d s.c.), displayed a significantly reduced response to both noradrenaline and acetylcholine, whereas the response to 5-HT, phenylephrine, guanfacine, and angiotensin II was unchanged.

These data suggest that SIM in vitro is not a vasoconstrictor but when administered in vivo can modify vascular responses to some agonists.

EFFECT OF EXTERNAL SODIUM AND OUABAIN ON FREE CYTOSOLIC Ca2+ (iCa2+) IN VSMC. **E. Munoz* and D.C. Batlle, VA Lakeside and Northwestern Univ., Chicago, IL.**

The existence of a Na+/Ca2+ antiporter has been demonstrated by measuring the rates of 45Ca2+ influx and 22Na efflux in VSMC (JBC 262:11988, 1987). The role of this exchanger on iCa2+ regulation, however, remains controversial. We sought to examine this issue by continuously monitoring iCa2+ in response to varying external Na and in response to ouabain. Studies were performed in cultured rat aortic VSMC (2-7th passage) grown in coverslips and loaded with Fura-2 AM (2 μM) for 45 min. at 37°C. Fura-2 fluorescence was monitored continuously at two excitation wavelengths (340 and 380 nm) using a superfused system adapted to a computerized spectrofluorometer. Substitution of external Na (Na_o) in the perfusion solution with choline (140 mM) resulted in a progressive increase in iCa2+ which peaked at 2-3 min. (from 176±25 to 285±36 nM, p<0.01) and then declined but remained higher than baseline (209±30 nM at 30 min.). The increase in iCa2+ was inversely related to Na_o over a range of 0-100 mM. The addition of ouabain ($2x10^{-3}$M) to the perfusion solution also produced a significant increase in iCa2+ which was apparent as early as 5 min. and increased progressively with time. The ouabain-induced increase in iCa2+ was seen in the presence of Na_o and was further accentuated in its absence. We conclude that VSMC in culture possess a Na+/Ca2+ antiporter which is activated by lowering Na_o and when intracellular Na2+ is increased by ouabain. The activity of this antiporter is capable of modulating iCa2+ within the physiologic range.

ANALYSIS OF RENAL VASODILATORY RESPONSES TO ACE INHIBITION IN RENAL HYPERTENSIVE RATS. L. Gabriel Navar, Jane Hazelrig and David Harvey. University of Alabama at Birmingham, Birmingham, Alabama 35294.

Localization of renal vasodilatory responses of the non-clipped kidney of Goldblatt hypertensive rats to ACE inhibitors was assessed with a hemodynamic model that predicts changes in glomerular and peritubular capillary dynamics in response to parameter adjustments. Resistances and K_f values were adjusted to match experimental data obtained from the non clipped kidney of 2-K, 1 clip Goldblatt hypertensive rats studied 3-4 weeks after clipping. Model responses to mechanical reduction in renal perfusion pressure from 170 to 130 mmHg could be matched to experimental values with minimal adjustments in vascular resistances. Following ACE inhibition (MK 422), we observed a 24±7% increase in RBF, an increased GFR and SNGFR, and maintenance of glomerular pressure. These responses were consistent with greater decreases in preglomerular resistance than in efferent resistance. Imposing a selective efferent arteriolar vasodilation to the model led to a profound reduction in glomerular pressure and SNGFR which was not consistent with the experimental data and could not be compensated for by any degree of K_f elevation. Thus, vasodilation of both pre and postglomerular resistance segments along with an increase in K_f explains the experimental data obtained following administration of ACE inhibitors to Goldblatt hypertensive rats.

ACUTE ELEVATION OF GLOMERULAR CAPILLARY PRESSURE IMPAIRS GLOMERULAR SIZE-PERMSELECTIVITY IN NORMAL RATS. J. Neugarten. Montefiore Med Cntr and Albert Einstein Coll of Med, Bronx, NY.

We sought to determine the effect of an acute elevation of glomerular capillary pressure (P_{GC}) on glomerular size-permselectivity in normal rats. Systemic hypertension was induced in 7 male Sprague-Dawley rats by bilateral carotid artery occlusion. Mean arterial pressure (MAP) rose to 166±4 mm Hg; P_{GC} rose to 54±3 mm Hg from a baseline value of 46±2 mm Hg ($p<0.01$). Urinary protein excretion, adjusted for filtration rate, increased from 0.011±0.002 to 0.131±0.039 mg/ml GFR, $p < 0.001$. Dextran sieving studies were performed during bilateral carotid artery occlusion at the elevated MAP and repeated at a lowered renal perfusion pressures averaging 108±1 mm Hg, achieved by suprarenal aortic ligature. GFR was unchanged (2.16±0.21 vs. 2.06±0.23 ml/min). However, urinary protein excretion declined from 0.131±0.039 to 0.046±0.007 mg/ml GFR, $p < 0.001$. In the hypertensive state, the fractional clearances of neutral dextrans (Θ_D) for molecular radii exceeding 46Å were elevated as compared to control values. With reduction in renal perfusion pressure, Θ_D for dextrans > 46Å uniformly declined ($p<0.05$). These data demonstrate that acute elevation of glomerular capillary pressure induced by bilateral carotid artery occlusion causes proteinuria and impairs glomerular size-permselectivity. Reduction in renal perfusion and glomerular capillary pressures reverses proteinuria and restores glomerular size-selectivity.

CHROMOGRANIN A (CGA) DISPOSITION AND DISTRIBUTION IN MAN: INSIGHTS FROM CGA KINETICS AFTER RESECTION OF PHEOCHROMOCYTOMA (PHEO). D. T. O'Connor and H. P. H. Neumann*. Dept. of Medicine, SDVAMC and UCSD, San Diego, CA and Albert-Ludwigs Universitat, Freiburg, FRG.

CgA is costored and coreleased with catecholamines from chromaffin granules, but little is known of disposition in man. We therefore studied plasma CgA, by homologous RIA, in 2 kindreds with familial pheo. In 8 subjects, CgA was measured before, during, and in an exponential time series after surgical resection of pheo. CgA was elevated preoperatively (141±26 ng/ml vs 28±1 ng/ml in controls) and increased further during operation (to 186±43 ng/ml). After resection, there was a rapid and then a prolonged fall in CgA. The data were fitted, by an interactive curve peeling program, to several models with best fit to a 2 compartment, biexponential model, yielding absolute and fractional transfer rates, pool mass, $t_{\frac{1}{2}}$, and residence time. The initial and subsequent $t_{\frac{1}{2}}$ values were, for pool 1 (plasma), 16 min, and for pool 2 (extravascular), 520 min, corresponding to mean CgA residence times ($=t_{\frac{1}{2}}/0.693$) of 23 min and 750 min. The CgA mass of pool 2 was 23.9 times that of pool 1, suggesting substantial extravascular tissue "binding" of CgA. We conclude: 1) Plasma CgA is markedly elevated in most patients with familial pheo. 2) The CgA decline after pheo resection best fits a 2 compartment model. 3) The relatively short (16 min) initial $t_{\frac{1}{2}}$ of CgA suggests that CgA may be responsive to acute perturbations of sympathoadrenal activity. 4) Extravascular vs intravascular compartment values support substantial tissue binding of CgA.

EFFECT OF HYPONATREMIA ON INTRACELLULAR ACTION OF ARGININE VASOPRESSIN (AVP) IN RAT VASCULAR SMOOTH MUSCLE CELLS (VSMC) IN CULTURE. K. Okada*, C. Caramelo*, P. Tsai* and R.W. Schrier. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

Non-osmotic stimulation of AVP has been demonstrated to occur in hyponatremic states associated with volume depletion and edematous disorders. This study was undertaken to examine the effect of decreased extracellular Na^+ ($[Na^+]_e$) on cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) and VSMC contraction. Incubation of the cells with decreased $[Na^+]_e$ for 60 min resulted in increased basal $[Ca^{2+}]_i$ (mM Na^+/nM Ca^{2+}, respectively: 140/91, 110/140, 80/178, 40/189 and 0/179, $p<.01$, n=7). These results were consistent with the findings of the ^{45}Ca uptake studies. 110mM $[Na^+]_e$ increased ^{45}Ca uptake from 10135 to 12084 counts/mg prot at 60 min ($p<.01$, n=4). This effect was blocked by 10^{-5} M verapamil. The preincubation of cells with 110 mM $[Na^+]_e$ for 60 min also increased basal $[Ca^{2+}]_i$ and AVP-induced mobilization of $[Ca^{2+}]_i$ with a submaximal dose of AVP (basal, 108 vs 148 nM, $p<.01$, n=16; 10^{-8} M AVP, 243 vs 311 nM, $p<.01$, n=8). The preincubation of cells with 110mM $[Na^+]_e$ for 60 min also enhanced submaximal AVP- and PMA-induced cell contraction (10^{-8} M AVP, 14.6 vs 28.8%; 10^{-8} M PMA, 8.1 vs 21.3%, $p<.01$, n=5) but did not alter the contractile response to the maximal dose of AVP and PMA (10^{-6} M). These results suggest that hyponatremia within a clinical range enhances the response of VSMC to a submaximal dose of AVP. This phenomenon may involve protein kinase C activation by increasing basal $[Ca^{2+}]_i$. These findings support a vascular role for the non-osmotic release of AVP with hyponatremia occurring as a pari passu event.

THE TISSUE RENIN-ANGIOTENSIN SYSTEM (RAS) IN THE 2K1C GOLDBLATT RATS: GENE EXPRESSION STUDIES. S. Rajaraman, A.S. Popatia,* C. Benedict,* and K. Graves*. Univ. of Texas Med. Branch, Div. of Renal Immunopathology and Cardiology, Galveston, TX.

We studied the alterations in the regulation of the genes for angiotensinogen and renin, the major components of the RAS, in the clipped and non-clipped kidneys and the liver, in the 2K1C rat model of renovascular hypertension. Total cellular RNA was extracted at 10 and 20 days after clipping and Northern and Dot Blot analyses were performed, using homologous renin and angiotensinogen cDNA, as molecular probes. We observed augmentation in the transcription of both the renin and angiotensinogen genes in the clipped kidneys, at 10 days, followed by suppression of both at 20 days. In the non-clipped kidneys, both remain markedly suppressed at 10 and 20 days. In the liver, angiotensinogen expression is suppressed at 10 days, but is markedly augmented at 20 days. We conclude that: (1) there is differential, tissue specific regulation of the components of the RAS, in renovascular hypertension; (2) the intrarenal modulation of the local RAS may play an important role in the maintenance of renal functions under adverse perfusion conditions; and (3) the increased biosynthesis of angiotensinogen by the liver at 20 days, when the plasma renin activity returns to normal levels, may be responsible for persistent hypertension.

PREVALENCE OF ATHEROSCLEROTIC RENAL ARTERY STENOSIS IN PATIENTS WITH GENERALIZED ARTERIOSCLEROSIS

Jeffrey W. Olin, Barbara Risius,* Jess R. Young,* Robert A. Graor* and William F. Ruschhaupt.* Cleveland Clinic Foundation, Cleveland, Ohio.

Three hundred seventeen consecutive aortograms were reviewed prospectively in order to determine the prevalence of atherosclerotic renal artery stenosis in patients (pts) with abdominal aortic aneurysm (AAA), aorto-occlusive disease (AOD), lower extremity occlusive disease (LEOD), and suspected RAS (S-RAS). The RAS in the first 3 categories was discovered as an incidental angiographic finding with an absence of the usual clues to suggest RAS.

	AAA n=91	AOD n=20	LEOD n=137	S-RAS n=69	P Value
Mean Age	69	61	66	59	<.001
Creatinine	1.3	1.3	1.3	1.9	<.001
Hypertension	57%	60%	61%	99%	<.001
Diabetes	12%	15%	36%	19%	<.001
>50% RAS					
Diabetic	55%	33%	48%	77%	.18
Non-Diabetic	40%	32%	36%	68%	
All patients	42%	35%	40%	70%	.001

High grade (75-100%) unilateral RAS occurred in 19%, 15%, 22% and 62% and bilateral RAS in 9%, 15%, 7% and 42% of pts investigated for AAA, AOD, LEOD and S-RAS. Significant RAS is common in pts with AAA, AOD and LEOD. An approach to the management of these pts with RAS discovered incidentally requires further delineation. The natural history of RAS needs to be determined and the role of medical vs. surgical therapy further defined.

ALTERED PROTEIN PROFILES OF THE AORTIC SMOOTH MUSCLE OF SPONTANEOUSLY HYPERTENSIVE RAT (SHR): Ramachandra M. Rao*, Eric W. Young, David A. McCarron, Div of Nephrology & Hypertension, Oregon Hlth. Sci. Univ. Portland, OR.

To explore a possible molecular basis for the cellular and subcellular defects in the vascular function of the SHR, we analyzed profiles of the expressed proteins in the aortic smooth muscle of SHR and normotensive WKY rats.

Thoracic aorta were dissected from the SHR and WKY (12 wk old) and smooth muscle was isolated and homogenized in Sucrose-MOPS buffer. The membrane proteins were extracted by adding SDS and β-mercaptoethanol. Twelve aliquots of four separate preparations of each strain (SHR & WKY) were subjected to SDS gel electrophoresis. The individual protein peaks as well as known quantities of standard protein were quantitated by densitometric scanning. A comparison of the protein peaks was done by ANOVA.

The vascular protein profile for SHR was significantly different ($p<0.01$) from WKY. Individual peak analysis revealed that a protein with a mol wt of 110 Kd was significantly ($p<0.001$) decreased in SHR (28.2 ± 1.8 μg/mg) compared to WKY (72.9 ± 7.4 μg/mg); a protein at 17 Kd was reduced significantly ($p<0.01$) in SHR (21.9 ± 6.1 μg/mg) compared with WKY (37.4 ± 8.1 μg/mg).

These results suggest that the vascular smooth muscle protein pattern is significantly altered in SHR compared with WKY. The reduction of two specific proteins in SHR might contribute to the altered function of aortic smooth muscle.

ETIOLOGY OF RENOVASCULAR HYPERTENSION(RVH)IN NORTH INDIA. Vinay Sakhuja, A.Gupta, N.Malik, S.R.Bhusnurmath, K.S.Chugh, (intr.by P.C.Singhal) Postgraduate Institute of Med.Ed.& Res.Chandigarh, India.

In the West,atherosclerotic narrowing of the renal arteries accounts for two-thirds of all cases of RVH and the remainder are due to fibromuscular dysplasia(FMD). We investigated our patients(pts)with RVH to determine its etiology in our country. Between 1982-1987,58 pts(24 males,34 females)whose age ranged from 13-60 yrs(mean 26)were confirmed to have RVH after renal angiography. Diastolic pressure was <104 mmHg in 8(13.7%),between 105-114 mm Hg in 15(25.8%)and >115 mm Hg in 35(60.3%). Twenty four pts(41.3%) had malignant hypertension. An abdominal bruit was present in 23(39.6%)and 10(16.3%)pts had renal insufficiency. Angiography confirmed that FMD was the commonest cause of RVH accounting for 30(51.7%)pts. Atheromatous ostial narrowing was encountered in only 3(4.9%) pts and extrinsic compression of the renal artery in 1. Twenty four pts(39.3%)had Takayasu's arteritis(TA). Renal artery stenosis was unilateral in 29/30 with FMD and all 3 with atheroma but only in 14/24 with TA. Eleven(45.8%)pts with TA had type II disease and 13(54.1%)had type III disease.

In summary, a comparison of the etiology of RVH in our country with that of the West reveals two striking differences viz.1)FMD is the commonest cause while autheroma is rarely encountered,2) TA is next in frequency only to FMD accounting for 39% of pts.

EVALUATION OF THE CONTROL OF HYPERTENSION IN THE ELDERLY. Sandra Schuyler,* and Mahendr Kochar. Zablocki VA Medical Center and Medical College of Wisconsin, Milwaukee, Wis.

The objective of this retrospective study was to evaluate the Hypertension Clinic's treatment among its elderly population (n=747, age ≥ 65 yrs). We selected the first 100 males fitting our criteria: Group 1 (G1) =65-74 years of age (n=50), Group 2 (G2) ≥ 75 years of age (n=50). G1 and G2 were further defined by type of hypertension (HTN) (measured in mmHg): Systolic HTN (SHTN) >160/<90; diastolic HTN (DHTN) <160/>90; systolic/diastolic HTN (S/DHTN) >160/>90; controlled HTN (CHTN) <160/<90. Drug regimens, lab values, heart rates, and weights were analyzed in conjunction with sitting and/or standing blood pressures for a period of 5 years. The mean age of the total population was 74 (SD 6.5) and a mean blood pressure of 176/103 mmHg (SD 22/11 mmHg) at entry. During the 5-year follow-up 72 patients had CHTN, 10 had SHTN, 10 had DHTN, and 8 had S/DHTN 60% of the time. The order of the amount of most frequently used medications was: diuretics 99%, potassium-sparing diuretics 56%, beta blockers 50%, sympathetic inhibitors 33%. The 3 most used non-HTN medications included antigout 18%, antiarthritics 14%, and digoxin 13%. The levels of fasting blood sugar, total cholesterol, and potassium did not change significantly during follow-up. We concluded that in the elderly the hypertension was controlled satisfactorily using the usual antihypertensive medications without any deleterious effects on the biochemical parameters.

NEPHROPTOSIS (NP)-RELATED HYPERTENSION (H): FEATURES OF A NEW MODEL OF RENOVASCULAR HYPERTENSION. F. Scanferla*, G. Bazzato*, A. Fracasso. Nephrology Div. Umberto I Hospital, Venice-Mestre (Italy)

Pathogenetic mechanisms which correlate NP and H were searched to demonstrate a causal relationship. 3 groups of women were studied: 14 hypertensives with nephroptosis (NP+H); 15 normotensives with nephroptosis (NP); 15 essential hypertensives (EH). Renal function and urinary excretion (U) were evaluated during 12 hrs in supine (SUP) and upright (UPR) position simultaneously with BP monitoring. PRA was also measured in SUP and UPR. Tc-99m-DTPA renography was performed comparing ptotic versus controlateral kidney in SUP and UPR. The study was repeated in NP+H after acute test and chronic therapy with Captopril (C) 50 mg. During basal condition NP+H showed some differences in UPR compared to the others: reduced NaU/KU ratio ($P<0.001$ vs NP, $P<0.005$ vs EH); increased PRA ($P<0.001$ vs NP, $P<0.005$ vs EH) and MAP ($P<0.001$ vs NP, $P<0.01$ vs EH). DTPA uptake of ptotic kidney was reduced in UPR of NP+H ($P<0.001$). After C, NP+H showed a normalization of BP in 11 subjects, and more marked difference of DTPA uptake between the two kidneys in UPR versus SUP from -20% to -31% ($P<0.001$). These findings were more evident in subjects (11) with ventral rotation of ptotic kidney in UPR. The pathophysiological relationship between NP and H can be explained as a "functional" renovascular hypertension, emphasized by ACE inhibition.

RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEPO) INCREASES MAP, TPRI AND SYSTOLIC AND DIASTOLIC DYSFUNCTION WITH INCREASED IMPEDANCE TO LV EJECTION DUE TO INCREASED HCT AND RBC MASS IN PTS WITH CRF. **Allan B. Schwartz, Gary S. Mintz,* Kwan E. Kim, John E. Prior,* S. Benham Kahn*. Hahnemann University, Department of Medicine, Philadelphia, PA**

A double blind placebo study was conducted with rHuEPO admin 3x/week to 7 pts with anemia of CRF, avg Hct 29% (28-31), avg GFR 14.4 ml/min (8-28). Hct ↑ to 40% at 43 days; retics ↑ from 1.4 to 7.4%. Bone marrow cellularity ↑ from hypo to hypercellular; ME ratio ↓ from 3.1:1 to 1.2:1. Blood volume ↑ from 3476 to 4592 ml ($p<.002$). MAP ↑ from 106 to 117 mm Hg ($p<.005$); TPRI ↑ from 3223 to 3607 dynes·sec·cm^{-5}/M^2 ($p<.02$). Radionuclide angio and 2D, M-mode, Doppler echocardiogram were performed by blinded investigators at time of target Hct 38% (8-12 weeks). LVEF ↓ from 52 to 47% ($p=.078$); LV end systolic dimension ↑ from 3.3 to 3.6 cm ($p<.005$) while LV end diastolic dimension showed no change; LA dimension ↑ from 4.0 to 4.5 cm ($p=.073$). Mitral valve flow velocity A/E ratio ↑ from 0.91 to 1.25 ($p=.063$). Ascend aortic peak velocity ↓ from 1.33 to 1.20 ($p=.11$).

Conclusions: rHuEPO administered to anemic pts with CRF ↑ Hct, ↑ RBC mass correlated with ↑ MAP, ↑ TPRI, ↓ LVEF and echo Doppler evidence of LV systolic and diastolic dysfunction.

EFFECT OF ALTERATIONS OF INTRACELLULAR pH (pH_i) ON INTRACELLULAR CALCIUM (Ca_i) IN VASCULAR SMOOTH MUSCLE CELLS (VSMC). M.S. Siskind* & J.H. Schwartz, Boston City Hosp., Boston, MA.

Ca_i and pH_i are important regulators of a variety of intracellular processes. Ca_i is a regulator of muscle contraction but the role of pH_i is unclear. The purpose of this study was to determine the effect of alterations of pH_i on Ca_i. A7r5 VSMC's were grown to confluence on glass cover slips. Ca_i was determined with the fluorescent probe fura-2, and pH_i with BCECF. Alkalinization of the VSMC's induced by exposure to 20 mM NH_4Cl (Δ pH 0.41 $\pm$ 0.07) resulted in a 57$\pm$12nM (n=5) rise in Ca_i in the presence of extracellular Ca (Ca_o) and a 26 $\pm$ 4 nM (n=5) rise in Ca_i in the absence of Ca_o. Similar changes in Ca_i were observed when cells were alkalinized by exposure to nigericin in a KCl buffer (pH 7.7). The rise in Ca_i was transient and the return towards basal Ca_i was not measurably affected by the presence of vanadate (10μM) or removal of extracellular Na^+. Neither 100μM verapamil or 100μM TMB-8 altered the alkaline induced changes. After cellular Ca^{2+} stores were partially depleted by exposure to AVP in a Ca^{2+} free solution, subsequent cell alkalinization induced no change in Ca_i. When cells were acidified by the removal of NH_4Cl (Δ pH 0.8), no change in Ca_i was observed. These results demonstrate that alkalinization of VSMC's leads to a rise in cytosolic Ca^{2+} via release of intracellular Ca^{2+} stores. The intracellular Ca^{2+} storage sites appear to be the same as those sites sensitive to AVP. Thus, pH_i may regulate Ca_i, and thereby play a role in the regulation of vascular smooth muscle tone.

CAPTOPRIL TEST VERSUS CAPTOPRIL-STIMULATED PLASMA RENIN AS SCREENING TESTS FOR RENOVASCULAR HYPERTENSION. Laura P. Svetkey* and Paul E. Klotman. Duke University Medical Center and the Durham VA Medical Center, Durham, North Carolina.

The "Captopril Test" (CT) first described by Muller et al (AJM 1986) has been proposed as a screening test for renovascular hypertension (RVH). We prospectively evaluated the CT and compared it to a single measurement of captopril-stimulated plasma renin activity (CS-PRA) in hypertensive patients being evaluated for RVH. In 80 subjects, CS-PRA was measured by RIA 60 minutes after ingesting 25 mg of captopril, dissolved in water. In 27 of these subjects, the CT test was also performed (i.e. pre- and post-captopril renin measurements were made). CS-PRA was elevated if the value was greater than 4 ng/ml/hr. CT was considered positive if it met all three criteria described by Muller et al. All 80 subjects then underwent conventional renal arteriography. All significant renal artery stenoses were treated with percutaneous transluminal angioplasty (PTA). RVH was defined as a reduction in blood pressure 2-4 weeks after PTA. Of 12 patients with RVH, 9 had an elevated CS-PRA (sensitivity 75%). Forty-four of 68 without RVH had a normal or low CS-PRA (specificity 65%). Of the 27 patients who also had the CT, the test was positive in 1 of 2 with RVH (sensitivity 50%), and was negative in 19 of 25 without RVH (specificity 76%). These data suggest that CS-PRA may be more sensitive, though less specific, than CT as a screening test for RVH.

CONTROL OF HYPERTENSION AMONG DIABETIC PATIENTS. Grigory Sorokin,* Arti Sheth,* and Mahendr Kochar. Zablocki VA Medical Center and Medical College of Wisconsin, Milwaukee, Wis.

It is estimated that more than 2.5 million Americans have both hypertension and diabetes. The Hypertension Clinic at the Zablocki VA Medical Center follows approximately 300 patients who have both these disorders. We conducted a retrospective study of 100 patients (75% white and 25% black) who were followed up for a 3-year period to see how well diabetes and hypertension had been controlled. Mean age in these patients was 64 years (SD ±10). In 82% of these patients, hypertension was controlled as defined by a sitting diastolic blood pressure < 90 mmHg. In 67% of the patients, diabetes was controlled as defined by a fasting blood sugar < 160 ml/dl. The antihypertensive medications used were: diuretics 92%, potassium-sparing diuretics 48%, beta blockers 68%, and others 20%. For controlling diabetes, insulin was used in 17% and oral hypoglycemics in 44% of the patients. The serum creatinine, potassium, triglycerides, and total cholesterol remained unchanged throughout the study. We conclude that it is possible to attain a satisfactory control of both hypertension and diabetes in a VA hypertension clinic.

EXOCYTOTIC CATECHOLAMINE AND CHROMOGRANIN A (CgA) CORELEASE DURING SELECTIVE STIMULATION OF THE SYMPATHETIC NERVOUS SYSTEM (SNS) IN MAN. M.A. Takiyyuddin,* P. Sullivan,* J. Cervenka*, and D.T. O'Connor. Dept. of Med, Univ. of California and VA Medical Center, San Diego, CA.

CgA is the major soluble protein that is co-stored and released with catecholamines by exocytosis from vesicles in the adrenal medulla and sympathetic nerves. We have found that selective adrenomedullary stimulation in man releases CgA into plasma. Since there is 5000 fold more CgA in the adrenal medulla than in sympathetic nerve endings, we hypothesized that selective activation of the SNS would result only in comparatively modest changes in plasma CgA. To test this, we measured plasma CgA and catecholamine responses to standing and to dynamic bicycle exercise (DBE), stimuli known to selectively stimulate the SNS. 19 male subjects were studied, 6 before and after 30 minutes of standing and 13 before, during and after DBE (50-200 watts) for 10-12 minutes. Following standing, plasma CgA did not change despite a 1.58 fold rise in plasma NE ($p<0.05$) and 0.54 fold rise in plasma E ($p<0.05$). During DBE the relative increases in plasma CgA and norepinephrine were 21% ($p<0.05$) and 82% ($p<0.05$) respectively, without a significant change in plasma E. Heart rate rose significantly in response to standing and DBE, reaching a maximum of 79±2 and 158±5 respectively. We conclude: 1) Catecholamine release is exocytotic during selective activation of the SNS in man. 2) Selective stimulation of the SNS results in comparatively modest changes in plasma CgA compared to changes attained during adrenomedullary activation. 3) Only rather intense SNS activation results in measurable changes in plasma CgA.

REDUCTION OF ARTERIAL PRESSURE AFTER REMOVING THE CLIP RESTORES RENAL FUNCTIONAL RESERVE IN GOLDBLATT HYPERTENSIVE RATS WITH RENAL ABLATION (GHT). E. Tapia*; N. Bobadilla*; L. Romero*; D. Amato*and J. Herrera-Acosta*. Dept. of Nephrology, Instituto Nacional de Cardiologia "Ignacio Chávez", México City.

Glycine infusion (GI) induces preglomerular vasodilation, rising glomerular plasma flow and filtration. This response is used to evaluate renal functional reserve (RFR). Glomerular damage in GHT is associated with increased glomerular pressure, hyperfiltration and loss of RFR.

To asses if the mechanism responsible of the loss of RFR is secondary to functional or structural factors, we studied 6 normal rats (N) and 11 with systemic hypertension induced by clipping right renal artery and partial renal ablation (3/4) of left kidney (GHT). After 40 days, 5 rats were made normotensive by removing the clip (PGHT). Normal rats, GHT rats 45 days after surgery, and PGHT rats 7 days after clip removal underwent micropuncture studies during control (C) and 15% glycine infusion (GI). Mean arterial pressure (MAP), single nephron glomerular filtration rate (SNGFR), single nephron plasma flow (SNPF), transcapillary hydrostatic pressure gradient (ΔP), afferent (AR) and efferent (ER) resistances were measured. Results are expressed as mean ± S.E.

Group	MAP	SNGFR	SNPF	ΔP	AR	ER
	mmHg	nl/min	nl/min	mmHg	dyn.s.cm-5	
N-C	104± 5.8	29±2.0	95± 7.1	33±1.3	3.11±.58	1.85±.24
N-GI	108± 4.7	51±3.9*	157±13.0*	35±1.6	1.81±.42*	1.16±.25
GHT-C	160±10.5¤	46±5.2	242±26.3¤	40±2.5¤	2.22±.37	0.91±.21
GHT-GI	160±11.1	46±4.5	249±29.7	40±2.7	2.79±.69	1.24±.39
PGHT-C	118± 5.2	24±3.4	81±14.5	36±2.8	3.63±.49	2.99±.79
PGHT-GI	124± 5.3	44±6.2*	144±26.5*	40±2.1	1.96±.22*	2.00±.42*

*$p < 0.05$ GI vs C. ¤ $p < 0.05$ vs N-C.

The response to GI was restored after reducing arterial pressure suggesting that loss of RFR is mediated by functional rather than structural factors, possibly related to sustained constriction of preglomerular induced by systemic hypertension or increased levels of circulating angiotensin II.

HOW DOES NaCl REGISTER IN NaCl HYPERTENSION? L Tobian, JY Lee, MA Johnson, S Hanlon & S Hamer. University of Minnesota, Minneapolis, Mn 55455

Using 8 gm of NaCl per 100 gm of soluble nutrients, we fed only a hypertonic drink (1.4% NaCl) or a hypotonic drink (.45% NaCl) to Dahl S rats. After 12 weeks, 11 S rats on hypertonic averaged 195 mmHg vs 195 in 12 S rats on hypotonic. Thus, the high NaCl signal seems not related to a higher NaCl concentration in body fluids, thereby suggesting volume signals. Most volume controls are around the third brain ventricle ("3V"). High dietary NaCl might swell the tissues surrounding "3V" which is slit-like. Such swelling would partially close the upper part of the slit & cause ependymal cells on opposite walls to touch, leading to hypertension in susceptibles. To test this, we stereotaxically blocked the aqueduct with inert silicone to produce hydrocephalus of "3V" in Dahl S rats & thus prevent ependymal cells from touching. After blocking or sham-blocking the aqueduct, a 6% NaCl diet was started. Intraarterial BP was taken after 6 weeks. Both 26 sham S rats & 20 blocked S rats, all on .23% low NaCl, had BPs averaging 130 mmHg. 34 sham S rats on 6% NaCl averaged 177 mmHg, whereas 17 blocked S rats on 6% NaCl averaged 149. Thus with 6% NaCl, BP rose 47 mmHg in sham S rats & only 19 mmHg in blocked S rats, a 60% reduction, $p<.001$. After 12 weeks on 6% NaCl, 61% of the sham S rats had died, vs 6% mortality in the blocked S rats, a 90% reduction in mortality ($p<.001$). Thus, preventing "3V" ependymal contact during 6% NaCl feeding in S rats abolished 60% of the BP rise and reduced mortality by 90%. 25 other Dahl S rats on 6% NaCl for 6 weeks underwent thermal lesions of periaqueductal fibers. Their BP was 10 mmHg higher than 13 S rats with sham lesions. Thus the aqueductal block lowered BP not through local injury.

BLUNTED VASCULAR REACTIVITY DURING CYCLOSPORINE (CSA) ADMINISTRATION IN CONSCIOUS RATS. Stephen C. Textor, Thomas Telles,* Leslie Smith-Powell,* City of Hope Med. Ctr, Duarte, CA.

Vasoconstriction and hypertension have been prominent during CSA Rx. To determine whether CSA alters sensitivity to vasoactive agents, we measured arterial pressure (MAP) in Sprague-Dawley rats and dose-response pressor curves to norepinephrine (NE), angiotensin II (AII) and bradykinin (BK) acutely (2 hrs) and after 7 days of CSA (10 and 20 mg/kg/d i.p. via osmotic pump). Pressor responses were compared to vehicle treated, pair-fed controls. Dose response curves were unchanged after 2 hrs of CSA infusion. After 7 days of CSA, however, basal MAP and pressure changes were as follows:

	Vehicle	CSA 10mg/kg	CSA 20mg/kg
MAP (mm Hg)	104±3	99±7	105±1
NE (500 ng)	37±2	2±3 **	11±2 **
AII (250 ng)	41±6	15±3 **	22±7 **
BK (5 ug)	-11±2	-18±6	-21±5

(Mean ± SEM, ** $p < .01$ vs Vehicle)

Maximal pressor responses in CSA rats at 10-fold higher doses were similar to controls. Pretreatment with verapamil did not prevent the attenuation of systemic vascular reactivity to CSA. These data demonstrate acquired reduction of vascular reactivity to norepinephrine and angiotensin II, but not to the vasodilator bradykinin during CSA. We interpret these results to suggest that vasoconstriction reflects direct vascular effects of CSA and is not mediated by enhanced vascular sensitivity to vasopressor substances in the conscious animal.

CAPTOPRIL IN PATIENTS WITH TYPE II DIABETES AND RENAL INSUFFICENCY. Enrico Valvo, Valeria Bedogna, Patrizia Casagrande, Leopoldo Antiga, Massimo Zamboni, Fares Bommartini, Lamberto Oldrizzi, Carlo Rugiu, Giuseppe Maschio. Div. of Nephrology, Univ. of Verona, VERONA, Italy.

Arterial and glomerular hypertension play an important role in both the development and progression of diabetic nephropathy. The ACEI could be considered "specific" antihypertensive drugs, since they reduce systemic and glomerular hypertension.

The effects of long-term treatment with captopril on systemic and renal hemodynamics and proteinuria were studied in 12 pts, aged 52 yr (40-66), with type II DM and the clinical syndrome of diabetic nephropathy. After 6 mo. the intrarterial BP fell(162/103±17/5 to 139/89±26/10 mm Hg) due to the reduction in TPR and no change in CO. Conversely, No change was seen in RVR and in FF. A slight, not significant, decrease in RPF(243±97 to 217±108 ml/min), in GFR(57±17 to 51±19 ml/min) and in proteinuria(4.50±3.10 to 3.40±2.31 g/day) was also observed.

Our findings suggest that captopril is an effective agent in patients with diabetic nephropathy, but the renal beneficial effects seem to be limited when this syndrome is complicated by renal insufficiency.

DIETARY SODIUM RESTRICTION ELEVATES MEAN ARTERIAL PRESSURE IN THE CONSCIOUS, UNRESTRAINED RAT Richard C. Vari, S.H. Valencia*, B.G. Hanss*, and K.F. Gordon*. Dept. of Physiology, Tulane University School of Medicine, New Orleans, Louisiana.

Systolic blood pressure measured, indirectly via the tail-cuff technique, has been reported to be elevated in the rat during dietary sodium restriction. In this study chronic femoral arterial catheters were utilized to examine the effects of dietary sodium restriction on blood pressure in the one-kidney conscious rat. The normal sodium diet (NS) contained 0.136±.001 mEqNa^+/gram compared to the low sodium (LS) diet of .003±.001 mEqNa^+/gram and were identical in every other constituent (TEKLAD). Comparison of mean arterial pressure is shown over time for NS and LS rats. *=P≤.05 LS vs NS; X±SE; ( ) = n per group

	Pre	Days after Nephrectomy			
GROUP	Neph	3	7	10	14
LS	116±2	118±4	129±4*	125±3*	133±1*
	(9)	(9)	(5)	(3)	(3)
NS	112±3	112±4	104±5	107±2	107±1
	(8)	(8)	(6)	(5)	(4)

Sodium restriction significantly elevated mean arterial pressure in the one-kidney rat. This data confirms, using direct measurements of blood pressure in consicous unrestrained one-kidney rats, previous observations of a significant and sustained elevation in blood pressure during dietary sodium restriction.

ENDOGENOUS INHIBITOR OF Na-K-ATPase INCREASES INTRACELLULAR FREE CALCIUM ($[Ca^{2+}]_i$) IN HUMAN PLATELETS (P) AND RAT VASCULAR SMOOTH MUSCLE CELLS (VSMC). C.Wanning,* H.Meyer-Lehnert, H.J.Kramer, Med. Poliklinik, Univ. of Bonn, West Germany.

An endogenous ouabain-like inhibitor (OLI) of sodium transport has been suggested to play a role in certain forms of hypertension by modulating vascular resistance. In the present study, 24 h urine was collected from salt-loaded healthy subjects and amounts of urine corresponding to 1 h urine collection were pooled and subjected to chromatographic separation after lyophylization. The post-salt fraction (F IV) eluted from Sephadex G-25 containing OLI was re-chromatographed on Sephadex G-10. For in vitro inhibition of Na-K-ATPase an enzyme preparation of hog cerebral cortex (Sigma) was used. The strongest Na-K-ATPase inhibition was observed in the late fraction (F IV/7) which showed a dose-related enzyme inhibition from 10 % (0.13 mg/ml) to 58 % (0.52 mg/ml). When this fraction was subjected to reverse-phase HPLC, OLI again was eluted in a late fraction (F IV/7g). Effects of OLI on $[Ca^{2+}]_i$ were examined in P and cultured rat aortic VSMC using fura 2. OLI rapidly increased $[Ca^{2+}]_i$ in P (70±8 vs 186±19 nM, p<.001) and in VSMC (82±9 vs 253±23 nM, p<.01). 45 min preincubation with OLI increased basal $[Ca^{2+}]_i$ in P (70±8 vs 229±12 nM, p<.001). For comparison, 45 min preincubation with ouabain (10^{-5}M) increased basal $[Ca^{2+}]_i$ to 379±7 nM (p<.001) in P; a similar effect was observed in VSMC. OLI increased vasopressin (10^{-8}M)-stimulated peak levels of $[Ca^{2+}]_i$ in VSMC from 310±8 to 377± 10 nM (p<.05). These results suggest that OLI may affect vascular resistance by modulating $[Ca^{2+}]_i$, a major determinant of smooth muscle contraction.

Na,K-ATPase, Na BALANCE, AND BLOOD PRESSURE IN RATS FED NaCl AND Na CITRATE. O Wagener* and AP Quintanilla, Dept. of Medicine, VA Lakeside Med. Center, and Northwestern Univ. Med. School, Chicago, IL.

Recent reports indicate that Na induces a higher BP when administered as NaCl than as an organic salt. The different effect on BP may be related to a difference in Na retention and volume expansion. Since Na,K-ATPase (ATPase) is found inhibited in volume-expanded hypertension, we investigated the relationship between BP, Na retention and ATPase activity in rats treated with NaCl and Na citrate. Ten uninephrectomized rats were randomly paired to receive a mixture (80%/ 20%) of NaCl/KCl, or equimolar amounts of Na and K citrate in the drinking water. Mean Na intake was 6.72 and 6.2 mmol per day in the chloride and citrate groups, respectively (p=NS). Mean Cl intake was 7.85 and 0.08 mmol/day (p=<0.001) and mean K intake 1.32 and 1.23 mmol (p=NS). After 8 weeks the rats were killed and ATPase measured in the red cells and kidney cortex. BP rose in both groups, more in the NaCl than in the Na citrate group, but the difference was not significant. At the end of the 8th week there was a mean cumulative Na balance of 68.7 and 112.7 mmol in the chloride and citrate groups, respectively (p=0.002), and a negative K balance of -105 and -64 mmol (p=0.001). ATPase activity in the RBC's was 262 (chloride) and 179 (citrate) nmol/mg/h (p=0.003), and 23.7 and 16.8 umol/mg/h in the kidney (p=0.095). We conclude that the citrate rats retained more Na than the chloride rats. There is a dissociation between Na retention and blood pressure in this model. ATPase correlates with Na retention but not with BP.

INTERACTION BETWEEN LOOP DIURETIC AND CALCIUM ANTAGONIST. C.S. Wilcox, N.R. Loon, M. Limacher* and B. Ameer,* Divisions of Nephrology, Cardiology and Pharmacy Practice, University of Florida, Gainesville, FL

Calcium antagonists (CA) and diuretics are often used together to treat hypertension. However, it is unclear if CA modify the short-term hemodynamic and salt-losing responses to diuretics. Therefore, Doppler echocardiographic, metabolic balance and pharmacokinetic studies were undertaken on 9 patients with mild essential hypertension during 2 weeks of regulated daily Na^+ intake of 120 mmol. Placebo (P) and nitrendipine (N: 20mg bid) were given for one week each (order randomized); bumetanide (B: 1 mg IV) was given for the last 3 days of each week. Although mean BP did not change with B during P (-0.3 ± 2.9 mmHg; ns) nor during N (-3.3 ± 4.7 mmHg; ns), N did change the hemodynamic responses to B. Thus, during P, B decreased cardiac output (CO: -24 ± 7%; p < 0.01) and increased total peripheral resistance (TPR: +40 ± 15%; p < 0.05). In contrast, during N, CO increased (+28 ± 9%; p < 0.02) and TPR decreased (-17 ± 7%; p < 0.05). N alone caused a negative Na^+ balance of -34 ± 15 mmol on the first day of N admnistration (p < 0.05). However, N did not modify the cumulative excretion of B (P, 263 ± 38 µg; N, 217 ± 21 µg), nor the increase in fractional Na^+ excretion with B (P, +7.0 ± 0.8%; N, +6.3 ± 0.8%), nor the relationship between Na^+ and B excretion (diuretic responsiveness). Therefore, cumulative negative Na^+ balance was significantly (p < 0.05) potentiated during the week with N + B (-108 ± 24 mmol) compared to P + B (-47 ± 17 mmol). Conclusions: 1. N reverses the short-term adverse effects of B on systemic hemodynamics. 2. N does not modify B pharmacokinetics or pharmacodynamics. 3. N and B have independent and additive salt-depleting actions in hypertension.

ACUTE HYPERTENSION DURING REDUCED UTEROPLACENTAL PERFUSION PRESSURE (UPP) IS INDEPENDENT OF THE RENIN-ANGIOTENSIN SYSTEM (RAS). Lori L. Woods. Division of Nephrology and Hypertension, Oregon Health Sciences University, Portland, Oregon.

Uteroplacental ischemia is known to cause hypertension in various species, but the mechanisms are unknown. The purpose of this study was to test the hypothesis that the RAS mediates the increased systemic arterial pressure (SAP) that occurs during reduced UPP, possibly due to release of renin or angiotensin from the ischemic gravid uterus. Five trained, chronically instrumented pregnant dogs (gestational age 49±2d, term=60d) maintained on a normal Na^+ intake (~80 mEq/d) were studied at rest. UPP was reduced to 60 mmHg using an inflatable aortic occluder positioned distal to the renal arteries but proximal to the uterine arteries, and precisely servo-controlled at this level for one hr. SAP increased by 13±2 mmHg, from 89±7 to a plateau of 102±7 mmHg ($p<.001$) after 30 min, and gradually returned to control after the occluder was released. On a separate day in the same animals, the RAS was fixed by infusing the converting enzyme inhibitor Captopril (14 μg/kg/min, i.v.) and sufficient angiotensin II to restore arterial pressure to normal (2-5 ng/kg/min, i.v.). Converting enzyme inhibition was verified by the absence of a pressor response to a bolus injection of angiotensin I (~250 ng). Reduction of UPP to 60 mmHg caused SAP to increase by 11±4 mmHg after 30 min when the RAS was fixed, a response which was not different from that in the control experiments. These data suggest that the increase in systemic arterial pressure during reduced uteroplacental perfusion pressure is independent of the renin-angiotensin system.

RENAL CLEARANCE OF ENALAPRILAT. M Zahid*, SK Mujais, A Quintanilla, K Koch*, W Shaw*, and T Gibson. Northwestern U and VA Lakeside, Chicago IL and Merck Sharp & Dohme Research Laboratories, West Point, PA.

Enalaprilat (Enal), active form of enalapril, is a converting enzyme inhibitor with widespread therapeutic use in hypertension and congestive heart failure. We investigated the renal clearance of Enal in 12 normal males. Simultaneous inulin, creatinine, and unbound Enal clearances were measured by standard techniques. Enal was given as a 1.5 mg bolus followed by a 0.475 μg/kg/min infusion. Levels of free Enal achieved during the infusion varied between 85 and 143 ng/ml but were stable for each subject. Renal clearances were: Inulin 119±18 mL/min, creatinine 127 ±15 mL/min (creat/inulin =1.07), and unbound Enal 222±49 mL/min (Enal/creat = 1.72; Enal/inulin =1.85). These results suggest that renal clearance of Enal occurs via a dual mechanism with significant tubular secretion and glomerular filtration. Altered clearance of the drug may occur when used in conjunction with other drugs that share or alter organic acid secretory pathway.

$1,25(OH)_2$ VITAMIN D IN THE YOUNG SPONTANEOUSLY HYPERTENSIVE RAT (SHR). Eric Young, Joan Torok*, Ann Marie Dolney*, David McCarron. Div. of Nephrology & Hypertension. Oregon Health Sciences University, Portland, Oregon.

We and others have reported that serum $1,25(OH)_2D_3$ levels are decreased in the SHR compared to the normotensive WKY in animals that are approximately 12 wks of age or older. However, others have reported that serum $1,25(OH)_2D_3$ is elevated in young SHR before the development of fixed hypertension. Therefore, we evaluated the vitamin D status of six wk old SHR and WKY rats maintain on a .1% calcium diet. Tail cuff blood pressure was 104.6±13.9 mmHg (mean ± SD) in the SHR and 96.5±15.5 in the WKY. There was no significant difference between SHR and WKY in serum total calcium (9.9±.3 vs 10.0±.3 mg/dl), serum ionized Ca (.90±.03 vs .91±.04 mM), and serum phosphorus (9.9±.6 vs 9.9±.6 mg/dl). However, serum $1,25(OH)_2D_3$ was significantly increased in the SHR compared to the WKY (387±58 vs 338±47 pg/ml, $p<.02$). Although the serum $1,25(OH)_2D_3$ concentration was elevated in the SHR, measurements of *in vitro* $1,25(OH)_2D_3$ synthesis by freshly isolated proximal tubules showed mildly decreased production in the SHR ($p<.01$ by ANOVA for incubation time and substrate concentration effect). Basal cyclic AMP production by proximal tubules tended to be decreased in the SHR (5.9±2.0 vs 8.3±1.6, $p<.10$), but incubation with PTH resulted in comparable stimulation of proximal tubular cyclic AMP in both strains (53.7±28.5 vs 54.4±22.0, NS). Thus, in accord with other reports, serum $1,25(OH)_2D_3$ is elevated in young SHR relative to WKY at the time they are developing hypertension.

HYPOHOSPHATEMIA IN ESSENTIAL HYPERTENSION. C.Zoccali, F.Mallamaci, F.Cuzzola, S.Parlongo, M.Postorino, G.Maggiore (introduced by G.Orlandini). CNR Centro Fisiologia Clinica,Reggio Cal ITALY.

Hypophosphatemia has been reported in patients with essential hypertension (EH), but the interpretation of this finding remains controversial. To study the relationship between hypophosphatemia and hypertension we have measured serum (s) and urinary (u) levels of Phosphate (P), Ca,Mg, plasma ionised Ca (pCa++), serum PTH (mid-region assay), in 45 treatment-free pts with mild to moderate EH and in 27 healthy subjects (HS). Serum P was lower ($P<.01$) in EH (2.96±SE.09 mg/dl) than in HS (3.51±.12 mg/dl) and 13 EH displayed frank hypophosphatemia (sP ≤2.5 mg/dl). This alteration was associated with a reduced TmP/GFR (2.7±.1 vs 3.3±.1, $P<.01$). Both sP and TmP/GFR were inversely related to systolic and diastolic pressure ($P<.01$). Calcium excretion was higher ($P<.01$) in EH but pCa++, PTH and s & u Mg did not differ in the two groups. Body weight was higher ($P<.05$) in hypophosphatemic EH (80±3 Kg) than in normophosphatemic EH (73±2 Kg). However, PTH and s & u levels of Ca and Mg in hypophosphatemic EH were identical to those in normophosphatemic EH.

Hypophosphatemia in EH results from impaired renal reabsorption of the anion but does not represent a mere consequence of parathyroid activation secondary to hypercalciuria. The relationship between sP and BP suggests that the development of hypophosphatemia is linked closely to the hypertensive process. Factors related to body weight excess play a role in reducing serum phosphate in EH.

CHARACTERIZATION OF A NOVEL GLOMERULAR EPITHELIAL CELL (GEC) GLYCOPROTEIN, GEC-GP140, AND ITS PARTICIPATION IN ANTIBODY (Ab)-MEDIATED GEC INJURY. Christine K. Abrass, VA Medical Center, Seattle, WA.

A glomerular antigen (gec-gp140) identified in glomerular eluates from rats with active Heymann nephritis (J. Immunol. 137:530, 1986) was isolated from normal rat glomeruli. gec-gp140 is a 140 kD heterodimeric, basic (pI 9.2) glycoprotein (gp) composed of 2 chains of 85 and 50 kD. Rabbit (Rab) anti-gec-gp140 binds to cultured GEC and only to the surface of GEC by immunoEM. Biochemical characteristics and cellular distribution of gec-gp140 distinguish it from gp330, podocalyxin and other GEC proteins. To study gec-gp140's role in the maintenance of glomerular capillary wall integrity, Ab to gec-gp140 was passively transferred to naive rats (n=8). The pattern of Ab deposition and cellular injury were studied for 49 days by IF, EM and immunoEM. Subepithelial electron dense deposits develop at 6 hrs when Ab binds to gec-gp140 on the base of podocytes. These subepithelial deposits do not persist beyond 7 days. Rab Ab is on the entire surface of GEC at 6 hrs. Rab IgG persists on the GEC surface throughout the study, but becomes more segmental over time. Ab binding is initially associated with segmental effacement of podocytes (6hr-16d); however, more extensive effacement is apparent after day 21, when C3 deposition occurs and proteinuria (>40 mg/d) develops. As rat anti-Rab IgG binds in situ, large aggregates of immune deposits are formed on the GEC surface with rearrangement of cytoskeletal elements underneath. Rats (n=8) given normal Rab IgG develop no abnormalities. gec-gp140 is a novel gp expressed on the surface of GEC that can participate in a unique form of Ab-mediated GEC injury.

CELLULAR ORIGIN OF ENHANCED LEUKOTRIENE B_4 (LTB_4) SYNTHESIS DURING ACUTE RAT NEPHROTOXIC SERUM (NTS) NEPHRITIS. C. Albrightson-Winslow*, D.E. Griswold* and K.F. Badr, Smith Kline & French Labs, King of Prussia, PA & Vanderbilt Univ., Nashville, TN.

Glomerular LTB_4 synthesis is enhanced 2 h after NTS administration in the rat. Its chemotactic and leukocyte (PMN)-activating properties markedly amplify early functional derangements in this model of glomerular injury. Cellular sources of LTB_4 biosynthesis, however, are undefined. Two h post-administration of NTS (n=5) or control rabbit serum (RS, n=5) to Munich-Wistar rats, kidneys were removed, ex vivo perfused, and effluent measured by RIA for LTB_4, PGE_2 and TxB_2 pre and post f-Met-Leu-Phe (fMLP, 1 µg) stimulation. Basal eicosanoid levels were not different between kidneys from NTS and RS-treated rats. fMLP increased mean LTB_4 generation in NTS kidneys from 120±22 to 310±50 pg/5 min ($p=.01$), but was without effect on LTB_4 release from RS kidneys: 130±20 to 160±40 pg/5 min. No stimulation of PGE_2 or TxB_2 release was noted in either group. To assess the contribution of infiltrating PMNs to LTB_4 release, we measured myeloperoxidase activity (MPOA), a marker of tissue PMN content. MPOA from NTS kidneys was 120±11 vs. 27±19 U/mg in RS-treated controls ($p<.005$). Regression analysis revealed a highly significant correlation between fMLP-stimulated increase in LTB_4 synthesis and renal tissue MPOA, $R=.79$, $p=.007$. These experiments: 1) suggest strongly that infiltrating PMNs are a principal source of leukotriene release during early experimental glomerulonephritis; and 2) provide evidence for the preferential activation of PMN 5-lipoxygenase (vs. cyclooxygenase) pathway during this phase of injury.

EPIDERMAL GROWTH FACTOR (EGF) REVERSES HEPARIN (H) INHIBITION OF RAT GLOMERULAR VISCERAL EPITHELIAL CELL (GEC) GROWTH AND STIMULATES PROLIFERATION. S. Adler, New York Medical College, Valhalla, NY

Proliferation of GEC is a feature of several types of glomerular disease but the factors regulating GEC growth are unclear. We have previously shown that H, heparan sulfate and other sulfated polysaccharides inhibit GEC growth (Am J Physiol, 1988,In press). The ability of known growth factors to reverse this inhibition and to regulate GEC proliferation was explored using a cloned line of rat GEC. EGF partially reversed the inhibitory effect of H (100 ug/ml) in a dose dependent, saturable manner (96.6±3.6% inhibition by H; 50.7±5.4% with EGF -1 ng/ml). Complete reversal of H inhibition could not be achieved even at low doses of H (lug/ml). EGF also directly stimulated proliferation of GEC growing in minimal media (2.0±0.3-fold increase in cell number at 1 ng/ml). Insulin-like growth factors 1&2 (IGF-1&2) and platelet derived growth factor (PDGF) alone or in combination with EGF did not reverse H inhibition or directly stimulate GEC growth suggesting a specific effect of EGF. Delay in addition of EGF for 2 hours following H resulted in a moderate decrease in the amount of reversal but no significant reversal occurred with a 24 hour delay. Treatment with H for 2 hours did not alter the amount of 125I-EGF binding to the cells.

These studies suggest that sulfated glycosaminoglycans inhibit GEC growth through both EGF-dependent and EGF-independent pathways. They also demonstrate that EGF stimulates proliferation of GEC, an effect which may be important in the pathogenesis of glomerulonephritis.

SCLEROSING GLOMERULOPATHY IN RHESUS MONKEYS WITH SIMIAN AIDS, Charles E. Alpers and Gary B. Baskin, Univ. of Washington, Seattle, WA and Delta Regional Primate Research Center, Covington, LA.

Human immunodeficiency virus (HIV)-associated nephropathy in man is characterized by focal glomerulosclerosis or mesangial hyperplasia and tubular dilatation with casts. Rhesus monkeys (n=19) inoculated with a simian immunodeficiency virus (SIV) genomically similar to HIV type 2 developed an immunodeficiency syndrome and lymphadenopathy, splenomegaly, diarrhea, weight loss, rash, and less frequently, encephalitis and hepatitis. The median survival was 44 weeks after infection.

Five monkeys developed a florid, sclerosing glomerulopathy. Glomeruli were enlarged, and showed segmental to global spongy expansion of mesangial matrix, modest cellular proliferation, and thickening and reduplication of glomerular basement membranes. One animal had focal features of a thrombotic microangiopathy involving glomerular capillaries and small arteries. Electron microscopy revealed capillary membranes with variable changes including reduplication, mesangial interposition, subendothelial lucency, and accumulation of particulate debris. Occasional foci of mesangiolysis were seen. Two cases showed scattered intraluminal and subendothelial fibrin tactoids. Crystalline endothelial tubulo-reticular structures, normally present in monkeys, were commonly present. No viral particles or immune deposits were identified. Five additional monkeys had less advanced glomerular changes consisting of mesangial hyperplasia. Foci of interstitial nephritis were present in 7 monkeys; focal tubular dilatation and cast formation were also seen. Uninfected monkeys and primates infected with other simian retroviruses (SRV) did not develop these lesions. These studies demonstrate an association between SIV infection and glomerular disease, and provide the first relevant model for the study of HIV-associated nephropathy.

INVOLVEMENT OF AN ANTIGEN DISTINCT FROM THE HEYMANN ANTIGEN IN MEMBRANOUS GLOMERULONEPHRITIS IN THE MOUSE. Karel J.M. Assmann*, Pierre Ronco*, Pierre Verroust*, and Robert A.P. Koene. Depts. of Pathol. and Nephrol., University Hospital Nijmegen, The Netherlands, and Inserm U 64, Hôpital Tenon, Paris, France

Membranous glomerulonephritis (MG) in the mouse can be induced by injection of a heterologous anti-serum against murine pronase digested renal tubular antigens (Kidney Int 24:303-312, 1983). The antigenic target in the glomeruli of mice is different from the Heymann antigen (gp 330). It shows the characteristics of a smaller antigen (gp 90) that can also be detected by a monoclonal antibody. We isolated the anti-gp 90 component from the polyclonal antiserum by absorptions and elution using mouse liver as a substrate, which lacks gp 330 but does express gp 90. Immunoprecipitation of radiolabelled renal proximal tubular brush borders (BB) demonstrated that the mouse liver eluate reacted with gp 90, but not with gp 330. In immunofluorescence the eluate bound to the glomerular capillary wall of both mouse and rat in a homogenous pattern. Injection of the eluate led to a transient, homogenous binding to the glomerular capillary wall of the mouse and also, though to a lesser extent, to that of the rat. Injection in mice presensitized against rabbit Ig caused a more prominent granular binding in a pattern typical of MG. The results demonstrate that an antigen with a molecular weight and a kidney distribution different from the Heymann antigen, can serve as a target for antibody-mediated MG in the mouse. Furthermore, they suggest that this antigen may also be involved in the MG of the rat in addition to the Heymann antigen.

GLOMERULAR LOCALIZATION OF PLATELET SECRETORY PROTEINS IN MESANGIAL PROLIFERATIVE LESIONS INDUCED BY HABU SNAKE VENOM (HSV). Jeffrey L. Barnes, Depts. of Pathol., Rhode Island Hosp. and Brown Univ., Providence, R.I..

Some platelet secretory proteins are growth factors for mesangial cells in culture and have been implicated in the pathogenesis of proliferative glomerular disease. In this study, the temporal association between glomerular localization of platelet secretory cationic proteins (PSCP) and progression of mesangial hyperplasia was examined in a model of mesangial proliferative glomerulonephritis induced by HSV. Intravenous injection of HSV (2mg/kg) leads to capillary dilatation into cysts filled with platelet aggregates at 8 hours which became more prominent at 24 hours. By 48 hours, lesions were heterogeneous, some cystic and others nodular (comprised of confluent proliferative cells). Most lesions were mixed showing features of cystic lesions containing clusters of proliferating cells. Lesions were mainly nodular at 72 hours. The above lesions were associated with persistent localization of PSCP as demonstrated by immunofluorescence microscopy. At 8 hours PSCP were restricted to platelets and their aggregates with limited diffusion into surrounding structures. PSCP localization was more diffuse at later time intervals and by 72 hours showed a homogeneous pattern throughout the nodules. Thus, prior to and throughout the proliferative phase of nodular formation, mesangial cells are exposed to a milieu replete with PSCP, some of which are presumably growth factors. This persistent association of PSCP with proliferating cells suggests a potential role for platelet secretory proteins and mesangial hyperplasia in this model.

MESANGIAL HYPERCELLULARITY AND UPTAKE OF MACROMOLECULES. W.M. Bagchus[x], M.F. Jeunink[x] and J.D. Elema[x]. Dept. Pathology, Univ. of Groningen, Groningen, The Netherlands. (Intr. by E.A. Lianos).

Monoclonal anti-Thy.1 antibodies (MATS) cause mesangiolysis, followed by mesangial proliferation leading to transient mesangial hypercellularity (MH). (Lab Invest 55:680, 1986). The accompanying proteinuria (UP) declines gradually after the 3rd day. The glomerular uptake of macromolecules was studied with iron dextran (ID) in male Wistar control (C) and MATS rats at 2, 3 and 5 weeks after induction of mesangiolysis. Serum iron levels at 24, 48 and 72 h after ID injection did not differ between C and MATS rats, indicating identical disappearance rates. Glomerular ion (in %) was measured in kidney sections 4 days after ID, using an image analyzing system. Glomeruli with overt MH were scored histologically. The incidence of focal sclerosis (FS) was studied histologically after 20 weeks in 2 additional groups (N>10) of C and MATS rats.

	UP (mg/24h)	% Glomeruli with MH	Glomerular iron (%)
MATS:			
2 weeks (N>5)	44 ± 16	23.0	167 ± 9[f]
3 weeks (N>5)	23 ± 12	9.8	129 ± 35[f]
5 weeks (N>5)	23 ± 7	2.5	109 ± 25
Control (N>5)	27 ± 12	0.0	100 ± 15

The significant (f=P<0.05 vs C) increase of glomerular ion in the MATS rats at week 2 and 3 correlated with the degree of MH. No significant UP was present anymore. Since after 20 weeks no difference was found between the incidence of FS in C and MATS rats, these data indicate that transient increased MH in itself does not lead to focal sclerosis.

CHARACTERIZATION OF A MONOCLONAL ANTIBODY TO GP 330 AND ITS ROLE IN THE PATHOGENESIS OF HEYMANN NEPHRITIS (HN). M. Behar*, R. Cameron* and M. Silverman, Membrane Biology Group, Univ. of Toronto, Toronto, Ontario.

A brush border membrane (BBM) constituent gp 330 localized in the glomerular epithelial cells (GEC) has been implicated in the pathogenesis of (HN) (J. Exp. Med. 157:6667,1983). By fusing the spleen of a nephritic Sprague Dawley (SD) rat with non secretory myeloma rat cells, a rat monoclonal antibody (Mab) to gp 330 was obtained. The present study was to investigate its role in the pathogenesis of HN. Mab gp 330 was injected intravenously in SD rats using identical experimental conditions (and the same protein load) by which intravenous injection of a rabbit polyclonal anti rat BBM results in subepithelial deposits and proteinuria (typical of passive HN). After 4 days the serum of the sacrificed animals show circulating anti gp 330 but no subepithelial glomerular deposits and no proteinuria. When a solid tumor secreting Mab anti gp 330 was transplanted in nude rats, after 14 days circulating anti gp 330 was present in the serum, there were small subepithelial deposits in the glomerulus but no proteinuria. We showed Mab anti gp 330 to be an IgM with capability of binding complement. When labeled with biotin the anti gp 330 Mab bound to the BBM of renal proximal tubular cells and to the GEC membrane facing the urinary space. The inability of Mab anti gp 330 to induce passive HN may be due to its inefficiency in producing subepithelial complexes or to the absence of other necessary immune complex(es).

BINDING OF MONOCLONAL ANTI-DNA TO HEPARAN SULPHATE (HS), GBM-HEPARAN SULPHATE PROTEOGLYCAN (GBM-HSPG) AND TO ISOLATED GBM-LOOPS. Jo H.M. Berden*, Rose-Marie Termaat*, Ruud J.T. Smeenk*, Peter Faaber*, Karel J.M. Assmann* (intr.by Robert A.P. Koene) Depts. of Nephrology and Pathology, Univ.Hospital Nijmegen, Netherlands

Previously we have shown (JCI 1986;77:1824) that polyclonal anti-DNA can crossreact with HS in ELISA. Forty-two monoclonal anti-DNA antibodies (MoAb) were obtained by fusion of spleen cells from MRL/l,(NZBxW)F_1, and GvH mice. Sixteen of the MoAb crossreacted in ELISA with HS. This binding to HS could be inhibited by DNA. Fifty percent of HS-crossreactive MoAb bound to human GBM-HSPG in ELISA and/or Western blots, but not to HSPG-core protein after removal of HS. Subsequently we isolated GBM loops (GBM-L) from human and rat glomeruli. Ultrastructurally we found a strong binding of cationic ferritin (CaF), indicating that anionic sites were well preserved. With indirect immunofluorescence on cryostat sections of these GBM-L, 7 of the 42 MoAb showed a fine granular staining along the GBM. Binding to GBM-HSPG in ELISA and to GBM-L could be inhibited by DNA. Heparitinase treatment of GBM-L diminished but did not completely prevent binding of either CaF or MoAb. Preincubation of GBM-L with CaF almost completely inhibited the subsequent binding of MoAb. Some MoAb showed a positive GBM-L staining although they did not bind in ELISA to HS or GBM-HSPG. These results demonstrate that monoclonal anti-DNA antibodies can bind directly to HS and to other not yet identified anionic sites in the GBM. The findings suggest that direct binding of anti-DNA to GBM might play a role in the initiation of SLE-nephritis.

TRANSFORMING GROWTH FACTOR ß (TGFß) UNIQUELY REGULATES PRODUCTION AND STRUCTURE OF GLOMERULAR EXTRACELLULAR MATRIX PROTEOGLYCANS. W. Border, S. Okuda*, L. Languino*, E. Ruoslahti*. University of Utah Health Sciences Center, Salt Lake City, UT and La Jolla Cancer Research Foundation, La Jolla, CA.

Accumulation of extracellular matrix (ECM) is a prominent feature of progressive glomerulonephritis. Since some growth factors are known to stimulate ECM production we examined the effects of TGFß, interleukin-1 (IL-1), platelet-derived growth factor (PDGF) and tumor necrosis factor (TNF) on the production of ECM by rat mesangial cells in culture. Cells were metabolically labeled with ^{35}S sulfate or ^{35}S methionine and conditioned media were analyzed by SDS-PAGE with fluorography combined with the use of enzymes or antibodies for specific molecular identification. In control experiments mesangial cells produced two species of proteoglycan identified as broad bands centered at 200 and 120 KD. These bands correspond in size to the small chondroitin/dermatan sulfate proteoglycans PG I and PG II (decorin) respectively; and, enzyme digestion showed both bands to be composed of chondroitin/dermatan sulfate. Exposure to TGFß for 48 h greatly increased the PG I band and induced a structural change detected as a shift in electrophoretic mobility. TGFß also produced a small increase in fibronectin but not laminin or type IV collagen. IL-1, PDGF or TNF had no substantial effects. These experiments show that TGFß is unique among growth factors in its metabolic effects on glomerular ECM. The release of a substance like TGFß in glomerulonephritis could stimulate the expansion of ECM and mediate the progression to glomerulosclerosis.

IMMUNE COMPLEX (IC)-ERYTHROCYTE COMPLEMENT RECEPTOR TYPE 1 (E-CR1) INTERACTIONS *IN VIVO* DURING INDUCTION OF GLOMERULONEPHRITIS (GN) IN NONHUMAN PRIMATES. D Birmingham*, LA Hebert, FG Cosio, J Mahan, R. Goel*, W Smead*. Ohio State University, Columbus, OH.

E-CR1, which are unique to primates, may be a key factor determining the fate of circulating IC. All previous studies examining the *in vivo* interaction of IC and E-CR1 used trace doses of IC. The present study was done to assess IC/E-CR1 interactions at high levels of IC formation in the circulation, levels that ultimately induced GN. Bovine gamma globulin (Ag) immunized cynomolgus monkeys (n=10) with E-CR1 levels ranging from 25 to 5000 CR1/E were studied first with low dose Ag (L-IC) resulting in trace IC formation, and then with high dose Ag (H-IC) causing IC levels capable of inducing GN. Under L-IC conditions (mean Ag dose=.04 mg/kg), IC formed in antibody excess and demonstrated peak E binding at 2-5 minutes (range=10-29%). Under H-IC conditions (mean Ag dose=5.8 mg/kg), IC formed at equivalence or in slight antigen excess and demonstrated peak E binding at 2-5 minutes (range= 6-36%). At L-IC, peak E binding correlated well with the log CR1/E ($r=.89$, $p<.0005$), while at H-IC there was more variation ($r=.70$, $p<.025$). The amount of Ag bound to E as IC was 50-200 times greater under H-IC conditions than under L-IC conditions. During H-IC, acute decreases in E-CR1 levels occurred (mean % decrease = 30% ± 6 SE, $p<.005$), and returned to near baseline within 24 hours. The decreased E-CR1 levels could not be explained by loss of E from the circulation. In summary, under conditions that lead to GN in the primate, IC that form in the circulation bind in large amounts to E-CR1 and cause acute reduction in E-CR1 levels. The present observations may explain the low E-CR1 levels seen in active SLE, and are consistent with the hypothesis that interactions of IC with E-CR1 are important in the pathogenesis of GN in the primate.

ANTI-LAMININ AND ANTI-GP90 AUTOANTIBODIES IN EXPERIMENTAL LUPUS NEPHRITIS. Jan A. Bruijn*, Ed H.G. van Leer*, Willem E. Corver*, Pancras C.W. Hogendoorn*, Emile de Heer*, Philip J. Hoedemaeker, and Gert J. Fleuren*. Univ. of Leiden, Dept. of Pathol. The Netherlands.

We studied the specificity of nephritogenic autoantibodies involved in murine chronic graft-vs-host disease (GvHD), an established model for human lupus nephritis (Bruijn et al. Am.J.Pathol. 130:639-641, 1988). Two wks after the induction of the disease, antibodies were found in a linear pattern along the glomerular basement membrane (GBM). This pattern changed to granular after 6 to 8 wks. At this stage, large electron-dense glomerular aggregates were present along the GBM both subepithelially and subendothelially. Proteinuria increased from 10 ± 2 to a maximum of 11,300 ± 2,140 µg/18hr. In sera and renal eluates antibodies were directed against GBM and renal tubular epithelial antigens (RTE). Injection in naive animals with affinity-purified anti-RTE antibodies from GvHD-serum resulted in an immunecomplex glomerulonephritis and proteinuria. Using absorption, ELISA, and Western blotting techniques the antibody specifities were further characterized. The anti-GBM fraction was mainly directed against laminin, whereas the main antigenic target in RTE was gp90 (dipeptidyl peptidase IV), an earlier identified nephritogenic glycoprotein. We conlude, that these antibodies play a nepritogenic role in GvHD.

RAT MESANGIAL CELLS (MCs) PRODUCE A CYTOKINE INDISTINGUISHABLE FROM GRANULOCYTE-MACROPHAGE-COLONY-STIMULATING FACTOR (GM-CSF).

K. Budde*, D.L. Coleman* and R.B. Sterzel; Dept. of Medicine, Yale U Sch of Med - VAMC, New Haven, CT.

Since glomerular inflammation can involve monocyte-macrophages (MPs) and T-lymphocytes, we have investigated whether products of MCs can stimulate the proliferative activity of these effector cells. We found that cultured rat MCs (subcultures # 2-15), maintained under serum-free conditions, secrete a soluble factor into the supernate (MC-CM), which supports growth of the T-helper cell-derived line, HT-2. This effect was completely inhibited by pretreatment of MC-CM with a polyclonal antibody to GM-CSF, but was not affected by a monoclonal antibody (11B11) to IL-4. MC-CM increased [^{3}H]thymidine incorporation by thioglycollate-elicited peritoneal MPs, but did not induce growth of the IL-2 or IL-4 dependent cell line, CTLL-2. By reversed-phase HPLC, the active fraction on HT-2 cells eluted at 48-50% acetonitrile. This fraction also stimulated proliferation of MPs but not of CTLL-2 cells. Gel filtration HPLC of the HT-2 stimulating factor revealed a molecular weight between 44 and 20kD and isoelectric focusing showed a pI of 4.55-5.0. These biochemical properties are consistent with those of GM-CSF from other cell sources. Western blot analysis using a polyclonal antibody to GM-CSF further confirmed, that cultured MCs produce a factor which is indistinguishable from this cytokine. Release of GM-CSF by MCs in vivo may play a role in the interaction of MCs with MPs, T-cells and neutrophils in glomerular disease.

ENHANCED IL-1 GENE EXPRESSION IS LOCALIZED BY IN SITU HYBRIDIZATION TO GLOMERULAR MESANGIUM IN EXPERIMENTAL GLOMERULONEPHRITIS(GN). S. Carey*, R. Ondash*, N. Njoku*,S.N. Emancipator, and J.R. Sedor. Case Western Reserve Univ, Cleveland, OH.

The IL-1 gene is expressed in kidneys of rats with immune complex GN induced by cationic bovine gamma globulin(cBGG). To localize the site of enhanced IL-1 mRNA, we hybridized 4 μm sections of kidney from normal and GN rats with complementary (antisense) or noncomplementary (sense) [^{35}S]IL-1β cRNAs, synthesized by in vitro transcription and size limited by alkali to 100 bp, or [^{35}S]end-labelled oligonucleotides (bp 776 - 810 of the murine IL-1β sequence). After autoradiography, grain counts over randomly selected fields of glomeruli and cortical tubules were quantified, corrected for background (emulsion not overlying tissue) and normalized for cross-sectional area. Glomeruli, but not tubules, of rats with cBGG GN specifically hybridized antisense (6.51 ± .01 grains/10 μm^2) but not sense probes. Normal rat kidney did not specifically hybridize either probe. Glomeruli from rats with cBGG GN hybridized with antisense probes contained 1-4 cells with ≥ 5 grains/cell, while nephritic glomeruli hybridized with sense probes or normal glomeruli incubated with either probe had only 0-1 cells with ≥ 5 grains/cell. Northern analysis of total RNA isolated from renal cortex of rats with cBGG GN demonstrated a 1.6 kb transcript which comigrated with macrophage IL-1 transcripts. Kidney sections from mice with mesangial proliferative GN and rats with heterologous phase nephrotoxic serum nephritis did not express IL-1β transcripts. We conclude in some models of GN, cells within the glomerular mesangium have increased levels of IL-1β expression.

EFFECT OF CYCLOSPORINE (CSA) ON THE ENDOTOXIN (E) INDUCED GENERALIZED SHWARTZMAN REACTION (GSR) IN THE DIABETIC (D) RAT. Timothy E. Bunchman, S. Michael Mauer, and Youngki Kim, Univ. of Minnesota, Minneapolis, Minnesota.

D rats are susceptible to E-induced GSR. Here we studied the effect of CSA on the GSR in rats made D with 75 mg/kg intravenous (iv) of streptozotocin. After 6 weeks of D, CSA (20 mg/kg) or intralipid (sham) was given intraperitoneally, daily for 10 days. Then, low dose E (0.1 mg/kg iv E, E. Coli 0.26:B6 Lipopolysaccharide B) was given 4 hours prior to sacrifice. The CSA/E treated D rats had an increase in glomeruli thrombi compared to the intralipid/E treated D rats (p <0.001,Table).

	NON-DIABETIC		DIABETIC	
	% with Thrombi		% with Thrombi	
	Rats	Glomeruli	Rats	Glomeruli
CSA	0	0	0	0
Sham/E	0	0	37	6+2*
CSA/E	14	1.4+1.4*	90	64+20*

Fewer glomerular thrombi were seen in the CSA treated D rats given high dose (2 mg/kg) E as compared to the D rats receiving high dose E alone. Daily insulin (4.5 i.u. ± 0.03*) resulted in reduced blood glucose in treated (206 mg/dl ± 30*) compared to untreated (690 mg/dl ± 70*) rats. Insulin treated rats given low dose E and CSA had fewer glomerular thrombi than non-insulin treated rats (p <0.001). Thus, CSA potentiates the GSR at low dose of E and diminishes the GSR at high dose E in D rats. Improved glycemic control diminishes the GSR in CSA treated D rats given low dose E. These interactions of CSA, diabetes, and E require further study. *(mean ± SEM)

EFFECT OF MONOPHOSPHORYL LIPID A (MPLA) ON PERITONEAL LEUKOCYTE FUNCTION

S. Carozzi[1*], M. G. Nasini[1*], M. Salit[2*], A. Cantaluppi[2*], S. Lamperi[1*] (intr. by S. Webster). Ospedale San Martino, Genova, Italy[1], Baxter Healthcare, Round Lake, Illinois[2].

Peritoneal leukocytes derived from CAPD patients who suffer high peritonitis incidence display a pattern of dysfunction when compared to cells taken from patients with lower infection rates. In the present study, MPLA, a relatively non-toxic, bacterial lipid A derivative, was tested for its ability to modulate the *in vitro* immune function of peritoneal cells taken from high infection rate CAPD patients. Peritoneal lymphocytes and macrophage were isolated from the overnight effluents of 6 patients (3 male, 3 female, average infection rate=1 case/7.2 PTM) and placed in culture. The cell cultures were incubated with varying concentrations of MPLA for 48 hours at 37°C. Culture supernatants were then tested for IL-1 (LAF assay), IL-2 (mitogenic assay), and gamma interferon (RIA). The cultured cells were tested for Fc receptor density (flow cytometry) and bactericidal capacity versus serum-opsonized *S. epidermidis*. MPLA at concentrations of 0.5 ug/ml to 50 ug/ml stimulated significant ($p<0.05$ vs. baseline) production of IL-1, IL-2 and gamma interferon. IL-1 and IL-2 production approached that inducible from control (laparoscopy) cells. Peak responses occurred in the presence of 5 ug MPLA/ml. MPLA at concentrations above 0.5 ug/ml elevated Fc receptor density from about 14,000 receptors/cell to about 25,000 receptors/cell; the level observed in control cells or those derived from low infection risk patients. Significant enhancement of bactericidal capacity was achieved by incubating macrophage in the presence of 0.5 ug MPLA/ml. MPLA, therefore, acts as a potent *in vitro* immunomodulator, capable of partially relieving the dysfunction associated with peritoneal leukocytes taken from high peritonitis rate patients.

FUNCTIONAL CHARACTERISTICS OF MACROPHAGES IN GLOMERULONEPHRITIS (GN). V. Cattell*, T. Cook*, J. Smith* and J. Salmon*. St. Mary's Hospital Medical School, London and Wellcome Research Labs, Kent, England. (intr.by R. Cotran)

The state of activation of infiltrating macrophages (GM) in gn may be central to their role in injury. We developed a method for isolating leucocytes from nephritic glomeruli (gl) and have studied GM function in rat in situ gn induced by cationized IgG. Leucocytes were characterised by monoclonal antibodies against leucocyte common (OX1), Ia (OX3/4) and macrophage (ED1) antigens. Superoxide (O_2) generation, zymosan (Z) and PMA stimulated, measured by ferricytochrome c reduction, and eicosanoid generation (PGE_2, TXB_2 and LTB_4) basal and ionophore stimulated (I) measured by RIA were studied in GM and in control thioglycollate-elicited (TM) and C.Parvum activated (CP) peritoneal macrophages. At maximal proteinuria (day 7) there were 534 ± 69 total leucocytes/ gl (normal rats 13 ± 1) with 407 ± 83 GM/gl. GM Ia expression rose from 15% on day 1 to 37% on day 7. Results of functional studies:

	No.	O_2 (nmol/mg) PMA	Z	PGE_2 (I stimulated ng/mg)	TXB_2	LTB_4
GM	3	131±34	312±79**	62±10*	109±28	UN
TM	3	45±17	89±31	663±128	201±53	119±56
CP	3	65±10	ND	442±39	150±4	UN

*=P<0.01; **=P<0.05 UN = undetectable (<13ng/mg)

Thus, large numbers of viable GM can be isolated from gl in gn for functional studies. GM in this model 1) produce large amounts of O_2, which may be a main mechanism of injury and 2) express high levels of Ia antigen and down regulated PGE_2 and LTB_4 production, characteristics described for immune activated macrophages.

IS NORMAL MHC EXPRESSION CONSTITUTIVE OR INDUCED? S.M. Cockfield*, J.R. Pleasants, P.F. Halloran, Department of Medicine, University of Alberta, Edmonton, Alberta.

Normal levels of renal MHC expression could be constitutive or induced by cytokines (ie. interferons) released by contact with foreign antigen. We have studied the relative contribution to normal expression by these mechanisms in murine kidney using a binding assay with radiolabelled anti-MHC mAb and immunohistology. Studies in T cell deficient nude and SCID (severe combined immunodeficiency) mice demonstrated normal levels of class I and II MHC expression, suggesting that functional T cells are not required for normal expression. Furthermore high doses of cyclosporine, anti-IFN-γ, or anti-IFN-α/β had little or no effect on expression. MHC products were not reduced in germ-free mice on a chemical, endotoxin-free diet, indicating that normal expression is not dependent on mediators induced by microbial flora. However class II expression was lowered by >50% with high dose irradiation, and this correlated with a decrease in staining of class II positive dendritic cells. Thus most normal MHC expression appears to be constitutive and independent of induced cytokines but a component of class II is expressed on or induced by a radiation sensitive cell. Therefore renal class II MHC may be independently downregulated which may have implications for clinical transplantation.

LIPOPOLYSACCHARIDE (LPS) FROM GRAM NEGATIVE BACTERIA INDUCES EARLY AND ACCELERATED NEPHRITIS IN NZB/W MICE. Tito Cavallo, Norman A. Granholm, Rodney Nunley*, Joseph Langevin*, Annette Ragosta*, and Colette Charland*. Department of Pathology, Rhode Island Hospital and Brown University, Providence, RI.

The NZB/W female mice develop spontaneous lupus nephritis by 5 to 6 months of age. To investigate the role of associated gram negative infections in the induction and progression of nephritis, we injected 2.0 month old NZB/W mice with LPS from *Salmonella minnesota* Re595, 50 mcg twice a week for 5 weeks. Compared to uninjected controls, at 3.5 months of age, LPS mice had features of polyclonal B cell activation [plasma concentrations of IgG, IgM, circulating immune complexes (IC), and antiDNA antibodies increased about 3-, 20-, 12-, and 3-fold, respectively], and nephritis with 6-fold increase in proteinuria (P≤ 0.01) and mild renal insufficiency. To investigate whether early nephritis induced by LPS resulted from impaired processing of IC, we gave LPS and control mice a subsaturating dose of BSA-antiBSA (0.12mg/g b.w.) containing greater than 60% nephritogenic IC (size larger than Ag_2Ab_2).

Groups	Proteinuria (mg/day)	Clearance (t 1/2, h)	Liver IC (mcg/g)	Kidney IC (mcg/g)
LPS	14.6±1.1	0.5±0.1	150.3±8.2	80.5±1.9
Control	2.6±0.1	0.7±0.1	159.9±9.7	71.1±5.2

As shown in the Table, the clearance, liver uptake, and renal localization of model IC were not significantly different in LPS mice compared to control mice. The data militate against impaired processing of IC as a mechanism of induction of and acceleration of nephritis by LPS. Instead, biologic characteristics of naturally occurring IC and/or *in situ* interaction of antibody with glomerular bound antigen are more likely to play a pathogenic role.

THE RELATION OF MESANGIAL CELL (MC) PROLIFERATION AND EXTRACELLULAR MATRIX (ECM) FORMATION IN PROLONGED CULTURE M. Coffee*, J. Grond*, M Kashgarian, R. B. Sterzel. VAMC-Yale Univ Sch Med, New Haven, CT.

The interaction of rat MCs and ECM was studied in prolonged (6-8 weeks) culture, where MCs grow three-dimensionally in hillocks with a core of ECM with scattered stellate MCs surrounded by fusiform MC. MC growth was assessed by 24 hr bromodeoxyuridine (BrdU) incorporation. After 4 days of serum-starvation in resting medium with .5% FCS, MCs were either kept in resting medium or switched to growth medium with 20% FCS for the subsequent 4 days. Both fusiform and stellate MCs showed persistent growth activity in resting medium with labeling indexes (LI) at day 5 of 9±3% and 4±2%, resp (p<.01,mean ± SD), and 5±2% and 3±2% at day 8 (NS). In growth medium, LI of fusiform MCs rose markedly to 39±9% at day 5 and further to 54±6% at day 8 (p<.01). In contrast, LI of stellate MCs did not change in growth medium: 4±1% and 4±2% at day 5 and 8 (NS), resp. LI of stellate MCs was independent of hillock size. BrdU-positive fusiform MCs were found embedded in ECM containing fibronectin, laminin, thrombospondin, and collagen type IV. The stable stellate MCs were surrounded by ECM with additional abundant collagen I and III. In short-term (<2 weeks) two-dimensional culture, confluent MCs had formed ECM and continued to incorporate BrdU in resting medium (LI: 10±3% at day 5, and 5±2% at day 8 (p<.01).In contrast, sparsely plated MCs completely lacked ECM, and BrdU labeling in resting medium was low at day 5 (2±1%) and very rare at day 8 (0.3±0.3%, p<.01). The response to growth medium of confluent MCs (LI: 36±7% at day 5 and 49±6% at day 8) was significantly greater than that of sparse MCs (21±8% at day 5 and 30±4% at day 8, p<.01). In conclusion, the presence of ECM both stimulates and maintains MC growth and ECM composition modulates MC response to serum growth factors.

ANCROD INCREASES SURVIVAL IN THE BXSB MOUSE. Edward Cole, Michael Glynn,* Carl Laskin,* Joan Sweet, Nancy Mason,* Gary Levy.* Univ. of Toronto, Toronto, Canada.

The effect of ancrod, a defibrinating agent, on glomerulonephritis (GN)in the male BXSB mouse was studied to determine the relationship between macrophage procoagulant activity (PCA), fibrin deposition, and GN. Similar high titres of anti-DNA antibodies and renal deposition of IgG were seen in both control and ancrod treated mice. However, marked GN and fibrin deposition were noted by three months of age in control mice, whereas little or no disease was seen in ancrod treated mice until five months of age. PCA rose with age in both ancrod treated and untreated mice, although it was significantly higher in control animals than in the ancrod treated group. Furthermore, ancrod therapy resulted in a decrease in plasma PCA inducing activity (PIF) and in a decrease in the effectiveness of PIF in inducing PCA in peritoneal macrophages in vitro. No mortality was observed in the 42 ancrod treated mice, whereas 22 of 42 control animals died. We conclude that defibrination with ancrod delays the development of renal fibrin deposition and GN, and improves survival in BXSB mice. These data are consistent with the concept that PCA contributes to injury in murine lupus GN by promoting fibrin deposition.

STIMULATION OF PROTEOGLYCAN (PG) SYNTHESIS IN RAT MESANGIAL CELLS (RMC) IN RESPONSE TO TUMOUR NECROSING FACTOR (TNF). M. Davies*, L. Shewring*, G. Thomas*, L. Jenner* (Intr. by R.B. Sterzel). KRUF Institute of Renal Disease, Royal Infirmary, Cardiff, Wales, UK.

Glomerulosclerosis is an important feature of certain glomerular diseases and results from the accumulation of abnormal amounts of different matrix components. In order to understand the mechanisms underlying glomerulosclerosis we have examined the effect of TNF on RMC synthesis and secretion of PG, an important component of the mesangial matrix.

Growth arrested RMC were treated for up to 24 h with TNF (1-100 U/ml) and then metabolically labelled with $Na_2{}^{35}SO_4$ for a further 24 h. ^{35}S-labelled PG in the cell layer (CL) and in the culture medium (CM) were then extracted, quantified and characterised. In control experiments the CM accounted for 60% of the total PG synthesized of which 44% was chondroitin sulphate (CS) and 16% heparan sulphate (HS). In the CL 23.5% was CS and 16.5% HS. TNF stimulated the PG synthesis in a dose and time dependent manner. All the increased PG was secreted and appeared in the CM. This increase was not accounted for by cell proliferation and not accompanied by an increase in PGE_2 generation. There was no qualitative difference in the PGs synthesized and the ratio of CS:HS remained the same in both the CM and CL in response to TNF.

These data suggest a possible role by which TNF may be involved in the pathogenesis of glomerulosclerosis independently of cellular proliferation.

EXPERIMENTAL GLOMERULONEPHRITIS (GN) INDUCED BY INJECTION OF FIBRONECTIN (FN) BINDING ANTIGEN (Ag) FOLLOWED BY SPECIFIC ANTIBODIES (Ab). FG Cosio, AP Bakaletz and JD Mahan. Dpts of Med. and Peds., Ohio State Univ., Columbus, OH.

We demonstrated that FN binding Ag and FN binding immune complexes (IC) localize to the glomerular mesangium by binding to mesangial FN (J.Clin.Invest. 80:1270,1988). Herein, we assessed whether the IC formed *in situ* from FN binding Ag can induce GN. S-D rats (n=14) received 20 mg/kg IV of phenylated gelatin (DNP-GL). GL binds to mesangial FN while DNP is a hapten for specific Ab. After 2 hours, 100 mg of IgG, isolated from a rabbit anti-DNP serum, was infused IV. Controls received DNP-GL (n=5) or Ab alone (n=3). Kidney biopsies were done at 1, 4, 8 and 30 days. Rats receiving DNP-GL and Ab (experimental group) showed: First, significant changes in glomerular scores, calculated by semiquantitative analysis of light microscopy (J.Lab.Clin.Med. 106:63,1985) and immunoperoxidase (0-4+):

	Control	Experimental (days)		
		1	8	30
Mesangial Matrix	11±1[+]	118±16*	150±15*	74±10
Glomerular Cells	49±6	91±9*	118±16[o]	64±19
Glomerular deposits				
Rabbit IgG	0	3.3±.2	1.3±.2	0.6±.1
C3	0	0.8±.3	2.0±.3	0.7±.1
Rat IgG	0	0.3+.1	0.9+.3	2.0+.4

[+] Mean±SEM; * p<.0001, [o] p<.05 vs control

Second, by EM, increased endothelial cell numbers on days 1 to 8 but no electron dense deposits; Third, all rats developed serum anti-rabbit IgG Ab on day 30; Fourth, proteinuria remained minimal (peak, mean 9.2±2.7 SE mg/24 h). In summary, infusion of DNP-GL plus anti-DNP Ab induces an active GN that has both an heterologous and an autologous phase. Conclusion, *in situ* IC formed with FN binding Ag are nephritogenic. Other FN binding Ag, such as DNA and bacterial Ag, could induce GN by a similar mechanisms.

A NEW MURINE FETAL KIDNEY DIFFERENTIATION ANTIGEN: BL26. Ira D. Davis,* Robert L. Vernier, Bonnie Lindman,* and Jeffrey L. Platt. University of Minnesota Medical School, Department of Pediatrics, Minneapolis, Minnesota.

The identification of kidney cell antigens that correlate with developmental events in the fetal kidney would contribute to our understanding of normal as well as pathological processes in renal differentiation and morphogenesis. We have developed a rat monoclonal antibody (BL26) to fetal mouse kidney cells which recognizes cellular antigens of the primitive ureter. The antigen is located within distal nephron epithelial cells, mesangium, and smooth muscle cells and resembles the distribution of the human CD9 (BA2) antigen. Gradient polyacrylamide gel/Western blotting and immunoprecipitation of ^{35}S-methionine labelled fetal mouse kidneys identify 24 Kd and 26 Kd components of this antigen. The BL26 antigen is expressed by the primitive ureter at 11.5 days gestation prior to nephron development. It is newly expressed by epithelial cells and lymphocytes participating in alloimmune phenomena. It is present in the epithelial lining of distal nephron cysts in Balb/c mice with autosomal recessive polycystic kidney disease. Given the array of circumstances for expression of BL26, this antigen may play a role in cell activation/proliferation which occurs during nephron development, cellular immune responses, and cystic proliferation in polycystic kidney disease.

INCREASED EXPRESSION OF A NOVEL MESANGIAL MATRIX PROTEIN IN MESANGIAL GLOMERULOPATHIES IN THE RAT. Emile de Heer*, Annemarie Arkema*, Pancras Hogendoorn*, #Christine Dijkstra*, 'Mohamed Daha* and Jan Weening* (intr. by Ph.J. Hoedemaeker). Univ. of Leiden, Dept. of Pathol. and 'Nephrol., #Free Univ. of Amsterdam, The Netherlands.

This study describes the biochemical and functional characteristics of a novel mesangial matrix protein, recognized by monoclonal antibody ED5. ED5 has been shown earlier to recognize rat follicular dendritic cells, that are involved in immune complex trapping in lymphoid organs. Partial immunochemical characterization indicated a protein with an apparent molecular weight of approximately 100 kD and subfragments of 50 and 25 kD as determined by SDS-PAGE analysis after affinity purification. We propose to call this mesangial matrix protein MMP-100. By immuno-EM MMP-100 was shown to be located in the extracellular matrix of the renal mesangium between mesangial cells and the GBM. Both in vivo and in vitro results indicate that MMP-100 does not appear to be a complement factor or an Fc- or a complement receptor. The functional significance of MMP-100 was studied in kidneys from rats with various glomerulopathies. In uninephrectomy-induced focal glomerular sclerosis and in BSA-induced chronic serum sickness nephritis the mesangial expression of MMP-100 was significantly increased, but not in passive Heymann nephritis or in adjuvant-treated controls. Double label IF demonstrated identical localisation of MMP-100 with mesangial immune complex deposits. We conclude that MMP-100 appears to be involved in the processing of mesangial immune complexes.

PLATELETS AND MESANGIAL CELL PROLIFERATION. J.D. Elema*, M.F. Jeunink* and W.M. Bagchus*. Dept. of Pathology, Univ. of Groningen, Groningen, The Netherlands. (Intr. by Ph.J. Hoedemaeker).

Monoclonal anti-Thy.1 antibodies (MATS) cause complement-dependent mesangiolysis and subsequent mesangial cell proliferation (Lab Invest 55:680, 1986). To study the involvement of platelets on mesangial cell proliferation we used a non-complement fixing monoclonal (MCA) anti-rat platelet antibody (PL.1). IV injection of PL.1 resulted in acute thrombopenia. Daily injections enabled ongoing thrombopenia. Cell proliferation was tested by incorporation of 5-bromodeoxyurudine (BrdUrd) in DNA. Immunohistology of kidney sections with anti-BrdUrd MCA was performed and the number of positively stained cells per glomerular crossection (cells/gcs) were counted. Kidney sections of MATS rats and thrombopenic MATS rats were tested at 48 h and 72 h after induction of mesangiolysis. Control rats had 0.23 ± 0.05 cells/gcs. At 48 h the number of cells/gcs in MATS rats and thrombopenic MATS rats were respectively 1.66 ± 0.35 and 1.32 ± 0.67. At 72 h they were respectively 2.03 ± 0.52 and 1.64 ± 0.52. Urinary protein excretion did not differ between these 2 groups. Immunohistology of glomeruli of MATS rats at 48 h and 72 h showed an increased influx of platelets in afflicted glomeruli compared with controls. Glomeruli of thrombopenic MATS rats were completely negative for platelets. Histological examination of kidney sections for mesangial hypercellularity did not show significant differences between the 2 groups. From these experiments it is concluded that in this model platelets do not play a major role in the induction of mesangial cell proliferation.

TUBULOINTERSTITIAL NEPHRITIS DURING THE HETEROLOGOUS PHASE OF NEPHROTOXIC SERUM NEPHRITIS (NTS). **Allison Eddy and Lori McCulloch* Dept. of Pediatrics, The Hospital For Sick Children, Univ. of Toronto, Toronto, Ontario.**

The pathogenesis of tubulointerstitial (TI) nephritis which co-exists with anti-GBM nephritis is unkown. We investigated TI immunopathology in a rat model induced by rabbit anti-rat GBM antiserum. Groups of animals were sacrificed at : 10 min; 1,2,8,24 hr; 2,3,5,7,14,21 and 28 days after NTS injection. The TI cellular infiltrate was quantitated and characterized using monoclonal antibodies as previously reported (Eddy et al, Kidney Int. 33:14, 1988). Nephritic rats developed TI nephritis which was most intense on days 3 to 7 and reversed by day 21. At 8 and 24 hours the TI cells were mainly polymorphonuclear cells ($OX42^+$, Ia^-, $W3/25^-$). By day 2 macrophages (MO) were present ($OX42^+$, $W3/25^+$, $OX19^-$). On days 3,5 and 7 all cell subsets were increased; MO were predominant and most lymphocytes ($OX19^+$) were cytotoxic T-cells ($OX8^+$). NTS reacted in vitro with all tubular basement membranes (TBM) as well as GBM. After in vivo injection, TBM binding was observed focally (<10%) but not until 24 hr. Prior to this period, proteinuria had developed and tubular protein droplets showed positive staining for rabbit IgG (8 hrs). Faint focal TBM staining persisted until day 21 but was most evident on days 3 and 5.Two rats rendered nephrotic by puromycin aminonucleoside prior to NTS injection developed more extensive TBM staining at 24 hr. At all time points TBM failed to stain for rat IgG and the pattern of staining for rat C3 was similiar to controls. Tubular cell injury, identified by the expression of vimentin intermediate filaments, was present focally, maximum on days 3 to 5 but did not co-distribute with linear TBM deposits of rabbit IgG. The results of this study suggest that NTS nephritis is an anti-basement membrane disease affecting tubules as well as glomeruli and that proteinuria may be a pre-requisite for adequate delivery of antibody to TBM.

DEXTRANASE REDUCES IMMUNE DEPOSITS IN MURINE IgA NEPHROPATHY INDUCED BY DEXTRAN IMMUNIZATION. S.N. Emancipator, L. Gesualdo*, S. Ricanati* and M.E. Lamm*. Institute of Pathology, Case Western Reserve University, Cleveland, OH.

We previously showed that proteases can reduce glomerular immune deposits, proteinuria and hyperlipidemia when given to rodents with glomerulonephritis (GN). We hypothesized that other classes of enzymes could be of benefit, even if they only cleave the antigen component of the deposits. We induced mesangial GN with predominantly IgA deposits in Swiss mice by intraperitoneal and intravenous immunization with 500 kD DEAE-dextran (Isaacs and Miller, Lab Invest 47: 198, 1982). After 11 weeks, groups of mice were injected twice daily for 5 days with: 375 μg chymopapain and 180 μg subtilisin per dose ip, 1 mg dextranase per dose iv, or plain saline. Only 33% of mice given dextranase had glomerular IgA deposits, compared to 100% of saline-treated and 83% of protease-treated mice (both $\chi^2 > 5.2$, $p < 0.02$). Dextranase also reduced the extent and severity of mesangial hypertrophy compared to saline treated mice, but did not significantly reduce the modest IgG or IgM deposits in the mesangium. While proteases reduced IgG and IgM mesangial deposits compared to saline ($p < 0.02$), they did not diminish IgA deposits significantly, and had no effect on the histologic appearance of the glomeruli. We conclude that digestion of antigen in an established experimental GN can ameliorate immune deposits and mesangial proliferation. The results also underscore the potential reversibility of acute GN.

LYMPHOCYTE FUNCTION OF HEMODIALYSIS PATIENTS AFTER EXPOSURE TO TOXIC MICROSOMAL INTERMEDIARY PRODUCTS OF CYCLOPHOSPHAMIDE AND PHENYTOIN. C.M. Erley*, B. Potjan*, I. Roots*, D. von Herrath*, K. Schaefer, Med. Abt. II, St. Joseph-Krankenhaus I, Bäumerplan 24, 1000 Berlin 42, Institut für klinische Pharmakologie, Freie Universität Berlin, FRG.

Various aspects of lymphocyte function have been studied in vitro in both nondialyzed and dialyzed patients. However, almost no data are available concerning the metabolic function of lymphocytes in these patients. We therefore studied the mortality rate of lymphocytes after exposure to toxic intermediary products of cyclophosphamide and phenytoin produced by rat liver microsomes during drug metabolism. This in-vitro assay showed that the metabolism of 1000 µM cyclophosphamide induced a lymphocyte mortality rate which was significantly lower in the lymphocytes from nine dialysis patients than in those from to four normal subjects: 33.5 % ± 11.0 dead cells versus 41.6 % ± 5.0, respectively. This pattern could also be observed after exposing the lymphocytes to 1000 µM phenytoin: 12.1 % ± 3.7 versus 14.0 % ± 1.3.

Conclusions: 1) Our results document that the metabolic function of lymphocytes from dialysis patients is not, as perhaps expected, diminished by chronic uremia.

2) As the enzyme activities studied in the above system play a major role in the detoxification of oxygen radicals produced during hemodialysis, it could well be that HD membranes influence the metabolic function of human lymphocytes.

LOCAL OXYGEN AVAILABILITY MAY BE AN IMPORTANT MODULATOR FOR THE GLOMERULAR MORPHOLOGY IN PROGRESSIVE RENAL DISEASES: A STUDY IN "BLOODLESS RATS". A Fogo*, F Nishijima* and I Ichikawa. Departments of Pathology & Pediatrics. Vanderbilt University Med. Ctr., Nashville, TN.

An increase in hematocrit (Hct) affects adversely the morphology of remnant glomeruli after subtotal nephrectomy (NPX). In this regard, increased renal lipid peroxidation products observed in the remnant kidney are suggestive of a potential toxic effect of O2 on the glomerular morphology. We studied the effect of experimentally manipulated local O2 availability on the early morphological changes of remnant glomeruli, i.e., hypertrophy, by using so-called "bloodless rats". In rats, a stroma-free hemoglobin, modified by cross-linking (SF-HgB), was transfused at a volume of 11% BW in exchange for 10% BW whole blood, resulting in 10 mg SF-HgB/100 ml. Hct immediately after transfusion averaged 7.0%, renal plasma flow rate (RPF) 3.90 ml/min/kidney, whole kidney GFR 0.95 ml/min/kidney (n=4). In 20 rats, SF-HgB exchange transfusion was performed immediately after NPX, and animals placed in room air (GR-1) or 80% ambient O2 concentration (GR-2) for the following 4 days until the following data were collected in hydropenia. (Mean; GR-3: non-transfused NPXed controls; † P<0.05 vs GR-2).

	MAP	PGC	RPF	GFR	SNGFR	Screat	%ΔPA	PO2	Hct
	----mmHg----		---ml/min---		nl/min	mg/dl	%	mmHg	%
GR-1	90	44	.74	.26	28	1.2	-3†	77†	25
GR-2	90	43	.76	.21	24	1.2	11	111	23
GR-3	125†	62†	.89	.38	34	0.7†	12	81†	53†

By day 4, Hct increased comparably in GR-1 and 2 despite a higher arterial PO2 maintained in the latter. Mean arterial pressure (MAP), glomerular pressure (PGC), RPF, GFR and SNGFR were lower, and serum creatinine higher, comparably in GR-1 and 2 vs GR-3, indicating that the typical abnormal glomerular hemodynamics of remnant nephrons were absent in GR-1 and 2 "bloodless rats". Glomerular planar area (PA) determined by thin-section histological analysis on ~100 glomeruli from the remnant kidney was essentially unchanged from previously removed kidneys in GR-1, contrasting to substantial increases in this parameter in both GR-2 and 3. The results indicate that the local level of O2 may be an important modulator for the degree of glomerular hypertrophy, which precedes the sclerosis in remnant glomeruli.

ANTI-NEUTROPHIL CYTOPLASMIC AUTOANTIBODIES (ANCA) STIMULATE NEUTROPHIL ACTIVATION IN VITRO. Ronald J. Falk, Regina Terrell*, Lisa Huneycutt-Calder* and J. Charles Jennette. UNC School of Medicine, Depts of Med. & Path., Chapel Hill, NC.

We have demonstrated a strong association between ANCA and pauci-immune necrotizing and crescentic glomerulonephritis (GN) with and without extrarenal manifestations of disease (N Engl J Med 318:1651-1657, 1988). The purpose of this study was to determine whether ANCA activate neutrophils in vitro. Serum and/or purified IgG (n=10) were examined from a total of 18 ANCA patients. Four lupus erythematosis IgG's and 6 normal human sera or IgG's (n=2) served as controls. Purified human neutrophils were treated with cytochalasin B. Singlet oxygen ($^1O_2$) was measured by luminol enhanced chemiluminescence after 50 minutes of incubation. Specific granule degranulation was measured by lactoferrin release using a sensitive ELISA. Primary granule degranulation was assessed by release of N-acetyl-glucosaminidase (NAG) using an autoanalyzer method. Phorbol myristate acetate or the calcium ionophore A23187 were used as positive controls.

ANCA sera stimulated $^1O_2$ production (30.0×10^3 cpms +/- 6.3 (mean +/- sem)) whereas normal sera had minimal effect (2.2×10^3 cpms +/- .88) ($p < 0.01$). When compared with unstimulated cells, ANCA sera enhanced $^1O_2$ release by a factor of 4.7, versus a factor of 1.3 for normal sera ($p < 0.01$). ANCA IgG's stimulated $^1O_2$ release (22.8×10^3 cpms +/- 4.9) by a factor of 3.5 times that of resting cells, values significantly greater than that of normal controls ($p < 0.01$). Three SLE IgG's had effects identical to controls; one SLE IgG (with a positive ANCA) caused $^1O_2$ emission. ANCA IgG's released 13.4 ng/ml of lactoferrin and 6.0 nmols/ ml/min of NAG, values no different from control sera or IgG's.

These preliminary results indicate that ANCA sera and purified IgG cause $^1O_2$ release, but do not cause degranulation of primary or specific granules. ANCA may stimulate a neutrophil respiratory burst releasing toxic oxygen radicals. ANCA may participate in the pathogenesis of necrotizing and crescentic GN.

NEPHRITIS-PRONE LUPUS MICE UTILIZE RELATIVELY 5' HEAVY CHAIN VARIABLE REGION(VH) GENES TO PRODUCE POLYREACTIVE IGG AUTOANTIBODIES. M.H. Foster*, M. MacDonald*, K.J. Barrett*, M.P. Madaio. New England Medical Center, Boston, MA.

To correlate V_H gene usage of B cells with their antigen binding properties in MRL-lpr/lpr mice, we produced 1128 monoclonal Ig-producing B cell hybridomas from 21 unimmunized and unstimulated 3 day, 1 mo, 3 mo, and 6 mo old mice. The antibodies(Ab) were analyzed for direct binding to 6 antigens: ssDNA, SmRNP, glomerular extract, mouse hemoglobin, bacterial levan and Salmonella LPS. 6% of 1025 Ab bound ssDNA, 15% of 794 bound SmRNP, and 83% did not bind any antigen. 119 anti-DNA and anti-SmRNP Ab were studied further. Among 56 anti-DNA Ab, there was a shift in isotype from IgM to IgG with age. This was associated with a high incidence of polyreactivity among IgG Ab: 92% of anti-DNA IgG Ab and 57% of IgM from 3 mo mice had activity against > 1 antigen, compared to 28% of anti-DNA Ab from 1 mo mice.

By hybridization to V_H gene family probes, a shift in V_H gene usage with increasing age was also observed. 33% of 1 mo anti-DNA Ab expressed a 3' V_H gene family (7183, Q52 or S107) and 28% used the more 5' family, J558. In contrast, 64% of anti-DNA Ab from 3 mo mice used J558. Of the IgG anti-DNA Ab, 84% used J558. J558 was also used by 83% of the anti-DNA Ab that crossreacted with glomerular extract. V_H gene usage by the 63 anti-SmRNP Ab that did not bind DNA was stochastic. In contrast to the 3' bias described for "natural" autoantibodies produced by immature B cells, only 5.9% of the 119 lupus autoAb used the 3'-most 7183 family. Both the anti-DNA and anti-SmRNP responses were polyclonal as determined by the expression of multiple V_H gene families in individual 3 mo old mice.

We conclude that in aging lupus mice the development of nephritis is associated with the emergence of B cells that use relatively 5' V_H genes to produce polyreactive IgG autoantibodies that share properties with those previously described for nephritogenic Ig.

THE DEVELOPMENT OF ANTI-DNA ANTIBODIES IN APOFERRITIN-INDUCED GLOMERULONEPHRITIS (APO-ICGN) IN NZB MICE. PA Frymoyer and J Gavalchin*. Dept. of Med., SUNY-HSC, Syracuse, NY.

Previous work with Apo-ICGN demonstrated that NZB mice develop a lesion similar to lupus glomerulonephritis (KI 33:312). This study sought a possible role for anti-DNA antibodies in Apo-ICGN in NZB mice.

NZB, B10.D2 and Balb/c mice were immunized with 4 mg apoferritin or saline i.p. q.d. for 28 days. For NZB mice, serum anti-ssDNA was not elevated in apoferritin relative to saline injected mice. Anti-dsDNA was O.D. 0.20 ± 0.02 vs. 0.15 ± 0.005 (p=NS) in the apoferritin vs. normal saline injected mice respectively and although the elevation was not significant, 7 out of 9 apoferritin injected mice had levels above the normal saline injected controls. To see if antibodies were being cleared from the circulation and deposited in the kidneys, antibodies were eluted from the kidneys. For the NZB mice, the renal eluate anti-dsDNA antibody was O.D. 0.093 ± 0.010 vs. 0.049 ± 0.007 ($p<0.01$) and for anti-ssDNA O.D. 0.102 ± 0.013 and 0.040 ± 0.004 ($p<0.01$) for the apoferritin vs. saline injected mice respectively. B10.D2 and Balb/c mice developed Apo-ICGN but without lupus like lesions. The renal eluate anti-ssDNA antibodies were not elevated in these strains. The renal eluate anti-dsDNA antibodies did not increase in B10.D2 mice although there was an insignificant increase in the Balb/c mice.

These data suggest that the lupus like lesions in Apo-ICGN in NZB mice may be associated with the development of antinuclear antibodies.

GLOMERULAR THROMBOXANE BUT NOT PROSTAGLANDIN OR INTERLEUKIN-1 IS AUGMENTED IN IgA NEPHROPATHY IN RATS. L. Gesualdo*, J.R. Sedor, M.E. Lamm* and S.N. Emancipator. Case Western Reserve Univ., Cleveland, OH.

We sought to adapt our established mouse model of IgA nephropathy to rats, thereby enabling study of glomerular pathophysiology. Two groups of 200 g Lewis rats, one continuously orally immunized with a 0.1% solution of bovine gamma globulin (BGG) in the drinking water, the other a nonimmune control, were studied. Both groups received 3 successive daily intravenous challenges with 10 mg of BGG after 6 weeks. After sacrifice, small portions of renal cortex were taken for microscopy. Glomeruli isolated from the remainder of the tissue were incubated with 3.0 μM calcium ionophore (A 23187) for 30 min at 37°C in Hanks' Balanced Salts. Hematuria ensued in 13/15 orally immunized rats, but in only 2/15 control rats ($p < 0.01$); 80% of orally immunized rats had IgA and IgG glomerular deposits, versus 20% of controls ($p<0.01$). Orally immunized rats had >32-fold increases in serum IgA and IgG anti-BGG vs controls ($p < 0.001$) and produced more glomerular thromboxane (TxB_2) (100 ± 5 vs 59.6 ± 5 pg/mg/min in controls, $p < 0.02$), but no significant increase in prostaglandin (PG) E_2 (169 ± 60 vs 190 ± 75 pg/mg/min in controls, p = NS). In parallel experiments, total renal cortical RNA revealed no hybridization with a probe for IL-1β by Northern analysis, in contrast to the enhanced IL-1β observed in a different model of glomerulonephritis that also has high glomerular TxB_2 but normal PGE_2 output. We conclude that chronic oral exposure to BGG induces IgA nephropathy in rats with concomitant increases of TxA_2, which may play a role in the pathophysiology of this experimental disease.

CHARGE-MEDIATED DEPOSITION OF IMMUNE COMPLEXES CONTAINING NATIVE GOAT ANTIBODIES. V. Joyce Gauthier* and Mart Mannik* (intro. Wm. Couser), Univ of Washington, Seattle, WA

Positive charges can mediate glomerular deposition of macromolecules and immune complexes (ICs) as previously shown with chemically cationized antibodies and antigens. To demonstrate that native antibodies with an isoelectric focusing spectrum similar to human IgG are capable of charge mediated deposition, affinity purified goat antibodies (AbG) were compared to native rabbit antibodies (AbR), which have a more anionic spectrum, either alone or as 5 times antigen excess ICs (AgAbG, AgAbR) prepared with human serum albumin. Clearance from circulation and size of these ICs were comparable. One mg was given IV to C57Bl/6J mice and glomeruli isolated and quantitated in ng per kidney (n=3-5 for each time point):

time	AbR	AbG	AgAbR		AgAbG
1 hr	13.2±0.1	36.9±9.6	53.2±14	p=.003	107.5±30
4 hr	12.1±1.8	61.6±26	69.7±8.3	p=.03	164.1±49
24 hr	nd	38.8±11	32.6±8.0	p=.03	62.7±12.5

Immunofluorescence microscopy confirmed the earlier and more extensive deposition of the more cationic AgAbG relative to AgAbR. This enhanced deposition of AgAbG was blocked at 1 hr by the prior administration of 5 mg of cationized rabbit serum albumin (RSA_{ED}) indicating the electrostatic nature of their initial interaction with glomeruli. In contrast, the initial deposition of AgAbR was not altered by RSA_{ED}. The deposition of immune complexes containing naturally occurring antibodies with an isoelectric focusing spectrum analogous to humans indicates that the charge of native antibody molecules is important in initiating deposition of immune complexes in glomeruli.

PROCESSING OF IMMUNE COMPLEXES IN MICE WITH AUTOIMMUNE DISEASE INDUCED BY POLYCLONAL B CELL ACTIVATION. Norman A. Granholm, Rodney Nunley*, Joseph Langevin*, Annette Ragosta*, Colette Charland*, and Tito Cavallo. Department of Pathology, Rhode Island Hospital and Brown University, Providence, RI.

It has been suggested that diminished clearance of immune complexes (IC) facilitates their localization in tissues. To test this hypothesis, we studied the clearance and the organ uptake of IC in a model of systemic lupus (Cavallo *et al.*, Am J Pathol 114:346, 1984). C57BL/6 mice exposed at 2.0 months of age to bacterial lipopolysaccharide, 50 mcg twice a week for five weeks, developed autoimmune disease, glomerulonephritis, and mild renal insufficiency. When challenged at 3.5 months of age with nephritogenic IC (BSA-antiBSA; 0.12 mg/g b.w.; Ag_2Ab_2 greater than 60%), LPS mice cleared IC at a diminished rate compared to controls. However, neither liver uptake nor kidney localization of IC were significantly different between LPS and control mice.

Groups	Plasma IC (mg/ml)	Clearance (t 1/2, h)	Liver IC (mcg/g)	Kidney IC (mcg/g)
LPS	3.0±0.1*	2.1±0.2*	125.0±18.8	57.0± 5.2
Control	0.7±0.2	1.0±0.2	170.8±20.6	61.8±12.2

* P less than 0.001.

The data demonstrate that in mice with autoimmune disease, an increased load of IC may result in a transiently decreased rate of clearance of IC without an impaired uptake of IC by the liver. Additionally, residual IC do not appear to localize readily in the kidney. Thus, specific biologic properties of naturally occurring IC, or *in situ* interaction of antibody with glomerular antigen may represent likely alternative mechanisms.

DISTINCT PLASMA LIPID CHANGES AND SYNERGISTIC FACTORS IN GLOMERULAR SCLEROSIS (GS) CAUSED BY DIETARY LIPIDS IN YOUNG WISTAR RATS (WR).
H.-J.Groene,* A.Walli*,E.Groene*,J.Thiery*,P.Niedmann* and U.Helmchen*. University of Goettingen,West Germany (intr.by C.J.Olbricht).

Hypercholesterolemia has been associated with GS in genetically obese rats and in rats with lipoid nephrosis. The current experiments were designed to test: 1. if a high fat diet induces distinct plasma lipid changes and GS in WR that do not have genetic defects and renal disease; 2. if arterial hypertension influences the renal effects of a fat diet. Male WR were fed a low fat (5%) diet N, a high fat (34%, cholesterol 5%) diet F without cholic acid and a high fat (34%) diet L with linolenic acid. Group I were WR with two kidneys, group II uninephrectomized WR, group III WR with two kidney, one clip (2K,1C) hypertension, group IV WR treated with the ACE-inhibitor enalapril. Rats fed diet F had an isolated elevated cholesterol concentration in very low density lipoproteins. After 6 months the GS-index was significantly higher with diet F than with diet L or N (group I: F(n=9) 13.2±4.1; L(n=9) 1.2±0.3; N(n=11) 1.8±0.6). Cholesterolester in isolated glomeruli was increased only with F. Uninephrectomy aggravated GS by diet F 3-fold. In 2K,1C hypertension,the unclipped kidney showed a 2.5-fold rise in GS-index after only 7 weeks of diet F; in the clipped,GS was not evident. Enalapril, without significantly lowering arterial pressure, inhibited GS in group IV with diet F. Thus, glomerular hemodynamic factors seem to play a pathogenetic role in GS induced by a lipid rich diet. The fact that dietary lipids aggravate GS in arterial hypertension may have implications for the progression of renal disease in humans.

EFFECT OF IMMUNE COMPLEXES (IC) ON RAT MESANGIAL CELLS (MC) IN CULTURE.
A.O. Haakenstad*, G.N. Marinides*, G.C. Groggel. University of Utah School of Medicine and VA Medical Center, Salt Lake City, UT

The effect of IC on ^{3}H uridine (3H-U) incorporation by rat MC was studied. P3 to P5 rat MC subcultures were used near confluence in microtiter plates for 16 hr incubation. Native (N) bovine serum albumin (BSA) (pI=6.25) and two species of cationic BSA (C_1) (pI=7.65) and (C_2) (pI=8.95) were used to prepare IC with affinity purified rabbit antiBSA at 5x antigen excess. In-situ IC were prepared by adding antiBSA to wells one hour after antigen. The dose of total IC protein ranged from 12.5 to 400µg per well. Experiments were performed in absence of serum, and in presence of either fresh serum (rabbit, bovine, human) as source of complement or heat-inactivated serum. IC binding to MC was compared to ^{125}I antiBSA alone in 15 min and 60 min incubations.

In the absence of serum, IC did not modify 3H-U incorporation compared to controls without IC. Only in the presence of human serum, either fresh or heat-inactivated, IC suppressed 3H-U incorporation at high doses (64% suppression at 400µg IC protein and 48% at 200µg). This effect was independent of charge or method of IC formation. The binding of C_2 IC was 20 fold greater, C_1 IC 10 fold greater, and N IC no different than the minimal binding of ^{125}I antiBSA alone.

In conclusion it appears that IC neither stimulate RNA-transcription nor probably protein synthesis by cultured rat MC inspite of significant binding of IC to MC on the basis of their charge.

FORMATION OF NEUTRAL PROTEINASE (NP), COLLAGEN IV (CIV), AND TISSUE INHIBITOR OF METALLOPROTEINASES (TIMP) BY RESTING AND PROLIFERATING MESANGIAL CELLS (MCs) IN CULTURE.
L. Grond*, D. H. Lovett, M. Coffee*, M. Kashgarian, R. B. Sterzel. VAMC-Yale Univ Sch Med, New Haven, CT & VAMC San Fransisco, CA.

Increased production and/or decreased degradation of extracellular matrix (ECM) by MCs may contribute to glomerulosclerosis. To study the effect of MC proliferation on ECM homeostasis, rat MCs (subcultures 2-10) were serum-starved for 4 days to halt growth and then exposed to the mitogenic stimuli FCS (10%) and arginine vasopressin (AVP,10-4 and 10-6 M), and to the differentiation cytokine transforming growth factor beta-1 (TGF-B, 1, 10, and 20 ng/ml). MC production of NP, CIV, and TIMP as well as the uptake of the thymidine-analogue bromodeoxyuridine (BrdU, 24 hr) were studied by immunocytochemistry using monospecific antibodies. FCS greatly increased the number of BrdU-positive MCs from 3±1% before exposure to 41±7%, 55±5%, and 63±7% at days 1, 2, and 3 in FCS ($p<.01$). Immunoreactivity for both NP, CIV, and TIMP intensified progressively during FCS feeding. AVP (10-6M) increased BrdU incorporation from 4±2% before exposure to 18±7%, 24±8%, and 31±4% at days 1, 2, and 3 ($p<.01$). However, both doses of AVP increased MC staining only for TIMP; NP and CIV remained at the low pre-AVP level. TGF-B increased TIMP staining at the 1ng/ml dose. In a dose of 10 and 20 ng/ml, this agent selectively enhanced CIV production, the TIMP staining being unaffected by these doses. NP immunostaining was not changed by TGF-B. With double staining no correlation between BrdU-positive cycling MCs and immunoreactivity for NP, CIV, or TIMP was observed. In conclusion: rat MCs in culture produce NP, its substrate CIV, and its inhibitor TIMP. The production of these proteins, as judged by immunocytochemistry, is unrelated to proliferative activity and appears to be independently regulated.

CYCLOSPORIN A (CsA) INHIBITS THE PROLIFERATION OF GLOMERULAR CELLS IN A MODEL OF IMMUNE MEDIATED MESANGIAL CELL INJURY (MCI).
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CsA ameliorates proteinuria in glomerular immune injury. A possible role of CsA on the proliferation of resident glomerular cells has not been studied. To adress this question, we evaluated the effect of CsA on glomerular cell growth in a rat model of MCI, applying an in vivo in vitro approach. MCI was induced by i.v. injection of 0.5 ml/kg of a rabbit-anti-rat-thymocyte serum (ATS). Rats were treated with CsA (15 mg/kg) beginning at day 2 after ATS. After glomerular isolation cell proliferation was evaluated by 3H-thymidin incorporation (3HTI) and estimation of total cell number. CsA in vivo inhibited 3HTI in glomerular cells in vitro by 40% compared to control nephritic rats. CsA (1.6 ug/ml) in vitro inhibited 3HTI by more then 50% in glomeruli from rats with MCI, which was associated with a significant reduction in total cell number. The inhibitory effect of CsA on cell growth was similar when glomeruli were grown in puromycin (2 ug/ml). The CsA effect on cell growth was not reversible when glomeruli were cultured with Interleukin 1 (10 ng/ml), but the cyclooxygenase inhibitor indomethacin partially prevented the inhibitiory effects of CsA on cell proliferation. The anti-proliferative effect of CsA on glomerular cells of rats with MCI, might be of significance in the treatment of glomerulonephritis.

TUBULAR SILENCING OF MHC CLASS II EXPRESSION BY TARGET ANTIGEN 3M-1-SPECIFIC ANTIBODIES ABOLISHES CD4+ T CELL-MEDIATED INTERSTITIAL NEPHRITIS. T.P. Haverty, E.G. Neilson, P. Whang*, R. Maki* and C.J. Kelly, Renal Sect., U. of PA., Phila., PA. & La Jolla Cancer Res. Found., La Jolla, CA.

Tubular epithelium secreting 3M-1 (MCT) also expresses small amounts of MHC class II molecules. Transient transfection assays with reporter gene constructs (CAT) in MCT cells demonstrated marked CAT activity with -385 bp 5' untranslated class II sequence which largely disappears when -2,700 bp are used. This 2,300 bp of 5' cis-acting element may be responsible for silencing class II expression. γIFN (interferon)-treated MCT cells overcome the normal silence of class II expression, but this visibility is reduced when MCT cells are co-treated with α3M-1-Ab:cell-surface class II by radioimmunobinding in the presence of α3M-1-Ab = 244 $\pm$ 54 vs control Ab = 1,558 $\pm$ 237 ($p<.001$). The functional effectiveness of α3M-1-Ab in vivo could be demonstrated by subcapsular transfer of class II-restricted 3M-1-reactive effector T cells into γIFN-treated mice. Interstitial lesions developed with co-treatment with control Ab = 1.4 $\pm$ 1.4 (scores 0-2) compared with α3M-1-Ab = 0.3 $\pm$ 0.3 ($P<.01$). Both groups developed severe injury when class I-restricted 3M-1-reactive T cells were used. α3M-1-Ab blocked γIFN-stimulated tubular class II expression without saturating 3M-1 binding sites by immunofluorescence. These findings collectively suggest that MHC class II expression in tubular epithelium is tightly regulated by transcriptional controls, but that limited expression is easily modulated by immunologic events related to kidney injury.

IMMUNOGLOBULIN HEAVY CHAIN SWITCH REGION GENE POLYMORPHISMS IN IDIOPATHIC MEMRANOUS NEPHROPATHY [IMN] AND IgA NEPHROPATHY [IgAN]. Hitman G*, Moore R*, Richards N*, Lucas E*, Venning M*, Goodship T*, Cunningham J*, Marsh F*, [intr. by Slatopolsky E]. The London Hospital, St Thomas' Hospital, London, and Freeman Hospital, Newcastle, UK.

Much evidence suggests that IMN and IgAN are immune complex mediated diseases. Moreover, genetic factors may play an important role in their pathogenesis. Recently, restriction fragment length polymorphisms [RFLPs] of the immunoglobulin heavy chain genes have been described which carry an increased risk for IMN and IgAN. We have therefore studied RFLPs of the switch region of the IgCμ [Sμ] and the IgCα1 [Sα1].

DNA was obtained from British Caucosoids with IMN [n=43], IgAN [n=64], and normal controls [n=144], digested with the restriction endonuclease Sac I, and studied using Southern blot techniques and hybridisation with a ^{32}P labelled oligomer DNA probe homologous to Sμ. This probe detects RFLPs of both Sμ [2.1 or 2.6kb] and Sα1 [6.9 or 7.4kb].

There were no significant differences in the genotypic frequencies of either the Sμ or Sα1 RFLPs in IMN or IgAN compared to normal controls. Sμ: 2.1 [23%, 27%, 25% respectively}; 2.1/2.6 [49%, 59%, 55%]; 2.6 [28%, 14%, 20%]. Sα1: 6.9 [7%, 14%, 15%]; 6.9/7.4 [49%, 46%, 48%]; 7.4 [44%, 40%, 37%]. The allelic frequencies of Sμ and Sα1 were similar in all groups.

These results suggest that immunoglobulin heavy chain switch region genes are not important in conferring disease susceptibility to IMN or IgAN, and do not support the recent finding by others of an association of Sμ polymorphisms in IMN, and Sμ and Sα1 polymorphisms in IgAN.

MACROMOLECULAR CHARGE INDEPENDENT OF SIGN LOWERS GLOMERULAR (G) TRANSPORT. L.W. Henderson and J.K. Leypoldt*, VAMC and UC, San Diego, CA.

Previous work in the rat has suggested that electrostatic forces alone can explain fractional clearance (FC) for cationic DEAE dextran (D+) > FC for neutral dextran (D) > FC for anionic dextran sulfate (D-). These observations may not reflect true G permselectivity since polycations like D+ can induce G structural alterations or themselves undergo charge-related changes in molecular configuration. We determined FC for D+, D, and D- in rabbits using fluorescent-labeled dextrans. Creatinine clearance (CC) and FC for a 25 Å Stokes radius (R) macrosolute were ($\pm$ SEM):

	D+ (n=14)	D (n=13)	D- (n=4)
CC (ml/min)	9.4 ± 0.9	11.7 ± 1.2	13.3 ± 1.4
FC	.63 ± .05	.94 ± .07	.36 ± .15

FC for D+ (R < 34 Å) and D- (R < 36 Å) were less than for D ($p < .05$). These results argue against any G structural alterations. For comparison, sieving coefficients (S) were determined in vitro across neutral (PS) and anionic (PA) synthetic membranes. S ($\pm$ SEM) for a 16 Å R solute were:

Membrane	D+ (n=3)	D (n=3)	D- (n=3)
PS	.70 ± .03	.68 ± .03	.76 ± .02
PA	.14 ± .02	.72 ± .01	.48 ± .06

S for D- and D+ were less than D with PA ($p < .05$), but S were not different with PS. These latter results support the former in vivo observations and argue against changes in molecular configuration as important. We conclude that reduced G transport of charged macrosolutes independent of sign cannot be reconciled with previous work but instead requires a new physiologic model that includes both electrostatic forces and the effects of coions and counterions.

EFFECTS OF EPIDERMAL GROWTH FACTOR ON COLLAGEN BIOSYNTHESIS BY CULTURED RAT KIDNEY MESANGIAL CELLS. Richard L. Hoover*, Raymond C. Harris and Michael A. Haralson*. Vanderbilt Univ. Med. School, Dept. of Pathology and Div. of Nephrology, Nashville, TN.

Altered mesangial cell matrix production may be an important component of a variety of glomerular diseases. Potentially, this process could be regulated by EGF and/or its functional homologue, transforming growth factor-α, as these growth factors have been demonstrated to affect matrix production by cells in other tissues. Thus, studies have been undertaken to assess the effects of both short-term (acute) and long-term (chronic) exposure to EGF on collagen biosynthesis of cultured rat kidney mesangial (RKM) cells. Incubation of RKM cells in medium containing EGF for either 24 hours (acute) or 120 hours (chronic) resulted in no substantial change in either the growth rate or the morphology of the cells. CM-trisacryl chromatographic analysis indicated that >90% of the collagen synthesized by RKM cells was type I and that this percentage was not altered by either short- or long-term exposure to EGF. The remaining collagens produced in all instances were found to be types III, IV and V. However, acute exposure of RKM cells to EGF resulted in the following specific decreases in the synthesis of the genetic types of collagen chains: α1(I) - 54%; α2(I) - 15%; type III - 27%; type IV - 55%; and type V - 64%. In contrast to these acute effects of EGF, chronic exposure of RKM cells to EGF resulted in no change in either the amount or distribution of the collagens produced. Thus, since in neither situation was collagen synthesis enhanced by exposure of mesangial cells to EGF, these findings suggest that the increased synthesis and deposition of extracellular matrix components observed in a number of glomerular diseases may not be the result of EGF's actions on the mesangial cell.

TRANSFORMATION FROM MESANGIAL TO DIFFUSE PROLIFERATIVE PATTERN IN THE GLOMERULAR LESION OF THE NEWZEALAND BLACK/WHITE(NZB/W) F1 MICE. Keiichi Inoue*, Kouju Kamata, Michihito Okubo*. Univ. of Kitasato Med. Sch., Dept. of Int. Med., Sagamihara, Kanagawa, Japan.

In human, mesangial lupus nephritis, its progression and transformation have been reported. Using spontaneously lupus prone NZB/W F1 mice, the transition from the mesangial to the diffuse proliferative peripheral lesion were studied. According to the IgG and C3 deposition pattern, glomerular lesions were divided into mesangial (Group M) and diffuse proliferative type (Group DP). In each type, IgG subclass distribution was studied with the immunofluorescence double staining technique using rat monoclonal anti-mouse IgG 1, 2a, 2b and 3, and FITC-labelled anti-mouse whole IgG. Age dependent progression and transformation of the glomerular lesion were observed from the mesangial in the mice of 24 to 28 weeks of age to the diffuse proliferative peripheral pattern in the mice of 36 weeks of age. Concerning the mesangial distribution, both groups M and DP showed diffuse and global deposition of IgG 2a and 3. On the other hand, although IgG 2b distribution was diffuse and global in the group M, its distribution was focal and segmental in the group DP. IgG 1 staining was essentially negative except in the wire loop lesion seen in the group DP. In the peripheral capillary wall of the group DP, IgG 2a>IgG 3 was stained in the decreasing order. C3 staining was positive in the mesangium of both groups M and DP, and in the peripheral wall of the group DP. Accordingly, IgG 2a and C3 constituted the major depositions both in the mesangial lesion of the group M and in the peripheral extension of the group DP. In conclusion, we observed transformation of the glomerular lesion in NZB/W F1 mice. Factors other than IgG 2a deposition and complement fixation may be contributory to the peripheral extension.

EXTRACELLULAR MATRIX (ECM) PRODUCTION BY CULTURED RAT MESANGIAL CELLS (MCs). E.Ishimura,* R.B.Sterzel, M.Kashgarian. Yale Univ Sch of Med. New Haven, Ct. 06510.

Formation of ECM by MCs contributes to progressive glomerulosclerosis. The production and distribution of ECM constituents by cultured rat MCs was investigated with immunocytochemistry, immunoelectron microscopy and ELISA assay. Immuno-staining for all ECM constituents increased after serum feeding. Before MC confluency, their localization was strictly intracellular and ER was heavily stained. Near confluency, extracellular deposition of collagen IV (C IV) and laminin (LM) appeared, followed by fibronectin (FN) and collagen III (C III), and increased in amount after confluency. In parallel, the intracellular staining for these proteins diminished markedly. Neither extracellular deposition nor intracellular loss was observed for collagen I (C I) and thrombospondin (TS). On culture slides coated with C IV or LM, extracellular deposition of the other ECM constituents clearly preceded confluency. ELISA assay demonstrated decreased C III and C IV production after confluency. In contrast C I production continued even after confluency. These results indicate that synthesis of C IV, LM, FN and C III parallels MC growth and that extracellular deposition of these ECM occurs at cell-to-cell contact. C IV or LM appears to accelerate production and facilitate secretion of other ECM constituents. Extracellular deposition of C IV, LM, FN and C III downregulates production of these constituents probably by a negative feedback. C I and TS are not deposited extracellularly and production continues independent of cell density and the presence of a secreted ECM.

PLATELETS (PLTs), NEUTROPHILS (PMNs) and COMPLEMENT (C): SEQUENTIAL ANALYSIS OF THEIR INTERACTION IN IMMUNE COMPLEX (IC) NEPHRITIS (GN). R. Johnson, C. Alpers, C. Pruchno*, M. Schulze*, P. Baker*, W. Couser. Univ. of WA, Seattle, WA.

In a model of in situ subendothelial IC GN in rats proteinuria is PMN-dependent but is also reduced by PLT depletion implying that PLT-PMN interaction is required for injury (JCI, in press 1988). To clarify this interaction we quantitated glomerular accumulation of ^{111}In-labeled PLTs and correlated this with PMNs by histology at 10 and 30 min, 1, 4 and 24 hrs after induction of GN. PLT accumulation preceded PMNs, peaking at 10 min (5733±756 PLTs/glom vs. 91±36 in controls) with a smaller peak at 4 hrs (2514±154 PLTs/glom) and then resolved (485±120). ^{111}In-labeled PLT infiltrates were associated with PLT bound fibrinogen and PLT phagocytosis of ICs by EM indicating PLT activation. In contrast, PMN accumulation did not occur until 30 min and plateaued at 1 and 4 hrs (15.9±1.9 and 16.5±4.2 PMNs/glom/2 μ section vs. < 1 in controls). Selective PLT depletion (< 10,000/ mm^3) did not alter the kinetics of PMN infiltration despite amelioration of disease but selective PMN depletion (< 25/mm^3) reduced PLT infiltration at 4 hrs. However, complement depletion (< 15% of normal) with cobra venom factor prevented both PLT and PMN infiltrates and reduced proteinuria (26±4mg/24 hr vs. 53±8, p < .02).

We conclude that PLT infiltration in this model is rapid, precedes PMNs and occurs by a C dependent mechanism which has not been previously described. The PLT-PMN interaction required for injury in this model occurs locally and does not appear to involve PMN chemotaxis.

EFFECT OF 1,25-(OH)$_2$D$_3$ ON SECRETION AND mRNA LEVELS OF LIPOPROTEIN LIPASE (LPL) IN CULTURED HUMAN MACROPHAGES. S.C. Jordan, U. Querfeld*, J. Ong*, J. Prehn*, J. Carty*, P. Kern*. Cedars-Sinai Med. Ctr., Dept. of Pediatr. & Endocr., Los Angeles, CA.

An atherogenic role of 1,25-(OH)$_2$D$_3$ has been reported in animal experiments. Normal macrophages express 1,25-(OH)$_2$D$_3$ receptors and are involved in the pathogenesis of atherosclerosis. We investigated the effects of 1,25-(OH)$_2$D$_3$ on spontaneous secretion of LPL, since this enzyme may facilitate lipid uptake by macrophages. Peripheral blood monocytes were obtained from healthy volunteers, isolated by Ficoll-Hypaque centrifugation, plated in culture dishes (10x10^6/dish), and incubated in RPMI 1640 medium/20% fetal calf serum with or without 1,25-(OH)$_2$D$_3$ (10^{-8}M). In 1,25-(OH)$_2$D$_3$-treated dishes, LPL mass (LPLm) in the medium measured by ELISA, exceeded controls by 22, 34, and 60% on day 3, 5, and 7, respectively. However, LPLm was decreased by 29 and 59% on day 9 and 12 in culture compared to controls. If 1,25-(OH)$_2$D$_3$ (10^{-10}M to 10^{-7}M) was added on day 6 in culture, a dose-dependent decrease of LPLm between 30 and 80% was observed on day 12. In preliminary experiments, LPL mRNA levels were decreased by 46 + 5% compared to controls in macrophages treated for 12 days with 1,25-(OH)$_2$D$_3$ (10^{-8}M). We conclude that 1,25-(OH)$_2$D$_3$ affects LPL secretion by human macrophages in vitro in a time-dependent and dose-dependent manner. Regulation of LPL secretion at the mRNA level could be part of a generalized pro-differentiating and antiproliferative action on human macrophages described for 1,25-(OH)$_2$D$_3$.

ISOLATION OF 330KD HUMAN KIDNEY PROTEIN SIMILAR TO THE RAT HEYMANN NEPHRITIS AUTOANTIGEN (GP330). John J. Kanalas and Sudesh P. Makker. Univ. of Texas Health Science Ctr., Dept. of Pediatrics/Nephrology, San Antonio, TX.

Heymann Nephritis (HN) of rat is the accepted morphological model of idiopathic membranous glomerularnephritis (MGN) in humans. Previously (Kid.Int. 33:164,1988) we have isolated human kidney glycoproteins that were similar to the HN autoantigen of rat. We have now purified the human 330kD immunodominant glycoprotein by electroelution. The isolated protein shows binding to the nephritogenic autoantibody eluted from the glomeruli of HN rats. Immunization of rats with the isolated protein produces classical HN, including proteinuria. Antibodies eluted from the glomeruli of the rats binds to both human and rat 330kD proteins. A polyclonal antibody raised against this isolated 330kD protein also binds to both human and rat 330kD proteins. Rats administered with this anti serum developed passive HN. Eluted antibodies from these glomerular deposits also bind to human and rat 330kD protein. These results demonstrate that: 1) there is a human kidney 330kD glycoprotein similar to the HN autoantigen of rat; 2) this protein is capable of producing active HN in rats; and 3) polyclonal antibodies against this protein are capable of eliciting passive HN.

QUANTITATIVE STUDY OF PROTEINURIA INDUCING MONOCLONAL ANTIBODY 5-1-6 Hiroshi Kawachi, Katsuyuki Matsui, Michiaki Orikasa, Tetsuo Morioka, Takashi Oite, Fujio Shimizu. (intr. by Tadashi Yamamoto) Dept.Immunol. Inst.Nephrol. Niigata Univ. Niigata, Japan.

A single injection of monoclonal antibody(mAb) 5-1-6 to rats was reported to cause massive though transient proteinuria. Immunoelectron microscopy indicated mAb 5-1-6 to bind in vitro to the surface of glomerular epithelial foot process, mainly to slit diaphragms (J.Immunol.141,1988 in press). We examined in vivo kinetics of mAb 5-1-6 and the relationship between the quantity of kidney binding antibody and proteinuria. The protein excretion started immediately after 2mg mAb injection and peaked on 5 day with average values of 138.5mg/24h, followed by a gradual decline and virtual normalization by day 15. On the other hand, the amount of total kidney binding antibody(TKAb) 1h after 2mg administration was 50.8±10.4 µg IgG/2 kidneys and the TKAb declined to 1.9±0.4 at day 15. The TKAb of rats 5 days after administration of 2mg mAb 5-1-6 was 6.1µg, though 108µg of circulating mAb with antibody activity was calculated to remain in blood. In contrast, TKAb of rats 1h after 125µg mAb injection was 12.8µg, when only 35.5µg of mAb was remained in blood. These results show that the amount of mAb 5-1-6 recognized antigen molecule decreased. The minimum dose of mAb to induce proteinuria is 125µg as injected dose. This dose corresponds to 12.8µg of TKAb at 1h and 0.34µg of TKAb at day 5. This amount is smaller than the minimum dose to induce nephtotoxic serum nephritis, passive Heymann nephritis or in-situ immune complex nephritis. It was also demonstrated the amount of mAb 5-1-6 binding to isolated glomeruli was over 81.7µg IgG/76,000 glomeruli, which indicates that proteinuria is considered to be induced, if more than 15.7%(12.8/81.7) of critical epitopes specifically occupied by this mAb. From these results, the mAb 5-1-6 recognized antigen molecule is considered to play an important role to regulate the permeability of glomerular capillary wall.

EXACERBATION OF LUPUS NEPHRITIS WITH ANTI-HEPARAN SULFATE-PROTEOGLYCAN (HS-PG) ANTIBODIES. N. Kashihara, H. Makino & Y. Kanwar*. Northwestern University, Dept. Path., Chicago, IL.

It is believed that anti-DNA antibodies cross-react with the HS-PG of GBM & conceivably the HS-PG plays a role in the pathogenesis of immune-complex nephritis in SLE. We investigated the role of anti-HS-PG antibodies in murine (NZB/NZW) lupus. Two groups (2 & 5 month old) of mice were selected. They received a single intravenous injection of anti-HS-PG (50 mg/100 g B.W.). The controls received normal rabbit IgG. The younger group of mice had mild proteinuria and knob-like GBM thickening. The anti-DNA antibodies were not detectable in the serum. The older group of mice had massive proteinuria (100-150 mg/100 g B.W/24 hr), knob-like thickening, and extensive subepithelial & mesangial immune-deposits made up of IgG & C3. The controls had minimal proteinuria (< 10 mg), no basement membrane changes, and a few mesangial deposits. Serum anti-rabbit IgG and anti-DNA antibody titers were 30-40% lower in the experimental group as compared to the control. These results indicate that the administration of the anti-HS-PG antibody accentuated the formation of immune-deposits in the mesangium, while the deposits in the subepithelium seem to be newly formed. The lower titers of the antibodies are probably related to their consumption in the kidney due to some conformational change in the GBM HS-PG which facilitated their deposition & ultimately caused accentuation of murine lupus nephritis. The cross-reactivities between various antibodies & antigens is being investigated.

IMMUNE COMPLEX (IC) EFFECTS ON HUMAN GLOMERULAR CELL FIBRONECTIN (FN) PRODUCTION. D. Kees-Folts,* J.D. Mahan, C. McAllister,* B. Shannon,* F.G. Cosio*. The Ohio State Univ., Dept. of Medicine, Children's Hospital, Columbus, Ohio.

Fibronectin, a structural component of glomerular basement membrane and mesangial matrix, is increased in most forms of glomerulonephritis (GN). Cultures of human glomerular endothelial cells (GCEC) and mesangial cells (MC) were exposed to 1 mg/ml human albumin-goat anti-albumin IC or heat aggregated gamma globulin (HAG) for up to 48 hrs. Cell supernatant FN and cell surface FN were determined by ELISA.

SUPERNATANT FIBRONECTIN (pg/cell ± SD)

GCEC	Control	HAG	IC
5 min	200.4 ± 105	205.9 ± 97	1080.8 ± 205#
6 hr	391.6 ± 197	416 ± 324	846.3 ± 223#
24 hr	737.4 ± 141	524.4 ± 181	460.3 ± 155*
48 hr	1677.8 ± 170	1286.5 ± 455	972.5 ± 439*
MC	**Control**	**HAG**	**IC**
5 min	55.9 ± 59	34.8 ± 203	162 ± 358
6 hr	283 ± 130	167.3 ± 118	180.6 ± 464
24 hr	968.6 ± 192	638.6 ± 204	453.2 ± 335*
48 hr	2831.4 ± 474	3070.2 ± 608	375.9 ± 632*

(* = $p < 0.05$ IC control)
(# = $p < 0.05$ IC control)

Mesangial cell surface FN was increased above control cells at all durations of HAG exposure but only after 48 hrs of IC exposure. Both GCEC and IC showed a decrease in supernatant FN after 24 hrs incubation with IC. The early increase in FN release after exposure of GCEC to IC may promote inflammatory cell attraction and adherence.

RECOGNITION OF MURINE SUPPRESSOR-INDUCER T CELLS (Ts_1) AND INHIBITION OF THEIR FUNCTION BY ANTI-2H4 ANTIBODIES. C.J. Kelly and E.G. Neilson, Renal Section, Univ. of Penna., Phila., PA.

Understanding the mechanism of antigen-specific suppression will require a thorough chemical and molecular analysis of the lymphokine(s) mediating this inactivated state. We have previously described a murine (SJL, H-2^S) Ts_1, $CD4^+$ cell line (M40) which, via a soluble protein (M40F), inhibits expression of murine anti-tubular basement membrane disease. M40F is antigen (SRTA) specific, antigen-binding, and reactive with $\alpha I\text{-}J^S$ antisera. M40F function is abrogated by $\alpha I\text{-}J^S$. Using flow cytometry and immunoprecipitation techniques, we have now shown recognition of a cell-surface molecule by $\alpha I\text{-}J^S$ antisera:precipitation of ^{125}I-labelled M40 cell membranes with $\alpha I\text{-}J^S$, but not $\alpha I\text{-}J^K$, demonstrates a 200+ Kd band by SDS-PAGE. Human $CD4^+$ Ts_1 cells express 2H4, a well-characterized T200 cell surface glycoprotein; its expression on human $CD4^+$ cells correlates with Ts_1 function. We found that α2H4 also abrogates M40 inhibition of effector cell induction (DTH to SRTA $4.0 \pm 0.5 \times 10^{-3}$ with M40F vs. $17.0 \pm 0.8 \times 10^{-3}$ in. with M40F and 1:500 α2H4, $p<0.001$) in a dose dependent manner. α2H4, like $\alpha I\text{-}J^S$, precipitates a 200+ kd protein from ^{125}I-labelled M40 cell membranes. We have extensively screened a lambda gt11 cDNA library, from M40 mRNA, with $\alpha I\text{-}J^S$ antisera. The protein product of a cDNA clone recognized by $\alpha I\text{-}J^S$ antisera, is also recognized by α2H4. This cDNA is approximately 9.5 kb and has been subcloned into a sequencing vector. Sequencing should provide important insights into this murine T200 protein and its relationship to previously characterized 2H4 isoforms.

CHONDROITIN SULFATE (CS) PROTEOGLYCAN (PG) SYNTHESIS AND REUTILIZATION OF CS/DERMATAN SULFATE (DS) GLYCOSAMINOGLYCANS (GAG) IS ASSOCIATED WITH BRANCHING MORPHOGENESIS IN FETAL KIDNEYS.

David J. Klein,* David M. Brown, Antoinette Moran,* Theodore R. Oegema, Jr.* and Jeffrey L. Platt, University of Minnesota, Departments of Pediatrics, Orthopaedic Surgery, Biochemistry, and Cell Biology and Neuroanatomy, Minneapolis, Minnesota

Branching morphogenesis in fetal mouse kidneys and CS PG synthesis were previously shown to be inhibited by p-nitrophenyl β-D-xylopyranoside (βDX) while glomerular development and heparan sulfate PG synthesis were uneffected. We therefore asked whether recovery of CS PG synthesis and renewed branching morphogenesis occurred after exposure to βDX in organ culture. Kidney tissue CS PG synthesis resumed within 4 hours of removal of βDX and was enhanced after βDX-initiated CS/DS GAG chains were released from the tissue (at 8 hr). Label-chase experiments showed that [^{35}S]sulfate incorporated into βDX initiated GAGs was re-utilized in the synthesis of CS proteoglycan after kidneys were transferred to control medium. Moreover, highly purified βDX-initiated GAG chains were taken up by kidneys more avidly than was free [^{35}S]sulfate. These ^{35}S-GAGs were degraded and reutilized in synthesis of ^{35}S-CS PG. Ureteric bud branching resumed within 17 to 48 hours after βDX was removed from the incubation medium. These findings are the first direct evidence that CS GAG processing as well as CS PG synthesis are intimately involved in branching morphogenesis.

DEMONSTRATION OF IV-COLLAGEN(C),LAMININ(L), PROTEOGLYCANS IN GLOMERULAR ADHESION.

Itaru Kihara*,Eisin Yaoita*,Katsutoshi Kawasaki*, Tadashi Yamamoto. Niigata Univ.School of Med. Dept. of Pathol.,Institute of Nephrology Niigata,Japan

Formation of new basement membrane(BM)-like material between the glomerular tuft and Bowman's capsule is defined as glomerular adhesion.This process could be responsible for progressive generalized sclerosis of many glomeruli. The BM-like material was electron microscopically pursued with polyehthyleneimine(PEI) perfusion and postembedding method for demonstration of L,III and IV-C,and heparan sulfate proteoglycan(HSPG) in aging male rats.

Glomerular adhesion appeared progressive(8-12%) in aged spontaneous hypertensive rats in their 19 months age. The visceral epithelial cells were segmentally enlarged with large vacuoles and phagozomes with a content of low density. Most of them were partially or completely detached from the glomerular basement membrane(GBM). The GBM where the epithelium was denuded lost its binding with PEI. There was the laying down of new and fribrillar BM-like material in the GBM where the visceral epithelium detached and in areas among segmentally proliferated parietal cells.The new material was positive for PEI,HSPG,IV-C and L,but not for III-C. The new BM-like material was surrounded by epithelial cells which were apparently derived from the parietal layer.

EXPRESSION OF HUMAN BASEMENT MEMBRANE ANTIGENS IN DEVELOPING KIDNEY AND EYE. Mary M. Kleppel* and Alfred F. Michael. Univ. of Minnesota Medical School, Departments of Laboratory Medicine and Pathology and Pediatrics, Minneapolis, MN.

The distribution of two novel basement membrane (BM) collagen chains (28 kDa parent chains) has been evaluated by immunohistochemical analysis of normal human tissue using monoclonal antibodies (Mabs) specific for the non-collagenous (NC1) domains of each chain. A more restricted distribution within tissues was observed, and distinctly different localization within the glomerular BM (GBM) when compared with type IV collagen. An autoantibody (FN) that developed in a transplanted patient with Alport syndrome co-localized with the 28 kDa antibodies and showed a slightly broader, but restricted tissue distribution. In the kidney, the 28 kDa parent collagen chains are late antigens, appearing at the early capillary loop stage of glomerular development while the Alport FN antigen is present in ureteric bud BM and at all stages of glomerular development. Both antigens are present in distal tubule BM whereas type IV collagen apppears in all BM of the developing kidney. In the human eye, the 28 kDa parent chains are, for the most part, late antigens in BMs which eventually contain them, appearing in trace amounts in fetal Bruch's membrane, but remaining absent in the inner limiting membrane and ciliary process BM until early childhood. Retinal capillaries, which are not present in fetal tissue, are reactive at birth while the lens capsule BM and the Descemet's membrane are very reactive early in development. The Alport FN antigen appears early in the eye whereas type IV collagen is present in all BM as well as loose matrix of the differentiating mesenchyme. We feel that the 28 kDa parent chain genes are regulated in concert with maturation of the BM, and that present data suggests that lens capsule BM and Descemet's membrane achieve functional and structural maturity earlier in gestation than other BMs specifically containing these restricted antigens.

ALBUMINURIA IN MURINE GRAFT VERSUS HOST NEPHRITIS IS NOT DEPENDENT ON LATE COMPLEMENT FACTORS. Robert A.P. Koene and Karel J. Assmann*. Depts. of Pathol. and Nephrol., University Hôspital Nijmegen, The Netherlands

The injection of parental strain lymphocytes into F1 hybrid mice produces a membranous glomerulonephritis in some F1 combinations during graft versus host disease. To study whether late complement (C) factors participate in the induction of albuminuria in this model we injected 10 male (A.THxA.TL)F1 hybrids i.v. at days 0 and 5 with A.TH donor lymphocytes. Both strains of mice are deficient of C5 as confirmed by us using a hemolytic assay of mouse C (J.Immunol.Meth.108; 1988:213). Ten non-injected hybrids served as controls. At week 15, when all mice were killed, 50% of the injected mice had developed albuminuria ranging from 155-10,085 g/18 hr, while the remaining and the control mice excreted normal amounts of albumin (70 g/18 hr). By light microscopy no glomerular proliferation was seen, while only 1 mouse had developed spikes on the subepithelial side of the GBM. Glomeruli of all proteinuric mice had subepithelial deposits of mouse IgG and C3 in IF and typical electron dense deposits in EM. Non-proteinuric injected mice showed a less intensive fine granular binding of mouse IgG only. Comparable results were obtained in 11 female F1 hybrids. The results demonstrate that typical lesions of membranous glomerulonephritis accompanied by substantial albuminuria can be induced in C5 deficient mice in which activation of late C factors cannot occur. Activation of early C components may be involved as suggested by the correlation between C3 deposition and albuminuria.

TRIPTERYGIUM WILFORDII (TW) INHIBITS AFFERENT IMMUNOLOGIC RESPONSES OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC). X-W. Li,* M.R. Weir. Univ. of Maryland Hospital, Dept. of Medicine, Renal Division, Baltimore, MD.

TW is an herbal medicine which has been used for the treatment of autoimmune disease in China. Little is known about is immunological effects, or its potential use in organ transplantation. TW in doses of 2.4, 1.2 and 0.6 ug/ml inhibited PBMC response to both phytohemagglutinin (PHA) (% inhibition: 89.8, 76.3, and 35.1, respectively (R)) and Concanavalin A (Con A) (% inhibition: 89.3, 47.3, and 9.9, R) in a concentration dependent fashion (measured after 72 hours of culture by thymidine incorporation). The earlier TW was added during the culture period the greater the inhibition of proliferation. TW (2.4 or 1.2 ug/ml) added 1 hour prior to the conclusion of a 72 hour culture of PBMC and PHA caused 29.1% and 21.9% inhibition, R. Trypan blue exclusion by the PBMC demonstrated no cytotoxic effect of TW. The generation of cytotoxic T cells (CTL) in 6 day allogeneic cultures was inhibited by TW in a concentration dependent fashion with 81% and 70% inhibition, R, with concentrations of 2.4 and 0.6 ug/ml (at killer to target: ratios of 25:1). However, no inhibitory effect of TW was noted on CTL effector function or on natural killer cell activity. TW appears to have concentration dependent inhibitory effects on the afferent limb of immunity and may be an important new immunosuppressive agent.

LOCALIZATION OF THE NEPHRON SITES RESPONSIBLE FOR THE NATRIURETIC EFFECT OF INTERLEUKIN-1. Donald Kohan,* Chris Merli,* and Eric Simon. Washington Univ. School of Med., Renal Div., St. Louis, MO.

Interleukin-1 (IL1) has been demonstrated to elicit an increase in renal sodium excretion. This effect occurs in the absence of any increase in filtered load of sodium, raising the possibility of an IL1-mediated decrease in tubule sodium reabsorption. In order to localize the nephron segment(s) responsible for the natriuretic effect of IL1, we performed micropuncture experiments on rats. Intravenous IL1 administration caused a marked increase in sodium excretion that was not accompanied by changes in glomerular filtration rate (GFR), plasma sodium concentration, or systemic blood pressure. Single nephron GFR (SNGFR), and absolute and fractional delivery of sodium (FD_{Na}) to the late proximal and mid-distal tubule were not affected by IL1. FD_{Na} to the early and late papillary collecting duct (PCD), however, was significantly enhanced by IL1 administration:

	Control	IL1	p<
SNGFR (nl/min)	21.7 ± 2.4	21.6 ± 2.8	NS
FD_{Na} (%)			
Late proximal	49.3 ± 5.3	53.8 ± 5.8	NS
Mid-distal	12.7 ± 2.0	13.2 ± 1.2	NS
PCD base	3.9 ± 0.5	9.3 ± 0.8	0.001
PCD tip	2.5 ± 0.5	7.5 ± 0.8	0.001
Urine	0.9 ± 0.2	3.7 ± 0.7	0.01

These findings demonstrate that the natriuretic effect of IL1 is due to inhibition of sodium reabsorption at a site beyond the superficial distal tubule. This suggests IL1 inhibits sodium reabsorption by the collecting duct and possibly in deep nephrons.

A NEW RAT MODEL OF IgA NEPHROPATHY (IgA-N) INDUCED BY STAPHYLOCOCCAL ENTEROTOXIN. L. Li* and Z. Liu* (intr. by R.W. Schrier). Dept. Nephrology, Jinling Hospital, Nanjing, China.

In order to demonstrate the important role played by the intestinal mucosa immune system in the pathogenesis of mesangial IgA-N, we successfully developed an IgA-N model in Sprague Dawley rats by weekly iv injections of staphylococcal enterotoxin B (SEB) for three weeks. Animals were sacrificed at 4, 8 and 11 weeks after SEB injection. Pathological and immunopathological studies were performed with LM, EM, IF and immunoperoxidase techniques for kidney, liver and jejunum tissues. IgA deposition in the glomerular mesangial areas and capillary walls with co-deposition of IgM (75%) and IgG (25%) were shown in 8 out of 12 rats. Serum IgA titer, circulating immune complexes and the number of IgA containing plasma cells within mucosa of jejunum of the experimental group were significantly higher than those of the controls. Specific IgA anti-SEB antibody was also detected in the blood. It is presumed that both the intestinal mucosa involved immune reaction and the immunological injury mediated by iv injection of SEB made contributions to the development of this model. This new animal model of IgA-A is of value in the study of the pathogenesis of IgA-N in man.

EFFECTS OF A THROMBOXANE SYNTHETASE INHIBITOR ON CONCANAVALIN A INDUCED GLOMERULONEPHRITIS. S.L. Longhofer,* D.D. Frisbie,* H.C. Johnson,* C.A. Culham,* K.T. Schultz* and G.F. Grauer. School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin.

We previously reported that dogs with concanavalin A (Con A) induced glomerulonephritis (GN) treated with a specific thromboxane synthetase inhibitor (TXSI), 3-methyl-2 (3-pyridyl)-1-indoleoctanoic acid (CGS 12970) had decreased urinary excretion of protein and thromboxane compared to untreated dogs (Longhofer, et al. Proc. Am. College Vet. Int. Med. 6:751, 1988). To further assess effects of this treatment, we examined glomerular histology and urinary prostaglandin E2 (PGE2) excretion in these same dogs.

Twelve beagles were immunized with Con A and after 4-fold increases in antibody titer, bilateral renal arterial infusions of 1 mg of Con A were performed. Six dogs were treated twice daily, starting the day after infusion, with 30 mg/kg of CGS 12970 per os. Twenty-four hour urinary excretion of PGE2 was determined for each dog prior to infusion and on days 4, 6, and 8 post-infusion. Urinary PGE2 excretion was significantly decreased in both groups on day 4, but returned to baseline by day 8. Light microscopy 8 days after infusion revealed epithelial crescent formation, mononuclear cell (MON) proliferation and polymorphonuclear leukocyte (PMN) infiltration. Glomerular MON and PMN profiles/um^3 were determined from 20 equatorially sectioned glomeruli from each kidney of each dog. The percent of glomeruli with epithelial crescent formation was also determined. Student's t tests were used to compare treated and untreated dogs. $P < 0.05$ was considered significant. Treatment with CGS 12970 did not effect urinary PGE2 excretion or PMN infiltration, however, CGS 12970 decreased MON proliferation and epithelial crescent formation. Results suggest that TXSI treatment may be beneficial in the developmental stages of GN, and effects associated with TXSI therapy are not due to endoperoxide shunting and increased production of PGE2.

EXPERIMENTAL GLOMERULONEPHRITIS (GN) IN THE NONHUMAN PRIMATE (P): GENERAL DESCRIPTION OF THE MODEL. J Mahan, LA Hebert, FG Cosio, D Birmingham*, X Shen*, R Goel*. Dept of Med and Ped, Ohio State University, Columbus, OH.

The erythrocyte (E)-complement receptor type 1 (CR1) system is unique to P. E-CR1 is an important part of an immune complex (IC)-clearing mechanism that may protect P against the development of IC-mediated GN. The present study was undertaken to develop a model of IC-mediated GN that later will be used to test critically the role of E-CR1 in the pathogenesis of GN in P. Cynomolgus monkeys (N=12) were chosen for study because they have E-CR1 levels that encompass the range seen in man. CR1/E ranged from <100 (N=4) to 1300-5000 (N=8) All cynos received 1 mg/kg of bovine γ globulin (Ag) in CFA and one or more Ag boosts at 4 wk intervals. All P developed precipitating antibodies (Ab, mean 98±51 S.E. mg Ag precipitated/ml plasma). Nine to 19 weeks after primary immunization, each P received, under light anesthesia, daily (5/wk) IV infusion of Ag (mean 11.4±2 S.E. mg/kg) to achieve or slightly exceed Ag/Ab equivalence for circulating precipitating Ab. Percutaneous kidney biopsies were performed at 0, 2 and 6 wk and at onset of GN. Nephrotic range proteinuria developed in 11 of 12 cynos between 3 and 7 wk of Ag infusion. By wk 6, all animals developed proliferative GN by light microscopy. Immunofluorescence was positive for cyno IgG and C3 in a mesangial and subendothelial distribution in all animals by wk 2 of Ag infusion. At the onset of GN, electron microscopy in 6 cynos demonstrated mesangial proliferation and large electron dense deposits in the mesangium, subepithelial and subendothelial spaces. Conclusion: A workable model of experimental GN has been developed in the nonhuman primate. Using this model, it should be possible to examine critically the role of the E-CR1-IC clearing mechanism in the pathogenesis of GN in the primate.

SYMPATHETIC NERVOUS SYSTEM AND RELEASE OF THROMBOXANE AND PROSTAGLANDINS WITH ANGIOTENSIN INFUSION IN RATS. F.C. Luft, C.S. Wilcox, Th. Unger, R. Kühn, P. Rohmeiss, R.B. Sterzel, Depts. of Physiol. & Pharmacol., Univ of Heidelberg, FRG; VAMC, Yale Univ., West Haven, CT; Dept. of Med., Univ. of FL, Gainesville, FL; & Depts. of Med. & Pharmacol., Indiana Univ, Indpls, IN.

To elucidate the role of the sympathetic nervous system (SNS) in angiotensin (Ang-II) hypertension, Ang-II was infused into rats (n=10) with minipumps (76 ng/min) for 10-14 days. Control rats (n=10) received sham pumps. We measured blood pressure (BP) by tail cuff and excretion of aldosterone (ALD), and PG's. Ang-II increased BP (day 2: +20 mmHg, day 10: +90, $p<0.05$), ALD excretion (from 10 to 70 ng/day by day 7; $p<0.05$). None of these variables changed in sham rats. Ang-II did not alter excretion of PGE_2 but rather increased $PGF_2\alpha$ and TxB_2. Groups of conscious Ang-II and sham rats (n=8) had direct measurement of splanchnic nerve records at day 14. The nerve activity (SpNA) in Ang-II rats was 13.5±5.5 vs 7.6±4.5 μV in shams ($p<0.05$). With incremental methoxamine, the decrease in SpNA for a given increase in BP was greater for Ang-II rats than sham rats ($p<0.05$). The role of SNS was tested further in groups which received phenoxybenzamine (300 μg/kg/d) throughout. This attenuated the rise in Ang-II-dependent hypertension by 50% without altering PG or TxB_2 excretion. We conclude that prolonged Ang-II infusion augments SNS tone despite the rise in BP and potentiates the baroreflex. Increased SNS tone does not mediate Ang-II-dependent release of PGI_2 or TxB_2, but contributes importantly to the hypertension.

RADIOSENSITIVE HOST CELLS MODULATE DISEASE EXPRESSION IN MURINE GRAFT-VS-HOST (GVH) DISEASE. R.A. Mann, A.B. Singh*, K. Hiehle*, and S. Nayar*. U.M.D.N.J.- Robert Wood Johnson Medical School, New Brunswick, N.J.

GVH disease is induced by the transfer of parental lymphocytes into semisyngeneic F1 recipient mice. The disease takes 2 forms depending on the parental/F1 strain combination. Acute lethal GVH, characterized by immunodeficency, occurs following C57BL/6 (B6) cell transfer to a B6D2 F1 mouse whereas DBA-2 cell transfer leads to lupus-like chronic GVH disease, auto-antibody production, and immune-complex glomerulonephritis. It has been suggested that the difference between the 2 forms of GVH reflects a relative deficiency of Lyt-2$^+$ anti-B6D2 cytotoxic cells in the DBA-2 donor inoculum. We now report that a radiosensitive host cell plays a key role in the immune response of chronic GVH disease.

B6 or DBA-2 cells, harvested from a 5 day mixed lymphocyte reaction (MLR) with either irradiated (IR) or nonirradiated (NIR) B6D2 stimulator cells, were tested for anti-B6D2 cytotoxicity (CTX). Irradiation of B6D2 stimulators increased DBA-2 CTX to a level comparable to that of B6 cells.

% Lysis of B6D2 Targets at 50:1 Effector to Target Ratio

Effector Strain	Irradiation of B6D2 cells during 5 day MLR: 0 rads	500 rads	2500 rads
B6	46	52	43
DBA-2	29	52	50

In acute GVH CTX against alloantigen is lost whereas in chronic GVH it persists. CTX against H-2s targets, for example, is a reliable predictor of ultimate disease expression. As expected, B6D2 recipients of DBA-2 cells maintained anti-H-2s CTX. IR B6D2 hosts lost this CTX following DBA-2 cell transfer.

% Lysis of H-2s Targets at 50:1 Effector to Target Ratio

DBA-2 Cell Transfer	Irradiation of B6D2 mice: 0 rads	500 rads
No	41	35
Yes	37	0

Lymphocytes harvested from sublethally IR and NIR B6D2 recipients of DBA-2 cells were placed in culture with IR (2500 rads) B6D2 stimulators. Thereafter, every 4-7 days, they were carried with fresh B6D2 cells. The culture supernatant from cells originating in a NIR mouse induced marked B6D2 B cell proliferation whereas that of cells from an IR mouse did not.

We conclude that a radiosensitive B6D2 cell (1) protects against DBA-2 anti-host CTX and (2) is necessary for the maintenance of anti-H-2s CTX and B cell hyperactivity characteristic of chronic GVH disease.

RABBIT COMPLEMENT IS ABLE TO BIND TO AND LYSE RAT MESANGIAL CELLS (MC) IN CULTURE. G.N. Marinides*, A.O. Haakenstad*, G.C. Groggel. Univ. of Utah School of Medicine and VA Medical Center, Salt Lake City, UT.

The ability of complement to bind to and lyse rat MC was investigated. Subcultures of P3 to P5 rat MC near confluence in microtiter plates were used. ^{3}H-uridine (3H-U) incorporation and LDH release as a marker of cell lysis were determined. Both normal (N) and heat-inactivated (HI) rabbit (rab), rat, human, bovine, and rabbit deficient in 6th component of complement (C6D) sera (S) were used. Immune complexes (IC) were prepared with BSA and rabbit antiBSA at 5x antigen excess. Only N rab S decreased 3H-U incorporation compared to HI S using serum-free media as control (100%). Table.

	Rabbit	C6D	Human	Bovine
N.	25± 6%	183±16%	281±15%	203±13%
H.I.	204±18%	207±12%	310±19%	207±14%
p value	<0.001	>0.05	>0.05	>0.05

N rabbit S caused a significantly greater release of LDH than all other sera, N rab S $61.2\pm4.7\times10^{-3}$ IU/L vs HI rab S $5.9\pm1.7\times10^{-3}$, P <0.001. The addition of IC (0-400µg IC protein) to N rab S reversed the decrease in ^{3}H-U incorporation in a dose dependent fashion, R=0.73, p=0.06. This protective effect of IC was lost by removing the IC prior to adding the N rab S. By IF staining there was binding of rabbit C3 to MC but no binding of immunoglobulin. No differences were found in susceptibility to N rab S induced lysis between early MC (P1) and late MC (P9).

In conclusion rabbit C binds to rat MC in culture and causes cell lysis. This effect of rabbit C is dependent on the terminal complement pathway.

EFFECT OF MACROPHAGE COLONY STIMULATING FACTOR (M-CSF) ON THE FUNCTION AND EXPRESSION OF Fc AND COMPLEMENT RECEPTORS (Fc-R, CR) ON U937 CELLS. R.L. Mehta, J.F. Leary,* and C.N. Abboud.* Univ. Of Rochester Med. Ctr., Dept. Of Medicine and Pathology, Rochester, New York.

Colony stimulating factors (CSF) mediate proliferation, differentiation and functional activation of granulocytes and macrophages. Both Fc-R and CR on human mononuclear cells participate in the handling of pathogenic immune complexes (ICx). We have studied the role of recombinant human macrophage CSF (rHuM-CSF) on Fc-R and CR mediated binding and catabolism of soluble ICx by U937 cells. C3b was incorporated (.C3b) into soluble heat aggregates of IgG (AIgG) and IgM (AIgM) using classical pathway of activation. U937 cells were cultured in medium alone or medium + 500u/ml of rHuM-CSF for 16-72 hours at 37.C. rHuM-CSF treated cells showed a 27% increase in the Fc-R mediated total binding of AIgG but no difference in C3b receptor (CR1) mediated binding of AIgM.C3b at 72 hours. Cells incubated with ligand in low ionic strength buffer failed to show an increment over control. Simultaneous experiments showed an increase in cell size associated with a decrease (-26%) in surface expression of the 40Kd Fc-R (Fc-RII) which was evident at 24 hours but significant at 72 hours in rHuM-CSF treated cells. CR1 and CR3 expression was reduced at 24 and 48 hrs but unchanged at 72 hours. We conclude that rHuM-CSF enhances Fc-R mediated uptake of soluble ICx by U937 cells. As Fc-RII binding is enhanced at low ionic strength our data suggests rHuM-CSF down regulates Fc-RII, however, does not appear to significantly influence CR1 mediated function.

PRODUCTION OF INTERLEUKIN 1 IN GLOMERULAR CELL CULTURES FROM RATS WITH NEPHROTOXIC SERUM NEPHRITIS. Koichi Matsumoto,* and Michinobu Hatano.* (intr. by N. Yoshizawa) Dept. Med., Nihon Univ., Tokyo, Japan.

To investigate the role of cellular immunity in the pathogenesis of experimental glomerulonephritis, we examined interleukin 1 (IL 1) activity in isolated glomeruli of rats with an accelerated autologous form of nephrotoxic serum nephritis (NTSN). The production of IL 1, measured by thymocyte stimulating activity, in the glomerular culture supernatants was measured at various stages throughout the evolution of NTSN. It was found that NTSN glomeruli early in the disease course (from day 1 to 5) had significantly increased levels of IL 1 activity when compared to the values obtained with normal controls. When glomerular culture supernatants and purified IL 1 were preincubated with an anti-IL 1β antibody, a parallel decrease of the biological activity was found, suggesting that the biological active binding sites of the culture supernatants and monocyte-produced IL 1 share some common structure. The administration of a rabbit anti-rat macrophage serum prevented the outgrowths of macrophages and mesangial cells and reduced the production of IL 1 activity in the NTSN rats. In this experimental model IL 1 synthesis, probably by multiple cell types, is present early in the disease process and perhaps may be an important mediator of glomerular immune injury.

ANTIBODY-INDEPENDENT UNILATERAL GLOMERULAR LESIONS INDUCED BY COBRA-VENOM-FACTOR (CVF)
R. Metz, M. Zeier, J. Nitsch, M. Raghunath, N.Gretz, E. Ritz, E.W. Rauterberg, Institute of Immunology and Dept.Int.Medicine, Heidelberg, FRG

Glomerular deposits of complement (C) components are found in the majority of patients with glomerulonephritis, but the relative contribution of local C-activation to glomerular lesions is unknown. We developed an unilateral antibody-independent model of glomerular injury in rats using the contralateral kidney as control. A bolus of highly purified CVF from Naja naja khaoutia, a potent activator of the alternative C-pathway, was administered via the right suprarenal artery. Trapping of CVF was enhanced by a preceeding bolus of concanavalin A (Con A). Controls received saline or Con A alone. Animals were sacrificed 2 h or 3d after injection. Cryostate sections of the left (untreated) and right kidney were examined by histology and immunohistochemistry (Mabs against rat granulocytes or Mo). 50µg CVF alone caused no and 200 mg Con A only marginal effects. In contrast, 50µg CVF, preceeded by 500 ug Con A, caused enlargement of glomerular diameter after 2 h (treated vs untreated kidney, $p < 0.0007$). In parallel, the number of nuclei per glomerulus was increased as was the number of granulocytes per glomerulus (22 ± 5.8 vs 14 ± 4.3; $p < 0.0001$). The lesions were still demonstrable after 3 d. Administration of saline gave consistently negative results. In vivo binding of Con A and CVF to glomerular sites was shown by immunohistochemistry. We conclude that (1) CVF provokes glomerular lesions in the absence of antibodies and (2) the CVF model is suitable to elucidate role of activated C-products in induction of glomerular lesions.

MONOCLONAL ANTIBODIES (MAB) TO A NOVEL 103 KD PODOCYTE MEMBRANE PROTEIN BIND TO AND INDUCE FOOT PROCESS SWELLING, OBLITERATION OF FILTRATION SLITS AND FORMATION OF SUBEPITHELIAL IMMUNE DEPOSITS (ID). Aaro Miettinen*+, Gerhard Dekan*, Eva Schnabel*, and Marilyn G. Farquhar, Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, and Department of Bacteriology & Immunology, University of Helsinki, Helsinki, Finland+.

MAB 27A (IgG_3 kappa-type, pI 6.9-7.6) recognizes a 103 kd protein by immunoblotting and by immunoprecipitation. By indirect immunofluorescence (IF) on 0.5 µm frozen sections of normal rat kidney it is apparent that staining is limited to podocytes, where a granular intracellular signal and a weaker cell surface staining is seen. By immunoelectron microscopy (EM) the intracellular labelling was seen to be due to the presence of the antigen throughout the entire biosynthetic pathway (rough ER, Golgi stacks and carrier vesicles), suggesting that this protein is synthesized at a high rate.

27A IgG (5 mg) was injected into rats, and the distribution of mouse IgG was followed for 1 h to 10 d postinjection. By 10 d the IgG had disappeared from the circulation. By direct IF, 27A IgG was bound to all glomeruli in a granular pattern at 1 h. Staining decreased but was clearly detectable at 1-10 d. At 10 d rat C3 was also detectable in the deposits. By immuno EM the bound 27A IgG was localized at 1 h along the base of foot processes and in filtration slits under the slit membranes. The foot processes were swollen and many of the filtration slits were obliterated. At 1 d the deposited IgG was seen mainly under the slit diaphragms, and morphologic changes of foot processes were less prominent. At 10 d glomerular morphology was normal, but subepithelial ID's persisted.

These results demonstrate the ability of injected MAB 27A to induce subepithelial ID, and they suggest that the 103 kd protein may play an important role in the maintenance of normal podocyte morphology.

HLA-D REGION GENE POLYMORPHISMS ASSOCIATED WITH IgA NEPHROPATHY [IgAN]. Moore R*, Hitman G*, Richards N*, Lucas E*, Venning M*, Goodship T*, Cunningham J*, Marsh F*, [intr. by Slatopolsky E]. The London Hospital, St Thomas' Hospital, London, and Freeman Hospital, Newcastle, UK.

IgAN has been associated with HLA DR4 but these observations are inconsistent suggesting that genes encoding the DR determinants are not those conferring susceptibility, but are markers for other closely linked disease associated genes. We have therefore studied genetic susceptibility to IgAN by examining restriction length polymorphisms [RFLPs] of the HLA D region genes.

DNA obtained from British Caucosoids with IgAN [n=27], membranous nephropathy [IMN; n=46, patient controls], and normal controls [n=141], was digested with restriction enzymes and studied using Southern blot techniques and hybridisation with ^{32}P labeled DNA probes; DRα, DQα, DXα, DQß, and DPα.

IgAN had an increased frequency of two non-allelic Taq I fragments designated T2 and T6. 44% of patients with IgAN possessed the phenotype T2+/T6+ compared to 15% normal controls [p=0.005] and 17.5% in IMN [p=0.03]. In addition, a Bgl II 3.5kb DPα fragment was decreased in IgAN 14.8%] compared to IMN [45.6%, p=0.015] and normal controls [34.9%, p=0.08]. No association of RFLPs of the DRα, DQα, and DXα genes was seen in IgAN. IMN had an increased frequency of the RFLPs of the latter genes which are known to be strongly associated with DR3.

These observations demonstrate that disease susceptibility genes may play a role in the pathogenesis of IgAN. Furthermore, the gene[s] conferring susceptibility are situated in the HLA D region, and are localised within or near to the DQ and DP subregions.

THE CONSTITUENTS OF CIRCULATING AND TISSUE-BOUND IMMUNE COMPLEXES IN STREPTOCOCCUS MUTANS-ASSOCIATED NEPHRITIS OF RABBITS. Masayuki Miyata*, Anthony Castellazzo*, Ingrid Glurich*, Murray W. Stinson*, Felix Milgrom* and Boris Albini. SUNY-AB, Department of Microbiology, Medicine and Oral Biology, Buffalo, New York.

Administration of disrupted Streptococcus mutans MT 703 (SM) thrice weekly i.v. over 4-8 wks leads to nephritis and mild proteinuria in 85-100% of New Zealand White rabbits; prolonged injections over up to 28 wks leads to a reduction of proteinuria and subsequent severe second proteinuric stage. In contrast, administration of S. mutans 10449 over 28 wks does neither induce nephritis nor proteinuria. Understanding of the composition of immune complexes (IC) in this experimental nephritis may yield some insight into the pathogenic role of microbial antigens (MA) on the one, and rheumatoid factor (RF), on the other hand. Circulating immune complexes (CIC) were dissociated using acid buffer or antigen excess. Both tests indicated presence of both MA and RF in CIC. Immunoblots of 3% PEG precipitates from CIC-positive sera revealed consistently a 53 kd MA. Tissue-bound IC (TIC) dissociated in antigen excess also indicated presence of MA and RF. In both TIC and CIC, participation of MA declined and that of RF increased over the development of the disease. These results demonstrate that MA are present consistently in TIC and CIC of the early disease stage; that from early on, but increasing with time, RF contribute to TIC and CIC; and that TIC and CIC have a comparable composition.

RECOMBINANT HUMAN INTERLEUKIN-2 (rIL2) INCREASES GLOMERULAR FILTRATION RATE (GFR) IN THE ISOLATED ERYTHROCYTE-PERFUSED RAT KIDNEY (IEPK). Ph. Moullier,* A. Altman*, and C. B. Wilson. Res. Inst. of Scripps Clinic, La Jolla, CA 92037.

To study renal effects of the T cell lymphokine IL2 as a potential mediator of renal injury, we used the IEPK perfused with 5 gm% albumin and bovine erythrocytes (Hct 45%). With a perfusion pressure of 75 mmHg, the IEPK has hemodynamics similar to in vivo values--flow: 4.99±0.42 ml/min/gm, vascular resistance (VR): 15.43±1.29 mmHg/ml.min-1, GFR: 0.9±0.2 ml/min/gm, filtration fraction: 18±4%, with well preserved glomerular morphology. After three (10 min) control periods (C), vehicle (V) or rIL2 (an acute local tissue concentration of about 3 nM) were injected over 5 min. With flow maintained constant, three (10 min) experimental periods (E) were compared as the % of mean C [(n=7) for V and (n=8) for rIL2)].

Period		E1	E2	E3
GFR	V	-0.5±22.7	7.7±14.7	23.3±34.4
	rIL2	77.4±60.5**	119.7±68.9**	144.5±112.7*
VR	V	-0.8±2.2	-0.4±2.6	0.5±4.5
	rIL2	3.3±6.7	9.3±8.9*	13.7±9.0**
% Na Reab.	V	98.8±0.9	99.1±0.9	99.1±1.1
	rIL2	99.1±0.9	98.8±0.9	98.1±1.6

C vs E: ** p<.01, * p<.05, Student's t test

No increase in urinary protein excretion was detected in rIL2 (n=4) compared to V (n=7) infused IEPK preparations. Partial inactivation of the rIL2 (murine CTLL-2 cell line assay), using rabbit anti-human IL2 antibody, proportionately reduced the increase of GFR. A second rIL2 and V produced similar changes. In conclusion, rIL2 produces a specific, progressive increase of GFR, no natriuretic effect, and a small delayed increase of VR.

THE NEPHROTOXIC EFFECTS OF CYCLOSPORINE-A (CY-A) ARE GREATLY ACCELERATED IN HYPERTENSIVE COMPARED TO NORMOTENSIVE RATS. Mahmood S. Mozaffari,* Dale R. Abrahamson, John J. Curtis, and J. Michael Wyss,* University of Alabama, Depts. of Cell Biol. and Anat. and Med., Birmingham, Alabama

CY-A therapy causes significant hypertension and nephrotoxicity in transplant recipients, but the etiology of these effects remains enigmatic, largely due to the lack of appropriate animal models. We tested the hypothesis that the adverse effects of CY-A are significantly accelerated by hypertension. Four groups of 8 week old uninephrectomized rats were included in the study: 1) deoxycorticosterone-acetate treated rats maintained on 1% saline (DOCA-NaCl) and receiving daily injections of CY-A (20 mg/kg), 2) DOCA-NaCl receiving the vehicle alone (Veh/DOCA-NaCl), 3) sham control (no DOCA-NaCl) receiving CY-A (CY-A/sham), and 4) Veh/sham. During the initial 3 days, systolic arterial pressure (SAP) increased significantly in the CY-A/DOCA-NaCl group (124 +/- 2 to 143 +/- 4 mm Hg), but their SAP did not increase significantly thereafter. In comparison, the Veh/DOCA-NaCl and CY-A/sham groups displayed delayed SAP increases (SAPs at 3 weeks, 167 +/-3, 133 +/-2 mm Hg, respectively). All three treatment groups were significantly proteinuric, but the CY-A/DOCA-NaCl group was affected most. Light microscopic examination revealed severe dilation of the renal distal tubules and a striking increase in the interstitial space between tubules in the CY-A/DOCA-NaCl group. Electron microscopic analysis confirmed these effects and also demonstrated large numbers of sloughed epithelial cells within the tubular lumen in this group. Only minor derangements of this type were noted in the Veh/DOCA-NaCl and CY-A/sham groups. These results suggest a significant nephrotoxic interaction between CY-A and hypertension.

CYST-DERIVED CELLS DO NOT EXHIBIT ACCELERATED GROWTH OR FEATURES OF TRANSFORMED CELLS. Sakie Nakamura, Phaibul Punyarit, Barbara Schumacher, Frank Carone. Northwestern Univ. Med. Ctr., Dept. of Path., Chicago, Illinois.

Progressive renal enlargement is a prominent feature in autosomal dominant polycystic kidney disease (ADPKD) suggesting that the disease is due to hyperplasia and/or preneoplastic transformation of renal epithelial cells. In this study in vitro methods were developed to grow and propagate large numbers of cyst-derived epithelial cells from ADPKD kidneys and cortical epithelial cells from normal human kidneys (NK). In order to study their biologic features during early cell passages, cells were grown on Vitrogen (bovine dermal collagen) - FCS (fetal calf serum) coated dishes and fed a basic medium (DME:F12) supplemented with 10% FCS or a defined medium (Sens) containing insulin, transferrin, selenium, hydrocortisone, tri-iodothyronine and epidermal growth factor (EGF). Both ADPKD and NK cells grew as monolayers, were positive for keratin by immunohistochemistry and flow cytometry and had ultrastructural features of renal epithelial cells. Confluent NK and ADPKD monolayers formed domes. In contrast to NK cells, the growth and propagation of ADPKD cells was not supported by defined medium alone but required serum supplementation and ADPKD cells did not respond to growth factors (insulin, transferrin, EGF) that promoted the growth rate, of NK cells. In serum supplemented media, the growth rate, cell doubling time and end cell number of ADPKD and NK cells were the same. Moreover, ADPKD cells did not exhibit any in vitro features of transformed cells: they were not immortal, they were sensitive to contact inhibition, they were anchorage dependent and they were not tumorigenic in nude mice. These findings do not support an increased rate of a cell growth or cell transformation as causative factors in ADPKD.

CHARGE AND MOLECULAR SIZE OF IgA IN SERA AND GLOMERULAR ELUATES FROM A MURINE MODEL OF IgA NEPHROPATHY. Eri Muso, Haruyoshi Yoshida, Eiji Takeuchi, Toshihide Shimada,* and Chuichi Kawai.* Kyoto Univ., Fac. of Med., 3rd Div., Dept. of Int. Med. & Dept. of Pathol. Kyoto. Japan.

To investigate the qualitative characteristics of IgA in the pathogenesis of IgA nephropathy, charge and size of IgA from sera and kidney eluates were analyzed in ddY mice which develop spontaneous IgA nephropathy with aging. Female ddY mice aged 12, 40 and 60 week old were used. Glomeruli were isolated using sieving technique and the bound proteins were eluted with citrate buffer (pH 3.2). The charge and the size of IgA in each sample were analyzed by isoelectric focusing (IEF) and by high performance liquid chromatography (HPLC), respectively. The IgA levels were detected by ELISA using anti-mouse IgA antibodies. Serum IgA levels were increased significantly in 40 and 60 week old mice with nephritis than in 12 week old mice without nephritis. The IEF analysis showed serum IgA in the pH range from 4.5 to 5.0 without changing with age. IgA in the eluates from 60 week old mice were focused in slightly limited acidic range from pH 4.5 to 4.8. HPLC fractionation revealed significant increase of polymeric IgA (>500kd) in the sera from 40 and 60 week old mice, especially in mice with severe glomerular damage. Further, almost all of the eluted IgA from 60 week old mice were polymeric form of more than 1000kd. These findings suggest that the polymeric quality of IgA, rather than electric charge, may be more pathogenic in the development of IgA nephropathy in ddY mice.

MONOCYTE SECRETE FACTORS THAT REGULATE GLYCOSAMINOGLYCAN(GAG) SYNTHESIS BY MESANGIAL CELLS(MCs) IN VITRO. Ichiei Narita, Testuo Morioka, Kazukiyo Yoshida, Takashi Oite, Fujio Shimizu, Masaaki Arakawa. Niigata Univ. School of Med., Dept. of Med(II) and Dept. of Immunol. Inst. of Nephrology. Niigata Japan. (Intr. by F. Marumo)

Proliferation of MCs and increased accumulation of mesangial matrices are histological hallmarks of chronic glomerulonephritis. But the mechanism involved in it remains uncertain. MC have been reported to synthesize various connective tissue and basement membrane components. This work was undertaken to elucidate the role of monocytes in icrement of mesangial matrices, particularly GAGs which are capable of interacting with several other matrix components such as collagens, laminine and fibronectin. MCs were used between third and fifth passages after primary culture of glomeruli. Monocytes, obtained from peripheral blood of normal adult human, were cultured for 72 h in D-MEM supplemented with 5% autologous human serum and 20 ug/ml of LPS. The cell-free culture supernatant was used as crude monocyte derived factor (MDF). The MDF was concentrated by diaflow membrane and fractionated by gel chromatography. ^{3}H-glucosamine incorporation to GAGs by rat and human MCs were measured by cetylpyridinium chloride precipitation method. At confluency, MDF had no effect on cell growth. But GAG synthesis by rat and human MCs was increased by addition of MDF in dose dependent fashion. MDF contained at least two components which exerted a stimulatory effect on GAG synthesis. Gel and ion exchange chromatography study showed that elution patterns of GAGs synthesized by MCs apparently same in the presence of MDF. Local infiltration of monocytes to glomerulus, which is often seen in various types of glomerular injury, may takes a role in increment of mesangial matrix accumulation through the factors we explored in this experiment.

MOLECULAR CLONING OF THE 3M-1 NEPHRITOGENIC ANTIGEN
Eric G. Neilson, Mae Jane Sun*, John Emery*, Carolyn Kelly, Tom Haverty, Michael Clayman, and Nancy E. Cooke*, Department of Medicine, University of Pennsylvania, Philadelphia, PA

We have a proximal tubular cell line (MCT) in culture that secretes the nephritogenic antigen 3M-1 which is the target ligand of αTBM disease producing interstitial nephritis in humans and mice. A cDNA library was prepared from this cell line and packaged in lambda gt11 phage. The library was screened with a monospecific polyclonal antibody to 3M-1. A positive clone obtained from this screening was used to rehybridize the library pulling out seven additional cDNA isolates. cDNA pM-2 hybridizes to a 1,750 bp mRNA transcript from MCT cells, but not from interstitial fibroblasts. Sequencing of these cDNAs revealed that 3M-1 is transcribed as four species of mRNA differing in their 5'-ends. One of these species contains 5' Gly-X-Y residues which may be a collagen foot integrating this isoform of 3M-1 into the extracellular matrix. A two-dimensional computer plot of the open-reading frame was used to identify potential antigenic epitopes for the nephritogenic T and B cell repertoires. Oligopeptides synthesized from this computer plot were found to bind to several monoclonal anti-3M-1 antibodies derived from animals with interstitial nephritis. One of these oligopeptides also strongly stimulates proliferation of cloned 3M-1-specific helper T cells growing in culture. Our findings clearly indicate that 3M-1 is cell-specific, polymorphic, and probably provides more than one antigenic epitope. The cloning of these cDNAs now allows for the isolation of their relevant genes and cis-acting elements.

REGULATION OF MOUSE MESANGIAL CELL PROLIFERATION BY 1,25,DIHYDROXYVITAMIN D_3 (1,25,D_3), B.S. Ooi, E.P. MacCarthy, S.Y. Hong,* A. Hsu,* Division of Nephrology and Hypertension, Department of Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio.

The monocyte/macrophage has been shown to be the principal effector cell infiltrating the glomerular mesangium in immune-mediated nephritis. Upon activation, the macrophage secretes a number of cytoregulatory substances, one of which has recently been shown to be 1,25,D_3. We have examined the effect of 1,25,D_3 on the growth of mouse mesangial cells in culture and found that 1,25,D_3 exerts a suppressive effect on the growth of such cells as assessed by [^{3}H]-thymidine uptake by the cells and by cell counts in a dose-dependent fashion. Additionally, 1,25,D_3 suppresses the mitogenic effect of epidermal growth factor (EGF) on the proliferation of the mesangial cells. Specificity of the effect was demonstrated by studies using 25,D_3 which had a significantly reduced effect compared to the effect of 1,25,D_3. 1,25,D_3 was also shown to suppress the growth of mesangial cells which had been committed to proliferate by prior incubation of the cells with EGF. The results of these studies are relevant to our understanding of the mechanisms regulating mesangial cell growth in nephritis and potentially to clinical states characterized by pertubations in the serum levels of 1,25,D_3.

TREATMENT OF LUPUS NEPHRITIS IN NEW ZEALAND F1 MICE (B/W MICE) BY 15-DEOXYSPERGUALIN (DSP). M. Okubo, N. Umetani,* K. Inoue,* and K. Kamata. Kitasato Univ. Dept. of Med., Sagamihara, Japan.

We already reported DSP prevented the development of spontaneous nephritis in B/W mice when the therapy was started at 14 wks of age just prior to the onset of the disease (Kidney Int. in press). The present study was done to evaluate the effect of DSP on the established disease. Twenty-eight-wk-old B/W mice with nephritis were left untreated (control group), or put on treatment either with cyclophosphamide, 15 mg/kg/wk (C Y group), or with DSP, 6 mg/kg/2 days (DSP group). Body wt. and proteinuria were periodically measured. The mice were serially sacrificed at 28, 32, 36 or 40 wks of age, their blood and spleen were taken to analyze lymphocyte subsets by flow cytometry, and kidneys were processed for light and immunofluorescent microscopy. Histological changes were semiquantitatively analyzed. Age-dependent decrease in body wt. was seen in the control group, but not in CY or DSP group. Less proteinuria was observed in both of the treated groups. Spleen wt. and L3T4+/Lyt2+ ratio of splenocytes at 40 wks of age were significantly lower in DSP group compared with CY or control group. Glomerular intracapillary cell proliferation at 32 wks of age, and crescent formation and tuft necrosis at 40 wks of age were significantly less in both of the treated groups, DSP group in particular. Deposition of IgG and C3 in the mesangium and along the capillary loops was also decreased by both treatment modalities. In conclusion, DSP treatment proved effective for the advanced nephritis in B/W mice, which was quite comparable to or even better than the action of CY.

THE CASCADING, INTERRELATED ROLES OF INTERLEUKIN-1, IL-2 AND IL-6 IN ANTI-CD3 DRIVEN MURINE T CELL PROLIFERATION. **Oleh Pankewycz*, Mary Yui*, Vicki Kelley, Terry Strom, Beth Israel and Brigham and Women's Hospitals, Harvard Univ., Boston, MA**

In vitro effects of antigen stimulation with T cells are mimicked by monoclonal antibodies (Mab) defining proteins of the T cell receptor-CD3 complex. We now define the requirements for activation and proliferation of purified resting murine T cells at varying cell densities upon stimulation with divalent (DV) or polyvalent (PV) forms of a mitogenic anti-CD3 Mab. Accessory cell (AC) depleted T cells do not proliferate to ConA nor to DV anti-CD3 at 100,000 cells/well. PV anti-CD3 provides a powerful signal for T cell proliferation at >5,000 cells/well. Recombinant (r) IL-2 strongly augments T cell proliferation to anti-CD3; whereas, rIL-1 exerts no direct effects on anti-CD3 stimulated T cells. rIL-6 promotes T cell proliferation only in response to PV anti-CD3. When added to adherent AC (95% macrophages) rIL- 1 stimulates IL-6. rIL-6 in turn amplifies accumulation of stable IL-2 mRNA transcripts in purified T cells after PV anti-CD3 stimulation. Thus, the maximum proliferative signal to T cells in low cell densities is PV anti-CD3 plus exogenous IL-2. IL-2 gene activation, critical for T cell proliferation, by antigen or anti-CD3, is potentiated by the cytokines IL-1 and IL-6. IL-1 indirectly supports T cell proliferation by stimulating IL-6 gene activation in AC. T cell stimulation by anti-CD3 Mab plus IL-6 induces transcription of T cell IL-2 mRNA. Thus, IL-1, IL-6 and IL-2 support T cell proliferation through cascading effects at the level of mRNA gene activation.

HEPARAN SULFATE (HS) AND HEPARIN SPECIFICALLY DELIMIT NEPHRON FORMATION IN EMBRYONIC KIDNEYS. Jeffrey L. Platt, Paul Trescony,* Bonnie Lindman* and Theodore Oegema, Jr.* University of Minnesota Departments of Pediatrics, Cell Biology and Neuroanatomy, Orthopaedic Surgery and Biochemistry, Minneapolis, Minnesota

Formation of nephrons from primitive mesenchyme is induced by ureteric buds. Nephron induction is closely coordinated with branching morphogenesis of the ureteric bud and is associated with de novo synthesis of chondroitin sulfate proteoglycan and release of free HS glycosaminoglycan (GAG) chains. We therefore asked whether GAG influence nephron development. Using a quantitative organ culture model, we demonstrated that up to 10-fold fewer nephrons were formed in kidneys incubated in heparin and HS than in control conditions or in kidneys incubated in chondroitin sulfate or hyaluronic acid. Inhibition was associated with altered expression of cell and matrix antigens. Development of previously induced nephrons, however, was unaffected suggesting heparin and HS acted on an early event in nephron formation. Branching morphogenesis was comparably impaired in kidneys exposed to heparin but not to HS or de-N-sulfated, N-acetylated heparin. Inhibition of nephron development depended on GAG sugar composition and O-sulfation but not on GAG chain size or charge density. The effectiveness of HS at concentrations as low as those existing in mature kidneys suggests these GAG may control formation of nephrons in fetal metanephric kidneys.

URINARY EXCRETION OF THE C5b-9 MEMBRANE ATTACK COMPLEX OF COMPLEMENT REFLECTS DISEASE ACTIVITY IN ACTIVE (AICN) AND PASSIVE HEYMANN NEPHRITIS (PHN). C. J. Pruchno*, M.W. Burns*, M. Schulze*, P. Baker*, R.J. Johnson, W.G. Couser, U. of WA, Seattle, WA.

In the Heymann nephritis models of membranous nephropathy (MN) formation of subepithelial immune complex deposits by reaction of antibody with a glomerular epithelial cell (GEC) antigen results in membrane insertion and transcellular transport of C5b-9 with excretion of C5b-9 in urine (U C5b-9). To test the hypothesis that U C5b-9 excretion measured by ELISA could be used as an index of active immune deposit formation by this mechanism, serial studies were carried out in both AICN and PHN. In PHN, U C5b-9 correlated with glomerular deposition of ^{125}I-anti-Fx1A (r=.88) in the heterologous phase. In the autologous phase, U C5b-9 paralleled serum levels of anti-sheep IgG. U C5b-9 returned rapidly to normal levels when circulating anti-Fx1A or anti-sheep IgG were no longer present or when kidneys from PHN rats with increased U C5b-9 were transplanted into normal hosts (n=3). In AICN at 20 weeks, rats with 4+ IgG deposits and persistent proteinuria exceeding 100 mg/day could be divided into groups with normal (A) or elevated (B) U C5b-9. Normal kidneys transplanted into group A rats (n=3) developed no deposits at 14 days whereas transplants in group B (n=3) all developed subepithelial deposits.

We conclude that in experimental MN induced by antibody to a GEC antigen, U C5b-9 is a sensitive index of on-going glomerular immune deposit formation and that proteinuria may persist for weeks in the absence of active immune disease.

ANTITHROMBOTIC MECHANISMS IN THE RAT KIDNEY; ASPIRIN ENHANCES GLOMERULAR ADP-ASE ACTIVITY. Klaas Poelstra, Machiel J. Hardonk and Winston W. Bakker. Univ. of Groningen, Dept. of Pathol., Groningen, the Netherlands.

Glomerular ADP-ase activity has been shown in the GBM of the rat kidney using cytochemical methods at the EM level. This activity could be reduced by adriamycin (ADR) injection leading to increased thrombotic tendency (Bakker et al. J.Lab. Clin.Med. 109:171, 1987). We now studied PGI_2 inhibition by injecting s.c. aspirin (2.5 mg/kg bw) in ADR (7.5 mg/kg bw) (n=10) or saline treated rats and compared glomerular ADP-ase activity and experimental intraglomerular thrombotic tendency with control rats (i.e. ADR treated n=8; saline n=7; saline + aspirin n=5). The results show decreased glomerular ADP-ase activity in combination with increased intraglomerular platelet aggregation following alternate kidney perfusion ex vivo of ADR treated animals compared with control rats (i.e. saline or saline + aspirin treated). In contrast, the ADP-ase inactivating effect of ADR could completely be abolished by subsequent aspirin treatment: normal glomerular ADP-ase was present while no intraglomerular thrombosis could be induced using the alternate perfusion system. Since aspirin treatment clearly enhances the glomerular ADP-ase activity in the rat kidney, its potential effect through endothelial PGI_2 inhibition cannot be deduced from the present data. However, this remarkable restauration of glomerular ADP-ase concomitant with the antithrombotic potential of the GBM reinduced by aspirin sheds a new light upon the antithrombotic action of this drug. Whether ADP-ase enhancement occurs via direct or indirect mechanisms remains to be investigated.

RENAL TUBULAR BASEMENT MEMBRANE CHANGE OCCURS PARI PASSU WITH THE DEVELOPMENT OF CYST FORMATION. Phaibul Punyarit, Paul Hollenberg, Raymond Novak, Walter Glogowski, Sakie Nakamura, George Flouret, Frank Carone. Northwestern Univ. Med. Ctr., Dept. of Path., Chicago, Illinois.

Our previous studies have shown that 2-amino-4, 5-diphenly thiazole hydrochloride (DPT) administered orally to rats induces a urine concentrating defect (within 1-2 days) and progressive, but reversible, cystic change of all collecting tubules (prominent between 4-8 weeks). Cystic change was characterized by tubular cell and basement membrane changes consisting of alterations in cellular biosynthetic/secretory organelles followed by thickening of the basement membrane with marked reduction (~50%) of the de novo synthesis of sulfated proteoglycans, suggesting that altered synthesis of tubular basement membrane plays a role in the development of cystic disease. In this study, following the administration of [14C]-DPT in vivo, a major urinary metabolite (>70%) was isolated by HPLC and characterized by gas chromatographic-mass spectral and NMR analyses as 2-amino-4, hydroxyphenyl-5 phenyl thiazole, designated as phenol II. Phenol II was synthesized and administered orally to rats for 4 days to compare its biological effects with DPT. Phenol II impaired concentrating ability significantly greater and induced tubular cystic transformation earlier and to a much greater degree than DPT. At day 5, in phenol II treated animals, basement membranes lining cysts were thickened several-fold and exhibited extensive loss and disorder of ruthenium red binding sites, indicative of loss of sulfated proteoglycans (heparin sulfate proteoglycan). The basement membrane changes occurred in tandem with the development of cystic transformation and strongly suggests that the basement membrane has a key role in the pathogenesis of PKD. The findings support the hypothesis that PKD may be due to a genetic or acquired defect in the synthesis/degradation of one or more basement membrane components (sulfated proteoglycans) resulting in faulty tubular morphogenesis.

SUPPRESSIVE EFFECT OF LYMPHOKINES ON SECRETION AND mRNA LEVELS OF LIPOPROTEIN LIPASE (LPL) IN CULTURED HUMAN MACROPHAGES. **U. Querfeld*, J. Ong*, J. Prehn*, J. Carthy*, P. Kern*, S.C. Jordan. Cedars-Sinai Med. Ctr., Dept. of Pediatr. & Endocr., Los Angeles, CA.**

Lipid loaded macrophages are part of atherosclerotic plaques which are frequently observed in patients with CRF. Spontaneous secretion of LPL occurs in cultured human macrophages and may facilitate lipid uptake. To study the effect of immunoregulatory lymphokines on LPL secretion, peripheral blood mononuclear cells obtained from healthy volunteers were isolated by Ficoll-Hypaque centrifugation, and plated in culture dishes ($10x10^6$/dish). After 6 days of incubation in RPMI 1640 medium with 20% fetal calf serum, recombinant human interferon gamma (rhγ-INF) and interleukin-2 (rhIL-2) were added to the medium (500 U/ml and 100 U/ml, respectively). LPL activity (LPLa) and LPL mass (LPLm) were assayed in the medium. Results (%Δ of mean from control mean):

		day 7	day 9	day 12
rhγ-INF	LPLa	-59	-73	-93
	LPLm	-43	-60	-40
rhIL-2	LPLa	-64	-100	-98
	LPLm	-21	-61	-100

The suppressive effect of rhγ-INF and rhIL-2 was dose dependent and threshold doses were 0.05 U/ml and 0.5 U/ml, respectively. Neither rhγ-INF nor rhIL-2 affected protein synthetic rate in macrophages (50U/ml). After 12 days, LPL mRNA levels were 22 + 5% of controls in rhγ-INF treated dishes (500 U/ml). Thus, LPL secretion by human macrophages in vitro can be suppressed by lymphokines.

RAT KIDNEY MESANGIAL CELLS SYNTHESIZE NOVEL COLLAGENOUS POLYPEPTIDES. N. Rosenblum, * M. Karnovsky* and B. Olsen* (intr. by W. Harmon) Div. of Nephrology. The Children's Hospital; Depts. of Pathology, Anatomy and Cell Biology, Harvard Medical School, Boston, MA.

Mesangial sclerosis is the principal pathogenic finding in many glomerular diseases such as inherited diffuse mesangial sclerosis. We have isolated and partially characterized collagenous polypeptides synthesized by cultured rat mesangial cells to develop probes to extracellular matrix components expressed in such diseases. Confluent cells, incubated with Na ascorbate and β-aminoproprionitrile, were labelled with ^{35}S-Met. Medium proteins were precipitated with ammonium sulfate, digested with bacterial collagenase and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. Collagenous polypeptides were identified by their sensitivity to bacterial collagenase. In addition to types I, III, IV and V collagen bands, we have identified collagenase sensitive bands of 300 kD and 200 kD. Bands of 250kD and 150kD were generated only after collagenase digestion. Digestion of the medium-derived proteins with pepsin led to degradation of these novel collagenous bands to low molecular weight peptide fragments. We conclude, therefore, that rat mesangial cells synthesize novel collagenous polypeptides with large nontriple helical domains and relatively small triple helical pepsin resistant domains. We speculate that these polypeptides are members of the nonfibrillar class of collagens which mediate interactions between fibrillar collagens and other components of the extracellular matrix.

GLOMERULAR SUBEPITHELIAL IMMUNE DEPOSITS (SID) INDUCED BY ANTIBODIES TO CULTURED RAT GLOMERULAR EPITHELIAL CELLS (GEC). R.J. Quigg, D.R. Abrahamson, A.V. Cybulsky, J. Badalamenti*, A.W.M. Minto* and D.J. Salant. Medical College of Virginia, Richmond, VA, Boston Univ. Med. Ctr., Boston, MA and Univ. Alabama at Birmingham, AL.

To investigate the role of GEC membrane antigens in SID formation, we produced a rabbit antiserum to cultured rat GEC (anti-GEC). By immunofluorescence (IF) on normal rat kidney, anti-GEC stained brush border (BB) of proximal tubules. Glomeruli of rats injected with anti-GEC contained diffuse, granular, capillary wall deposits of rabbit IgG at 5 days. Rabbit IgG was localized to SID beneath slit diaphragms and in coated pits on the GEC membranes by immunoelectron microscopy. IgG was also identified in cytoplasmic vesicles, indicating that membrane-bound immune complexes had been endocytosed by GEC. By immunoprecipitation and gel electrophoresis of radiolabelled, solubilized BB, anti-GEC shared reactivity with three proteins immunoprecipitated by anti-Fx1A (M_r 98 and 120 kd as well as a high M_r protein that corresponded to gp330). Anti-GEC reacted with five of seven proteins immunoprecipitated by anti-Fx1A from surface-labelled GEC (M_r 42-172 kd) including the 98 and 120 kD proteins present on BB. Both antisera also precipitated gp330 from biosynthetically-labelled, but not from surface-labelled, GEC. Anti-gp330 did not stain the cell membranes of GEC by IF. Thus, antibodies to cultured rat GEC antigens induce formation of SID that resemble those in passive Heymann nephritis produced by injecting anti-Fx1A. Cultured rat GEC synthesize gp330 but appear not to express it on their plasma membranes.

THE FUNCTIONAL AND PHENOTYPIC DISTINCTION BETWEEN CD4+2H4+ AND CD4+2H4- CELLS PERSISTS AFTER ACTIVATION WITH CON A. D Rothstein*, S Sohen*,SF Schlossman*, and C Morimoto*. (intr. by CB Carpenter). Dana Farber Cancer Inst. Boston Ma.

The monoclonal antibodies anti-2H4 (CD45R), and anti-4B4 (CDw29) along with UCHL1, identify reciprocal subsets of CD4 lymphocytes with suppressor-inducer (2H4+UCHL1-4B4-), and helper-inducer (2H4-UCHL1+ 4B4+) activity. Alterations in the relative numbers of these cells have been documented in a number of clinical settings including active SLE, and renal transplantation. Recent reports suggested that upon activation, 2H4+ cells become 2H4- and gain UCHL1 and 4B4 antigens. This, plus the ability of 2H4-, but not 2H4+ cells to respond to recall antigens suggested that 2H4+ and 2H4- subsets represent naive and memory cells, respectively. We now report on the phenotypic and functional changes of CD4+ cells separated into 2H4+(4B4-UCHL1-) and 2H4-(4B4+ UCHL1+) subsets and then activated with ConA (n=6). On days 2,7,14, and 21, aliquots of these cells were evaluated for phenotype and for their ability to provide suppressor-inducer or helper-inducer activity by decreasing or increasing PWM-driven IgG synthesis, respectively. The 2H4- cells remained >95% 4B4+ and UCHL1+, and 5-10% low density 2H4+, after activation. On day 2, the 2H4+ cells expressed high density 2H4 (>90%) as well as 4B4 and UCHL1(>70%). After day 2, the density and the % of cells positive for 2H4 decreased but plateaued with 45-50% of the cells positive on days 14 and 21. UCHL1 and 4B4 expression remained >90%. 5-10% of the cells expressed only 2H4, whereas 40-45% coexpressed UCHL1. Despite these phenotypic changes, the cells that were originally 2H4+ continued to provide suppressor-inducer function, and did not provide increased levels of help. The 2H4- cells provided significant help and minimal suppression. We conclude that although activation causes a down-regulation of 2H4+, and increased expression of UCHL1 and 4B4, these cells remain distinct, both functionally and phenotypically from 2H4- cells.

GLOMERULAR PRODUCTION OF A CHEMOATTRACTANT FOR WHITE BLOOD CELLS. B. Rovin*, J. Lefkowith*, and G. Schreiner, Renal Division, Washington Univ., St. Louis, MO.

Glomeruli(GLOM) possess a resident population of macrophages(MAC) which increase in inflammation in a complement-independent fashion. To characterize the factors regulating WBC migration, GLOM were isolated from normal rats, and rats made nephritic by IV injection of nephrotoxic serum. The GLOM were incubated in RPMI-1640 and the cell free supernatants were tested for WBC chemotactic activity in a multiwell microchemotaxis chamber. Responder cells were rat peritoneal macrophages(MAC), rat PMN, or human peripheral monocytes(MONO). GLOM from normal rats elaborated a chemoattractant for MAC and MONO, which increased in a time dependent fashion. Nephritic GLOM demonstrated enhanced production of chemoattractant relative to normal glomeruli:

	NET CELLS MIGRATED(±SEM)		
Glomeruli	MAC	PMN	MONO
Normal	850±170	1080±328	2131±169
Nephritic	1786±69	4205±246	5414±189

This factor retained 85% activity after incubation at 56°C for 30 min., or after heating to 100°C for 5 min., and was resistent to freeze-thawing. Activity was retained on an octadecylsilane column after ethanol extraction, and could be eluted with methanol. In summary, normal GLOM produce a chemoattractant for WBC during short term culture, which is increased by an inflammatory stimulus. Initial characterization suggests this factor is heat stable and resembles a lipid.

CYTOKINES PROMOTE NEUTROPHIL (N) ADHERENCE TO CULTURED HUMAN GLOMERULAR CELLS. K. Samudra,* J.D. Mahan, C. McAllister,* B. Shannon,* The Ohio State Univ., Depts. of Pediatrics & Medicine, Children's Hospital, Columbus, Ohio.

Leukocyte adherence to glomerular capillary structures appears to be an early step in the development of many forms of glomerulonephritis (GN) in man. Endothelial cells actively participate in inflammatory processes and, by altering cell surface properties may promote leukocyte adherence. The ability of cells to modify N adherence was investigated after exposure of human glomerular capillary endothelial (GCEC) and mesangial cells (MC) to 5 U/ml Interleukin 1 (IL-1), 5 U/ml Interleukin 2 (IL-2) or 100 U/ml gamma Interferon (gIFN). Normal human N were isolated and adherence was determined after 15 or 60 min incubation. Data is expressed as # N/# GCEC or MC.

	NEUTROPHIL ADHERENCE (15 MIN)			
	Control	IL-1	IL-2	gIFN
GCEC	.05 ± .01	.78 ± .09*	.11 ± .09*	-
MC	.19 ± .08	.91 ± .26*	1.20 ± .37*	.35 ± .11

(* = $p < 0.05$ vs control)

After 60 min only GCEC and MC exposed to IL-1 showed greater adherence for N over controls. This data suggests that exposure of glomerular cells to cytokines may agument N adherence and enhanced adhesiveness of GCEC and MC may be involved in the induction of inflammation in GN.

EVIDENCE THAT CULTURED MESANGIAL CELLS (MC) ARE OF MESANGIAL ORIGIN. Marc J. Sadovnic, and W. Kline Bolton. Univ. of Virginia Med. Ctr., Dept. of Med., Charlottesville, Va.

Functional and structural parameters have been used to define cells of presumed mesangial origin. We have cultured presumed MC from chickens (ch) by modifications of methods for mammalian MC. Ch MC have similar features - morphology, resistance to PAN, susceptibility to mitomycin C, growth in L-valine free medium, non-staining for factor VIII antigen, staining for fibronectin, myosin and desmin, and contraction with angiotensen II. These characteristics have defined these cells and mammalian MC as mesangial. However, direct evidence is lacking. We have produced a monoclonal antibody (MAb) by standard techniques. MAb 6H12, an IgG 2a isotype, binds to ch mesangium *in vivo* after iv injection and *in vitro*. It stains glomeruli in culture and mesangial cells in the first, second, and third passage. It does not react with fibrous tissue or fibroblasts, monocytes/macrophages, lymphoid cells, smooth or skeletal muscle, liver or lung. It does not bind to human, rat, mouse, bovine, canine or rabbit mes. After IV injection 6H12 binds avidly to the mesangium, slightly to peritubular tissues, but not to other organs. It is undetectable by 72 to 96 hrs. 6H12 does not fix ch complement *in vitro* or *in vivo*, but strongly fixes mammalian complement.

These findings confirm for the first time that cells in culture previously identified by indirect parameters to be mesangial cells are indeed derived from the mesangium.

TRANSCYTOSIS AND TRANSTUBULAR MOVEMENT OF HUMAN IMMUNOGLOBULIN LIGHT CHAINS (LC) IN THE PROXIMAL TUBULE (PT) OF THE RAT IN VIVO. Paul W. Sanders, Guillermo A. Herrera*, and John H. Galla. Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, AL.

To investigate the PT handling of LC's, male Sprague-Dawley rats were prepared for microperfusion experiments in standard fashion. Isolated rat nephrons were perfused in vivo at 20 nl/min for 20 min with artificial tubule fluid that contained two different κ LC's with similar molecular weights. Subsequently, the tubules were processed for immunoelectron microscopy using polyclonal antibodies to human κ LC. Perfusion with LC1 produced no ultrastructural alterations in the PT. The majority of this LC was localized to the luminal brush border membrane (30.7±4.0 %) and the endolysosomal system (65.1±4.4%); a small amount (3.7±0.7%) was also present along the tubular basement membrane and interstitium adjacent the perfused tubule. All of these values were different ($p < 0.001$) from background (mitochondria and nuclei) (0.5±0.1%). Perfusion with LC2 resulted in PT damage that manifested as disruption of the microvillus border, vacuolation, and cell fragmentation and sloughing. This LC was also noted on the microvillus border, in the endolysosomal system, and along the tubular basement membrane. Thus, endocytosis and transtubular movement of both LC's was noted. Transcytosis with exocytosis of the nontoxic LC was demonstrated; the majority of transtubular movement of the toxic LC is likely due to disruption of epithelial integrity. Transepithelial movement of LC's may be responsible for the tubular basement membrane deposition of LC's that can occur in monoclonal LC deposition disease.

IDENTIFICATION OF A SPECIFIC Fc RECEPTOR (FcR) FOR IgG ON CULTURED RAT MESANGIAL CELLS (MC). A. Santiago*$, DeCandido*, H. Holthofer, D. Schlondorff. Albert Einstein College of Medicine, Bronx, NY.

Recently we and others have shown that cultured mesangial cells bind and take up immunecomplexes in an Fc-dependent manner leading in turn to generation of PGE_2, reactive oxygen, and platelet activating factor. The present studies were designed to further characterize potential FcR on mesangial cells. Binding assays with either monomeric or aggregated [^{125}I] labeled rat subclass-specific IgG were performed at 4°C for 2 hrs on subcultured rat mesangial cells. Monomeric rat IgG2a, IgG2b IgG_1 and aggr. IgG2a and IgG1 bound only nonspecifically. In contrast saturable binding of aggr. [^{125}I] IgG2b occurred, which was displaceable by excess (100ug/ml) IgG2b, but not IgG2a, IgG, or F(ab')2 fragments of IgG2b. The presence of an FcR was confirmed by surface protein iodination of MC and immunoprecipitation with either a polyclonal or monoclonal antibody prepared against murine FcR (kindly provided by Dr. J. Unkeless). Both antibodies precipitated a broad 45-55 kDa iodinated protein band that comigrated with the FcR from mouse macrophage J774 cells on SDS-PAGE. This protein band also reacted with the polyclonal anti FcR Ab on immunoblots. In contrast rat papillary epithelial cells were negative. By immunofluorescence microscopy all MC stained positive with polyclonal FcR-Ab. These results further characterize FcR on rat MC. The receptor is of the low affinity type with specificity for agg. rat IgG2b, the major IgG subclass in rat.

GLOMERULAR C3c DEPOSITION REFLECTS ACTIVE IMMUNE DISEASE IN EXPERIMENTAL GLOMERULONEPHRITIS (GN). M. Schulze*, M. Burns*, C. Pruchno*, P. Baker*, R. Johnson and W. Couser. U. of WA, Seattle, WA.

On-going glomerular immune complex formation is associated with C3 activation followed by covalent binding of C3b to immune deposits. Plasma factor I degrades C3b to C3d which remains bound and C3c which is released. We tested the hypothesis that glomerular staining for C3c might therefore distinguish on-going C3b deposition from previous C3b deposition as reflected by C3d deposits alone. On-going C3b deposition was arrested by treating rats during various types of GN (passive Heymann nephritis, PHN; cationized IgG anti-IgG, cat IgG; con-a anti-con-a, con-a) with cobra venom factor. Quantitative fluorescence densitometry for C3c and C3d deposits was performed before and after arresting C3b deposition and the % fluorescence remaining 24 hrs after C3 depletion calculated (mean ± SEM; *$p < 0.001$ vs C3c).

Model	day	n	C3c(%)	C3d(%)
PHN	2	3	-3.1±10.0	48.1±12.1*
	4	3	13.4±16.5	42.0±13.9*
	7	4	16.4± 8.2	85.8±12.5*
cat IgG	1	3	-8.4±10.8	35.5± 9.1*
con-a	1	3	6.7±17.6	36.8±12.9*

Abrogation of antibody deposition by transplanting PHN kidneys into normal hosts also resulted in a marked reduction in C3c staining compared to controls at 6 days.

We conclude that C3c is rapidly released from immune deposits in GN and that detection of glomerular C3c indicates on-going complement activation and immune deposit formation.

ESSENTIAL FATTY ACID DEFICIENCY INHIBITS MONOCYTE MIGRATION IN GLOMERULONEPHRITIS (GN). George F. Schreiner, Brad H. Rovin*, James B. Lefkowith*. Depts. of Med. & Path., Washington University Medical Center, St. Louis, Missouri.

Rats maintained on a diet deficient in essential fatty acids (EFAD) display marked depletion of normally resident glomerular and interstitial macrophages (Mϕ) to the extent that EFAD kidneys can be allografted without immunosuppression of the recipient (Science 240:1032). EFAD kidneys are also resistant to induced Mϕ infiltration in immune and non-immune models of glomerular injury. 24 hrs after induction of nephrotoxic serum GN in rats (n=6) control (C) diet glomeruli and EFAD glomeruli had comparable antibody deposition (568 pg/glom vs 607 pg/glom, EFAD vs C). Yet EFAD glomeruli manifested a 71% depletion of Mϕ (16.1 ± 1.0 vs 40.5 ± 1.5 Mϕ/glomerulus, EFAD vs C) and a 74% inhibition of TxB_2 synthesis (4.9 fmole/10^3 glomeruli vs 13.6, EFAD vs C). Proteinuria/24 hrs was 26 mg (EFAD) vs 120 mg (C). Analogous results were observed in a model of non-immune glomerular injury induced by deposition of ferritin complexes, in which C glomeruli accumulated 31.4 ± 2.3 Mϕ/glomerulus compared to EFAD glomeruli with 6.5 ± 0.9 Mϕ/glomerlus. EFAD rats have normal levels of circulating leukocytes. EFAD Mϕ are normal with respect to phagocytosis and chemotaxis. In microwell Mϕ chemotaxis assays, EFAD glomeruli release less of a lipid chemoattractant. Thus the EFAD state appears to uniquely inhibit Mϕ infiltration into the kidney under basal and inflammatory conditions.

THYMOSIN BETA-4 (TB) AND IN VIVO CELLULAR IMMUNITY (CI). S.Y. Shen, O. Corteza,* D. Revie,* S. Feng,* M. Torres,* K. O'Neil,* P. Chretien.* Div. of Neph., Div. of Ped. Immun., Univ. of MD., Balto., MD.

TB is a peptide isolated from thymosin fraction 5. In a mouse-oxazolone model, we evaluated the effects of TB, cyclosporine (CS), and dexamethasone (DM) on in vivo CI measured by serum interleukin-2 level (IL-2) and by quantitative delayed-type hypersensi- tivity (DTH). Female 12-wk old C3H mice were randomized into 7 groups of 15. Day 1 to day 9, one group was given daily SQ injections of 0.1 mg NaCl (sham), while each of the remaining groups received daily a test drug, or 2-drug, 3-drug combination. The daily doses were TB 0.86 ug/0.1 ml, CS 0.5 mg/0.1 ml, and DM 0.0022 mg/0.1 ml in a single or combination regimen. Each mouse was sensitized to oxazolone by a smear on the shaved abdominal wall on day 2, challenged by smear to the back of the right ear on day 8. DTH was quantitated after 48 hours by the difference in thickness of the right and left ears. Serum was collected from each mouse after DTH measurement and each group pooled. Serum IL-2 was measured in duplicate by a modified colorimetric assay using CTLL-2 cells and multiwell scanning spectrophotometer. Effects of each test drug and each combination of DTH and IL-2 were expressed as (mean of group-mean of sham)/mean of sham x 100%. A negative % indicates a suppressive effect. The results % are:

	CS	DM	TB	CS+TB	DM+TB	CS+DM+TB
DTH	-53	-37	-53	-43	-32	-57
IL-2	-47	-6	-45	-44	-29	-54

These data suggest that TB suppresses CI, which is similar to CS and is not synergistic to CS or DM.

AFFINITY PURIFICATION OF URINARY CRYSTAL GROWTH INHIBITOR (CGI). Hiroshi Shiraga,* Michael D. Clayman, Eric G. Neilson, John R. Hoyer. Dept. of Pediatrics & Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA

Monoclonal antibodies (McAb) to human calcium oxalate (CaOx) CGI were produced to allow further characterization of the major macromolecular inhibitor of stone formation in human urine. A McAb column was used for affinity purification without loss of either the functional activity or immunologic reactivity of this protein *in vitro*. CGI was semipurified from human urine by DEAE-cellulose column chromatography (J.Biol.Chem.258:12594-12600, 1983). Column fractions were segregated according to inhibitory activity (IA) assayed by inhibition of ^{14}C- Oxalate incorporation into CaOx seed crystals. Clones from fusions of Lewis rat B cells with Sp2/0-Ag14 myeloma cells were selected on the basis of their differential reactivity by ELISA with DEAE column fractions having IA. IgG fractions of ascites from nu/nu mice carrying hybridoma cells were coupled to Sepharose to prepare anti-CGI columns. Affinity purified human CGI (AfPuCGI) showed a dose dependent inhibition of CaOx crystal growth. AfPuCGI also showed immunologic reactivity with polyclonal antibodies (PcAb) and McAb to CGI on ELISA and Western blots. AfPuCGI was not reactive with either PcAb or McAb to human Tamm-Horsfall protein. The specificity of these anti-CGI antibodies will facilitate isolation and more precise biochemical characterization of CGI and evaluation of patients with CaOx stones.

C1q MODIFIES Fc RECEPTOR (FcR) MEDIATED PLATELET AGGREGATION BY IGG IMMUNE COMPLEXES (Icx). J. Sloand, R. Mehta, and G. Schmer*. Univ. of Rochester Med. Ctr., Dept. of Med., Rochester, NY, and Univ. of Washington, Dept. Lab. Med., Seattle, WA.

Platelet participation in Icx diseases can incite or aggravate injury to the glomerular microcirculation. Platelet activation by Icx's is mediated by FcR-Fc interaction. C1q, a subunit of C1, can potentially influence this interaction either by competitive interference with Fc-FcR or by involvement with platelet collagen receptor. We studied the role of C1q in Icx-mediated platelet aggregation. Soluble Icx's consisting of IgG molecules covalently bound to a polyacrylic acid backbone were prepared (VI Int'l Cong. Immunol 3.42.8, 1986). Icx's (.004-.008ug/ul) were incubated alone or in the presence of varying doses of C1q (.01-.05ug/ul) with washed human platelets (300,000/mm3) in an aggregometer at 37C. Aggregation responses were quantitated and compared with controls. Icx-induced platelet aggregation was FcR mediated as it was completely inhibited by monoclonal antibody to the 40kd FcR but not by other antibodies. All doses of C1q totally abrogated Icx-mediated aggregation, but had no effect on aggregation in response to equivalent aggregating doses of thrombin. Much higher doses of C1q (7.2ug/ul) were needed to inhibit equal aggregation responses to collagen. Pre-incubation of platelets with heat-aggregated IgM-Icx (.1-.2ug/ul) had no effect on IgG-Icx mediated platelet aggregation. We conclude that C1q specifically inhibits IgG-Icx mediated platelet aggregation by interfering with FcR-Fc interaction. C1q content of IgG-Icx present in the circulation or deposited in endocapillary beds may influence platelet participation in certain Icx diseases.

EFFECTS OF VERAPAMIL (V) ON IN VITRO MURINE IMMUNE RESPONSE. A.B. Singh*, K. Hiehle*, S. Nayar*, and R.A. Mann. U.M.D.N.J.-Robert Wood Johnson Medical School, New Brunswick, N.J.

The calcium channel blocker V has been reported to inhibit both T cell proliferation in response to mitogen and the induction of cytotoxic (CTX) effectors. We now report that V is a potent suppressor of both B and T cell mediated immune responses in mice.

V, in a concentration dependent manner: (1) inhibited B cell proliferation in response to (a) anti-mouse IgM, (b) culture supernatant (C-SPNT) from a B6 anti-H-2d mixed lymphocyte reaction (MLR), or (c) C-SPNT from a cell line known to induce B cell proliferation, (2) inhibited the proliferation of C57BL/6 (B6, H-2b) splenocytes in response to H-2d alloantigen in a MLR, and (3) abrogated the induction of B6 anti-H-2d CTX cells in a 5 day MLR. The latter inhibition was only seen when V was added on days 0, 1, or 2 and persisted when V was removed after day 2 (% lysis at 50:1 effector:target ratio = 54% without V vs 0% with 4 X 10^{-5}M V present on days 0-2 vs 49% with 4 X 10^{-5}M V present on days 3-5). V mediated suppression of CTX was synergistic with cyclosporin and largely reversible by the addition of C-SPNT from a B6 anti-H-2d MLR.

Reversal of V Mediated Suppression of CTX by C-SPNT
(% lysis at 50:1 effector to target ratio)

	V concentration X 10^{-6} M			
	0	5	10	20
V w/o C-SPNT	56	45	35	0
V w C-SPNT	54	50	43	45

Finally, V does not inhibit the induction of Concanavalin-A (Con-A) induced T suppressor cells (Ts) but, in fact, may promote the induction of Ts. After 48 hrs in either: (1)media, (2) Con-A at a concentration known to induce Ts, (3) V (2 X 10^{-5}M), or (4) Con-A + V, cells were washed, irradiated, and added to syngeneic cultures containing T cell mitogen. Cells cultured in media alone suppressed mitogen induced proliferation by 19% whereas the additon of cells cultured with Con-A, V, or Con-A + V resulted in 93-98% suppression.

Taken together, our data suggest that V may be a potent modulator of many immune responses.

FAILURE OF AFFINITY MATURATION WITHIN THE IDIOTYPIC NETWORK AS A MECHANISM FOR INCREASED AUTO ANTIBODY PRODUCTION. Roger E. Spitzer and Ann E. Stitzel. State Univ. of New York Health Science Ctr., Dept. of Pediatrics, Syracuse, NY.

C3NeF is an autoantibody found in patients with membrano-proliferative glomerulonephritis (MPGN). One mechanism of control of C3NeF production is by the idiotypic network whereby internal image anti-idiotypic antibody (Ab2 beta) stimulates and non-internal image anti-idiotypic antibody (Ab2 alpha) suppresses C3NeF B cell activity. Ab2 alpha and beta were, therefore, isolated from the serum of 3 patients with MPGN and two normal adults. The affinity of each for C3NeF isolated from patients, normals, and newborns was then determined by ELISA. For any given Ab2 alpha or beta preparation, affinities were 20-100X greater with C3NeF from patients as opposed to normals or newborns. For any C3NeF preparation, however, affinities were the same for Ab2 alpha or beta from normals or patients. These data indicate that autoantibodies like C3NeF, in contrast to their anti-idiotypic antibodies, affinity mature and that this "maturation" is both idiotypic and paratopic. The relative increase in affinity of C3NeF for the internal image anti-idiotypic antibody, therefore, may mediate a sequence leading to antigenic mimicry and increased autoantibody production.

PREDOMINANT FUNCTIONAL ROLE FOR CYCLO-OXYGENASE (CO) PRODUCTS OF ARACHIDONIC ACID (AA) IN MESANGIOPROLIFERATIVE AUTOLOGOUS NEPHROTOXIC SERUM (NTS) NEPHRITIS: K Takahashi*, GF Schreiner, J Ebert* and KF Badr. Vanderbilt Univ. Nashville, TN and Washington Univ. St. Louis, MO.

While much is known regarding acute NTS-induced glomerular injury, the functional characteristics and pathophysiologic mediators of the more relevant chronic autologous phase remain undefined. Studies were performed in Munich-Wistar rats 14 days post-injection of rabbit serum (Gp 1, n=4), NTS in the absence (Gp 2, n=6), or presence (Gp 3, n=6) of a CO inhibitor, ibuprofen (20 mg/kg ip x 4 ds). A strictly mesangial macrophage/monocyte infiltrate was noted with equal intensity in Gps 2 and 3. Urinary protein (mg/24 hr) was 8.5 in Gp 1, 17.2* in Gp 2, and 9.7† in Gp 3. Mean values for SNGFR, glomerular plasma flow (Qa), transcapillary hydraulic pressure difference (ΔP), afferent (Ra) and efferent (Re) arteriolar resistances, and glomerular ultrafiltration coefficient (Kf) were: *: $p<0.05$ vs Gp 1; †: $p<0.05$ vs Gp 2.

	SNGFR	Qa	ΔP	Ra	Re	Kf
	-----nl/min------		mmHg	(10^{10}dyn·s·cm^{-5})		nl/(s.mmHg)
Gp 1	42.2	171	33	1.67	0.91	0.062
Gp 2	33.5*	170	42*	1.53	1.01	0.027*
Gp 3	38.5†	136*†	36†	2.39*†	1.26*†	0.060†

During chronic autologous mesangioproliferative glomerulitis (Gp 2), despite preservation of Qa and increased ΔP, GFR is depressed due to the profound reduction in Kf. CO inhibition results in intense affferent vasoconstriction and decreases in Qa and ΔP, but GFR is preserved due to absence of the fall in Kf (Gp 3). CO inhibition also reverses the associated proteinuria. Thus, vasodilator and Kf-lowering AA-CO products mediate glomerular functional changes during chronic autologous NTS-induced glomerular injury, thereby contrasting the central pathophysiologic role of lipoxygenase products in the early heterologous phase.

CHRONIC HIGH PROTEIN INTAKE MAINTAINS PROTEINURIA IN A UNILATERAL RAT MODEL OF GLOMERULAR IMMUNE INJURY (ICGN).

Friedrich Thaiss, Wilhelm Schoeppe, Rolf A.K. Stahl, Dept. of Medicine, Division of Nephrology, Frankfurt, F.R.G.

High protein (hp) intake enhances proteinuria and progression of renal disease in rats with reduced renal mass. It is, however, unclear whether protein intake influences proteinuria and glomerular hormones in immune mediated glomerular diseases. To adress these questions we examined the role of hp intake on proteinuria, glomerular hemodynamics and prostanoid formation in an unilateral rat model of glomerular immune injury. Rats were unilaterally nephrectomized and ICGN was induced in the remaining kidney employing cationized human IgG.

At 2 weeks (w) rats with in situ ICGN had significant proteinuria which was uneffected by the dietary regimen (lp 412±87; hp 454±87 mg/24h). 8w following induction of disease the animals on hp had significantly higher proteinuria when compared to animals on lp (320±76 vs 44±6 mg/24h). Glomerular PGE2 formation increased from 2 to 8w (2w: lp 327±46, hp 424±65; 8w: lp 516±49, hp 584±70 pg/mg protein/min). GFR (inulin clearance) was not different at 2w (lp 223±30, hp 287±32 ul/min/100g bw), however, increased at 8w (lp 382±47, hp 524±55 ul/min/ 100g bw). The cyclooxygenase inhibitor indomethacin did not influence GFR and proteinuria in either lp or hp diet. Our data demonstrate that in this model of glomerular immune injury hp diet maintains proteinuria. Increased proteinuria and elevated GFR in animals on hp appear independent of changes in glomerular prostanoid formation.

HETEROGENEITY OF PROTEINURIA IN NEPHRONS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

Coleman H. Terrell,* Margaret M. Delaney,* John R. Hoyer. The Children's Hospital, Philadelphia, PA.

Proteinuria in individual long and short looped nephrons of 14-month-old male SHR was directly assesed by a method based on the luminal immune complex formation that results from passage of antibodies of antibodies to Tamm-Horsfall protein (TH) into the glomerular filtrate (KI 33:325, 1988). These luminal immune deposits in the thick ascending limbs of Henle (TAL) identify proteinuric nephrons. Since the TAL of long looped nephrons are adjacent to vascular bundles (VB) in rats, they may be distinguished from short looped nephrons in sections perpendicular to the axis of medullary rays in the inner stripe of the outer medulla. Renal tissue obtained 24 hours after injecting rabbit anti-TH was studied quantitatively by immunofluorescence for presence and semiquantitatively for intensity of rabbit IgG deposits in TAL adjacent to VB (TALa) and distant from VB (TALd) in the inner stripe. An average of 522.3±56.7 (mean±SEM) TAL/rat were evaluated in 14 SHR. Luminal rabbit IgG deposits were nearly twice as frequent in TALa as TALd (30.8±3.4% vs. 15.9±2.2%, $p<.001$), and TALa deposits were more intense, ($p<.001$). Albuminuria ranged from 4.2 to 61.4 mg/24 hr and significantly correlated with the proportion of TAL with deposits within each zone (TALa: $p=.025$; TALd: $p=.002$ by Spearman rank). The ratio of TALa/TALd with deposits (2.75±.73) was greater in the 7 rats with albuminuria <20 mg/24 hr than in 7 rats with >20 mg/day (1.68±.14), ($p=.022$). Only 2 of the 13 rats with focal glomerulosclerosis (FGS) in the inner cortex also had FGS in the outer cortex. A common mechanism(s) probably causes both the greater contribution to proteinuria by long looped nephrons shown in these studies of SHR and the greater FGS in deep glomeruli also observed in earlier studies of this model.

THE C-MYC ONCOGENE INDUCES KIDNEY CYSTS (KC) IN TRANSGENIC MICE

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The murine c-myc oncogene, driven by an SV40 enhancer, and human β-globin promoter, was injected into mouse eggs(F1: C57Bl/6J X CBA/J), producing transgenic mice positive for the c-myc construct on the basis of DNA analysis. Twenty independent transgenic lines were established. Transgenic mice carrying this construct consistently developed two different phenotypes, polycystic kidney disease (PCKD) and hematopoietic malignancies. Transgenic founders died of renal failure at age 3 weeks to 5 months (mean serum creat. 1.4 mg/dl; BUN 276 mg/dl). Heterozygous transgenic mice demonstrated multiple bilateral kc, accompanied by tubular hyperplasia, focal segmental and global glomerulosclerosis. The severity of PCKD was progressive with age. By ultrastructural study, kc predominantly affected collecting tubules. In addition, most adult kidneys demonstrated myeloma, or other lymphoid malignancies, often confined to the kidney. In some transgenic lines, kc were detected at birth; tubular hyperplasia at day 5-7; microadenomas and parietal epithelial cell hyperplasia at day 10, without associated hematopoietic malignancies. By RNA analysis, strong c-myc transcript was detected in kidney, but weaker or absent signal in other organs. We conclude that in this model 1) the morphology and autosomal dominant inheritance of kc closely resemble human adult PCKD 2) renal expression of c-myc oncogene may be a cause of kidney cysts.

SPECIES SPECIFICITY OF ACTIVATION OF THE ALTERNATIVE PATHWAY OF COMPLEMENT BY POLYMERIC IgA.
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Codeposition of IgA, C3 and properdin in the mesangium of patients with IgA nephropathy, suggests the activation of the alternative pathway by IgA. Since this subject is still controversial, we have analysed the potential of polymeric and aggregated IgA (AIgA) to activate complement. Plastic Elisa wells were coated with AIgA of various sizes, washed and incubated with fresh human serum in the presence of 10 mM Mg EGTA. After washing the amount of C3 bound was measured with goat anti-human C3. A dose dependent activation was observed in the presence of Mg EGTA. The degree of activation was related to the size of AIgA and it did not occur with factor D-deficient serum, indicating activation of the alternative pathway. The activating capacity of rat polymeric IgA was studied in a similar fashion. Monomeric, dimeric and polymeric rat IgA anti-DNP antibodies were obtained from fusions of mesenteric lymph node cells. Their activating capacity was studied using rat, human and guinea pig serum as a complement source. Complement activation was only seen with rat serum. Dimeric IgA was 10 x as active as monomeric IgA and polymeric IgA was at least 3 x more active than dimeric IgA. These results may explain some of the controversies around the complement activating capacity of IgA.

COMPLEMENT ACTIVATION AND FIXATION BY HUMAN IgA RHEUMATOID FACTOR (IgA RF). FB Waldo, and RE Schrohenloher. Depts of Pediatrics and Medicine, Univ of Alabama at Birmingham, B'ham, Al.

IgA RF is an auto-antibody to IgG commonly found in patients with rheumatoid arthritis and other conditions, including IgA nephropathy. We have previously shown in a heat aggregated system that human IgA inhibits the activation and fixation of C3 by IgG (KI 33,326a, 1988). Therefore, we have examined the effects of IgA RF on complement activation. Human polymeric IgA1 RF was purified from the serum of a patient with multiple myeloma. Polystyrene wells coated with normal human IgG caused < 5% C3 consumption. Addition of RF caused a maximum of 18% C3 activation and this was not inhibited by classical pathway blockade with Mg/EGTA. No C3 fixation to IgA RF heavy chain (HC) could be detected. Wells coated with tetanus toxoid (TT) and human IgG anti-TT caused 30 % C3 activation and C3 fixation to the IgG HC. Addition of IgA RF reduced C3 activation to 12 % and reduced but did not eleminate C3 fixation to IgG HC. Similarly results were found in a fluid phase system. Heat aggregated IgG caused 35-40 % C3 activation and C3 fixation to IgG HC. IgA RF caused 50-75% inhibition of C3 activation and fixation. We conclude that IgA RF may cause limited alternative pathway C3 activation but fixes too little C3 to be detected by our method. More importantly, IgA RF partially inhibits IgG mediated complement activation and fixation. Therefore, IgA RF may limit C3 fixation to IC and thus their clearance. IgA RF containing IC deposited in tissue, however, may still cause alternative pathway C3 activation.

MONOCYTE INVOLVEMENT IN PASSIVE HEYMANN NEPHRITIS. Arnold Vogt*, Masanori Hara*, Dieter Bitter-Suermann*, Stephen Batsford*, (Intr. by Jeffrey Kreisberg). Dept. of Immunology, Freiburg, Inst. of Med. Microbiology, Mainz, F.R.G.

A role for complement but not polymorphonuclear granulocytes (PMN) has been demonstrated in passive Heymann nephritis (PHN) in rats (Salant et al. JCI 66: 1339, 1980). We have now identified monocytes as another important mediator in this model. PHN was induced in Wistar rats by i.v. injection of sheep anti-rat FxIA. Four groups were studied: (A) rats given normal sheep IgG (nephritic controls), (B) rats given sheep anti-rat PMN IgG (PMN depleted), (C) rats given sheep anti-rat monocyte IgG (monocyte depleted), (D) rats injected with cobra venom factor (complement depleted). In vitro specificity controls for anti-cell antisera were made by cytotoxicity tests and inhibition of phagocytosis; in vivo specificity controls were performed in heterologous Masugi nephritis (PMN dependent) and accelerated Masugi nephritis (monocyte dependent). Complement and monocyte depletion significantly delayed the onset of proteinuria, PMN depletion had no effect. Monocyte infiltration was seen in control nephritic rats (mean 24/glomerulus), monocyte depletion prevented this influx (mean 7.9/glomerulus) (normal rats mean 5/glomerulus). In the monocyte depleted group no differences in glomerular deposition of C_3, C_9 and MAC were seen in comparison to the nephritic control rats. Serum C_3 levels were comparable in all groups and the amount of anti-FxIA antibody bound was the same. This shows that, besides complement, monocytes are necessary to induce renal damage in passive Heymann nephritis, a finding not previously reported. The concept of a sole role for complement in glomerular immune injury involving subepithelial deposits should be reconsidered.

A GOODPASTURE-LIKE SYNDROME IN RABBITS INDUCED BY THE DISSOCIATED HUMAN NC1 MOLECULE. **M. Weber*[1], K. Sorger*[2], K.-H. Meyer zum Büschenfelde*[1], H. Köhler*[1] (intr. by E.J. Feinstein). 1st Dept. of Internal Medicine[1] and Inst. of Pathology[2], University of Mainz, FRG**

Animal models mimiking human Goodpasture's syndrome have rarely been described. We here report of a pulmo-renal syndrome induced in rabbits by the dissociated human NC1 globule.

Human glomerular basement membrane was prepared as previously described. The globular domain NC1 of collagen IV was isolated after collagenase digestion by chromatographic purification (DE 52 column, BioGel A 1.5m column). Purity was controlled by SDS-PAGE and electron microscopy after "rotary-shadowing". Various concentrations (50, 100, 300 µg/ml) of NC1 were dissociated by 6 M guanidin-HCl, TrisHCl, pH 7.5 (GuHCl), homogenised in Freund's adjuvans (1:1), and injected subcutaneously in young rabbits. In addition, crude collagenase-solubilized GBM (300, 900 µg/ml) was used as antigen. Freund's adjuvans in 6 M GuHCl was used in controls. Anti-NC1 antibodies were estimated by ELISA.

All but the control animal developed anti-NC1 antibodies, proteinuria, and elevated s-creatinine levels. Four animals died between day 7 and day 28 due to lung failure. The remaining animals were killed after 40 days. Glomerulonephritis and massive hemorrhagic pulmonary edema were demonstrable on light microscopy in animals receiving NC1 or the collagenase-digested BM antigens. On immunofluorescence microscopy linear deposition of rabbit IgG along GBM, alveolar BM, and capillary BM could be seen. Disruption of alveolar and capillary BM with evidence of blood extravasation was detectable.

The epitopes recognized by anti-GBM antibodies are sequestered inside the NC1 hexamer. The number of accessible epitopes increases 20-50-fold upon dissociation. We therefore suggest, that dissociation of the NC1 hexamer by GuHCl may be critical to stimulate the immune response in a way that a pulmo-renal syndrome may develop in rabbits.

MECHANISMS OF ADDITIVE INHIBITION OF IMMUNOLOGICAL RESPONSES BY CYCLOSPORINE AND VERAPAMIL. M.R. Weir, D. Gomolka,* R. Peppler,* and B.S. Handwerger,*. Univ. of Maryland Hospital, Dept. of Med., Renal Div., Baltimore, MD.

Verapamil (V) and cyclosporine (CSA), additively impair in vitro immunological responses of human peripheral blood mononuclear cells (PBMC). Additive inhibition may, in part, be related to additive impairment of interleukin 2 (IL2) responsiveness. V and CSA additively inhibited HT-2 (IL 2 dependent T cell clone) response to recombinant IL 2. This effect was not due to additive inhibition of IL 2 receptor acquisition as determined by flow cytometry. This may be related to the block of a post-receptor event. The additive inhibition of the 2 drugs was not due to additive inhibition of protein (measured by leucine incorporation) or RNA (measured by uridine incorporation) synthesis, interleukin 1 production (determined by ELISA), or IL 2 production (determined by ELISA) since CSA, alone, almost completely inhibited these activities, and little or no additive effect was seen with V, even though V had a significant concentration dependent inhibitory effect of its own. Nor was the additive inhibition due to additive blockade of slow calcium channels (as determined by ^{45}calcium uptake) which was blocked by V, but not by CSA. CSA did have an ability to block ^{45}calcium uptake, only when the PBMC were pre-incubated in it for at least 30 minutes before exposure to stimulus. Thus, the additive inhibitory effect of the 2 drugs likely occurs through the additive block of a post-IL 2 receptor event.

INCREASED GLOMERULAR m-RNA FOR TYPES I AND IV COLLAGEN PRIOR TO CELLULAR CRESCENT FORMATION IN RABBIT ANTI-GBM DISEASE. Roger Wiggins, Steve Merritt*, Sem Phan*, Paul Killen. Ann Arbor, MI.

An accelerated rabbit model of anti-GBM disease was used to examine the time course of glomerular collagen metabolism. Glomeruli were isolated (> 93%) by iron embolization and magnetization from control, day 4 (monocyte influx, onset of proteinuria), day 7 (fibrin containing casts in Bowman's space) and day 14 (cellular crescents). Glomerular RNA was prepared by sonication in guanidine isothiocyanate, centrifugation to remove GBM followed by ultracentrifugation through a CsCl gradient. Mean RNA recoveries for control and days 4, 7 and 14 were 1.08±0.14, 2.20±0.46*, 1.62±0.33 and 1.10±0.13 ng per glomerulus. Specific transcripts for β actin, αI(IV) and αI(I) collagen chains were detected by Northern Blot with 32-P labeled cDNA probes and quantitated from dot blots. All samples were hybridized with a 32-P labeled genomic DNA fragment coding for 28s rRNA. All mRNA levels were normalized to the 28s rRNA and expressed as percent of control. The following values were obtained:

	Actin	αI(IV)	αI(I)
Control (n=5)	100±8	100±22	100±16
Day 4 (n=5)	1719±570**	150±20	127±52
Day 7 (n=4)	391±124*	488±127**	1278±274**
Day 14 (n=7)	351±66**	297±46**	306±60**

(*p < 0.05, **p < 0.02).

These results show that mRNA for types I and IV collagen increases markedly in glomeruli within 4 days of influx of monocytes and T cells and onset of proteinuria and before the cellular crescent forms.

IMMUNE COMPLEX-INDUCED MONOCYTE-DEPENDENT ENDOTHELIAL CELL TISSUE FACTOR SYNTHESIS IS MEDIATED BY INTERLEUKIN-1. Bryan Wharram*, Katherine Fitting, Daniel Remick, Joseph Fantone, Steven Kunkel, Roger Wiggins. Ann Arbor, MI

Since immune complex (IC) associated organ injury is often associated with intravascular coagulation we determined how monocytes (M) and human umbilical vein endothelial cells (EC) interact to produce procoagulant activity (PCA) when incubated in the presence of IC (aggregated IgG). 1 M per 320 EC resulted in significantly increased PCA production. PCA was > 90 % tissue factor-like by clotting assay. PCA appeared after 2 hours of incubation, peaked by 6-8 hours, persisted at high levels through 24 hours, and was prevented by cycloheximide. PCA was M-dependent but produced by EC as shown by finding that supernatants from M preincubated with IC and then incubated with EC induced PCA synthesis but not vice versa. Anti IL-1 and TNF prevented synthesis of PCA by 88.5 and 50.4 %, respectively, but these antibodies were shown to be only partially specific. Assay for IL-1 and TNF bioactivity showed large amounts of IL-1 (approximately 1 ng/ml equivalent of recombinant IL-1 beta) was present in supernatants but TNF levels were detectable only by WEHI assay (0.01 ng/ml). Dose response curves for EC PCA production using recombinant IL-1 α and β and TNFα and various mixtures of IL-1 and TNF showed that this amount of TNF was below that which would be required to amplify IL-1-induced PCA synthesis by EC. These results show that IC-induced M-dependent synthesis of tissue factor by EC is caused by IL-1 released by M into the supernatant. Direct cell: cell contact is not required and TNF does not contribute significantly to induction of the PCA signal under the conditions used.

ANTIGEN PRESENTING CAPACITY OF MHC CLASS II POSITIVE PROXIMAL TUBULAR CELLS IN MRL/*lpr* MICE WITH LUPUS NEPHRITIS. R.P. Wuthrich*, L.H. Glimcher*, and V.E. Kelley. Dept. of Med., Brigham and Women's Hospital, Boston, MA.

Enhanced MHC class II antigen (Ia) expression is observed in the proximal tubules (PT) of MRL/*lpr* mice with lupus nephritis, and precedes the onset of proteinuria and overt glomerulonephritis. We studied the pathogenic role and the regulation of Ia on PT in murine lupus nephritis by immunoperoxidase, Northern blot analysis and *in situ* hybridization. Enhanced Ia expression is prominent on PT and in the perivascular mononuclear infiltrates. Increased Ia begins focally on PT adjacent to invading mononuclear cells. PT have a lower level of mRNA than the mononuclear cells in the infiltrates, while class II surface product is prominent in both sites, suggesting differences in the post-transcriptional regulation for class II or reabsorption of shed Ia by PT. We generated PT cell lines from normal C3H/FeJ and from MRL/*lpr* mice by transfection with origin-defective SV40 DNA. Transfected cell lines form adherent monolayers, show alkaline phosphatase and non-specific esterase activity, and produce PGE_2. These PT do not constitutively express I-A and I-E, nor mRNA for class II. Stimulation (24h) with interferon-γ (IFN-γ) (10-1000U/ml) effectively induces a dose-dependent increase in Ia mRNA and surface product, whereas IL-4 and TNF have no effect. IFN-γ stimulated class II positive PT are capable of presenting foreign antigen and autoantigen to Ia^k-restricted T cell hybridomas, whereas unstimulated Ia-negative PT do not present antigen. Thus, by presenting a yet undefined autoantigen to T cells, Ia positive PT might play a crucial role in the initiation and progression of lupus nephritis in MRL/*lpr* mice.

TUMOR NECROSIS FACTOR (TNF) AUGMENTS PROLIFERATION OF RAT MESANGIAL CELLS (RMC). Tadashi Yamamoto, Eishin Yaoita*, Katsutoshi Kawasaki* and Itaru Kihara*. Dept. of Pathology, Inst. of Nephrology, Niigata Univ Sch of Med, Niigata, Japan.

TNF produced by activated macrophages as a cyto toxic cytokine against some types of malignantly transformed cells is on the contrary a potent growth factor for normal fibroblasts. The present study was designed to determine if RMC in culture proliferate in response to TNF (recombinant,human) and to compare with the effect of interleukin 1 (IL-1, recombinant,human) or 24 hour-culture super natant (sup) of rat peritoneal macrophages (Mp) stimulated (s-Mp) or unstimulated (u-Mp) with LPS (10 ug/ml) and silica (50 ug/ml). RMC after several passages in 10% fetal bovine serum (FBS) medium were placed in 96 well-plates ($5x10^3$/well) and cultured for 6 days in 1% FBS(quiescent RMC) or for 24 hours in 5% FBS medium (proliferating RMC). The response to TNF, IL-1 or Mp culture sup was assesed by incorporation of ^{3}H-thymidine.

Stimulation: medium Index		TNF 50 U/ml	IL-1 50 U/ml	s-Mp 1:40	u-Mp 1:40
quiescent	1.00	1.11	1.16	1.10	1.08
quies.+5% FBS	1.00	1.55*	1.58**	1.97***	1.41**
proliferating.	1.00	2.76***	1.53***	1.42***	1.16

*p<0.05, **p<0.01, ***p<0.001 (Student's t test)

TNF, as well as IL-1 and Mp sup (s-Mp>u-Mp), augmented the proliferation of RMC in the presence of 5% FBS, but not RMC in quiescent stage. Mp released a larger amount of TNF (1500 vs 2.5 U/ml) and IL-1 (1125 vs 1.4 U/ml) when activated, but might produce other factors affecting RMC prolifera tion concomitantly.

EXPERIMENTAL GLOMERULONEPHRITS (EXGN) INDUCED IN RATS BY A LECTIN AND ITS ANTIBODIES (II). Futoshi Yoshida*, Atsushi Fukatsu*, Seiichi Matsuo , Yuzo Watanabe* and Nobuo Sakamoto*. Nagoya Univ. , 3rd Dpt. of Med., Nagoya, Japan.

To study the glomerular pathology in a model in which immune injury is initiated on the surface of glomerular endothelium (GEN), EXGN was induced in rats by implantation of Lentil lectin (LcH) by left kidney perfusion and intravenous injection of rabbit anti-LcH serum (A-LcH). 15 minutes after A-LcH injection, immune complexes (ICs) containing rabbit IgG, rat C3 and LcH were localized on the cell surface of GEN forming thick cell coats. 3 to 24 hours later, subendothelial deposits (SEND) appeared with disappearance of cell coats. 4th day, marked cellular increase in mesangial area with " mesangial interposition" was observed. At day 7, there was partial healing of mesangial change but large SEND were still seen together with tiny sub-epithelial deposits (SEPD). Rats injected with normal rabbit serum instead of A-LcH didn't show any deposits or mesangial pathology. LcH, when exposed to cultured glomerular epithelial cells (GEP) at 37°C, bound to the cell membrane diffusely . Subsequent exposure to A-LcH caused rapid redis-tribution of ICs showing "capping".

These results suggest that persistence of SEND is related to the mesangial pathology since the previously reported model (Kidney Int. 33;320, abstr.) showed rapid disappearance of SEND and minimal mesangial pathology. Our data also suggest that SEPD in the present model are formed by trans-GBM movement of SEND and/or by local formation of ICs through the mechanisms of "patching/shedding" of ICs on GEP surface.

FUNCTIONAL PROPERTIES OF THE C1q RECEPTOR ON U937 CELLS. Kenjiro Yamanaka* and David W Knutson. Penn State College of Medicine, Dept. of Medicine, The Milton S. Hershey Medical Center, Hershey, PA.

U937 cells, a human monocytoid cell line, are known to express both Fc-receptors for the Fc portion of IgG and receptors for the C1q subcomponent of C1, the first component of complement. Fc receptors mediate binding and endocytosis that leads to digestion of immune complexes containing IgG. However, the functional properties of the C1q receptor have not been completely determined. We studied the function of C1q receptors on U937 cells using ^{131}I-labeled C1q (C1q) and ^{125}I-labeled heat-aggregates of IgG (A-IgG). U937 cells ($2x10^6$) were incubated with C1q and/or A-IgG for 2h at 37°C and binding and catabolism of both were measured as cell associated and acid soluble cpms. C1q (1.76 µg) was bound (15.4%) but not digested (<0.1%) by U937 cells. Baseline binding (2.5%) of A-IgG (0.5 µg) was increased to 17.2% by the presence of 0.11-1.76 µg of C1q. However, baseline digestion of A-IgG (1.5%) was only increased to 1.6%. Conversely, the presence of A-IgG (0.5 µg) increased the bind-ing of C1q (1.76 µg) only slightly (from 15.4% to 17.2%) and caused only a small increase in diges-tion of C1q (from 0% to 1.6%). We conclude that the C1q receptor on U937 cells can bind C1q and increase the cell binding of model soluble immune complexes containing C1q, but that the C1q recep-tor does not support endocytosis, nor does the in-creased binding of model complexes mediated by C1q result in increased endocytosis and digestion of bound complexes. These data suggest that the C1q receptor may be immobile on the surface of U937 cells and thus "protected" from endocytosis.

COLONY-STIMULATING FACTOR PROMOTES GLOMERULAR MACROPHAGE PROLIFERATION IN LUPUS NEPHRITIS. M. Yui*, W. Brisette*, R. Wuthrich*, and V.E. Kelley., Dept. of Med., Brigham & Women's Hosp., Boston, MA., & Pfizer Central Res., Groton, CT.

Our previous studies report an increase in interleukin-1 (IL-1) and tumor necrosis factor (TNF) gene expression and product proportional to the severity of renal injury in autoimmune MRL-lpr mice. A source of these cytokines are the glomerular macrophages (macs) which appear prior to proteinuria but are most prominent in mice with impaired renal function. Since IL-1 and TNF stimulate colony-stimulating factor (CSF), a potent mac growth factor, the interplay between these cytokines may promote renal injury. Therefore, we measured CSF levels in MRL-lpr mice by determining the development of granulocyte/ mac colonies from bone marrow in soft agar. CSF activity was detected in serum of MRL-lpr mice by 4 mo. of age (> 55 colonies), while age-matched congenic MRL-++ and C3H mice with normal kidneys rarely had detectable amounts (< 5 and 0 colonies, respectively). Glomeruli isolated from nephritic MRL-lpr mice stimulated with LPS produced CSF. Mac lines were created by trans-fecting glomerular macs from nephritic MRL-lpr mice with origin defective SV40 DNA. CSF (M and GM) and IL-3 increased mac proliferation in a dose dependent response. We speculate that IL-1 and TNF induce CSF production, which in turn stimulates mac growth and proliferation and leads to the generation of additional intrarenal IL-1 and TNF. The interplay of these cytokines maybe responsible for a viscious cycle resulting in progressive renal injury.

LEUKOCYTES SUBPOPULATIONS IN SLE WITH AND WITHOUT RENAL MANIFESTATIONS. Yoshihiro Akashi*, Nobuyuki Yoshizawa, Akihiko Takeuchi*, Takao Kubota*, Takashi Oda* and Hirohumi Niwa*. Dept. of Medicine, National Defense Medical College, Saitama, Japan.

Renal involvement in SLE has a wide variety of morphological spectrum ranging from normal to DPLN and it is often accompanied by tubulo-interstitial change. However, the significance of T-I change in SLE is still unclear. In the present study, we identified the infiltrating immune cells within both the interstitium and the glomerulus and studied its significance contrasting the SLE with urinary findings with SLE without it. Frozen biopsy sections from 20 pts with SLE without renal manifestations in group I, 20 pts with renal manifestations in group II and 10 normal controls were examined using monoclonal antibodies by indirect immunoalkaline phosphatase labelling. The pts in group I and II were morphologically classified as MGA(10:5), Mes LN (6:3), DPLN(3:8), FLN(0:1) and MLN(1:3). In the interstitium there was a significant increase in total T cell and monocyte/Mϕ numbers, most numerous in DPLN, intermediate in MLN, and no increase in MGA and Mes LN. They correlated with the histological activity but not with serological activity nor renal manifestations. Helper T cells constituted the predominant infiltrating T cell type, whereas, suppressor T cells were only occasionary present in DPLN. Activated T cells were also proportionally increased to the histological activity, B cell and NK cell were rarely seen. In the glomeruli there was no significant increase in the number of leukocytes in any type of LN. These data suggest that interstitial leukocytic infiltration may not directory mediate proteinuria but they may participate in the glomerulo-interstitial change in histologically active lupus nephritis.

RENAL DISEASE IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED CHILDREN. P. Chander, I. Sagel, R. Weiss, C. Pimentel, A. Gupta, M. Bamji, G. Treser. New York Medical College, Departments of Pathology, Pediatrics, & Medicine Valhalla, N.Y.

Focal and global glomerulosclerosis (FGS) is the dominant lesion in IV drug users and also in HIV positive adults with severe proteinuria. To study renal lesions occurring in nondrug-related HIV infected individuals, we followed 66 children with perinatal HIV infection of which 38 had evidence of renal disease. This was defined by proteinuria >1+ or urine protein/creatinine ratio >0.25, and/or microhematuria, and/or serum creatinine >1.0 mg/dl. One patient had nephrotic syndrome and 1 severe hypertension. LM was done on renal tissues from 16 of the 38 patients (12 autopsies & 4 biopsies). EM was performed on 12 & IF on 5 tissues. Proliferative glomerulonephritis (PGN) was present in 14 and mild FGS (mean, 7% glomeruli) in 12. FGS as the sole lesion was present in only 1 patient. CMV glomerulopathy, suggested by glomerlar viral inclusions and confirmed by EM & DNA hybridization, was present in 1 patient. Extra glomerular CMV and virus-like particles on EM resembling Herpes Viruses were noted in 2 others. Frequent tubuloreticular inclusions were seen in 4/4 biopsies. Abundant mesanigial and focal capillary wall deposits were present in 7/12, corresponding with IF deposits of IgG, M, C_1 & C_3 in 4/5 and ↓ C_3 and ↑ IgG levels in the same patients. Renal disease, albeit mild, appears to occur frequently in children with HIV infections. PGN, possibly immune complex mediated, is more prominent than FGS in children and may represent early mild HIV Nephropathy.

C4A LOCUS NULL ALLELES AND SEVERE RENAL DISEASE IN PATIENTS WITH HENOCH-SCHONLEIN PURPURA (HSP). Bettina H. Ault,* Robert J. Wyatt, F. Bryson Waldo, Bruce A. Julian, F. Bruder Stapleton, Robert H. McLean. Univ. Tn., Memphis, TN., Univ. Alabama-Birmingham, AL., Johns Hopkins Univ. School Med., Baltimore, MD.

The fourth component of complement, C4, is encoded by 2 loci (C4A and C4B) in the major histocompatibility complex. Previously, we have shown an association between homozygous null C4A or C4B phenotype (no detectable gene product) and HSP. The majority of children with HSP glomerulonephritis (GN)have a favorable clinical outcome; however, some pts have acute renal failure (GFR <50 ml/min/1.73m^2) or eventual progression to end stage renal disease (ESRD). To determine whether C4 phenotype is associated with ARF or ESRD, we studied 39 pts with childhood onset HSP (ages 3-15 years at onset, mean 7.6±3.2 years). Six pts had normal urinalyses. Twenty-six had hematuria+/-proteinuria and a favorable outcome. Five presented with ARF; two recovered completely, three progressed to ESRD. Two other pts without ARF later developed ESRD. Data for C4 null alleles are shown below.

	Number of C4 null alleles			
	A		B	
	1 null	2 null	1 null	2 null
ARF and/or ESRD (7)	2	2	1	2
GN; good outcome (26)	2	0	4	3
Normal UA (6)	2	0	2	0

Pts with ARF and/or ESRD were more likely to be heterozygous or homozygous for C4A null alleles than those with GN and a good outcome (chi-square with Yates' correction: P<0.02, odds ratio 5.95). Severe renal disease in HSP appears to associate with the presence of null alleles at the A locus. We conclude that C4 phenotype may be useful in determining pts with HSP at risk for more severe renal disease.

OMEGA-3 FATTY ACID DIETARY SUPPLEMENTATION IN LUPUS NEPHRITIS. W.F. Clark, A. Parbtani,* B. Holub,* M. Huff,* and P. Falardeau.* Univ. of Western Ontario, Univ. of Guelph and Institut de Recherches Cliniques, Montreal, Canada.

The effect of dietary fish oil (Omega-3 fatty acids - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) on several mechanisms involved in immune, inflammatory and atherosclerotic vascular disease was determined in 12 subjects with systemic lupus erythematosus (SLE) and nephritis. These out-patients supplemented their usual diet for 5 weeks with daily doses of 6 g of fish oil, followed by a 5 wk washout period, then 5 wks of 18 g of fish oil daily. The platelet EPA content rose 6-fold with the lower and 15-fold with the higher dose of fish oil and similar changes occurred to the platelet DHA content. The platelet arachidonic acid incorporation was reduced by 16 and 20% respectively. These changes were associated with a reduction in collagen-induced platelet aggregation and an increase in red cell flexibility and a decrease in whole blood viscosity. Prostacyclin (PGI_2) production was unaffected by the fish oil but PGI_3 formation correlated with its administration and dosage. Neutrophil leukotriene B_4 release was reduced 78 and 42% respectively by the low and higher doses of fish oil. The higher fish oil dose induced a 38% decrease in triglyceride and a 39% reduction in VLDL cholesterol associated with a 28% rise in HDL cholesterol. The fish oil had no effect on immune immune complex or anti-DNA antibody titre, albuminuria or platelet serotonin. We conclude that in patients with lupus nephritis, fish oil effects mechanisms involved in inflammatory and atherosclerotic vascular disease.

PATHOGENETIC ROLE OF ANTI-VASCULAR ENDOTHELIAL CELL ANTIBODY IN SLE. X.H. Du*, S.F. Zhou*, X.M. Wang*, H.P. Zhong* (Intr. by F. Llach). China-Japan Friendship Hospital, Dept. of Nephrology and Immunology, Beijing, P.R.O.C.

In order to study the relationship between vascular endothelial cell (VEC) antigen system and SLE, including lupus nephritis, Terasaki's microcytotoxicity test and indirect immunofluorescence were used to detect anti-VEC antibody. Sera of 21 SLE patients, previously absorbed with lymphocytes, and of 100 healthy donors were examined. Among the 21 SLE patients evaluated, 17 had kidney injury and 13 were in active stage SLE. Results showed that anti-VEC antibody was found concordantly in 76.2% of 21 SLE patients, while only 1% of the donors were positive ($p<0.05$). This antibody was detected in 84.6% of patients in active stage SLE and in 62.5% of patients in the inactive stage ($p>0.05$) while in patients with and without kidney injury, it was detected in 82.4% and 50%, respectively ($p>0.05$%).

These data show that anti-VEC antibody indeed exists in the sera of SLE patients, the presence of which is closely related to SLE onset and is independent of whether the patient is in active stage SLE or has kidney injury.

In summary, our data suggest that anti-VEC antibody may be one of the triggering factors in the vasculitis of SLE. It is proposed that renal VEC may act as a target cell which can be attacked by anti-VEC antibody to constitute an in-situ immune complex formation in the kidney, leading to lupus nephritis.

MONOCLONAL ANTIBODY-BASED URINARY DIAGNOSIS OF RENAL DISORDERS. Dieter Gassner and Gerhard A. Müller*: (intr. by H.G. Rennke). Medical University Clinics, Dept. 3, Tübingen, FRG.

Urinary shedding of renal antigens (ra), specific for distinct parts of the nephron could be a reliable indicator of kidney damage. Establishing specific urinary assays using monoclonal antibodies (moabs) detecting ra would constitute a non-invasive second generation test system for the diagnosis of renal injury. Although moabs generated by immunization with kidney homogenates or isolated renal cells can detect epitopes of kidney cells, it is uncertain, whether antigens recognized by the selected moabs are shed into the urine. Moreover, proteolysis of renal antigens in the urine may influence the reactivity of the moabs. To overcome these problems new moabs were produced by a direct intrasplenic immunization of mice with pathologic urine samples. Hybridoma were screened for antibodies recognizing ra shed into the urine by use of immunoperoxidase staining of frozen sections of a normal human kidney. Moabs reactive with structures of the normal kidney should indicate the anatomical site of the antigen shed into the urine by the injured kidney. A panel of moabs was established which allows an immunohistologic dissection of the nephron. First data point to the usefulness of these moabs in urinary enzyme immunoassays. Among the latest trends is the development of urinary sandwich-immunoassays, which will be useful to detect the anatomical region of a renal disorder, the course of the disease, its response to therapy and probably may serve as an early indicator of renal graft rejection.

EVIDENCE OF CYTOKINE-LIKE SUBSTANCES AND INHIBITOR(S) IN HUMAN RENAL CYST FLUID. K.D. Gardner, M.D. Sadick*, L.W. Elzinga, W.M. Bennett, and R.M. Locksley*. Departments of Medicine, University of New Mexico School of Medicine, Albuquerque NM; Oregon Health Sciences University, Portland OR; and University of California, San Francisco, San Francisco CA.

Epithelial cell hyperplasia, neutrophil margination, abnormal basement membrane, fibrosis, and salt wasting characterize cystic kidneys. In other settings these abnormalities are ascribed to cytokine effects. Accordingly, we assayed 23 cyst fluids from 11 autosomal dominant polycystic kidneys for their mitogenic effects on thymocytes from C3H/HeJ mice. Fluids, even those from the same kidney, evoked differing responses. Four provoked proliferative responses that were equal to or greater than that produced by 5 units/ml of recombinant human interleukin-1 beta (IL-1) in the same assays. One mitogenic fluid was tested further. It lost 30% of its mitogenic activity when incubated with antiserum to IL-1. Of five non-mitogenic fluids from one kidney, three inhibited the mitogenic response of thymocytes to IL-1 while two did not. One of two non-mitogenic fluids from yet another kidney potentiated the thymocyte response to IL-1 in the absence but not in the presence of murine monoclonal antibody to interleukin-2. These findings demonstrate that cytokine-like substances and an inhibitor of IL-1 are variably present in and among cyst fluids from polycystic kidneys, even among fluids from the same kidney. They raise the possibility that disordered intrarenal cytokine production, distribution, or binding plays a role in the expression of polycystic kidney disease.

THE DIAGNOSTIC SENSITIVITY OF SERUM C3 LEVELS IN SLE IS AFFECTED BY THE METHOD OF C3 DETERMINATION. LA Hebert, J Clough*, J Neff*, R Rohde*, EJ Lewis, M Pohl, Ohio State Univ, Columbus, OH, Cleveland Clinic, Cleveland, OH, and the Lupus Nephritis Collaborative Study Group (LNCSG), Chicago.

The lower limits of normal (LLN) for C3 vary widely from Center to Center. For example, for the 14 Centers in the LNCSG, the LLN for C3 ranges from 55 to 119 mg/dl. We examined whether differences in the LLN for C3 affects the diagnostic sensitivity (S) of this test in SLE. Seventeen consecutive SLE pts with GN followed by one of the LNCSG Centers (OSU) were evaluated. Thirteen pts were selected for study because each was followed long term (28-72 mo) and had multiple (15-55) sera analyzed (total of 402 sera) for C3 and C4 by both a standard nephelometric (N) technique and by a standard radial immunodiffusion (R) technique. Twenty-five SLE relapses, renal (15) or nonrenal (10) occurred in 9 pts. Ten remissions (4-12 mo periods of stability without SLE activity (ignoring C3, C4 levels), separated by a period of at least 4 mo from the nearest relapse) occurred in 5 pts. During remission C3-N and C3-R were comparable. During relapse C3-N (LLN = 97 mg/dl) was abnormal in 24/25 (S = 96%); C3-R (LLN = 55 mg/dl) was abnormal in 16/25 (S = 65%), $p < 0.005$. During SLE relapse the mean C3 level, as % of LLN, was 71 $\pm$ 3 SE% for C3-N, and 96% $\pm$ 5, for C3-R, $p < 0.001$. The tendency for C3-R to decline relatively less than C3-N during SLE relapse was not seen for C4-R (LLN 20 mg/dl) and C4-N (LLN 13 mg/dl): C4 as a % of LLN was 110 $\pm$ 11% for C4-N and 100 $\pm$ 12% for C4-R. Regression of C3-R on C3-N for each pt showed linearity and a high degree of correlation (mean $r = 0.86 \pm 0.03$), indicating that the insensitivity of C3-R is not related to aberrations in detection of C3 by R. Conclusion: Compared to a standard widely used C3-N assay, a standard widely used C3-R assay has an inappropriately low LLN resulting in reduced sensitivity in identifying SLE relapse.

ANTI-NEUTROPHIL CYTOPLASMIC AUTOANTIBODY-ASSOCIATED GLOMERULONEPHRITIS (ANCA-GN): PATHOLOGIC FEATURES. J. Charles Jennette and Ronald J. Falk. U.N.C. School of Medicine, Dept. of Pathol. & Med., Chapel Hill, NC.

We have demonstrated a strong association between ANCA and pauci-immune GN with necrosis and crescents (New Eng. J. Med. 318:1651-1657, 1988). The purpose of this study was to precisely define the pathologic characteristics of ANCA-GN. Forty eight patients with GN diagnosed by renal biopsy and ANCA detected by ELISA, and no evidence for lupus, were studied. ANCA-GN patients had a mean ANCA of 89.3 compared to 23.2 (SD 9.5) in 83 normal controls. By light microscopy, 96% had glomerular crescents, 42% >50% crescents, 98% segmental glomerular necrosis and 10% necrotizing arteritis in the renal biopsy. Three had a medullary capillaritis. By immunofluorescence microscopy, 95% had no more than trace glomerular immunoglobulin, and none had greater than 2+ (out of 0-4+). C3 was observed in 68%, but was not >2+ in 90%. By electron microscopy, 90% had no electron dense deposits, and those that did had only small deposits. Of the 48 patients, 21 had renal-limited disease, 12 Wegener's or microscopic polyarteritis, and 15 nondiagnostic systemic disease. Extrarenal pathologic findings included necrotizing arteritis, respiratory tract granulomatous inflammation, acute sinusitis, necrotizing alveolar capillaritis and cutaneous necrotizing leukocytoclastic vasculitis. The severity of renal and extrarenal pathologic findings did not correlate with ANCA levels or ANCA immunofluorescence patterns, although there was a trend toward greater frequency cytoplasmic versus perinuclear pattern with lung involvement.

We conclude that ANCA-GN is characterized by a paucity of immune deposits, segmental necrosis and crescent formation; and is frequently but not always accompanied by a variety of extraglomerular necrotizing vasculitides. Included in the ANCA-GN category are patients currently diagnosed as pauci-immune (idiopathic) crescentic GN, Wegener's granulomatosis and microscopic polyarteritis.

EARLY T CELL ACTIVATION IN IgA NEPHROPATHY (IgAN). Kar Neng LAI* and Fernand Mac-Moune LAI.* (intr. by J.P. Bosch). Dept. of Medicine and Morbid Anatomy, Chinese Univ. of Hong Kong, Hong Kong.

The expression of interleukin-2 receptor (IL2R) and production of interleukin-2 (IL2) by cultured lymphocytes were studied in 21 patients with IgAN, 17 patients with other nephropathies, and 17 healthy subjects. T cell subsets were determined by double immunofluorescence technique using fluorochrome conjugated monoclonal antibodies. IL2 was measured by RIA in the supernatant taken from 24-hour culture of lymphocytes stimulated with lectin mitogens. These tests were performed during an infection-free period. The percentages of circulating CD4+ and CD8+ cells, and total activated lymphocytes did not differ between the 3 groups of subjects. Following pokeweed mitogen stimulation, the individual T cell subsets bearing IL2R were distinctly different in IgAN compared with the others. IgAN patients had increased activated CD4+ cells and reduced activated CD8+ cells. The IL2 production was also increased in patients with IgAN. 10 patients received cyclosporine A (5 mg/Kg/day) for 3 months. Cyclosporine therapy resulted in significant reduction of lymphocyte proliferation and IL2R expression mainly affecting the T helper than the T suppressor cells. IL2 production from cultured lymphocytes was not affected by maintenance cyclosporine therapy. Our study suggests a defective immunoregulation in IgAN with enhanced T helper and reduced T suppressor activities, and increased IL2 production by lymphocytes when stimulated with mitogen and probably, during clinical exacerbation.

LOW-DOSE INTERLEUKIN-2 THERAPY NORMALIZES THE NON-RESPONDER STATE OF DIALYSIS PATIENTS TO HEPATITIS-B VACCINATION. **Hans Köhler[1], Hubert Dumann[1], Karl-Hermann Meyer zum Büschenfelde[1], Stefan C. Meuer[2] (intr. by E. J. Feinstein). [1]1st Dept. of Int. Medicine Mainz and [2]Dept. of Appl. Immunology, DKFZ Heidelberg, FRG.**

Recent data suggest, that the secondary immunodeficiency of uremic non-responders to Hepatitis-B (HB)-vaccination is due to a failure of the monocytes to support the process of primary T-cell activation (Meuer et al, J Clin Invest 80:743-749, 1987). This defect results in a lack of interleukin-2 (IL-2)-production and an enhanced sensitivity of the IL-2 receptor system. Addition of low dose IL-2 fully reconstitutes the deficient immune response in vitro. In this study we investigated the systemic immune response of uremic non-responders to HB-vaccination, who received HB-vaccine in combination with a local injection of natural IL-2 (n IL-2). 10 HBsAg-negative dialysis patients who had not responded to several previous vaccinations with HB-Vax over a number of years received 40 µg HB-Vax (Merck, Sharp and Dohme) and four hours later 1 ml (2,5 x 10^5 U/ml) of n IL-2 (Biotest AG, Ffm) i.m.. In addition, 8 non-responders to HB-vaccination were revaccinated without adding IL-2. Prior to the clinical trial T-cell functions were studied following antigen receptor triggering with a monoclonal antibody directed at the T-cell receptor. After four weeks, prior to a second treatment with HB-Vax and n IL-2, 6 out of 10 non-responders had developed protective anti-HBs titers (> 10 U/l). One additional patient showed seroconversion 4 weeks subsequent to the second treatment. There was a good correlation between in vitro data and in vivo responses. 7 out of 10 patients showed a high IL-2 receptor sensitivity in vitro and these patients consequently developed antibody titers following substitution with IL-2. Contrary, only one out of 8 controls who had received another HB-vaccination without IL-2, developed anti-HBs of > 10 U/l. Thus, the local application of low dose IL-2 during a standard vaccination induces anti-HBs titers in those patients who show a high IL-2 receptor sensitivity in vitro.

ULTRASTRUCTURAL (EM) CHANGES IN IgA NEPHROPATHY (IgAN) IN RELATION TO HISTOLOGIC AND CLINICAL DATA. Hyun Soon Lee and Haeng Il Koh. Seoul Nat'l Univ., and Inje Med. Coll., Seoul, Korea.

We previously reported that modified classification of Meadow et al for Henoch-Schoenlein nephritis can be a useful prognosticator in IgAN (Clin Nephrol 27: 131, 1987). In order to evaluate the EM changes in IgAN in relation to the histologic grading and clinical data, renal biopsy material from 239 patients with IgAN was analyzed. 41 were children and 198 were adults. 43 adults (18%) exhibited histologic grades IV and V lesions in association with high levels of proteinuria, serum creatinine and blood pressure when compared to the remaining 196 patients with histologic grades I to III lesions. A significant difference was noted between children and adults in the severity of the glomerular lesions ($p < 0.01$). Mesangial deposits were observed in all (100%), subendothelial deposits in 37%, subepithelial deposits in 18%, glomerular basement membrane (GBM) abnormalities with splitting, thinning, thickening and/or deposition in 20%, mesangiolysis in 44%, and mesangial interposition in 25%. The frequency of GBM abnormalities and subepithelial deposits in children was significantly higher than that seen in adults ($p < 0.01$). All of the above EM changes except for mesangial deposits were associated with more advanced histologic grading ($p < 0.025$). Yet none of these EM parameters were associated with more severe clinical findings when our analysis was restricted to grade III lesions. These results suggest that the 5 EM parameters described here may merely be epiphenomena of more severely or actively affected glomeruli, with poor predictive values for prognosis if applied to individuals.

BLOOD GROUP SECRETOR STATE IN RELATION TO P-BLOOD-GROUP PHENOTYPE AND RENAL FUNCTION IN PATIENTS WITH PYELONEPHRITIC RENAL SCARRING. H.Lomberg*, S.Jacobson*. (Intr. by CM.Kjellstrand). Dept. Clin.Immunol. Gothenburg and Dept. Med. Karolinska Inst.Stockholm, Sweden.

ABH blood-group non-secretors are overrepresented among children developing renal scarring. An increased incidence of the P1 blood-group phenotype was found in children with recurrent pyelonephritis without vesicoureteral reflux.

We analyzed ABH blood-group secretor state and P blood-group phenotype in relation to glomerular filtration rate (GFR) and presence of renal scarring in 84 patients (mean age 48, range 31-76 years) with urinary tract infections. Among 43 patients with renal scaring and no obstructive mal-formations, 37% were non-secretors compared to 22% in a healthy population (n=954, p=0.059). The highest incidence of non-secretors (38%) was observed in patients of the P1 blood-group phenotype (p=0.05 vs healthy controls). This overrepresentation of non-secretors was not seen in a control group of 41 patients with a recent episode of acute pyelonephritis not developing renal scars (17%, NS vs healthy controls). GFR was similar in secretors and non-secretors of patients with renal scarring (80 ml/min x 1.73 m^2 vs 79 ml/min x 1.73 m^2). Pyelonephritic renal scarring most often develops during early childhood. Older patients in this study have probably received inadequate antibiotic treatment during childhood. The incidence of non-sectretors in patients who were 39 years or younger was 55% (p=0.019 vs healthy controls) compared to 28% (NS vs healthy controls) among patients who were >40 years old.

Conclusion: We found an association of blood-group non-secretor status with risk for development of renal scarring after acute pyelonephritis in patients without predisposing factors in the urinary tract.

ABNORMAL EXPRESSION OF MHC CLASS II ANTIGENS IN GLOMERULONEPHRITIS (GN). Claudia A. Müller, Teut Risler, Adalbert Bohle, and Gerhard A. Müller*: (intr. by H.G. Rennke). Medical Univ. Clinics and Dept. of Pathology, Tübingen, FRG.

Various forms of GN show an association with HLA antigens or are otherwise suspected to involve abnormally regulated immune responses. For these reasons the expression of defined MHC class II antigens DQ, DR, DP and of a putatively new determinant DY was analysed within the normal and diseased kidney using monoclonal antibodies. Immunoperoxidase staining was performed on frozen kidney sections of 17 normal and 80 kidneys with histologically different forms of GN. In normal adult kidneys HLA-DR, -DQ, -DP and -DY antigens are expressed on subpopulations of glomerular and interstitial cells and vascular endothelia. Proximal tubular cells variably express HLA-DR and always -DY but not -DQ or -DP antigens in normal kidneys. In rapidly progressive GN (RPGN), membranoproliferative GN or focal glomerular sclerosis (FGS), however, proximal tubular cells often do express HLA- DR, -DQ and -DP antigens. In RPGN a reduction of all MHC class II antigens on glomerular cells was observed, whereas glomerular crescents were strongly HLA-DR/DQ/DY/DP positive. In FGS, however, glomerular expression of HLA-DQ antigens seemed to be increased. Abnormal expression of HLA-DQ or -DP antigens on tubuli was not always correlated with T-cell infiltrates in the diseased kidney. Altered expression of MHC class II antigens during disease may point to anatomical regions in the kidney for immunologically mediated injuries in GN.

CLEARANCE KINETICS AND FATE OF MODEL IGA OLIGOMERS IN PATIENTS WITH IGA NEPHROPATHY. Vincenzo Montinaro,* Abdalla Rifai,* Marino Melle,* Paolo Schena,* Luigi Nitti,* and Angelo D'Addabo.* Rhode Island Hospital, Providence, RI, and Polyclinic, Univ. of Bari, Bari, Italy.

To determine the pathophysiologic mechanisms involved in IgA nephropathy, the clearance kinetics and fate of model IgA oligomers were investigated in IgA glomerulonephritis patients and normals. Model IgA oligomers were prepared by covalent cross-linking of purified human IgA with a heterobifunctional reagent, N-Succinimidyl 3-(2-pyridyldithio) proprionate, SPDP. After intravenous injection, large-sized (>2 x 10^3 kD) radiolabeled IgA oligomers were removed rapidly from the circulation of patients ($t_{1/2}$ = 3.17± 0.48 min) and normal controls ($t_{1/2}$ = 3.88 ± 1.28 min). Dynamic gamma camera scintigraphy showed the liver was the major organ involved in removal of the IgA oligomers with an equal peak uptake time in patients (10.3 ± 0.7 min) and controls (10.5 ± 0.4 min). Insignificant amount of radioactivity was detectable in the spleen and kidneys. The small-sized IgA oligomers (<1x10^3 kD), predominantly dimers and trimers, had a slower and similar clearance rate for patients ($t_{1/2}$ = 30.38 ± 8.69 hr) and controls ($t_{1/2}$ = 30.27 ± 10.1 hr).

These findings have specific importance in showing that patients with IgA nephropaty do not suffer from an IgA Fc-receptor dysfunction. In addition, the findings have general significance in showing the liver as a major organ for removal of IgA immune complexes.

DOES T-CELL ACTIVATION (TA) OCCUR DURING DIALYSIS? A-J Neusy, A. M. Valeri* and L. Liebes*. NYU Medical Center, NY, NY.

TA is accompanied by binding of Interleukin-2 to its membrane receptor (IL2R), followed by shedding of an IL2R subunit (sIL2R) into the plasma. We studied whether TA occurs during dialysis and renal insufficiency (RI) by measuring IL2R and sIL2R in 18 HIV-seronegative ESRD patients (pts) on hemodialysis (HD - group I),9 pts on peritoneal dialysis (PD - group II), 5 RI pts (group III) with a mean serum creatin- ine of 4.2 mg% and 6 healthy donors (group IV). IL2R was measured by fluorescence-activated cell sorting and sIL2R by an ELISA assay (T-cell Sciences kit). Pts in group I were studied pre and post 4 hours of HD. The results were:

Groups	IL2R (% lymphs ± SEM)	sIL2R (U/ml ± SEM)
I(pre)	9.7 ± 2.7	1205 ± 67
I(post)	11.7 ± 2.6	1261 ± 88
II	4.7 ± 1.3	1276 ± 138
III	15.8 ± 6.6	548 ± 82
IV	5.7 ± 1.4	188 ± 26

Plasma sIL2R in group I was unchanged by HD and did not differ from group II; both groups were markedly higher than groups III ($p < 0.01$) and IV ($p < 0.001$). IL2R was not affected by the presence of RI nor by HD or PD.

In summary, TA does not occur during HD. It is unclear whether the increase in sIL2R is due to increased production or decreased renal clearance. Further studies to clarify this question are being pursued.

MESANGIAL CELL AUTOANTIGEN(S) IN IgA NEPHROPATHY AND HENOCH SCHONLEIN PURPURA. D J O'Donoghue, A Darvill, P E C Brenchley, F W Ballardie, (intr. by G Berlyne), Dept. of Renal Medicine, Manchester Royal Infirmary, UK.

We have described IgG autoantibodies to a glomerular antigen associated with nephritis in IgA nephropathy (IgAN)[1]. Antigen localisation has been studied by immunofluorescence, immunoblotting and by ELISA using ligands from cultured human mesangial cells or whole glomeruli.

Antimesangial IgG antibodies were detected in the sera of 13/20 IgAN and 9/14 Henoch Schonlein nephritis (HSP) patients compared with 20 normals (mean OD controls 0.1±0.02, IgAN 0.34±0.2, $p<0.01$; HSP 0.21±0.18, $p<0.05$), and were absent in other nephropathies. IgA and IgM binding was not detected. SDS-PAGE and immunoblotting of denatured and reduced mesangial cell ligand showed affinity of IgG from IgAN and HSP patients to 48 and 58 kD components, comparable to those using ligand from whole glomeruli, where specificity of autoantibody was confirmed in $F(ab')_2$ studies. IgG-biotin conjugates from ELISA positive sera in both diseases showed binding to the mesangium and capillary loops of normal glomerular tissue and a speckled membrane pattern on mesangial cells.

IgG autoantibodies bind to components of mesangial cells and whole glomeruli of similar molecular weight in both IgAN and HSP, supporting autoantigen identity, and suggesting a common autoimmune component in the pathogenesis of these nephritides.

1 Nephrol. Dial. Transplant., 1987, 2, 5, 422; Lancet, 1988, (ii), in press).

CHARACTERIZATION OF C3 NEPHRITIC FACTOR (C3NeF) IN NON-HYPOCOMPLEMENTEMIC MPGN. Hiroyuki Ohi, S. Watanabe, A. Yamada, Y. Tanuma, T. Fujita, M. Seki, M. Hatano, Nihon University Dept. of Int. Med., Tokyo, Japan.

C3NeF is an autoantibody of C3 convertase (C3bBb), and the cause of hypocomplementemia (low C3) in the serum of MPGN patient. The sera of 202 MPGN patients was examined and C3NeF was found in the sera of 6 patients with non-hypocomplementemic MPGN. We examined C3NeF in the purified IgG from 5 non-hypocomplementemic sera (non-hypo C3NeF) and 5 hypocomplementemic sera (hypo C3NeF) for the purpose of determining why C3NeF does not make hypocomplementemia. C3 was not converted to C3c, and C3d can not be detected in the patient's sera of non-hypo C3NeF. Five purified IgG from non-hypo C3NeF can stabilize EAC4b3bBb Cells the same as hypo C3NeF's IgG in the EDTA gelatin veronal buffer, but non-hypo C3NeF's IgG can not stabilize C3 convertase (EAC4b3bBb Cell) in the control protein (H and I) where as hypo C3NeF's IgG can stabilize. C3NeF in the hypocomplementemic serum has two charactors. One is the inhibition of intrinsic decay of C3 convertase (C3bBb) and another is the inhibition of extrinsic decay by H and I. C3NeF in the non-hypocomplementemic sera can inhibit the intrinsic decay the same as hypo C3NeF IgG but can not inhibit the extrinsic decay. This is different from hypo C3NeF IgG, therefore non-hypo C3NeF can not activate C3. In conclusion C3NeF has heterogeneity and these findings are very important for the consideration of the pathogenesis of MPGN.

SIGNIFICANCE OF TUBULO-INTERSTITIAL (T-I) CHANGE IN IgA-GN. Takashi Oda*, Nobuyuki Yoshizawa, Akihiko Takeuchi*, Takao Kubota*, YoshihiroAkashi*, Osamu Hotta*, Yasuhiro Oshikawa*, Satoshi Ohshima* and Hirohumi Niwa*. Dept. of Medicine, National Defense Medical College, Saitama, Japan.

We previously studied the significance of renal hyaline arteriolosclerosis and T-I change in 66 pts with IgA-GN and 12 pts with FGS. A close correlation was observed between glomerular sclerosis and T-I change only in pts with IgA-GN. In the present study we identified the infiltrating immune cells within both the interstitium and the glomerulus and studied the relationship between the infiltrating cells and the glomerular damage. Frozen biopsy sections from 30 pts with IgA-GN; 10 with grade 1, 10 with grade 2, 10 with grade 3 and 10 normal control sections were examined using 10 kinds of monoclonal antibodies by indirect immunoalkaline phosphatase labelling. In the tubulo-interstitium there was a significant increase in total leukocyte numbers in grade 2 and grade 3 when compared with normal. Most of these infiltrating cells were identified as T cells and as monocytes/Mø. Helper T cells constituted the predominant infiltrating T cell type and they proportionally increased to the degree of glomerular damage, where as, a small number of suppressor T cells were present without a discernable trend. Monocytes/Mø and activated T cells were also proportionally increased to the grade of the disease. B cell and NK cell were rarely seen. In the glomeruli there was no significant increase in the number of leukocytes in any of the grade. These data suggest that interstitial leukocyte infiltration, particularly T and Monocyte/Mø may participate in the glomerulo-interstitial change in the progressive stage of IgA-GN.

THE GLOMERULAR CAPILLARY AS AN ACTIVATOR OF THE ALTERNATIVE PATHWAY OF COMPLEMENT IN NEPHRITIS. Cynthia Pan, C. Frederic Strife, Jean Snyder and Clark D. West. Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Evidence for alternative pathway activation in the nephritic glomerulus has been assessed by quantifying C3b deposition. Deposited C3b, which initiates formation of the membrane attack complex, is normally inactivated to C3bi by factors H and I. However, when C3b is deposited on an alternative pathway activator, up to 50% resists inactivation. As a measure of alternative pathway activation, the ratio of glomerular C3b:C3bi was determined. Autoradiography on frozen renal biopsy sections reacted with buffer and then with ^{125}IMoAbC3c allowed quantitation of C3b + C3bi. Because C3bi is exquisitely sensitive to trypsin, C3b could be quantitated by treating a serial section first with dilute trypsin (2 ug/ml) and then with ^{125}IMoAbC3c. To assure that the trypsin removed all the C3bi, a trypsinized section was reacted with ^{125}IMoAbC3bi (neoantigen); data for the biopsy were discarded if the glomerular label with this antibody was over background. The study has shown significant amounts of C3b in the glomeruli of 6/6 patients with MPGN (1 with MPGN II) and 1/4 with SLE. In the three with SLE and no or minimal C3b, two had mild glomerulitis and one had glomerular scarring. The glomeruli of a patient convalescent from HSP contained large amounts of C3bi and virtually no C3b. This study indicates that the glomerular capillary can become an activator of the alternative complement pathway in nephritis. This could be secondary to injury or a primary event in pathogenesis.

BIOCHEMICAL CHARACTERIZATION OF DEPOSITS IN LIGHT CHAIN DEPOSITION DISEASE. Maria Picken*, Blas Frangione*, and Gloria R. Gallo. New York University Med Ctr., Dept of Pathol., New York, NY.

Systemic deposits of monoclonal light chain distinct from light chain amyloid (AL) is one of the deleterious effects of myeloma and plasmacytic dyscrasias. We studied tissue from a patient with light chain deposition disease (LCDD). Immunopathologic examination revealed Kappa light chain deposits in systemic basement membranes of all organs. The light chain deposits from myocardium were extracted in 6M guanidine-HCl under reducing conditions and purified by column chromatography. The recovery of kappa light chain was monitored by immunofluorescence microscopy of homogenized muscle smears and by Western Blot. The immunoblot of the extract demonstra ted 4 main bands reactive with antiKappa antibody: intact K (M.W. 28 kda), and 3 fragments (M.W. 20, 16 and 15 kda). As revealed by aminoterminal microsequencing on electroblotted bands 3 of the 4 bands tested belonged to the $Kappa_1$ subgroup. These findings confirm the monoclonality of the Kappa light chain deposits in LCDD which are composed of intact LC and fragments. The difference thus far demonstrated brtween AL and LCDD is the lack of fibrils and amyloid P component in LCDD, suggesting that local tissue factors may be responsible for different processing of the light chain deposits.

CHARACTERIZATION OF A REGULATORY SYSTEM FOR SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) PRODUCTION IN STEROID-RESPONSIVE NEPHROTIC PATIENTS. H. William Schnaper, April L. Wade*, Robin L. Wesselschmidt*, and Deborah Kees-Folts*. Dept. of Pediatrics, Washington Univ. Sch. of Med. St. Louis, MO.

The association of immune suppression with steroid responsive nephrotic syndrome suggests a common origin for albuminuria and abnormal immunity. Previously, we reported that nephrotic patients who experience remission after corticosteroid therapy produce SIRS, an inhibitory protein secreted by activated suppressor T lymphocytes. Patient sera contain a second protein which activates lymphocytes to produce SIRS (Schnaper and Aune, J. Clin. Invest. 79:257, 1987). To determine the origin of the SIRS-inducing protein, supernatants of peripheral blood lymphocytes were tested for ability to activate SIRS production by normal lymphocytes. CD4+ cells from steroid-responsive patients, but not from steroid-unresponsive patients or healthy controls, secreted a SIRS-inducing protein which was identical to the serum SIRS-inducing activity by molecular weight (13,000-18,000 daltons), acid resistance and elution pattern on HPLC. This protein can be distinguished by physical and antigenic characteristics from SIRS and from interferons, which have previously been found to induce SIRS production. T-cell hybridomas which secrete the SIRS-inducing protein were produced from patient lymphocytes; the hybridoma-derived material has similar characteristics to those of serum and lymphocyte-derived SIRS-inducing activity. These findings define a novel regulatory pathway for suppressor lymphokine production in SRNS which may be further elucidated by characterization of the hybridoma-derived protein.

INCREASED SERUM LEVELS OF IgA1 IMMUNE COMPLEXES(ICs) CONTAINING ANTIIDIOTYPIC IgG ANTIBODIES IN PATIENTS WITH PRIMARY IgA NEPHROPATHY(IgAN). F.P.Schena,A.Pastore,V.Montinaro,N.Ludovico,(intr.by S.N.Emancipator) Chair of Medical Therapy, Univ. of Bari,Italy.

A new solid phase ELISA was used to detect IgA1-ICs containing IgG and IgM(IgA1-IgG ICs and IgA1-IgM ICs)in 49 serum samples from 32 patients (pts) and 16 subjects with non-IgAN. A jackfruit lectin, Jacalin, was used as substrate to bind selectively human IgA1 ICs in serum PEG precipitate(7%). The presence of antiidiotypic IgG, A and M antibodies against the F(ab')2 region of IgG was investigated by solid phase ELISA on polyvinylchloride plates coated with purified F(ab')2 fragment derived from normal human pepsin-digested IgG. Six pts were studied during the remission and relapse(fever, upper respiratory tract infection and macrohematuria).

Our results showed significant high serum levels of IgA1-ICs($p<0.001$)in 34% of IgAN pts,IgA1-IgG-ICs ($p<0.005$)in 30% and IgA1-IgM-ICs($p<0.005$)in 21% of pts.Significant high values of IgG and IgA antibodies directed at F(ab')2 of IgG were found. A significant increase of IgA1-IgG-ICs($p<0.005$)occurred during the relapse. Our data suggest that an increased production of IgA1-ICs occurs in IgAN pts; ICs are mainly IgA1-IgG in the relapse. The presence of high serum levels of antiidiotypic IgG and IgA antibodies against the F(ab')2 region of IgG indicate that in addition to multiple anomalies of IgA regulation, described in IgAN pts, there may be further aberrancies.

RENAL DECAY-ACCELERATING FACTOR (DAF): CORRELATIONS WITH DISEASE PARAMETERS. DD Sedmak* and FG Cosio. Depts of Medicine and Pathology. Ohio State University. Columbus, Ohio.

DAF is a cell membrane associated glycoprotein that inhibits C3 activation thereby protecting cells against complement mediated damage. Previously, we demonstrated that in the normal kidney DAF is present mainly in the region of the juxtaglomerular apparatus (JGA). Herein, we examined the distribution of DAF in 76 diseased human kidney biopsies. DAF was stained by peroxidase using a mouse monoclonal anti-DAF antibody. The following abnormalities were observed: First, the percent JGA/DAF positive glomeruli was significantly decreased in patients with SLE (n=9, 33±21%), RPGN (n=10, 33±21%), HUS (n=4, 38±29%) and interstitial nephritis (n=4, 37±13%) compared to normal kidneys (n=15, 64±9%) ($p<.0001$ by ANOVA). In addition, the decrease in the percent of JGA/DAF positive glomeruli correlated significantly with the presence of glomerular C3, IgM and/or fibrinogen. Second, in 51 specimens (67%) DAF was present in the mesangium and this finding was significantly more common in patients with glomerular C1q, C3, IgM, crescents and/or electron dense deposits. Third, DAF was present in the interstitium in 52 specimens (68%) and in blood vessels in 29 specimens (38%). Conclusion: in diseased kidneys, DAF tends to be lost from the JGA but is often present in the glomerular mesangium, interstitium and blood vessels. This pattern is especially prominent in patients demonstrating complement deposition in the glomeruli. We postulate that DAF may play a role in protecting the kidney against the products of complement activation.

IMMUNOPEROXIDASE IDENTIFICATION OF NUCLEATED CELLS IN URINE IN GLOMERULONEPHRITIS. M. Segasothy*, K.F. Fairley*, D.F. Birch* and P. Kincaid-Smith. Dept of Nephrol., Royal Melbourne Hosp., Melbourne, Vic., Australia.

The morphology and number of urinary erythrocytes is a valuable guide to diagnosis and prognosis in glomerulonephritis. Nucleated cells in urine of patients with glomerulonephritis have not been studied. We used the immunoperoxidase technique using monoclonal antibodies(Segasothy et al,Am J Clin Pathol.In press). to identify and quantify nucleated cells in urine of patients with crescentic glomerulonephritis (CN)(n=14) and noncrescentic glomerulonephritis (NCN)(n=11). The diagnosis was confirmed in all cases by renal biopsy. Chi squared tests were performed to determine significant differences in the cell types between the 2 groups. CN was distinguishable from NCN by total cell numbers exceeding 30000/ml ($p<0.001$) and counts of granulocytes exceeding 10000/ml ($p<0.05$), monocytes exceeding 3000/ml ($p<0.001$), T4 lymphocytes exceeding 1500/ml T8 lymphocytes exceeding 1500/ml ($p<0.001$), glomerular epithelial cells exceeding 4000/ml ($p<0.001$), proximal tubular cells exceeding 8000/ml ($p<0.001$), loop of Henle cells exceeding 1500/ml ($p<0.01$) and urothelial cells exceeding 1500/ml ($p<0.05$). High numbers of glomerular epithelial cells and monocytes in CN suggest that both may be involved in crescent formation. Presence of large numbers of inflammatory and tubular cells in CN suggest that associated tubulointerstitial damage is an important contributory factor towards renal failure. It is concluded that the identification of nucleated cells in urine is helpful in the diagnosis of glomerulonephritis.

AN ULTRASTRUCTURAL STUDY OF THE EVOLUTION OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FGS). Akihiko Takeuchi*, Nobuyuki Yoshizawa, Akira Seno*, Takao Kubota*, Takashi Oda*, Yoshihiro Akashi*, Yasuhiro Oshikawa* , Yusuke Fuse*, and Hirohumi Niwa*. Depts. of Med. and Pathol., National Defense Med. College, Saitama, JAPAN.

To study the evolution process of the glomerular disease of FGS, it was electron-microscopically compared to minimal change nephrotic syndrome (MCNS) and mild proliferative IgA nephropathy (IgA). The findings assessed semi-quantitatively were as follows : epithelial cytoplasmic vacuolization, foot process fusion, epithelial detachment, GBM abnormality, endothelial swelling, and electron dense deposits (EDD). The epithelial cytoplasmic vacuoles were mainly consisted of the dilated rough endoplasmic reticulums (RERs), and some of those contained proteinous flocculent material, probably indicating the existence of disturbance in the transport system of the products. Epithelial detachment could be divided into the two types, incomplete type that some of foot processes detached from the GBM and complete type that epithelial cell was completely detached or abolished. Epithelial detachment, particularly complete type, tended to be more frequently found in FGS than the others. EDD was markedly observed in IgA, moderately in FGS, and negatively in MCNS. The other findings were similar among the three. In FGS, the mesangial and endothelial changes were not so remarkable compared to the epithelial one. The results suggest that in FGS, epithelial cell is the most severely damaged, resulting in foot process fusion, RER dilatation and eventually epithelial detachment which probably leads to segmental sclerosis.

IMMUNOELECTRONMICROSCOPY (ImEM) IN MESANGIOPATHIC GLOMERULONEPHRITIS (mesGN) RELATED TO ALCOHOL ABUSE (AA). SM Smith* WE Hoy and A Welford* Pathology and Biology Depts, Univ. of New Mexico, and Lovelace Medical Foundation, Albuquerque, New Mexico.

We have shown that subjects with AA frequently have mesGN, with mesangial hypercellularity, deposits of IgA and IgM, and often with mesangial electron dense deposits (EDDs). These EDDs can be dark and distinct, or small, pale and ill-defined. This study was undertaken to confirm that these EDDs contain immuneglobulins (Igs), as indicated by routine IF.

Renal samples from forensic autopsies showing AA-related mesGN (7 cases), and without AA-related mesGN (4 cases), were fixed in Carson Millonig phosphate-buffered formalin, and embedded in gelatin capsules in LR White resin, without osmification. Thin sections were incubated with rabbit anti-human IgA or IgM sera, followed by goat anti-rabbit IgG conjugated to colloidal gold (Jansen AuroProbe), then stained with uranyl acetate and lead citrate. Labelling was excellent, with virtually no background.

ImEM confirmed IgA deposition in 3 of 4 cases of mesGN. It confirmed strong IgM deposition in one case, and was equivocal in one case, and was negative in another case with trace IgM by routine IF. IgA by ImEM concentrated in the large, well defined EDDs, whereas IgM conglomerates were smaller and did not localize in predictable areas. None of the control biopsies showed labelling by ImEM, although two had traces of IgM on routine IF.

This demonstrates the validity of routine IF and EM studies on carefully handled autopsy tissue. It proves that EDDS in AA-related mesGN contain Igs, with good correlation with routine IF findings. The significance of traces of IgM by routine IF remains uncertain.

DECREASED EXPRESSION OF C3b RECEPTOR (CR1) ON THE ERYTHROCYTES OF PATIENTS WITH ACUTE GLOMERULONEPHRITIS. Suresh C. Tiwari, G. Panchamoorthy, Nalini S. Bora, Lalit M. Srivastava, Departments of Nephrology and Biochemistry, All India Institute of Medical Sciences, New Delhi 110 029, INDIA.

Human C3b receptor (CR1) which plays a role in the clearance and processing of immunecomplex and immune regulation has been analysed for its binding with its ligand, C3b at low ionic strength ($u = 0.0513$) and its distribution on the erythrocytes of 18 healthy Indian subjects and 24 patients with various histological types of glomerulonephritides both acute and chronic. The mean CR1 number on the erythrocytes of acute glomerulonephritides patients (244 ± 82) is significantly lower ($P < 0.001$) than that of the normal subjects (640 ± 211), recovering AGN (434 ± 54) and chronic gomerulonephritides patients (498 ± 146) without changes in their affinity constants (Ka). However, there is no statistically significant difference in the CR1 number of normal subject, recovering AGN and CGN patients which suggests that the reduced CR1 level is associated with acute glomerulonephritides only, thus suggesting ongoing immunological activity. The increase in CR1 number in recovering AGN and chronic glomerulonephritides suggests the burnt out immunological injury. The loss of CR1 in the AGN is not an inherited but is of acquired type, because on recovery of AGN its number improves.

This study thus suggests the diagnostic utility of CR1 estimation in the patients with glomerulonephritides and more so to visualize and monitor the course of the disease during follow up.

MHC CLASS I AND II ANTIGEN EXPRESSION IN RENAL DISEASE - AN IMMUNOHISTOCHEMICAL STUDY ON KIDNEY BIOPSIES. Ruediger Waldherr,* Michael Rambausek,* Irene Noronha,* and Eberhard Ritz. Depts. Pathol. and Int. Med., University of Heidelberg, F.R.G.

MHC class I and class II molecules serve as restriction elements for the presentation of antigens to antigen-specific T cell reactions and may be implicated in the process of cellular transformation. We have analysed the expression of MHC products in normal renal tissue (n=15) and in 100 consecutive renal biopsies (cryostate sections) by a sensitive immunoperoxidase method using monoclonal antibodies against HLA-ABC (clone W6/32), HLA-DR (2.06 and L243), HLA-DQ (SK10) and HLA-DP (B7/21). HLA-ABC was expressed by almost all parenchymal cells. Staining for HLA-DR was found consistently in glomerular and interstitial capillary endothelial cells, in "dendritic" (LCA positive) and most infiltrating interstitial cells but not in mesangial and most extracapillary cells. Similar but weaker positivity was observed for HLA-DQ and HLA-DP. HLA-DR positive infiltrating mononuclear cells in glomeruli were noted in acute GN, anaphylactoid purpura and SLE associated with enhanced staining of endothelial cells. In addition, HLA-DR was uniformly expressed by proximal tubules in 25 specimens. In 57 biopsies, only focal positivity was observed confined to atrophic or regenerating tubules, or tubules surrounded by mononuclear infiltrates. HLA-DP (n=29) and/or HLA-DQ (n=23) were only expressed by a limited number of HLA-DR positive tubules. The differential (neo)expression of HLA class II molecules, particularly by tubular cells, suggests that specific HLA-dependent (immune) reactions are involved in various glomerulonephritides.

INTERLEUKIN-3 (IL-3) LEVELS IN UREMIC AND DIALYSIS PATIENTS. T. Weinstein*. P. Fishman*, M. Djaldetti*, J. Levi. Depts. of Nephrology and Medicine "B", Hasharon Hosp., Petah-Tikva; Tel-Aviv Univ. Med. Sch., Israel.

Cell mediated immunity is depressed in patients with chronic renal failure (CRF). IL-3 displays a variety of biological activities including proliferation of pluripotent hematopoetic stem cells and augmentation of cytotoxic cell activity. In this study we examined the ability of peripheral blood mononuclear cells to spontaneously release IL-3 in 15 patients pre and post hemodialysis (HD) treatment, 15 patients on CAPD, 15 patients with advanced CRF (creatinine 6.1±1.5) and 15 healthy subjects. IL-3 levels were measured using a IL-3 dependent 32-D-d-23 line and expressed as counts per minute (±SEM). Control values were 102269±10013, CAPD 93529±11346, CRF 102025±11664. Pre-HD 126951±9258, which is significantly higher than the control ($p<0.05$). Post-HD 87051±9011, which is significantly lower ($p<0.005$) than pre-HD levels.

In conclusion, IL-3 levels in CAPD and CRF patients are similar to control, whereas IL-3 levels are increased in pre-HD patients. However, we found a marked decrease in IL-3 levels post-HD, perhaps due to dialysis membrane related effects. This may lead to a compensatory production of IL-3 in the post-HD period.

ABSENCE OF CLONAL LYMPHOID POPULATIONS IN PERIPHERAL BLOOD OF CHILDREN WITH THE NEPHROTIC SYNDROME. Barry L. Warshaw, Leonard C. Hymes, and Irene J. Check. Emory University School of Medicine, Department of Pediatrics and Pathology, Atlanta, Georgia.

It has been postulated that the idiopathic nephrotic syndrome is caused by an abnormal clone of T lymphocytes which produces a lymphokine toxic to the glomerular basement membrane. Using recombinant DNA probes which recognize constant regions in the immunoglobulin and T-cell-receptor gene sequences, we studied peripheral blood lymphocytes from children with nephrotic syndrome for the presence of clonal T or B lymphocyte populations. Nine children, ages 4-18 years, with active nephrotic syndrome were studied. Four patients were steroid responsive, and 5 were resistant with lesions other than minimal change. DNA was digested with EcoRI, BamHI, and HindIII restriction enzymes and hybridized against probes J_H, HuC_TB and J_K. Control studies using cell lines to assess sensitivity indicated that a clonal population comprising as few as 3% of total cells could be detected. Results: None of the test samples showed detectable rearrangement of immunoglobulin or T-cell-receptor genes. We conclude that clonal populations of T or B lymphocytes are not present in peripheral blood of children with nephrotic syndrome. Lymphoid involvement in the pathogenesis of this disease is more likely on a polyclonal basis.

IN VITRO INTERLEUKIN - 2(IL2) EFFECT ON SPONTANEOUS IMMUNOGLOBIN G (IgG) PRODUCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE).
D.R.WIGFALL*,O.E.BROWN*,K.L.SHAUGER*(intr. by J.I.Scheinman). Div.of Pediatr.Nephrol.,Duke University,Durham,NC.

We have previously demonstrated the spontaneous presence of increased numbers of IL2 receptors (IL2R) on peripheral blood lymphocytes (PBL), and purified B-lymphocyte preparations in patients with active SLE compared to normal controls. Since increased spontaneous IL2R expression, T cell dysfunction, and B cell hyperfunction all characterize active SLE, we undertook a study to determine whether the IL2R presence was functionally significant. PBL from four patients with active SLE, diagnosed by revised ARA criteria, and two normal donors were cultured in media alone, or media supplemented with Pokeweed mitogen, or supplemented with IL2 in low dose (.01U/ml) or high dose(100U/ml). The cells were cultured for seven days with the supernatants assayed for IgG by EIA. In normal donors, there was marked enhancement of IgG production by PWM, low dose IL2, and high dose IL2 (700%,500%,>1000% respectively), which was accompanied by a proliferative response measured by ^{3}H-thymidine incorporation. In patients with active SLE, there was a marked reduction in spontaneous IgG production by PWM, or IL2 in low or high dose (25%,10%,70% respectively), with no change in ^{3}H-thymidine incorporation. The mechanism of inhibition of IgG production remains to be clarified.

LEUKOCYTES SUBPOLULATIONS IN GLOMERULI OF PATIENTS WITH ACUTE POSTSTREPTOCOCCAL GLOMERULONEPHRITIS. Nobuyuki Yoshizawa, Takashi Oda*, Akihiko Takeuchi*, Takao Kubota*, YoshihiroAkashi* and Hirohumi Niwa*. Dept. of Medicine, National Defense Medical College, Saitama, Japan.

We have previously proposed that preabsorbing antigen (PA-Ag) initiates the glomerular injury via in situ immune reaction in APGN. In the present study we identified the infiltrating immune cells both within the glomerulus and in the interstitium. Frozen sections from 24 pts with APGN (18 overt, 6 asymptomatic; 1 to 40 days after onset) were examined using monoclonal antibodies (Becton Dickinson) by indirect immunoalkaline phosphatase labelling. Sections from 103 pts with other glomerulonephritis and 10 normal controls were also studied for comparison. There was a prominent increase in the total number of intraglomerular monocyte/Mϕ (Leu M-5) infiltration with slightly increased T cells (Leu 4). It correlated with time after the onset; namely the more leukocytic infiltration was observed when the tissue was taken earlier. Helper T (Leu $3a^+$, Leu 8^-) tended to be more increased in the earlier stage, whereas, suppressor T (Leu $2a^+$, Leu 15^+) was even throughout the course. B cell (Leu 16) and NK cell (Leu 7) were not seen in the glomeruli. In asymptomatic pts, total number of the intraglomerular leukocytes was fewer than in overtpts, however, the proportions of the infiltrating cells were similar. There was a significant increase in total interstitial leukocyte numbers in APGN when compared to normal, however, it was not distinctive. These data suggest that monocyte/Mϕ may interact with PA-Ag planted in GBM and mesangium resulting in glomerular hypercellularity.

ELEVATED PLASMA INSULIN-LIKE GROWTH FACTOR I (IGF-I) CORRELATES WITH BONE FORMATION IN UREMIC HYPERPARATHYROIDISM. Dennis L. Andress, M.R. Pandian*, David B. Endres*, Donald J. Sherrard, Jeffrey B. Kopp.* V.A. Medical Center, Seattle, WA; Nichols Laboratory, San Juan Capistrano, CA.

Bone formation (BF) in uremia is regulated in part by parathyroid hormone (PTH). However, while low levels of PTH are often associated with low rates of BF elevated PTH does not always correlate with increased BF. To identify other factors that may regulate BF in uremia, we compared plasma immunoreactive IGF-I, IGF-II and PTH to bone histology in 15 dialysis patients without aluminum-related reductions in BF. Plasma levels of IGF-I, but not IGF-II or PTH, were higher in patients with high rates of BF when compared to those with low or normal BF ($p < 0.02$). While the BF rate at the tissue level correlated with plasma PTH ($r=0.53$, $p < 0.05$) and with IGF-I ($r=0.67$, $p < 0.01$), only for plasma IGF-I were there significant correlations with bone apposition ($r=0.57$, $p < 0.05$) and BF rate at the BMU level ($r=0.62$, $p < 0.02$), parameters which reflect cellular mineralization activity. Among the static histologic parameters, osteoblastic osteoid correlated only with plasma PTH ($r=0.76$, $p < 0.001$), while osteoclast number correlated with both PTH ($r=0.56$, $p < 0.05$) and IGF-I ($r=0.67$, $p < 0.01$). There were no correlations of IGF-II levels with bone histology. We conclude that elevated IGF-I may promote BF in uremic patients with hyperparathyroidism. The mechanisms in which IGF-I and PTH increase BF may be different, however, since PTH correlates better with the index for osteoblast number, and IGF-I correlates better with parameters of bone mineralization.

IS THERE A HEPATITIS B VIRUS (HBV) ASSOCIATED GLOMERULONEPHRITIS? EVALUATION OF THE SIGNIFICANCE OF HBV ANTIGEN DEPOSITION IN THE KIDNEY. J. Zhang* and L. Li* (intr. by R.W. Schrier). Dept. Nephrology, Jinling Hospital, Nanjing, China.

To clarify the relationship between HBV infection and glomerulonephritis (GN), and to explore the significance and mechanisms of HBV deposition in kidney, renal biopsies from 60 patients with various forms of GN and HBV antigenemia (Ag_b) were compared with 59 age-, sex- and renal histology-matched controls. The biopsies were studied with layer PAP immunoperoxidase and immunofluorescence techniques using monoclonal antibodies to HBV surface (HBsAg), core (HBcAg) and e antigen (HBeAg). All 3 HBV antigens were detected in kidneys of >67% of patients with membranous nephropathy and in >64% of cases with lupus nephritis regardless of the presence of HBV Ag_b. In certain patterns of primary GN, including mesangioproliferative GN, IgA nephropathy and membranoproliferative GN, HBsAg in kidney was more common in patients with HBs Ag_b than those without Ag_b (47.7% vs 20.5%, $p<.05$). No difference in HBcAg or HBeAg deposition was observed in patients with or without HBV Ag_b. Granular deposition of HBV antigens was shown in the same pattern as that of Ig(s) and complement deposition. Renal HBV deposition correlated closely with the extent of other deposits. We concluded that the deposition of HBV antigens in kidney is often non-specific, although HBsAg is more commonly seen in some HBsAg carriers with primary GN. Massive immune complex deposits seem to be the prerequisite of renal HBV deposition. The role of HBV in the development of GN is therefore not confirmed and "HBV-IC nephritis" is in need of further evaluation.

EFFECT OF ONE YEAR RENAL TRANSPLANTATION (Tx) ON SEVERE ALUMINUM ASSOCIATED BONE DISEASE (AABD). *Elias David-Neto, *Vanda Jorgetti, *Emil Sabbaga, *Aurelio Borelli, *Marcello Marcondes: (intr. by Roberto Zatz). Univ. of São Paulo, São Paulo, Brazil.

Bone histomorphometry(BHMP)(iliac crest) of 10 adult patients(pts)with severe AABD before Tx(X±SD) (osteoid relative vol.(ORV):25±16%;osteoid width OsW:27±12μ;osteoid surface(OS):71±7%;Aluminum(Al) covered surface(AlS):89±24%;no tetracicline label (TL)),was compared to BHMP after 1 yr of Tx with creatinine clearance:50ml/min,to check whether good renal function was enough to remove Al and restore BHMP. Two distinct patterns were seen:1) In 5 pts AlS disappeared and BHMP was normalized, unlike bone formation surface(BFS):25±7%*(*$p<0.05$ T-Paired test) and reabsorption surface(RS):8±5% (Group I). 2)In 5 pts AlS fell to 42±20% and other signs of AABD still remained(ORV:17±12%;OsW:22±17μ OS:51±25% and no TL was seen in 3 out of 5 pts (Group II). Prednisone dosage was similar at 1, 6,and 12 months in both groups. PTH(N-terminal) before Tx was raised in 2 out of 3 pts in Group I and in none of 3 pts in Group II (34±9.9 vs 17±3.4 pmol/L*). Before Tx BHMP data in Group I but not in Group II, suggested elevated PTH activity (active bone formation surface:0.04±0.08% vs zero; RBS:9±0.9% vs 4.7±4.7%*;fibrosis:4.6±0.8 vs zero*).

It is concluded that AABD improves after one year of Tx, but the rate of improvement depends on the bone remodeling rate which is faster in pts with associated hyperparathyroidism at the time of renal transplantation.

LACK OF HISTOLOGIC SIGNS OF VIT D DEFICIENCY IN THE EARLY DEVELOPMENT OF RENAL OSTEODYSTROPHY. M-C.Faugere,* R.M.Friedler, P.Fanti, H.H.Malluche, Div.of Nephrology, Bone & Mineral Metabolism, Univ. of Kentucky Medical Center, Lexington, KY.

Even though several pathogenetic factors for renal osteodystrophy (ROD) have been well described, no information is available on the initial sequence of events taking place in the development of ROD. To investigate this, 5/6 nephrectomy was done in 54 Beagle dogs. Another 11 dogs were sham operated. Bone biopsies and determinations of PTH and 1,25D were done before and four months after surgeries and Ca, P and Creat in serum (s) were studied at baseline, 2,4,8,12, and 16 wks. Creat and Ca rose at 2 wks and remained elevated. P was elevated at wks 2 and 4 and fell thereafter to normal. PTH increased and 1,25D did not change.

Histological Results	Before	After
Cancellous bone volume	18.4 ± 0.7	17.5 ± 0.7
Lamellar osteoid volume	24.6 ± 2.0	53.2 ± 3.1
Osteoid seam thickness	6.9 ± 0.2	7.9 ± 0.2**
# of Osteoblasts	677 ± 46	1332 ± 81**
# of Osteoclasts	72.5 ± 4.9	117 ± 8.7**
Double labelled trab.	11.0 ± 1.0	20.6 ± 1.0**
Mineralization rate	0.81 ± .03	0.95 ± .03*
Activation Frequency	6.80 ± 0.8	15.3 ± 1.3**
Mineralization Lag Time	9.1 ± 0.5	9.2 ± 0.3

(*p<0.05 **p<0.01)

No changes were seen in sham operated dogs.

The data show that early renal failure in dogs results in increased sCa, transient rises in sP and higher bone turnover without a mineralization defect. This suggests that enhanced PTH effects on bone represent the only identifiable histologic lesion in the development of early ROD.

THE PROTECTIVE EFFECT OF PARATHYROID HORMONE ON ACUTE ALUMINUM TOXICITY. M.A. Frutos,* M. Rodriguez, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla.City, OK.

Aluminum (AL) administration decreases osteoblast surface and bone formation rate (BFR) in the rat. The presence of parathyroid hormone (PTH) may be protective. To evaluate this question, pair-fed rats were given varying doses of intraperitoneal (IP) AL and 1-34 PTH was continuously infused via an Alzet pump at 2 units/hr. Eight groups were evaluated; in 4 groups, doses of AL, 0,5,10, and 20 mg IP, were administered over 2 days. In the other 4, PTH was given simultaneously with the same AL doses. After double tetracycline labeling, rats were sacrificed at 12 days. Shown below are osteoblast surface (OB) and BFR ($\mu m^3/\mu m^2$/day).

AL (mg)	0	5	10	20
%OB (PTH-)	15±2[a]	15±3[a]	7±1[a]	8±4[a]
%OB (PTH+)	46±7[a]	38±6[a]	50±5[a]	22±6[a]
BFR (PTH-)	.31±.05	.12±.09	.01±.01	0
BFR (PTH+)	.75±.2[b]	.48±.2[b]	.04±.02	0

X±SE;a P<.02 vs OB (PTH-);b P<.05 vs BFR (PTH-)

For comparable serum AL levels, stainable trabecular bone AL was less with PTH; significant correlations between serum and stainable AL were observed in both PTH-, r=.87 and PTH+, r=.96.

In summary, 1) increasing doses of AL decrease OB and BFR; 2) PTH increases OB and BFR; 3) increasing doses of AL reduce the effect of PTH on OB and BFR; and 4) PTH decreases stainable trabecular bone AL. In conclusion, 1) acute AL administration produces low bone turnover; 2) PTH has a protective effect against acute AL toxicity; and 3) even with PTH, BFR is markedly reduced at high doses of AL.

LONG TERM EFFICACY AND SAFETY OF ORAL Ca ± Mg(OH)2 AS PHOSPHATE BINDER VERSUS Al(OH)3 IN DIALYSIS PATIENTS.

Albert FOURNIER, Philippe MORINIERE, Martine COHEN SOLAL CHU Amiens

32 patients on chronic hemodialysis whose plasma phosphate had been controlled without Al(OH)3 thanks to 60 mmol/day of oral calcium in association in half of them with Mg(OH)2 (2.5 g/d) for at least 2 years were matched regarding age (52 years), sexe ratio (1) and previous duration on dialysis (30 versus 34 months) to a group of patients who had been previously dialysed and receiving only 22 mmol/d of oral Ca, 0.5 µg of 1αOH vit D (in a third of them) and Al(OH)3 (6.5 ± 3g/d). Dialysate Mg was decreased from .75 to .37 mmol/L when Mg(OH)2 was used whereas dialysate Ca was always 1.65 mmol. Each year arterial calcifications were measured linearly on the anterior and posterior walls of the aorta in front of L2-L3-L4 on a profile X rays of the lumbar spine and on the lateral walls of the iliac and femoral arteries seen on a frontal pelvis.

mean	Ca ± Mg(OH)2				Al(OH)3 ± 1αOHD3			
Time months	T0	T24	T36	T48	T0	T24	T36	T48
n	32	32	17	9	30	30	15	8
Vascular calcification cm	14	21	35	30	19	27	37	35
P Calcium mmol/L	2.3	2.4	2.5	2.4	2.3	2.3	2.3	2.3
P PO4 mmol/L	1.6	1.7	1.6	1.6	1.8	1.7	1.6	1.6
Alk Pase IU (<170)	123	130	107	111	141	158	152	144

P aluminium was very low (0.3 µmol/L) even after 4 years and P Mg only slightly increased to 1.4 mmol in the Ca group. Bone biopsies performed in 14 of the Ca group showed 10 osteitis fibrosa with higher C-PTH levels (348 ; normal < 45 pg/ml) and 4 non aluminic, non ferric, non diabetic aplastic bone disease associated with lower PTH levels (185), whereas those performed in 17 of the Al(OH)3 group showed 14 osteitis fibrosa and 3 aluminic aplastic bone disease but no osteomalacia.

Conclusions : Moderately high doses of oral calcium associated in half of the cases to Mg(OH)2 allow a similar control of CaPO4 metabolism and of hyperparathyroidism (as assessed on plasma alkaline phosphatase) as the classical approach using Al(OH)3 and do so without higher risk of vascular calcifications. Furthermore they prevent aluminic bone disease and allow the emergence of an idiopathic anosteoblastose with relative hypoparathyroidism, the etiology and clinical significance of which remain to be established.

BONE CELLS MEDIATE THE INHIBITORY EFFECT OF ALUMINUM ON MINERALIZATION IN VITRO. WG Goodman, Medical and Research Services, Sepulveda VAMC and UCLA School of Medicine, Los Angeles, CA.

Aluminum (Al) deposition in bone can cause osteomalacia, and Al impairs the seeding and growth of bone crystals in cell-free systems in vitro. However, the role of the osteoblast as a modifier of Al-induced decreases in calcification remains uncertain. Thus, the effect of Al on the uptake of ^{45}Ca in 12 day embryonic chick bone was evaluated in both live and devitalized hemi-calvaria grown in serum-free BGJ media containing 4 mM phosphorus (P). Media Al levels were <3 ug/L before additions of Al as Al citrate; paired control (C) bones were maintained in equimolar Na citrate, and ^{45}Ca uptake was measured during the final 2 hrs of all incubations. ^{45}Ca uptake (cpm/ug bone, $\bar{x}$±SD) decreased after 24 hrs to 77±8% of C values at 10 uM Al (158±14 vs 121±15, p<0.001) and to 38±2% of C at 100 uM Al (210±21 vs 80±9, p<0.05) whereas lower media levels of Al did not impair ^{45}Ca uptake (93±9% of C at 0.1 uM Al and 99±11% of C at 1 uM Al). Decreases in ^{45}Ca uptake were also evident after 8 hrs at both 10 uM Al (87±11% of C, p<0.05) and 100 uM Al (76±16% of C, p<0.05), but not after 4 hrs. In contrast, neither 10 uM Al (301±27 vs 283±27, NS) nor 100 uM Al (252±12 vs 272±24, NS) reduced ^{45}Ca uptake at 24 hrs in devitalized hemi-calvaria. Lowering the media P level from 4 mM to 2 mM unexpectedly attenuated the inhibitory effect of 10 uM Al on ^{45}Ca uptake at 24 hrs (86±11% of C, p<0.05) and eliminated the response to 100 uM Al (98±10% of C, NS). These data suggest that bone cells mediate Al-induced reductions in tissue calcification. P may modify the response of bone to Al, possibly by altering osteoblastic activity.

ASSESSMENT OF BONE MASS IN DIALYSIS PATIENTS. S Gouge, A Howard, J Moore, W Duncan*: Nephrology & Endocrinology Svcs, Walter Reed Army Medical Center, Washington, DC.

We used photon absorptiometry to measure bone mass of the proximal radius (PR) and spine (S) in 68 dialysis patients (33 men, 35 women, mean age = 48) who have been dialysis dependent for a mean of 37 months. We also evaluated the patients' symptoms (pain, weakness, pruritis, fractures), serum chemistries, including PTH and aluminum concentrations, and results of skeletal radiographs. PR mass was reduced compared to age, weight, and sex matched controls (p less than [LT] 0.05), and correlated only with duration of dialysis (DD) (r = -0.53), C-PTH (r = -0.42), and N-PTH (r = -0.50) [all p LT 0.01]. S mass was related only to C-PTH and N-PTH (both r = -0.35, both p LT 0.05). Neither PR nor S mass correlated well with symptoms or x-ray findings. We then performed step-wise discriminant analysis of all demographic, clinical, and chemical variables to characterize their contribution to bone mass more completely. We found that DD, sex and race contributed most significantly to reduced bone mass (all p LT 0.001). Thus caucasian women had significantly lower PR and S mass than other dialysis patients, despite the fact that 75% of these women had been on dialysis less than 2 years, 52% were less than 50 years of age, and 43% were premenopausal. These data indicate that caucasian women on dialysis, even those young and premenopausal, have reduced bone mass compared to other dialysis patients. These patients may be candidates for aggressive efforts to attenuate the effects of osteodystrophy.

PROTON MEDIATED CALCIUM EFFLUX FROM BONE IS INDEPENDENT OF CELLULAR CITRATE PRODUCTION. M.M. Kaleta* and D.A. Bushinsky. Department of Medicine, University of Chicago, Chicago, IL.

Whether proton mediated calcium efflux from bone is dependent on increased citrate production is unclear. To compare calcium and citrate flux, we cultured neonatal mouse calvariae in control (Ctl, pH ≅ 7.40), acidic (Ac, pH ≅ 7.20) and very acidic (VAc, pH ≅ 7.00) medium and determined net calcium (JCa) and citrate flux (JCit).

Compared to Ctl, JCit was not altered in Ac (JCit = 63 ± 3 nmol/bone/ 24h in Ctl vs. 58 ± 3, p = ns) but fell in VAc (46 ± 3, p < 0.01 vs. all others) while JCa increased in both Ac and VAc (JCa = 35 ± 7 nmol/bone/24 h in Ctl; 315 ± 20 in Ac; 342 ± 20 in VAc; Ac and VAc p < 0.001 vs. Ctl). JCit was correlated directly (r = 0.54, p < 0.001) and JCa inversely (r = −0.88, p < 0.001) with pH. $1,25(OH)_2D_3$ (1,25D) stimulates both JCit and JCa from bone. With 1,25D, JCit was not altered in Ac (JCit = 80 ± 3 in Ctl vs. 70 ± 4, p = ns) but fell in VAc (56 ± 4, p < 0.01 vs. others) while JCa increased in Ac and VAc (JCa = 209 ± 20 in Ctl; 389 ± 41 in Ac; 590 ± 30 in VAc; each p < 0.01 vs. all others). Again, JCit was correlated directly (r = 0.58, p < 0.001) and JCa inversely (r = −0.89, p < 0.001) with pH. Citrat is produced by osteoblastic cell lines. To determine if citrate production is pH dependent, we cultured 1,25D stimulated UMR-106 osteosarcoma cells. Compared to Ctl, Ac decreased citrate production (26 ± 5 nmol/mg protein/24 h vs. 7 ± 2, p < 0.001). Citrate production was correlated directly with pH (r = 0.53, p < 0.001).

Lowering the medium pH consistently decreases citrate and increases calcium efflux, indicating that calcium efflux from bone is not dependent on production of the organic acid, citrate.

LOW TURNOVER BONE DISEASE WITHOUT ALUMINUM IN DIALYSIS PATIENTS G. Hercz, W.G. Goodman, Y. Pei, G.V. Segre*, J.W. Coburn and DJ Sherrard. Depts of Med, Univ of Toronto, Toronto, Canada & Univ of Wash., Seattle, WA.

Low turnover bone disease due to aluminum (Al) deposition occurs in patients on hemodialysis; its prevalence in CAPD patients is not well known. In 31 patients treated solely with CAPD we performed a bone biopsy, deferoxamine (DFO) infusion test to assess for tissue Al loading and measurement of serum calcium (SCa), and parathyroid hormone (PTH) by both mid-molecule and immunoradiometric (IRMA) assays. These patients had a mean age of 56.6±2.9 (SE) years, with 6/31 over the age of 70. They had been on CAPD for 30.0±4.4 months (range 3 to 103 mos). On bone biopsy, 24/31 (77.4%) had decreased bone formation rates (BFR) (normal 108 to 600 um^2/mm^2/day). Serum PTH levels in this group were lower by both mid-molecule (382.0±44.3 vs 843.9±210.4 pmol/L, p < 0.005) and IRMA (117.9±27.5 vs 378.4±158.6 pg/ml, p < 0.01) assays, as compared to the patients with normal/high BFR. Twelve of the 24 patients with low BFR had evidence of substantial bone Al accumulation (Al+) with > 30% surface stainable Al. In contrast, the other 12 patients showed minimal or no evidence of bone Al deposition (Al-). As well, both plasma Al (13.1±2.9 vs 76.5±28.9 ug/L, p < 0.025) and the rise in plasma Al after DFO (25.5±10.4 vs 100.3±39.7 ug/L, p < 0.05) were lower in Al-. In 8/12 Al- patients, other known causes of low BFR were not identified. Hypercalcemia, SCa > 11.0 mg/dl, was common during calcium carbonate therapy in both Al+ (10/12) and Al- (9/12). In conclusion, the prevalence of low turnover bone disease is high in adult patients on CAPD. In half, the disorder cannot be attributed to bone Al deposition. The long-term consequence of low turnover bone disease in asymptomatic dialysis patients warrants further evaluation.

ACCELERATED DEVELOPMENT OF HYPERCALCEMIC ALUMINUM ASSOCIATED BONE DISEASE IN A PATIENT WITH PROTRACTED ACUTE RENAL FAILURE. Lederer ED, Gillum DM, Dept. of Medicine, Baylor College of Medicine, Houston, Texas.

Having observed hypercalcemia in patients dialyzed on a REDY machine for prolonged periods of time, we undertook complete evaluation in one individual. A 63 year old white female underwent emergency repair of a Type I aortic dissection. Postoperative course was complicated by acute renal failure, severe ileus requiring total parenteral nutrition (TPN), and respiratory failure. Hemodialysis was initiated one week postoperatively on a REDY SORB system and continued until her death six months later. Despite the absence of exogenous calcium, she developed hypercalcemia after one month that persisted on a zero calcium bath. Ionized calcium levels ranged from 1.38 to 1.55 mmol/L (nl 1.19-1.29). 25hydroxyVitamin D was 10 ng/ml (nl 9-52). Vitamin A level was elevated at 267 ug/dl (nl 30-95), as was serum midrange PTH at 2410 nl eq/ml (nl<200). Serum aluminum (Al) was 99 ng/ml and dialysate aluminum 38 ng/ml. Bone biopsy performed after 5 months of dialysis and preceded by double tetracycline labeling demonstrated severe osteopenia, no detectable tetracycline, and abundant aluminum deposited along the junction of calcified bone and osteoid, consistent with aluminum associated aplastic bone disease. Potential sources of aluminum included TPN, oral aluminum hydroxide, sucralfate and intravenous albumin of which she received in excess of 150 vials (1875 g). We conclude that aluminum associated bone disease can occur in the setting of protracted acute renal failure requiring repeated dialysis on a REDY.

BASAL AND STIMULATED OSTEOCALCIN DURING ALUMINUM EXPOSURE. M.E. Martinez*, M. Rodriguez, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, Okla.

Osteocalcin (OC) is thought to be a specific marker for osteoblast (OB) activity. Calcitriol (CTR) stimulates OC. Since aluminum (AL)administration decreases OB, the present study evaluates the effect of AL administration on OC production in pair-fed rats before and after CTR. Four groups were studied: Normals (N); N+AL-20 mg of AL given during first 3 days; Renal failure (RF)-2 stage 5/6 nephrectomy; and RF+AL. On Day 11, intravenous CTR, 44 ng/kg, was administered and rats were sacrificed 24 hrs later. Changes observed in osteoid volume (OV), and basal (sOC) and stimulated serum OC (ΔOC) were:

	N	N+AL	RF	RF+AL
sOC (ng/ml)	110±6	100±6	137±7[a]	154±11[b]
ΔOC (%)	103±21	134±22	104±16	52±8[b,c]
OV (%)	1±.2	1.7±.4	2.7±.5[a]	16±1.4[a,b,c]

X±SE p<.05 a vs N; b vs N+AL; c vs RF.

Significant correlations were: a) N, basal, OB vs OC, r=.72; b) N, stimulated, OB vs ΔOC, r=-.76; and c) N+AL, stimulated, OB vs ΔOC, r=-.79.

In summary, 1) basal OC is greater in renal failure; 2) ΔOC is less in RF+AL; 3) in N, OB and basal OC correlate positively; 4)in N and N+AL, OB and ΔOC correlate inversely; and 5) AL administration increases osteoid volume only in RF+AL. In conclusion, 1) only in N rats, basal OC increases as the number of osteoblasts increases; 2) AL may impair OC production; and 3) the inverse correlation between OB and ΔOC indicates that stimulated OC decreases as the number of osteoblasts increases, which may reflect greater reserve in low OB states.

$24,25\ (OH)_2D_3$ IN COMBINATION WITH $1,25\ (OH)_2D_3$ AMELIORATES RENAL OSTEODYSTROPHY IN RATS WITH CHRONIC RENAL FAILURE (CRF). D. Rubinger,* A. Moscovitz,* M.M. Popovtzer, I. Bab* and D. Gazit.* Hadassah University Hospital, Jerusalem, Israel.

The present study was undertaken to examine the effects of $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ on bone histology in rats with CRF (5/6 nephrectomy). The following groups (G) were studied: 1)control, and rats treated with: 2)$1,25(OH)_2D_3$. 3)$24,25(OH)_2D_3$ and 4)$1,25(OH)_2D_3$+$24,25(OH)_2D_3$. Plasma calcium levels (X±SE) were in: 1)4.7±0.11, in 2)6.17±0.18 ($p<0.001$ vs 1) in 3)4.51±0.18 ($p<0.001$ vs 2) and in 4)5.76±0.08mEq/L ($p<0.001$ vs 1, $p<0.02$ vs 2). Resorption surface (RS), active resorption surface (ARS), osteoclast numbers (OS), and relative osteoid volume(ROV) were as follows:

G	RS(%)	ARS(%)	OS(no/mm²)	ROV(%)
1	24.1±3.5	17.4±2.82	4.32±0.5	3.16±0.47
2	21.3±1.6	8.56*±1.78**	3.03±0.6	4.87±0.82
3	22.7±2.5	9.35±1.69**	2.62±0.4	2.28±0.51+
4	11.3±2.9*	1.95±0.43*	0.78±0.16*	6.57±0.88

$p<0.02$ *vs 1,2,3; **vs 1; +vs 1,2,4.

These results confirm our previous observations that $24,25(OH)_2D_2$ blunts the hypercalcemic effect of $1,25(OH)_2D_3$ in rats with CRF. Only the combined treatment of $1,25(OH)_2D_3$ with $24,25(OH)_2D_3$ reduced significantly all bone resorption parameters.

$24,25(OH)_2D_3$ alone, however, was most effective in reducing ROV. The above findings suggest that the combination of $24,25(OH)_2D_3$ with $1,25(OH)_2D_3$ is the most effective treatment in ameliorating uremic osteodystrophy in rats with CRF.

EVALUATION OF PARAMETERS TO ASSESS BONE DISEASE IN HEMODIALYSIS PATIENTS. Leonida L. Rasenas*, Maria V. DeVita*, Paul M. Zabetakis, Michael F. Michelis. Section of Nephrology, Lenox Hill Hospital, New York, New York.

A prospective study of 25 patients on hemodialysis for more than 2 years is described. Serum levels of calcium(Ca), phosphorus(PO4), alkaline phosphatase(AP), and N-terminal parathyroid hormone(N-PTH),hand x-rays,dual photon absorptiometry (DPA), deferoxamine(DFO) stimulation tests, and bone biopsies are reported. Ca was normal in 64%, low in 12%, high in 24%. PO4 was high in 68%. AP was high in 64%. N-PTH was high in 76%.X-ray studies showed secondary hyperparathyroidism(SHP) in 52%. DPA showed normal bone density in 56%, low density in 36%, and high density in 8%. DPA studies were normal in 7 of 13 patients who had SHP by x-ray. DFO tests were positive for aluminum(Al) in 47%. Bone biopsies were done in 8 patients. Five had osteitis fibrosa cystica(OFC), 1 had osteoporosis, 1 had OFC and aluminum deposition(AD), and 1 had AD alone, despite + DFO tests in 4 of 8.

DATA FROM PATIENTS WITH OFC BY BONE BIOPSY

	Ca	PO4	AP	N-PTH
Normal	8.5-10.5	2.5-4.5	<115	8-24
Mean±SE	10.0±0.3	7.3±1.4	288±96	97±3

The patient with OFC and AD had Ca 9.4,PO4 5.5, AP 415, and N-PTH 79. The patient with AD alone had Ca 10.2, PO4 5.0, AP 72, N-PTH 14.Conclusions: 1. DPA correlated poorly with chemical, biopsy and x-ray data. 2. Despite + DFO studies, bone biopsy revealed Al in only 50% of patients biopsied, suggesting extraosseous deposition. 3. Most patients had OFC. 4. Chemical data and DFO tests help to detect bone disease, but biopsy best reveals type and degree.

ALUMINUM INDUCES CELL MEDIATED CALCIUM EFFLUX FROM BONE. S.M. Sprague* and D.A. Bushinsky. Dept. of Medicine, University of Chicago, Chicago, IL.

The mechanism by which aluminum (Al) produces bone disease in patients with renal failure is not clear. To determine if Al induces calcium efflux from bone, we cultured neonatal mouse calvariae with and without graded concentrations of Al (10^{-8} to 10^{-5} M). Al caused a dose dependent net calcium flux (JCa) from bone (JCa = 31 ± 10 nmol/bone/24 h in control; 183 ± 18 in 10^{-8} M Al; 283 ± 16 in 10^{-7} M Al; 440 ± 67 in 10^{-6} M Al; 550 ± 92 in 10^{-5} M Al; each $p < 0.001$ vs. control). To determine if calcium efflux was cell mediated we cultured dead (freeze-thaw cycles) calvariae with and without Al (10^{-7} M). There was calcium influx (JCa = -461 ± 13) into dead bone which was not altered by Al (JCa = -488 ± 14, p = ns vs. dead).

Release of β-glucuronidase (β-glu), a lysosomal enzyme which correlates with cell mediated calcium efflux, increased after 24 h incubation in 10^{-7} M Al (1.7 ± 0.1 μg/bone/h vs. 0.9 ± 0.1 in control, $p < 0.001$). Calcitonin (3×10^{-9} M), an inhibitor of osteoclastic bone resorption, abolished the increase in β-glu (0.9 ± 0.2 in calcitonin + Al, $p < 0.001$ vs. Al). Calcitonin also nullified the Al induced JCa (JCa = 136 ± 36 in control; 346 ± 32 in 10^{-7} M Al; -41 ± 26 in calcitonin + Al; each $p < 0.01$ vs. others). Alkaline phosphatase, a marker of osteoblastic activity, was not altered by Al.

Thus, Al induces calcium efflux from bone which is cell mediated. Al increases β-glu; calcitonin inhibits the increased calcium efflux and β-glu, suggesting that Al stimulates osteoclasts to resorb bone. The mechanism by which Al stimulates cell mediated net calcium efflux and whether this efflux contributes to the development of bone disease requires further study.

URINARY CITRATE (U_{cit}) AND CALCIUM (U_{Ca}) CONCENTRATIONS IN FUROSEMIDE-TREATED INFANTS WITH AND WITHOUT NEPHROCALCINOSIS (NC). N. D. Adams, J. C. Rowe*, A. M. Lazar*, E. Horak*, S. D. Hopfer*, and T. B. Condren*. Univ. of Connecticut Health Center, Farmington, CT.

Increasing numbers of very low birth weight infants (VLBW, <1500 grams) are surviving. Bronchopulmonary dysplasia (BPD), a frequent complication in these infants, is treated with furosemide (F) because of its diuretic effect and its inhibition of fluid transport in the airway epithelial cell. NC has been described in up to 64% of these infants (Jacinto, Ped 81: 31,1988). F-induced hypercalciuria has been proposed as pathogenic. We have proposed that a decrease in $U_{cit}V$ is an additional risk factor for NC, but in a previous study we did not find an absolute decrease in $U_{cit}V$ (µmol/kg/day) in 9 F-treated VLBW infants compared to 7 not treated with F (Adams et al, J Bone Min Res 3:S165, 1988). In the present study of 18 VLBW infants treated with F, we have compared the 7 who developed NC with the 11 who did not. NC infants were smaller: 0.83 ± 0.28 (SD) vs 1.1 ± 0.35 kgs ($p < 0.05$) and tended to be more immature: 26.4 ± 2.9 vs 28.2 ± 2.7 weeks gestational age. There was no difference in $U_{cit}V$ between NC and non-NC infants, but the interrelationship of U_{cit} and U_{Ca} in these subsets of F-treated infants was different. In non-NC infants, U_{cit} increased as U_{Ca} increased: $y = .269x + .47$, where $y = U_{cit}$, µmol/ml and $x = U_{Ca}$, µmol/ml ($r = .572$, $p = .001$). In contrast, in NC infants, U_{cit} fell as U_{Ca} increased: $y = -.356x + 1.91$ ($r = -.441$, $p = .009$) a change in urine composition which would increase stone risk. The mechanism(s) for these differences may be multifactorial. In 53 measurements of creatinine clearance (C_{cr}) in these and other VLBW infants, both on and off F, C_{cr} increased with postconceptional age: $y = .195x - 6.5$, where $y = \ln(C_{cr}$, ml/min) and x = postconceptional age, weeks ($r = .726$, $p = .0001$). Thus, the lower C_{cr} in the smallest and most immature infants may account in part for the diminished citrate excretion in infants with NC. Other possible factors; eg. potassium depletion and metabolic alkalosis occuring in chronic F therapy may further explain differences between those VLBW infants who do and do not develop NC. Further dissection of these factors may offer insights into pathogenesis, prevention and treatment of NC.

ALUMINUM INHIBITION OF MUCOSAL TO SEROSAL CALCIUM TRANSPORT IN EVERTED DUODENAL SACS. Andrew J. Adler and Geoffrey M. Berlyne, Brooklyn V.A.M.C., Dept. of Med. Bklyn, NY.

We previously reported that Al inhibits Ca absorption from in vivo perfused rat duodenum (A.J.P. 249:G209, 1985). To investigate this relationship further, we studied the effect of Al on bi-directional Ca flux in everted duodenal and ileal gut sacs, using dual Ca isotopes. Gut sacs from 250g male Sprague Dawley rats were everted and filled with 0.5 ml of buffer consisting of NaCl 120 mM, KCl 4mM, $CaCl_2$ 1mM, Tris HCl 30mM,pH 7.2, ^{3}H-PEG 2µCi/ml,and ^{45}Ca 2µCi/ml and either Al-free (Al-) or 2µM $AlCl_3$ (Al+). Sacs were incubated for 1h at 37°C in 350 ml of the identical buffer except that ^{3}H-PEG was omitted and ^{47}Ca, 6µCi/ml, was the Ca tracer used. Solutions were gassed with 5% CO_2, 95% O_2. Results are reported as µMCa/hr/g WW. N=7/gp.

	J_{ms}	J_{sm}	J_{net}
Duodenum (Al-)	2.21 ±0.50	0.37 ±0.10	1.84 ± 0.45
Duodenum (Al+)	1.07 ±0.26*	0.55 ±0.17	0.52± 0.16*
Ileum (Al-)	0.33 ±0.07	0.37 ± 0.08	-0.04 ±0.09
Ileum (Al+)	0.37 ±0.10	0.39 ± 0.09	0.02 ± 0.05

*$p<.001$ compared to Al-free control.

Similar studies were carried out to determine the effect of Al on Na-dependent glucose transport using ^{14}C-glucose. There was no effect observed in either duodenum or ileum. Conclusions: 1)Al suppresses J_{net} Ca by inhibiting J_{ms} Ca in duodena but not ilea. 2)Al has no effect on J_{sm} in either segment. 3) The effect Al is relatively specific for Ca as it does not inhibit glucose transport.

A NEW FLUORESCENT TECHNIQUE FOR MEASURING MAGNESIUM. B. Bandari*, K. Golchini*, and I. Kurtz, Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

A new technique for measuring the free magnesium concentration fluorescently in aqueous solutions has been developed using phenylalanine tRNA ($tRNA^{Phe}$), which was purified from whole yeast tRNA. $tRNA^{Phe}$ possesses a fluorescent base (Wye base) at position 37 and binds magnesium in the region of its anticodon loop. Excitation spectra (250 nm to 400 nm) and emission spectra (330 nm to 600 nm) of 8 µM $tRNA^{Phe}$ were acquired in a HEPES-buffered solution, pH 7.4, in the presence of various concentrations of magnesium. Increasing the magnesium concentration from 0 to 3 mM caused an 80% increase in the fluorescence intensity (excitation 311 nm, emission 445 nm). Calcium (3 µM) or a decrease in the solution pH to less than 7.0 quenched the fluorescence by only 4%. The fluorescent measurement of magnesium agreed favorably with the standard atomic absorption technique. To monitor magnesium flux across an epithelial membrane, rabbit red blood cell ghosts were loaded with 0.42 mM $tRNA^{Phe}$ (0 mM magnesium in and out). The addition of magnesium (2mM) to the suspension of red cell ghosts resulted in a rapid influx of magnesium at an initial rate of ~50 µM/sec. Removal of Na^+, K^+ and Cl^- did not affect magnesium influx, suggesting that the major pathway for magnesium transport in this preparation is a leak pathway. Conclusions: 1) A new method has been developed for measuring magnesium fluorescently in aqueous solutions using $tRNA^{Phe}$. 2) This probe can also be used to measure magnesium transport across epithelial membranes in real time.

NMR MEASUREMENTS OF INTRACELLULAR CONCENTRATION OF PHOSPHATE $[Pi]_i$ IN THE KIDNEY DURING DEVELOPMENT. M. Barac-Nieto, T. Dowd*, R.K. Gupta*, A. Spitzer. A. Einstein Coll. of Med., Depts. of Pediatr. and Physiol. and Biophys., Bronx, NY.

Fractional reabsorption of Pi and the Vmax of the Na^+-Pi symport are higher in rapidly growing than in mature animals; this difference is not accounted for by extracellular factors. This prompted us to explore the relationship between $[Pi]_i$ and the rate of growth which, in turn, correlates with the rate of Pi transport. Kidneys of 2-3, 8-9 and 12-13 wks old rats and of newborn (2-3 days old) and adult (>57 day old) guinea pigs were isolated, cannulated and placed in a 15mm sample tube of an XL 200 NMR spectrometer, while being perfused with nutrient supplemented media containing 2% ^2H_2O. The ^{31}P-spectra were stable for 4-6 hrs. The extracellular volume of the kidney was derived from the ^{35}Cl and ^{2}H signals. In the rat, there were no age related differences in the intracellular concentrations (in mM) of: ATP (2.2±.3, 2.5±.4, 2.4±.3), phosphodiesters (3.7±.6, 3.5±.5, 3.6±.6), and phosphomonoesters (1.3±.3, 1.5±.3, 1.7±.5 in the three age groups, respectively). The $[Pi]_i$, on the other hand, was 0.65±.1 in the 2-3 wks old, 0.70±.1 in the 8-9 wks old and 1.4±.3 mM in the 12-13 wks old rats. The $[Pi]_i$ correlated inversely ($r=-0.9$) with the growth rate (7.5±.6, 6.5±.4, and 0.4±.2 g/day in the three age groups, respectively). $[Pi]_i$ was also lower in the newborn (0.35 mM) than in the adult (1.2 mM) guinea pigs. We conclude that during growth $[Pi]_i$ is low in the kidney and speculate that this may enhance the rate of Pi transport.

MECHANISM FOR ACTIVE Ca^{++} TRANSPORT ACROSS RAT INTESTINAL EPITHELIUM. J.L. Borke*, A. Caride*, J.T. Penniston*, and R. Kumar. Mayo Clinic and Foundation, Rochester, MN.

In order to determine why active Ca^{++} transport occurs only in certain segments of the intestine, we studied the distribution of epitopes of the plasma membrane Ca^{++}-pump along the entire intestine of rat. We used monoclonal antibodies prepared against the erythrocyte Ca^{++}-pumping Ca^{++}-Mg^{++} ATPase and immunohistochemistry to study this distribution. Sections of paraffin-embedded intestinal segments from rat duodenum, jejunum, four segments of ileum, cecum and colon were analyzed by immunohistochemistry. We found that staining for epitopes to the Ca^{++}-pump was the most intense in the basolateral membranes of cells in the duodenum. Basolateral membranes were also stained to a lesser degree in the cells of the jejunum and cecum. Staining was absent in the cells of the ileum and colon. Western blot analysis of rat intestinal homogenates showed that the monoclonal antibody bound to a major band at Mr $\approx$ 140,000 which corresponds to the molecular weight of Ca^{++}-pumps identified in other tissues.

Conclusion: These results demonstrate that epitopes of the plasma membrane Ca^{++}-pump are distributed along the rat intestine in the same regions where active Ca^{++} transport is most rapid. These studies suggest that a Ca^{++}-Mg^{++} ATPase Ca^{++}-pump of the red cell type may be part of a mechanism for the active transport of Ca^{++} in the mammalian intestinal epithelium.

MALEIC ACID (MA) INDUCED PHOSPHATURIA IS DUE TO DIRECT INHIBITION OF PHOSPHATE (Pi) TRANSPORT AT THE LEVEL OF THE PROXIMAL TUBULAR BRUSH BORDER MEMBRANE (BBM). V.B. Delaney, J. Guntupalli. Emory University School of Medicine, Division of Nephrology, Department of Medicine, Atlanta, Georgia.

MA administration to rats (50mg/kg/hr) causes proximal tubular dysfunction in the form of a Fanconi syndrome with a reduction in the tubular reabsorption of Pi (TRPi/GFR) from 63- 3 to 32- 3 ug/ml, $p<0.0001$ without alteration in plasma Pi. BBM were prepared and Pi transport characteristics studied in 3 groups of rats treated thus with MA and compared with saline treated controls. Results of Na dependent transport at 2 minutes are expressed in nmol/mg protein.

		Control	MA
Group 1	PTH:intact, Diet:normal Pi	1.04 - 0.08	0.812 - 0.04, $p<0.05$
Group 2	PTH:intact, Diet:low Pi	1.88 - 0.4	1.54 - 0.19, $p<0.05$
Group 3	PTX:chronic, Diet:low Pi	1.4 - 0.14	1.15 - 0.10, $p<0.05$

BBM prepared from normal rats incubated in vitro with MA (9mM) for 30 minutes also resulted in inhibition of Na dependent Pi transport: control 1.13 - 0.12, MA 0.68 - 0.09, $p<0.05$. The degree of inhibition was directly related to the MA concentration (0-9mM). A similar degree and type of inhibition was observed following incubation with equimolar concentrations of the trans-isomer, Fumaric acid. In all experiments, inhibition of Pi uptake was confined to the Na dependent component with no effect on Na independent, non-gradient or voltage clamped Pi transport.

Conclusions: MA induced phosphaturia appears to: 1)result from a direct inhibitory effect of MA on the transport characteristics of the proximal tubular BBM; and 2)be independent of PTH and dietary Pi content.

INCREASED CYCLIC AMP IN RENAL PROXIMAL TUBULES IS ACUTE EFFECT OF NICOTINAMIDE ANALOGS. PI Campbell* and SA Kempson. Dept. of Physiology, Indiana Univ., Indianapolis, IN.

Nicotinamide is a rapidly acting and potent specific inhibitor of the Na^+/phosphate (Pi) cotransporter in the renal brush border membrane (BBM). A transient increase in renal cortical cyclic AMP occurred in vivo within 1 h after injection of rats with a phosphaturic dose of nicotinamide. The possible role of cyclic AMP was tested in vitro using proximal tubule (PT) suspensions prepared from rat kidney cortex by collagenase digestion and percoll gradient centrifugation. In the presence of 0.5 mM IBMX the cyclic AMP content of PT was increased 2-5 times ($p<0.005$) after incubation for 1 h with nicotinamide, 5-methylnicotinamide or picolinamide. The increase was dose-dependent at drug concentrations up to 3 mM. Pi transport was determined in BBM vesicles isolated from PT suspensions. A 30-40 % inhibition of the initial uptake phase (10 s) of Na^+/Pi cotransport accompanied the increase in cyclic AMP in PT incubated with nicotinamide or picolinamide ($p<0.05$). Pi uptake at 120 min was not different from controls. Na^+/leucine cotransport by BBM vesicles was not changed indicating the specificity of the inhibition of Pi transport. The inhibitory effect of nicotinamide occurred with no change in PT content of NAD. Picolinamide increased NAD content only 25 %. An increase in cyclic AMP rather than NAD may mediate the acute inhibition of Na^+/Pi cotransport by nicotinamide. NAD may be more important for maintaining the chronic inhibitory effect of nicotinamide.

PREVALENCE AND PATHOGENESIS OF HYPOCALCEMIA IN CRITICALLY ILL CHILDREN. B.Gauthier, J.Schaeffer*, A.Steele*, H.Trachtman, F. Di Carmine*, M.Urivetsky*, J.Tobash* and F.Chasalow*. Schneider Children's Hospital of LIJMC, New Hyde Park, NY.

To determine the prevalence of hypocalcemia in critically ill children and obtain information about its pathogenesis, we did ionized serum calcium (iCa) in 48 consecutive patients admitted to a pediatric ICU and in healthy controls. The iCa in controls was 1.18±0.04 mmol/L (mean±SD) and we therefore defined hypocalcemia as a iCa < 1.1 (i.e. the mean-2SD). We also measured serum PTH (pg/ml), 25(OH)D3 (ng/ml), 1-25(OH)2D3 (pg/ml) and calcitonin (pg/ml) in patients with and without hypocalcemia and in healthy controls.

Seven ICU patients were hypocalcemic (15%). Other results are shown on the table below as mean±SD.

	Hypocalcemic patients (n=6)	Normocalcemic patients(n=6)	Controls (n=20)
iCa	0.96±0.11*	1.24±0.06	1.18±0.04*
PTH	108±127*	92±164	18±5*
25(OH)D3	1.12±0.33*	1.37±0.37	1.47±0.51*
1-25(OH)2D3	29.8±23.4	35.0±12.0	35.1±11.9
Calcitonin	388±135*	78±23	74±25*

*$p<0.05$

We conclude that hypocalcemia occurs frequently in critically ill children. It is associated with calcitonin and PTH levels more than 5 times greater than normal. Those abnormalities suggest that the most probable primary event is the elevation of calcitonin causing hypocalcemia and secondary hyperparathyroidism. The reason for the increase in calcitonin is not apparent.

THE EFFECT OF ACUTE METABOLIC ACIDOSIS (AMA) ON RENAL PHOSPHATE (Pi) HANDLING IN THE RAT: ITS RELATIONSHIP TO PTH, DIETARY PHOSPHATE AND CORTICOSTEROIDS. J. Guntupalli, V.B. Delaney, E. Bourke. Emory Univ. Sch. of Med., Renal Div., Atlanta, GA.

AMA was induced by HCl loading in 4 groups of chronically PTX rats (E). Control animals (C) within each group were given equivalent volumes of 0.9% NaCl. The fractional excretion of Pi (FEPi) and Na dependent Pi transport in proximal tubule brush border membranes (BBM) (expressed as nmol/mg protein at 2 min.) were each measured in the final stages of the acid infusion. Group 1: rats on a normal diet were infused with 50mM HCl in 0.9% NaCl at 2 ml/kg/hr for a 3 hour period. No change in FEPi (4.8±1.7% (C); 5.2±1.3% (E)) or Na dependent Pi transport was seen (1.04±0.23 (C); 0.96±0.19 (E)). Group 2: rats on a normal diet, infused with 25mM HCl in 0.9% NaCl at 2 ml/kg/hr for a 6 hour period with the production of a similar degree of acidosis as Group 1 (systemic pH 7.11±0.12; plasma HCO_3 12±3 mmols/L) showed an increase in FEPi (4.3±1.7% (C); 25.3±2.1% (E), p <0.001) and a decrease in Na dependent BBM Pi uptake (1.22±0.06 (C); 0.90±0.04 (E), p <0.05). Group 3: rats on a low Pi diet (0.03% for 4 days) infused with HCl as in Group 2, showed an elevated FEPi (0.58±0.15% (C); 19.73±1.70% (E), p <0.001) and a depressed Na dependent BBM Pi uptake (2.10±0.08 (C); 1.45±0.06 (E), p <0.05). Group 4: adrenalectomized rats (76 hours prior to the study) maintained on DOCA, were infused with HCl as in Groups 2,3. The defect in the Na dependent BBM Pi transport was sustained (2.10±0.15 (C); 1.58±0.10 (E), p <0.05). Conclusions: 1) Acute HCl acidosis requires a minimum sustaining period of >3 hours to produce a demonstrable Pi transport defect in vivo and in vitro, and 2) the production of this defect is independent of PTH, dietary Pi and endogenous glucocorticoids.

EFFECT OF pH UPON Na^+-DEPENDENT PHOSPHONOFORMIC ACID (PFA) BINDING ON RENAL BRUSH BORDER MEMBRANE (BBM) AND ITS INHIBITORY EFFECT ON Na^+-GRADIENT-DEPENDENT UPTAKE OF Pi BY BBM VESICLES (BBMV). A. Hoppe and T.P. Dousa, Nephrol. Res. Unit, Mayo Clinic, Rochester, MN.

PFA is a specific competitive inhibitor of Na^+/Pi cotransport across epithelial BBM. We examined the effect of $[H^+]$ upon the ^{14}C-PFA binding and ^{32}Pi uptake by BBMV in pH range 5.5 - 7.5. Both ^{14}C-PFA binding and ^{32}Pi uptake by BBMV increased in parallel with increasing pH, but only in the presence of Na^+ in the medium:

Na^+-dependent:	pH 5.5	pH 6.5	pH 7.5
^{14}C-PFA binding (nmol/mg prot/20′)	2.38	3.25*	3.69*
^{32}Pi-uptake (pmol/mg prot/5″)	29	161*	351*

* sign. different from value at pH 5.5 (t-test).

Increased ^{14}C-PFA binding at higher pH was due to an increase in affinity. The relative (Δ%) extent of the inhibitory effect of PFA on Na^+-dependent ^{32}Pi uptake was also enhanced with increasing pH and the inhibition remained competitive. Present results suggest that two dissociated OH groups are required for ^{14}C-PFA binding on BBMV and for inhibitory effect of PFA on Na^+/Pi cotransport across renal BBM. PFA likely competes with binding of Na_2HPO_4 on the luminal surface of BBM. This finding also supports the notion that dibasic Pi is the ionic form cotransported with Na^+ across renal BBM.

STRUCTURAL ABNORMALITIES OF URINARY TAMM-HORSFALL GLYCOPROTEIN (THP) AMONG FAMILY MEMBERS OF A CALCIUM OXALATE KIDNEY STONE FORMER (SF). B. Hess*, Y. Nakagawa* and F. L. Coe. Nephrology Program, University of Chicago, Chicago, IL

We have found that THP from SFs is a defective inhibitor of calcium oxalate crystal aggregation at pH 5.7/200 mM NaCl (Kidney Int.33:342(1988); with an additional 5 mM $CaCl_2$, THPs from SF show an abnormal increase of intrinsic viscosity. We have studied THP from the wife (W) and 2 sons (S_1, S_2) of one severe SF, to determine family occurrence pattern.

THP viscosity was measured at pH 5.7/200 mM NaCl/5 mM $CaCl_2$/25°, using a capillary viscometer. Amino acid (AA) analysis was performed after 24/48 /72 h acid hydrolysis. Cysteine was determined by either S-carboxymethylation or performic acid oxidation. Circular dichroism (CD) spectra of THP were measured between 320 and 190 nm, and mean residue ellipticity, [θ], was calculated (deg.cm^2/dmole).

Axial ratios of THP (long axis/short axis), derived from viscosities, were 310 (SF),135 (S_1), 160 (S_2) and 15 (W). AA analyses showed microheterogeneity; contents (mol% AA residue,±SEM) of Thr (7.4±0.9), Gly (10.3±0.7) and Ala (9.0±0.7) varied most between individual THPs. At 216 nm,[θ] was between -5133 (SF) and -800 (W), indicating more helical structure in SF-THP. At 273 nm, [θ] was between -132 (SF) and +45 (W), indicating different optical activities of Tyr and Trp; sons' THP were always intermediate between SF and W.

Thus THPs from individuals are structurally heterogeneous and functionally defective among SFs. Loss of crystal aggregation inhibition is related to formation of more elongated THP aggregates with more helical structure.

BILE SALTS AND ILEAL CALCIUM (Ca) ABSORPTION: A NEGLECTED BUT IMPORTANT NON-VITAMIN D REGULATION? **M.S. Hu*, P. Abdella* and D.B.N. Lee. VA Medical Center, Sepulveda and UCLA School of Medicine, Los Angeles.**

Bile salts have Ca ionophoric and tight-junctional activities and may be responsible for ileum as the major site for Ca absorption. We studied the effect of taurodeoxycholate (TDC) on Ca fluxes in ileum from male Wistar rats (400g), using conventional Ussing chamber techniques. Mucosal (M) TDC (2mM, EXP) or vehicle (C) was added at 60 min and study continued to 120 min. PreTDC M-to-serosal (S, Jms) and S-to-M (Jsm) fluxes were 19±2[SEM] and 41±3 respectively (N=7), giving a net (Jnet) secretory flux of -21±2 nmoles/cm2/hr. TDC caused immediate rise in conductance (G) which peaked at 75 min (EXP 39±2, C 19±2 mS/cm2, P<0.001) and then dropped to C levels by 105 min. Jsm exhibited identical changes, i.e., rapid increase to peak at 75 min (86±2 vs 21±1, P<0.001) and then dropped to level of C by 120 min. Jms rose slower and peaked at 90 min (51±3 vs 23±2, P<0.001) but thereafter remained stable as G and Jsm waned. The rapid but transient increase in Jsm and the slower but sustained increase in Jms led to an initial increase, followed by reduction and final elimination of the basal secretory flux. This TDC-induced reduction in Ca secretion in intestine of mature animal mimics the action of vitamin D. The sustained, conductance-independent increase in absorptive flux (Jms) may be mediated transcellularly through the Ca ionophoric effect of TDC. The increase in Ca flux through the conductance pathway is most likely the result of TDC action on junctional complexes and could increase Ca absorption when luminal [Ca] is high, e.g., after feeding. Because ileum is not the primary intestinal target for vitamin D, bile salts may play important regulatory roles in Ca absorption.

EFFECT OF CISPLATIN ON RENAL TUBULAR TRANSPORT OF PHOSPHATE. Paul N. Kintziger* and Henri E. Küntziger. Cours Universitaire, Luxembourg and Hôpital Pasteur-Vallery-Radot, Paris, France.

Cisplatin (CPT) nephrotoxicity might be initiated by alterations of transport events at proximal tubule apical or basolateral membranes. CPT effect on Na-dependent phosphate (P..i..) uptake by brush border membrane vesicles (BBMV), isolated from rat renal cortex by Mg precipitation, was studied with the rapid filtration technique. With mM medium CPT, P..i.. uptake at 20 s, in % of equilibrium, as compared to controls (C : 625 +/- 43), increased initially (CPT..in.. : 913 +/- 114), but decreased after prolonged preincubation (CPT..prol.. : 230 +/-69), $p < 0.01$, N = 5, mean +/- SD. Kinetic analysis showed no apparent Km change ; Vmax, nmol/mg Protein /10 s, increased with CPT..in.. (C : 2.15 +/- 0.06 ; CPT..in.. : 7.55 +/- 0.57, N = 3, $p < 0.01$), and decreased with CPT..prol.. (C : 2.32 +/- 0.53, CPT..prol.. : 1.36 +/- 0.22, N = 3, $p < 0.01$). CPT dose-response analysis disclosed a K..s.. of 0.21 mM for CPT..in.., and a K..i.. of 0.09 mM for CPT..prol.. In animals injected 24 hours previously with CPT, urinary P..i.. excretion, µmol/ml GFR, increased from 0.44 +/- 0.10 to 0.56 +/-0.10, N = 4, $p < 0.01$; BBMV P..i.. uptake at 20 s, in % of equilibrium, decreased from 271 +/- 41 to 187 +/- 8, N = 6, $p < 0.01$. To conclude : CPT might have a biphasic action on P..i.. tubular transport, a transient initial stimulatory effect, but a sustained inhibitory one, disclosed at the BBMV as well as for whole kidney.

PHOSPHATE TRANSPORT BY INNER MEDULLARY COLLECTING DUCT CELLS: RESPONSE TO CHANGES IN EXTRACELLULAR PHOSPHATE. BS Levine, KA Knibloe,* DR Mishler,* & JA Kraut. VA Med Ctrs, WLA & UCLA Sch Med, Los Angeles, CA

The proximal tubule, the prime site of renal phosphate (P) transport, plays a pivotal role in the renal response to P-restriction. Recent studies suggest that the distal nephron including the inner medullary collecting duct (IMCD) may also be an important site of P transport and data from this laboratory have demonstrated Na-dependent P transport by cells from this nephron segment. To examine if IMCD cells also contribute to increased P reabsorption following P-deprivation, IMCD cells in culture for 3 days (subconfluent) were incubated in low P media (-P, P < 0.3mM) for 2, 4, 18 or 24 hrs and compared to controls (+P, P = 1mM). P uptake was significantly higher in cells incubated in -P for 24 hrs than in controls (8.1±0.6 nmol/mg prot/5 min in -P vs 5.2±0.6 in +P, $p<0.01$) but were no different with shorter incubation periods. To examine if more rapid adaptation occurs when cells are pre-adapted to -P, cells were incubated for 24 hours in -P, the media then changed to +P for 40-60 min and -P then reintroduced. P uptake was suppressed by 20.6±3.0 % by +P, $p<0.01$. Re-introduction of -P for only 20 min increased P uptake by 15.9±4.8%, $p < 0.01$. These studies show that IMCD cells adapt to changes in extracellular P. Although initial adaptation requires many hours, rapid adaptation can occur following a period of "pre-adaptation". These data are consistent with a requirement for synthesis of new P carriers during the initial adaptation which can then be rapidly inserted or removed following changes in media P.

THERAPY OF HYPERPHOSPHATEMIA IN CHRONIC HEMODIALYSIS PATIENTS WITH SUCRALFAT. +R.W. Kurz, F. Stockenhuber, G. Sunder-Plassmann,++V. Meisinger, P. Balcke. 1st Dep. of Medicine,++Dep.of Occupational Medicine,Univ. of Vienna,+KFJ Hospital.

Hyperphosphatemia is a most common problem in patients on chronic hemodialysis treatment beeing a major factor of the development of secondary hyperparathyreoidism. In 17 out of 60 patients on regular dialysis treatment conventional therapy of hyperphosphatemia with aluminium hydroxides, aluminium carbonates and dietary phosphorus intake restrictions was of insufficient effect only. In these 17 patients additional therapy with sucralfat (daily dose = 3 g) was started. The mean serum level of phosphorus before sucralfat therapy was 2.7 ± 0,5 mMol/l and decreased significantly within 4 weeks of sucralfat therapy to 1,79 ± 0,4 mMol/l. Four months after starting sucralfat therapy the mean serum level of phosphorus was 1.6 ± 0,5 mMol/l. The remaining 43 patients on regular dialysis treatment served as control group. They had tolerable serum phosphorus levels with conventional therapy already (mean serum phosphorus = 1.6 ± 0,4 mMol/l) and no significant change of the mean serum phosphorus during the observation time of 4 months was observed. In all 17 patients who received sucralfat therapy serum aluminium levels were within normal range.

We conclude that sucralfat is a potent phosphate binder, which can be administered successfully at least in addition in dialysis patients whose hyperphosphatemia cannot be treated effectively with conventional therapeutic approaches.

MAGNESIUM (MG) BIOAVAILABILITY FROM MAGNESIUM CITRATE (MgCit) AND MAGNESIUM OXIDE (MgOx). Jill Lindberg* and C.Y.C. Pak. Letterman AMC, San Francisco, CA., Southwestern Med School, Dallas TX

Mg salts should be soluble and bioavailable to treat calcium oxalate (CaOx) nephrolithiasis. We compared in vitro solubility (sol) and in vivo gastrointestinal absorbability of MgCit vs MgOx. Sol. of 25 mmoles of each salt was examined in solutions containing varying amounts of hydrochloric acid (HCL), (0-24.2 mEq) in 300ml distilled H_2O intended to mimic achlorhydric to peak acid secretory states. Mg absorption from MgCit and MgO was measured in normal volunteers (NV) by assessing the rise in urinary Mg (UMg) following oral Mg load.

MgO required acid for dissolution, as it was insoluble in H_2O and only 43% soluble in simulated peak acid secretion (24.2 mEq HCL/300ml). MgCit had high sol. even in water (55%). MgCit was more soluble than MgO in all states of acid secretion. Titration of filtrates from sol. studies to pH of 6 and 7 (mimicing pancreatic bicarbonate secretion) did not cause reprecipitation of either salt. Approximately 65% of MgCit was complexed as soluble MgCit, whereas Mg complexation was not present in the MgO system. The amount of ionic Mg was greater following MgCit dissolution than following MgO dissolution, in all solutions except at the highest concentration of HCL.

The increment in UMg following MgCit load (25 mmoles) in NV was significantly higher than that obtained from MgOx load (during 4 hours post-load, 0.22 vs 0.006 mg/mg creatinine, $p < 0.05$; during second 2 hours post-load, 0.035 vs 0.008 mg/mg, $p < 0.05$). MgCit was more soluble and bioavailable than MgO and may be useful in prevention of recurrent CaOx nephrolithiasis.

INTERACTION OF SULFHYDRYL (SH) MODIFYING AGENTS WITH Pi TRANSPORT IN RENAL BRUSH BORDER MEMBRANE. Mahmoud Loghman-Adham. Univ. of Utah Medical Center, Salt Lake City, UT.

SH groups are essential for D-glucose transport by intestinal BBM vesicles (BBMV). To explore the role of SH groups in Na^+-Pi cotransport across renal BBM, we used $HgCl_2$, an agent which penetrates membranes freely and converts SH to S-S. Rat renal BBMV were prepared by a Ca^{++}-precipitation method and transport measured by a rapid filtration method.

$HgCl_2$ inhibited the initial Na^+-Pi cotransport in a dose-dependent manner (IC 50 = 50uM). Na^+-independent transport (Na^+ replaced by K^+) was not affected. The overshoot of Na^+-Pi cotransport was reduced without a significant change in equilibrium uptake. Maximum inhibition was reached within 5 min. and did not increase thereafter. Inhibition decreased with increasing BBMV protein concentration. Therefore, protein concentration was kept at 7 mg/ml for all experiments. Dithiothreitol completely protected against inhibition, but Pi, foscarnet, and Na^+ gave no protection. BBMV volume was not affected at low $HgCl_2$ concentrations, but decreased at higher concentrations (>100uM). The inhibitory effect persisted under Na^+ equilibrium-exchange conditions, indicating a mechanism distinct from an effect on Na^+ conductance. Kinetic studies were consistent with non-competitive inhibition with a reduction in Vmax from 1.5 to 0.6 nmoles/mg prot/5 sec without a change in Km.

SH groups are essential for the function of Na^+-Pi cotransporter of renal BBM, but are not part of the Pi binding site.

$1,25(OH)_2D_3$ DIMINISHES RENAL CELL PROLIFERATION AND COMPENSATORY RENAL GROWTH

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Normal and malignant renal cells express receptors for $1,25(OH)_2D_3$ and the secosterol inhibits proliferation of malignant renal cells, but whether compensatory growth of normal renal tissues is responsive to $1,25(OH)_2D_3$ has not been examined. We studied 4 week old male SD rats with uni-NX or sham-OP which received $1,25(OH)_2D_3$ or solvent (2.5 ng i.p. daily) for 5 days after uni-NX. Kidneys were removed for receptor studies (Scatchard analysis), histomorphometry and measurement of mitotic activity (after pretreatment with colchicin). Rats with uni-NX had unchanged S-Cr (0.37 ± 0.03 vs 0.48 ± 0.1 mg/dl), S-Ca and S-$1,25(OH)_2D_3$ (231 ± 51 vs 188 ± 38 pg/ml). Renal $1,25(OH)_2D_3$ receptors increased with time in sham OP animals (N_{max} from 32.5 fmol/mg protein on day 1 to 57 on day 5), but less so in uni-NX (from 30 on day 1 to 40 on day 5). Mean renal weight increased more in uni-NX (from 0.64 g on day 1 to 0.93 on day 7) than in sham-OP (0.58 g on day 1 to 0.73 on day 5); the increment in renal weight was less in uni-NX treated with $1,25(OH)_2D_3$ (0.73 g on day 1 and 0.72 on day 5).

We conclude that compensatory renal growth after uni-NX is modulated by $1,25(OH)_2D_3$.

CALCIUM ACETATE (CaAc), AN EFFECTIVE BINDER OF DIETARY PHOSPHORUS (P) IN PATIENTS WITH CHRONIC RENAL FAILURE (CRF). Martin L. Mai, Michael Emmett, Mudassir S. Sheikh, Carol Santa Ana, John S. Fordtran, Baylor University Medical Center, 3500 Gaston, Dallas, Texas 75246.

The calcium (Ca) salts are being increasingly used as P binders in patients with CRF due to the toxicity of aluminum salts. Theoretical calculations confirmed by in vitro and in vivo (in normal subjects) results indicate that CaAc has important advantages over other Ca salts as a P-binder. CaAc is readily soluble in both acid and alkaline solutions (unlike $CaCO_3$ which requires an acid pH) and does not contain a complexing anion which competes with P for binding (such as citrate).

We used a one meal technique (JCI 73:640, 1984) to measure net intestinal P and Ca absorption in 6 dialysis dependent patients. CaAc (1 gm Ca) was compared with $CaCO_3$ (1 gm Ca) and placebo in randomized double-blind fashion.

	Ca		P		
	Ingested	Absorbed	Ingested	Absorbed	
	mg	mg	mg	mg	%
Placebo	201±5	-28±28	346±4	181±31	(53)
$CaCO_3$	1197±6	355±46	341±4	138±21	(40)
CaAc	1202±7	314±41	346±5	75±16*	(22)

* = P<0.05 compared with placebo and with $CaCO_3$.

For a given dose of Ca salt more P will be bound and less Ca absorbed with CaAc. Hypercalcemia, a limiting toxic effect of Ca salt therapy, should therefore be less common with CaAc. CaAc appears to be an optimal P-binding Ca salt in patients with ESRD; it is manufactured by Braintree Laboratory (Braintree, Mass.) under the name of Phos-Lo.

HCO_3 ABSORPTION STIMULATES ACTIVE CALCIUM ABSORPTION BY THE DISTAL TUBULE (DT).

S. Miyakawa* and K. Bomsztyk. Univ. of Washington, Seattle, WA.

To determine whether luminal HCO_3 has an effect on distal Ca absorption rat surface DT were perfused in vivo. Total Ca concentration was measured by atomic absorption, total CO_2 by microcalorimetry and luminal Ca ion concentration, $[Ca^{2+}]_L$, and transepithelial voltage, V_{TE}, with microelectrodes. With HCO_3-free or HCO_3 (20 mM) perfusate rates on Ca absorption were the same averaging 1.9 ± 0.3 (n=12) and 1.9 ± 1.0 (n=14) pmol/min. This indicates that luminal HCO_3 per se has no effect on distal Ca transport. With HCO_3 solution rate of HCO_3 absorption was not different from zero, -12 ± 26 (n=4) pmol/min, but when carbonic anhydrase (C.A.) was added to the HCO_3 perfusate (HCO_3 + C.A.), HCO_3 absorption increased to 56 ± 13 (n=4) pmol/min. C.A. - stimulated HCO_3 absorption was associated with increased Ca absorption, 9.6 ± 3.4 (n=5) pmol/min, and as a result $[Ca^{2+}]_L$ at the end of the perfused segment fell from 0.94 ± 0.03 (n=12) mM with HCO_3 to 0.30 ± 0.08 (n=12) mM with HCO_3 + C.A. The enhanced Ca absorption was due to active transport because V_{TE} was the same with HCO_3 or HCO_3 + C.A. perfusate averaging, -20 ± 6 and -22 ± 7 (n=6) mV.

We conclude, that HCO_3 absorption, but not luminal HCO_3 per se, stimulates distal Ca absorption. This is consistent with our previous observation that acidification of DT fluid stimulates active Ca transport (Clin. Res. 30:523A, 1988).

TIME COURSE OF THE DECREASE IN RENAL Pi REABSORPTION FOLLOWING SUPPRESSION OF GROWTH HORMONE RELEASE IN IMMATURE RATS. Susan E. Mulroney*, Michael D. Lumpkin*, and Aviad Haramati. Department of Physiology, Georgetown University School of Medicine, Washington, D.C.

We recently developed a new model of growth hormone (GH) deficiency, using an antagonist to GH-releasing factor (GRF-AN: [N-AC-TYR^1-D-ARG^2]-GRF-(1-29)-NH_2). A single injection of GRF-AN suppresses the surges of GH release (up to 6 hr), and chronic 4 day treatment of GRF-AN (2x daily) was found to reduce both somatic growth and the maximum capacity for phosphate reabsorption (TmPi) in immature rats. However, since positive Pi balance was reduced by 50% after only 1 day of GRF-AN treatment, it is possible that the TmPi may also be rapidly altered. Therefore, we sought to examine the time course between the suppression of GH surges and the decrease in TmPi. Chronic silastic catheters were placed in 29 immature (28-32 days old) Wistar rats. Control animals were injected with 0.1 ml saline only, whereas experimental rats received GRF-AN (100 ug/kg) in saline at 8 am and 1 pm daily (to precede two major peaks of GH release). The TmPi was determined with Pi infusions at 0 hr, 3 hr, 6 hr, 24 hr, and 4 days after the first GRF-AN injection. Acute (0 hr) GRF-AN treatment did not alter the TmPi (4.4±0.4 vs. 4.5±0.2 umol/ml in controls). However, a significant decrease in the TmPi (3.8±0.2 umol/ml, $P<0.05$) was seen after 3 hr (reflecting suppression of 1 GH surge). After 6 hr of treatment (i.e. suppression of 2 GH surges) the TmPi was maximally reduced (3.3±0.2 umol/ml, $P<0.05$ vs. 3 hr) and no further decrease in the TmPi was evident at either 24 hr (3.4±0.1 umol/ml) or 4 days (3.3±0.1 umol/ml). Thus, inhibition of the TmPi occurs within hours of GH suppression, supporting the notion that growth hormone plays a key role in regulating renal Pi transport during development.

BLUNTED EFFECT OF DIET ON Pi TRANSPORT IN BBMV OF NEWBORN GUINEA PIG. Richard Neiberger, Mario Barac-Nieto and Adrian Spitzer. A. Einstein College of Medicine, Department of Pediatrics, Bronx, N.Y.

We have reported that, at any filtered load below Tm, the newborn reabsorbs at least 3-fold more Pi/g kw than the adult (V. Johnson and A. Spitzer, Am. J. Physiol. 251:F251, 1986). To assess for age related differences in the renal response to variations in Pi intake we studied the kinetics of the Na^+-Pi cotransport in BBMV of guinea pigs given standard, high, or nominally Pi free diet for 5 days.

	Standard		High Pi		Pi free	
Age (days)	3-14	>57	3-14	57	3-14	>57
Vmax ($pmol \cdot mg^{-1} \cdot s^{-1}$)	650^b ±77	144^b ±17	$472^{a,b}$ ±53	$81^{a,b}$ ±13	596^b ±72	318^b ±32
Serum Pi (mM)	1.8 ±.2	2.1 ±.2	$3.5^{a,b}$ ±.2	2.3 ±.1	$0.8^{a,b}$ ±.1	2.0 ±.2

a = P < .05 compared to same age group on standard diet.
b = P < .05 3-14 vs >57 days old guinea pigs on same diet.

An increase in Pi intake resulted in a smaller relative decrease in the Vmax of the newborn than of the adult (27 vs 44%, $p<.05$); a low Pi intake did not affect the Vmax in the newborn ($p>.8$) but it did up-regulate the system in the adult. Km values were not influenced by age or Pi intake. Thus, in the newborn, the Na^+-Pi cotransport system is characterized by a high transport capacity but a low adaptability to changes in dietary Pi. As a result, changes in Pi intake are associated with large variations in serum Pi.

ELUCIDATION OF NEPHROCALCIN (NC) ISOMERS BY ^{31}P-NMR. Y. Nakagawa* and F. L. Coe, Nephrology Program, Univ. of Chicago, Chicago, IL and T. Otsuki*, Chem. Dept. Occidental College, Los Angeles, CA

NC, an acidic urinary glycoprotein inhibitor of calcium oxalate crystal growth, contains phosphorus (P), and that NC is excreted in 3 or 4 isomers (J.Biol.Chem.,258:12594(1983)). We have used ^{31}P-NMR to investigate the type of P binding and to quantify the amount of P bound to NC.

NC was purified from bovine kidneys, and separated into 3 fractions (A, B, and C), suing a DEAE-cellulose column eluted with a linear gradient of NaCl from 0.05 M to 0.4 M. P contents were measured by a modified Fisk-Sabbarow method.

P contents (ug/mg NC) were 48.5 (A), 471 (B), and 3586 (C). Fraction C was then dissolved in D_2O, and the ^{31}P-NMR spectrum was measured using a Bruker 500 NMR spectrophotometer. The chemical shifts (ppm) were +1.7458, -0.7644 and -1.1630. After alkaline phosphatase digesting, P content reduced to 1229 and the shift of +1.7458 disappeared, indicating phosphoserine was involved. Dephosphorylated NC inhibited calcium oxalate crystal growth 10-fold less ($k_d = 10^{-6}$M) than native NC. Rechromatographed dephosphorylated fraction C eluted from DEAE cellulose column in A and B positions. After deglycosylation by glycosidase, P content was 168, and NMR showed no chemical shift, indicating P attachment to carbohydrate, not the protein.

NC molecules contain phsophoserine and phosphorylated carbohydrates. Removal of P from phosphoserine decreases NC inhibition of calcium oxalate crystal growth 10-fold; therefore, P seems to be important for crystal growth inhibition.

COMPARATIVE EFFECTS OF CALCIFEDIOL (25OHD) AND CALCITRIOL (1,25(OH)$_2$D) SUPPLEMENTATION ON GROWTH IN EXPERIMENTAL UREMIA. Steve Prager, Carolyn Abitbol, Gaston Zilleruelo, Jose Strauss. Univ. of Miami Sch. of Med., Dept. of Pedi., Miami, Fl.

Growth failure in uremia has been attributed to poor nutrient intake and endogenous deficiency of vitamin D (VD). Experiments were designed to test the differential effects of supplementation with the VD analogues, 25OHD and 1,25(OH)$_2$D. Young male Wistar rats were rendered uremic (U) by 7/8 nephrectomy while a double number of controls were sham operated. Half of the controls were fed ad libitum (AC) whereas the others were restricted to that quantity of feed consumed by uremic partners (PC). All were divided into 3 groups receiving 3 weekly injections of placebo, 0.1 mcg 1,25(OH)$_2$ or 1.0 mcg 25OHD for 21 days. Linear growth, nutrient intake and mortality were recorded. Statistical differences are indicated by unshared superscripts:

	Nutrient Intake (g/d)		Mortality (%)		
	U/PC	AC	U	PC	AC
Placebo	11 ± 2^a	26 ± 3^c	38	10	0
25OHD	18 ± 1^b	18 ± 4^b	41	71*	17*
1,25(OH)$_2$D	17 ± 3^b	24 ± 3^c	24	12	0

	LINEAR GROWTH (cm)		
	U(n)	PC(n)	AC(n)
Placebo	$1.2\pm1.4(6)^a$	$0.9\pm0.4(9)^a$	$5.0\pm1.0(10)^c$
25OHD	$2.1\pm0.4(10)^b$	$2.2\pm0.7(6)^b$	$3.4\pm1.2(10)^c$
1,25(OH)$_2$D	$1.8\pm1.2(13)^{ab}$	$4.2\pm1.6(14)^c$	$4.8\pm1.2(10)^c$

Supplementation with either VD analogue improved U appetite. Increased mortality was noted in controls given 25OHD. A similar growth response was noted in U with each VD analogue.

USE OF MUTAGENESIS TO EXPRESS A NOVEL PHOSPHATE TRANSPORT SYSTEM IN AN ESTABLISHED RENAL CELL LINE. Gary Quamme and Heini Murer*. Dept. of Medicine, University Hospital, University of British Columbia, Canada and Institute of Physiology, University of Zurich, Zurich, Switzerland.

Phosphate (Pi) transport across the apical membrane of opossum kidney (OK) cells is similar to the brush-border membrane of the proximal convoluted tubule, i.e. completely sodium-dependent and greater at pH 7.5 (10.7±0.1 nmole·mg^{-1} prot·5min^{-1}) than pH 6.5 (5.9±0.04). Cloned OK cells (genetically uniform) were treated with ethyl methane sulfonate (EMS, 700 ug/ml, 16 hr) to induce mutagenesis. After a period of 8 days, a positive selection procedure was divised to select cells of interest. As arsenate is a substrate for the Na-Pi cotransporter it was used (100 mM, 1 hr, pH7.5, 3 cycles) to select cells (killing efficiency 99.9% of wild OK cells per cycle) with alternate means of Pi uptake. Survivors were cloned and screened for Pi transport characteristics. One clone expressed Pi transport which was Na-independent and greater at pH 6.5 (3.3±0.1 nmoles·mg^{-1}·5min^{-1}) than 7.5 (0.9±0.2). In addition, Na-independent Pi uptake was inhibited by DIDS (0.8±0.15 at 0.1 mM). The stilbene-sensitive inhibitable transport was not evident in the apical membrane of wild parent OK cells. Residual Na-dependent Pi transport was insensitive to PTH and cAMP which is consistent with the disappearance of PTH sensitive component of Pi transport. These studies provide evidence for a novel apical Pi transporter which is sodium-independent and may transport the acid form ($H_2PO_4^-$) in preference to the alkaline form (HPO_4^{2-}).

RED CELL PHOSPHATE TRANSPORT IN X-LINKED HYPOPHOSPHATEMIA (XLH). N. Senol Runyan*, Georgia State University, Bio. Dept, Atlanta, GA 30303. Edmund Bourke, S.U.N.Y. Health Science Center, Brooklyn, NY.

A "mutant transport allele" has been postulated which implies a transport impairment in cells other than epithelial cells. Studies were therefore undertaken to quantitate Pi transport in red cells of healthy volunteers and a XLH patient using radiolabelled ^{32}P. Results are expressed as millimoles of Pi per kg hemoglobin per hour (mmPi/KgHgb*hr). Values for total Pi uptake in the XLH patient (XLH) and a normal volunteer (C) are 2.53±0.12 and 2.47±0.07, respectively. Total Pi uptake represents that mediated by Band 3, the sodium (Na) dependent pathway and the diffusion pathway. Following inhibition of Band 3 by DNDS, Pi uptake via the Na dependent and diffusion pathways is measured to be 0.48±0.04(XLH) and 0.49±0.03(C). Pi transport via Band 3 and diffusion pathways was measured in a Na free medium without the addition of DNDS; the values are 2.10±0.17 (XLH) and 2.02±0.04(C). When DNDS was added to this medium, values of 0.18±0.01(XLH) and 0.21±0.01(C) are obtained; this reflects Pi transport via the diffusion pathway. Serum from the XLH patient and normal controls was studied to determine its affect on Pi transport. There is no significant difference between the XLH patient and normal controls when either serum is used as the influx media. Thus, red cell Pi transport via all three pathways is not different in XLH when compared with control red cells. The data do not support a circulating factor in the XLH patient which impairs total Pi transport.

CALCIUM EFFLUX AND INFLUX IN THE RAT PROXIMAL TUBULE, THE ROLE OF SODIUM. J. Rothrock* and J. Dominguez. Dept. of Med. Ind U. Med. School and V.A. Med. Ctr. Indianapolis, IN.

The Na^+/Ca^{2+} (Na/Ca) exchanger in the rat proximal tubule (PT) may exchange extracellular Na (Na_o) for intracellular Ca (Ca_i). In that case, Na/Ca exchange may promote Ca efflux and regulate Ca_i homeostasis. Although, Na/Ca exchange may also exchange extracellular Ca (Ca_o) for intracellular Na (Na_i). In this case, Na/Ca exchange may not activate Ca efflux. Yet, in both cases, Na_o substitution may increase Ca_i. To define the orientation of Na/Ca exchange, we measured Ca_i in PT loaded with aequorin, and Ca efflux and Ca influx, in 150 mM Na_o and in Na_o isosmotically reduced to 15 mM in rat PT. The change in Na_o from 150 to 15 mM increased Ca_i from 104± 39 to 175± 48 nM, $p < 0.001$, (mean ± S.D., $n = 22$). Ca efflux was measured as the fractional efflux ratio of experimental and control PT labeled with ^{45}Ca (Ca FER), $n = 5$. When both groups were exposed to 150 mM Na_o, Ca FER was 1.02 ± 0.06. To eliminate the effect of Ca_o on Ca efflux, we then removed Ca_o in both groups, and Ca FER was 1.04 ± 0.12; the subsequent isosmotic reduction of Na_o to 15 mM in the experimental group lowered Ca FER to 0.78 ± 0.12, $p < 0.05$. We then restituted 150 mM Na_o in the experimental group and Ca FER surged to 2.50 ± 0.38, $p = 0.002$. When Na_o is substituted, the fall in $\Delta\mu Na^+$ may reverse Na/Ca: Ca influx at 15 and 600 seconds was 2.3 ± 0.5 and 5.2 ± 3.5 nmoles/mg protein when Na_o was 150 mM, and increased to 3.4 ± 0.9 and 10.6 ± 3.7, respectively, when Na_o was isosmotically reduced to 15 mM, $p < 0.05$ for both points, and for intermediate points at 30, 60, 120, 180 and 300 seconds, $n = 7$. We conclude that in rat PT surges in Ca_i caused by Na_o substitution are due to inhibition of Ca efflux and activation of Ca influx. The results support the notion that in PT Na/Ca exchange may be oriented Na_o/Ca_i, and may participate in Ca_i homeostasis by promoting Ca efflux.

CALCIUM TRANSPORT MECHANISMS IN THE TURTLE BLADDER. S. Sabatini and N.A. Kurtzman, Depts of Internal Medicine and Physiology, Texas Tech Univ HSC, Lubbock, TX.

The turtle bladder is a complex epithelium which transports sodium and secretes protons. The mucosal cell layer consists predominantly of granular (G) cells and mitochondrial-rich (MR) cells. The former are thought to be the sodium transporting cells, while the latter are believed to be the proton secretory cells. We have separated the two cell populations and have shown that G cells contain very high activity of Na-K ATPase, while MR cells have very high levels of NEM-sensitive ATPase. In the present study we examined the Ca transporting properties of these two cell populations. MR cells contain both high and low affinity binding sites for calcium, whereas G cells do not. ATP-dependent Ca transport (Ca-ATPase) is 4-5 fold higher in MR cells as compared to G cells. In MR cells, the calcium ionophore (A23187) increased Ca-ATPase activity, and in G cells it stimulated ATP-independent calcium transport. Verapamil decreased ATP-independent calcium transport in both G and MR cells. These results demonstrate that the calcium transporting properties of turtle bladder epithelial cells are highly subspecialized. The MR cells have high Ca-ATPase activity, whereas, the G cells display ATP-independent calcium transport, likely mediated by a Na/Ca exchanger.

INTRACELLULAR pH AND CALCIUM TRANSPORT IN TURTLE BLADDER. S. Sabatini and N.A. Kurtzman, Depts of Internal Medicine and Physiology, Texas Tech Univ HSC, Lubbock, TX.

In a series of in vitro experiments we examined unidirectional Ca fluxes in the turtle bladder membrane under conditions simulating metabolic acidosis and alkalosis (pH 6.4, 7.4, and 8.4). In some experiments the ionized Ca of the mucosal and serosal baths was kept constant, whereas in others it was not. Serosal acidosis markedly inhibited the mucosa-to-serosa Ca flux, while alkalosis stimulated it. This was true despite the presence of an unfavorable 2 mM Ca gradient. The effect of acidosis on Ca flux was not dependent on sodium transport as it was still seen in the presence of ouabain. When the serosal pH was 7.4 and mucosal pH was lowered to 4.4, a maneuver which inhibits luminal acidification, the mucosa-to-serosa Ca flux was again inhibited. Changing pH had no effect on the serosa-to-mucosa Ca flux. In stripped mucosa, total tissue Ca concentration, as measured by atomic absorption spectrometry, was identical in all the groups regardless of acid-base status. Ca^{45} uptake doubled when buffer pH was varied from 4.4 to 8.4. These results demonstrate that decreased serosal or mucosal pH alters mucosal membrane Ca permeability and suggest that Ca uptake at the mucosal membrane is directly proportional to pH and that Ca extrusion at the serosal membrane changes in parallel with uptake so that the cell calcium concentration remains constant.

CALCIUM TRANSPORT ACROSS THE PARS RECTA OF CORTICAL SEGMENT 2 PROXIMAL TUBULES:ROLE OF IONIC DIFFUSION. Paul Sacks* and James E. Bourdeau. Michael Reese Hospital and Medical Center & University of Chicago, Chicago, Illinois.

The purpose of this work was to evaluate the diffusional component of the net Ca absorptive flux across the pars recta of cortical segment 2 proximal tubules studied under physiologic conditions. Single partes rectae were dissected from rabbit kidneys and perfused in vitro. The bath was an artificial ultrafiltrate of plasma, whereas the perfusate simulated late proximal tubular fluid (high $[Cl^-]$, low $[HCO_3^-]$, no Na^+-cotransported solutes). Transepithelial voltages at the perfusion (ψ_o) and collection (ψ_L) ends were measured simultaneously in doubly-cannulated tubules. Ionized Ca concentrations in perfusates ($[Ca^{2+}]_o$), collectates ($[Ca^{2+}]_L$), and the bath ($[Ca^{2+}]_b$) were calculated from measurements of Ca^{2+} activities made in situ with Ca^{2+}-selective microelectrodes. Epithelial Ca permeability (P_{Ca}) was estimated from the bath-to-lumen movement of ^{45}Ca. The total [Ca] of perfused and collected fluids was measured by continuous-flow microcolorimetry. Under these conditions, $\psi_o = \psi_L = +2.5\pm0.7$ mV. $[Ca^{2+}]_o = 1.85\pm0.01$ mM, $[Ca^{2+}]_L = 1.58\pm0.04$ mM, and $[Ca^{2+}]_b = 1.41\pm0.01$ mM. Using the measured P_{Ca}, $1.85\pm0.32\times10^{-7}$ cm^2/s, we calculated the diffusional component of the net Ca absorptive flux from the mean luminal $[Ca^{2+}]$ and the measured ψ and found that ionic diffusion, 0.104 pmol s^{-1} cm^{-1}, accounted entirely for the measured Ca absorption, 0.081 ± 0.012 pmol s^{-1} cm^{-1}. We conclude that Ca^{2+} diffusion is the primary mechanism of Ca absorption in the pars recta of cortical segment 2 proximal tubules of the rabbit.

NORMAL VARIATIONS OF DIET POTASSIUM INFLUENCE SET-POINT AT WHICH KIDNEYS MAINTAIN SERUM PHOSPHORUS CONCENTRATION. A Sebastian, RE Hernandez,* AA Portale, J Colman,* J Tatsuno,* RC Morris, Jr. University of California, San Francisco, CA.

In six healthy men ingesting a constant diet [27 mmols phosphorus (PO_4), 52 meq K^+/day], we increased diet K^+ within the normal range to 156 meq/day, first with KHCO3 (8 days), then with KCL (8 days), with intervening 8-days of no K^+-supplement (RECOVER). Urine PO_4 decreased promptly on increasing diet K^+ with either K-salt, each inducing a persisting 7-10 mmoles cumulative PO_4 retention. During RECOVER, the retained PO_4 was dumped. Serum PO_4 (mmol/L) was higher during KHCO3 (1.25±0.03) and KCL (1.25±0.03) than during CONTROL (1.17±0.02) and RECOVERY (1.17±0.04) ($p=0.022$: repeated measures ANOVA). Diet K^+-induced increases in serum PO_4 correlated with plasma K^+ increases ($r=+0.64$, $p=0.027$). Serum calcitriol (pg/ml) was lower during KHCO3 (30±3) and KCL (32±4) than during CONTROL (36±4) and RECOVERY (39±3) ($p=0.020$), the changes correlating inversely with those in serum PO_4 ($r=-0.69$, $p<0.02$). ANOVA revealed no significant effect on serum PTH, ionic calcium, urine cAMP, plasma renin, body wt, serum albumin, or creatinine clearance; plasma volume decreased with KCL but not with KHCO3. Thus, normal diet K^+ variations exert an anion-independent regulatory effect on renal handling of PO_4 that influences the set-point at which serum $[PO_4]$ is maintained at a constant diet PO_4. That effect is directionally appropriate and quantitatively sufficient to provide fine modulation of serum calcitriol levels under ordinary conditions of normal diet K^+ variations and normal diet PO_4.

CHRONIC EFFECTS OF PARATHYROID HORMONE (PTH) ON RENAL TUBULE CYTOSOLIC FREE Ca LEVELS ($[Ca^{2+}]_i$): DEPENDENCY ON RETAINED Ca BUT NOT ON SERUM Ca. Jyoti Solanki,* Kong Lau,* Bonnie Eby,* Sally Tan,* Patricia Rodrigues,* and Kai Lau. Michael Reese Hospital & University of Chicago, Chicago, IL.

Acutely, PTH is known to increase $[Ca^{2+}]_i$ in proximal and distal tubules by a process in part dependent on extracellular fluid (ECF) Ca. We evaluated its chronic effects and mechanism of action in stable parathyroidectomized (PTX) Wistar rats by infusing 1.4 units per h x 6d of bovine 1-34 PTH via subcutaneous mini-osmotic pump (Group I) or its vehicle, 2% cysteine HCl (Group II), both fed a normal (0.87%) Ca diet. In group III, PTH was infused, but diet Ca was reduced to prevent Ca retention. In group IV, vehicle was given but diet Ca was increased 4 folds to normalize serum Ca. $[Ca^{2+}]_i$ was measured by the fura-2 technique using dual wavelength excitation and Mn to quench signals from ECF dye. All but group III experienced positive Ca balance. Other data (mean ± S.E) are: (§, $p<0.05$ vs group I)

	PTH (u/h)	Diet Ca (g %)	Ca (mg/dl)	Mean $[Ca^{2+}]_i$ (nM) after Mn 1 min	2 min	3 min
I	1.4	0.87	10.7±0.2	205±21	141±20	104±16
II	0	0.87	6.8±1.6 §	147±16 §	87±15 §	54±15 §
III	1.4	0.43	10.9±0.2	153±10 §	66±6 §	35±7 §
IV	0	3.3	9.4±0.2 §	153±9 §	70±6 §	39±4 §

Conclusions: (1) Similar to acute infusion, chronic low doses of PTH (reproducing primary hyperparathyroidism) also raise tubular $[Ca^{2+}]_i$. (2) These effects, however, depend on a positive external Ca balance instead of serum Ca, corroborating and extending our previous findings that hyperparathyroidism secondary to diet Ca deprivation was associated with a reduced $[Ca^{2+}]_i$. (3) Oral Ca loading increased serum Ca to normal in PTX rats but failed to increase $[Ca^{2+}]_i$ beyond that achieved by a normal diet adequate to produce in Ca retention. (4) $[Ca^{2+}]_i$ is regulated by multiple factors, hormonal, dietary and ionic Ca levels.

CALCIUM-KETOVALIN (KV), A NEW, VERY EFFECTIVE ALUMINUM-FREE INTESTINAL PHOSPHATE BINDER. D. von Herrath*, C. M. Erley*, G. Asmus*, K. Schaefer, Med. Abt. II, St.Joseph-Krankenhaus I, Bäumerplan 24, 1000 Berlin 42, FRG

Hyperphosphatemia and secondary hyperparathyroidism are regular complications in patients (pts) suffering from end-stage renal failure. Aluminum (Al)-containing drugs are widely used to control the serumphosphate (P), but this therapy carries the well-known risk of Al-toxicity. Previously we demonstrated that a mixture of ketoacids (KA) is very effective in lowering increased serum P and serum PTH levels, (ASN 1986). Since recent studies have revealed that the mixture of KA acts as an intestinal P-binder, we became interested in investigating whether KV alone, when provided as calcium salt, would also lower P. In-vitro experiments showed that KV lowered P as efficiently as calcium carbonate ($CaCO_3$): reduction in the solution by 82.6 % and 83.3 %, respectively. Without any additional Al-containing drugs, KV stabilized P in $CaCO_3$-resistent HD pts at 5.6 + 0.3 mg/dl.

In conclusion: 1) KV is a new intestinal P-binder which seems to be as potent as other Al-free P-binders.

2) In-vitro and in-vivo studies show that KV is as efficient as $CaCO_3$ although it provides less calcium.

3) It is of further note that a combination of $CaCO_3$ and KV might be of special clinical value as $CaCO_3$ binds P predominantly when the pH is 2.0 or 5.0, whereas KV reached greatest binding efficiency when the pH is 7.0.

THE ROLE OF BRUSH BORDER MEMBRANE (BBM) PHOSPHOLIPID COMPOSITION AND FLUIDITY IN THE AXIAL HETEROGENEITY OF RAT RENAL PHOSPHATE (Pi) TRANSPORT. P. Wilson*, B. Baird*, and M. Levi. VAMC and UT Southwestern Med. Ctr., Dallas, TX.

Axial heterogeneity of renal tubular Pi transport was expressed at the level of BBM isolated from superficial (SC) and juxtamedullary (JMC) cortex, as BBM Na-Pi cotransport activity was higher in SC (1104 vs 438 pmole/mg/5sec, p<.01). Kinetic measurements revealed a higher Vmax (2759 vs 674 pmole/mg/5sec, p<.01) and a higher Km (111 vs 73 uM, p<.01) in SC. The differences in Pi transport were independent of endogenous PTH activity and dietary Pi. We then determined if differences in BBM fluidity and lipid composition exist, as recent evidence suggests an important role for fluidity in modulating Pi transport. BBM fluidity was increased in SC, i.e. the fluorescence anisotropy of diphenylhexatriene was lower in SC (0.247 vs 0.254, p<.01). The increase in SC-BBM fluidity was caused by a lower sphingomyelin (SPH, 38.9 vs 48.5 molar %, p<.01) and a higher phosphatidylcholine (PC, 21.7 vs 13.5 molar %, p<.01), resulting in a markedly lower SPH/PC molar ratio (1.80 vs 3.68, p<.01). Differences in BBM phospholipid composition and fluidity may therefore play an important role in the axial heterogeneity of renal tubular Pi transport.

DIFFERENTIAL EFFECTS OF pH ON SODIUM-DEPENDENT PHOSPHATE UPTAKE IN OUTER CORTICAL AND OUTER MEDULLARY BRUSH-BORDER MEMBRANE VESICLES. Jennifer Walker*, Tim Yan* and Gary Quamme, Dept. of Medicine, University Hospital, University of British Columbia, Vancouver, B.C., Canada.

Two kinetically distinct sodium-dependent phosphate (Pi) transport systems have been identified in early and late proximal tubules; a high capacity process (G_1) located only in outer cortical tissue, and a high affinity (G_2) in both cortical and outer medullary brush-border membranes. Studies were designed to determine the effect of pH on Pi uptake and on Na^+ interaction of both systems. BBM vesicles were prepared from porcine outer cortical and outer medullary tissue and Pi uptake was performed at 21°C, 4s, voltage-clamped, and $pH_{in}=pH_{out}$, over the pH range 8.0 to 6.0. In G_1, the V_{max} decreased from 6.6±0.1 to 3.8 nmoles·mg^{-1} protein·min^{-1} in stepwise fashion with acidification. Km was unchanged, 4.6±0.2 mM (n=6). The K_D for Na was also not altered (17±3 mM). These results (G_1) are distinct from the high affinity system (G_2) in which the V_{max} decreased from 1.1±0.25 (pH 8) to 0.2±0.1 nmoles·mg^{-1} prot.min^{-1} (pH 6) but the Km decreased 0.30±0.04 to 0.04±0.01 mM, respectively, with the inflection point, 6.85±0.03. The K_D for Na was not altered by pH (48±6 mM, n=11). These data indicate a differential sensitivity of G_1 and G_2 to external pH and support the previous conclusion, based on kinetic data, of two distinct Na-Pi transporters located in early and late proximal tubules. The increase in affinity of G_2 with acidification would be appropriate for a system located in the late proximal tubule.

AIDS-RELATED HYPERCALCEMIC CRISIS: A POTENTIAL MECHANISM. Demetrios Zikos, R.D. Bloch*, J.C. Cheng, H.A. Skopicki*, D.R. Peterson and K.A. Fisher. Univ. of Health Sciences/The Chicago Med. School, Dept. of Medicine and Physiology & Biophysics, and VA Med. Ctr., North Chicago, Illinois.

This study was undertaken to determine the cause of hypercalcemia in a patient with the acquired immunodeficiency syndrome (AIDS). An abberation of calcium regulation has been previously reported, but its pathogenesis is poorly understood. We thus examined the clinical and laboratory data of a 40-year-old man with AIDS who developed hypercalcemic crisis, characterized by severe hypercalcemia (Sca=14.1 mg/dl), acute non-oliguric renal failure (Scr=3.6 mg/dl) and profound lethargy. Biochemical studies, six days after initiation of crisis therapy, revealed that despite hypercalcemia (Sca-ionized=6.8 mg/dl),immunoreactive iPTH, C-terminal PTH, 25-hydroxy-vitamin D and 1,25-dihydroxyvitamin D values were normal. However, urinary cAMP/GFR was markedly elevated, implicating the effect of a parathyroid hormone (PTH)-like substance in the pathogenesis of hypercalcemia. Since no other causes for the hypercalcemia and/or the increase in urinary cAMP/GFR could be elicited by clinical, laboratory and post-mortem findings, it is suggested that the hypercalcemia is directly related to HIV infection. Further studies are needed to elucidate the nature and the origin of the PTH-like substance.

PARATHYROID HORMONE (PTH) INHIBITS B-CELL PROLIFERATION: IMPLICATIONS IN CHRONIC RENAL FAILURE (CRF). Jadwiga M. Alexiewicz*, Marian Klinger*, Thomas O. Pitts, Zbigniew Gaciong*, Mariana Linker-Israeli*, and Shaul G. Massry. Div. Nephrol. Univ. So. Calif., Los Angeles, CA.

B-cell proliferation is impaired in uremia but the mechanisms of this defect are not known. Lymphocytes have receptors for PTH, and it is possible that excess PTH in CRF is responsible for this B-cell defect. We examined T-cell independent B-cell proliferation induced by Staphylococcus aureus Cowan I (SAC) after 5 days culture of lymphocytes from normal subjects and CRF patients. B-cell proliferation in CRF patients was lower (<54% $p<0.01$) than normals. Both 1-34 and 1-84 PTH ($1,2,4,\times10^{-7}$ M) inhibited B-cell proliferation in a dose-dependent manner with the effect being greater with 1-84 PTH (-61%) than with 1-34 PTH (-20%) ($p<0.02$). Also, the effect of PTH was less in CRF patients (-47%) than in normals (-58%), ($p<0.05$). Both forskolin which causes a rise in cAMP levels and calcium ionophore (0.5uM) mimicked the effect of PTH. TPA, an activator of protein kinase C, reversed the effects of both PTH and forskolin. The data show that PTH inhibits B-cell proliferation and this action is mediated most likely by PTH-receptor interaction leading to cAMP production and by the ionophoric property of the hormone. The results are consistent with the notion that inhibition of B-cell proliferation in CRF is at least in part mediated by excess PTH. The data expands the role of PTH in the uremic syndrome.

EFFECT OF ACUTE PHOSPHATE DEPRIVATION ON PHOSPHODIESTERASE (PDIE) AND PARATHYROID HORMONE (PTH) AND FORSKOLIN (FK)-STIMULATED ADENYLATE CYCLASE (AC) ACTIVITIES IN MICRODISSECTED RAT PROXIMAL CONVOLUTED (PCT) AND PROXIMAL STRAIGHT TUBULES (PST). T. J. Berndt*. S. Homma*, A. Yusufi, T. P. Dousa and F. G. Knox, Mayo Medical School, Rochester, MN

The effect of changes in dietary phosphate on PTH or FK-stimulated AC activity was studied. Rats were fed normal (NPD, 0.7% P) or low (LPD, 0.07% P) phosphate diet for 4 days prior to the experiment. In each experiment, 8 rats fed LPD and NPD were anesthetized and acutely TPTX. Two hours after TPTX, the kidneys were perfused and segments of PCT and PST were microdissected.

	Adenylate cyclase (fmol/mm/30 min)		
	Basal	PTH (10 U/ml)	FK (10^{-4}M)
PCT			
NPD	28.8±4.6	420.4±68.2†	386.2±73.0†
	NS	NS	NS
LPD	27.2±3.5	367.6±58.2†	353.4±45.8†
PST			
NPD	13.2±5.1	52.1±14.5†	122.2±22.2†
	NS	NS	p<.05
LPD	10.1±1.7	37.4±8.3†	65.9±15.4†

†p<.05, Basal vs stimulated.

PDIE activities in PCT were 39.1±2.6 in NPD rats and 49.1±2.7 fmol/mg/min, p<.05) in LPD rats. PDIE activity in PST was 10.7±1.4 in NPD rats and 9.8±0.8 fmol/mg/min in LPD rats. We conclude that increased PDIE in PCT and blunted AC response in PST in LPD rats may contribute to the altered regulation of phosphate reabsorption by PTH in acutely phosphate deprived rats.

METABOLIC ACIDOSIS STIMULATES PARATHYROID HORMONE (PTH) SECRETION, WHICH CONTRIBUTES TO THE RENAL RESPONSE AGAINST ACIDOSIS. M.Bichara*, O.Mercier*, and M.Paillard. Lab. Physiologie Renale, Hôp. L.Mourier, 92 Colombes, and INSERM, Paris, France.

We have recently shown that PTH acutely stimulates urinary acidification (AJP 251:F444,1986). The aim of the present study was to determine whether an acid load stimulates PTH secretion. Intact, adrenalectomized (ADX), and ADX-thyroparathyroidectomized (ADX-TPTX) rats were studied during 3-h HCl-loading (30 nmolH^+/min/g body wt). Metabolic acidosis was associated with increases in plasma iPTH concentration in both intact (from 21.4±3.2 to 30.6±3.5 pg/ml,P< 0.05) and ADX rats (from 22.0±1.7 to 39.1±4.4 pg/ ml,P<0.05), despite a significant increase in the plasma ionized calcium concentration in these animals. Plasma iPTH concentration remained stable in time-control rats and decreased from 21.8 ±1.0 to 14.5± 1.5 pg/ml (P<0.05) in $CaCl_2$-infused non-acidotic rats. Acidosis-induced increases in fractional excretion of phosphate and in titratable acid and ammonium excretion rates were observed in intact and ADX rats, but not in ADX-TPTX rats. Net acid excretion increased 43% in intact (from 1,281±73 to 1,837±205 pmol/min,P<0.001) and 56% in ADX rats (from 727±72 to 1,132±31 pmol/min, P< 0.001) after 3-h HCl-loading, but not in ADX- TPTX rats (from 614±49 to 739±53 pmol/min, NS). We conclude 1) that acute metabolic acidosis stimulates endogenous PTH secretion ; 2) that PTH markedly contributes to the renal response against metabolic acidosis ; and 3) that the latter is blunted in the absence of adrenal and parathyroid glands.

CHARACTERIZATION OF THE 1,25-$(OH)_2$D RECEPTOR IN CULTURED BOVINE PARATHYROID CELLS. A.J. Brown*, C. Ritter*, E. Slatopolsky. Renal Division, Washington University School of Medicine, St. Louis, MO.

Several laboratories, including ours, have shown that the 1,25-$(OH)_2$D receptors are decreased in extracts from parathyroid glands of uremic patients and experimental animals. In order to determine the factors involved in this regulation, we have examined the receptor in cultured bovine parathyroid cells. Cytosols isolated from confluent cells contained high amounts of receptor (60 to 100 fmol/mg cytosolic protein), whereas cytosols from sub-confluent cells contained no detectable receptor, despite the fact that 10 nM 1,25-$(OH)_2$D suppressed PTH secretion by 40%. Inclusion of the protease inhibitors, diisopropylfluorophosphate and Trasylol, and receptor stabilizer, sodium molybdate, in the homogenization buffer had no effect. Also, mixing of sub-confluent cells with confluent cells before homogenization did not decrease receptor binding suggesting the absence of protease activity in the rapidly growing cells. Finally, it is unlikely that the lack of binding in cytosols from subconfluent cells is due to receptor occupancy, since the concentration of 1,25-$(OH)_2$D in the medium is 5 times less than the Kd. On the other hand, intact cells incubated for 2 h with 0.5 nM [^{3}H]-1,25$(OH)_2$D showed specific binding of this label (20 to 25 fmol/mg total cell protein) regardless of the cell density. The reason for this discrepency between the intact cell and broken cell assays for receptor in the rapidly growing cells requires further investigation.

CALCITRIOL METABOLISM IN PATIENTS WITH CHRONIC RENAL FAILURE. B Buchsbaum, S Patel*, C H Hsu. Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

Calcitriol metabolism is poorly understood in chronic renal failure (CRF). In this experiment, we attempted to study calcitriol metabolism in age and sex matched Caucasian patients with CRF and normal subjects. Patients with diabetes, nephrotic syndrome, cirrhosis of liver, and endocrinological diseases were excluded from the study. Group 1 (n=4) was normal subjects (creatinine clearance, Ccr= 122 ± 9.7 ml/min). Group 2 (n=7) was patients with CRF (Ccr=35.1 ± 6.1). Group 3 (n=3) was patients on hemodialysis (serum creatinine=14.2 ±.4 mg/dl). Plasma levels of calcitriol were significantly lower in gr. 3 (4.5 ± .20 pg/ml, $p < 0.001$), and slightly lower in gr, 2 (23.6 ± 3.3 pg/ml, p=0.06) when compared to gr. 1 (34.6 ± 3.7 pg/ml). Calcitriol production rates (PR) were significantly reduced in gr. 2 (15.3 ± 2.1 ng/kg/day, $p < .01$) and gr. 3 (4.7 ± .7, $p < .01$) patients in comparison to gr. 1 (29.5 ± 4.7). Metabolic clearance rates (MCR) of calcitriol were significantly decreased in Gr. 2 (0.45 ± .01 ml/min/kg, $p < 0.02$), but they were not decreased in gr. 3 (0.73 ± .12, vs normal subjects, 0.58 ± .03). Since calcitriol can induce its own degradation enzymes, MCRs of calcitriol were measured again in group 2 patients after they had received orally 1 ug of calcitriol daily for two weeks. The MCRs of calcitriol were normalized in two patients (pre, 0.44 and 0.45 to post, 0.61 and 0.53, respectively) and remained unchanged in the other 5 patients. Plasma concentrations of calcitriol were slightly increased after receiving oral calcitriol (30.2 ± 4.2 pg/ml, p=ns)). We conclude that PR and MCR of calcitriol are decreased in patients with moderate renal failure, but the MCR remained normal in patients on hemodialysis despite a decreased PR of calcitriol.

INTERMITTENT INTRAVENOUS 1-ALPHA-HYDROXYCHOLECALCIFEROL REDUCES INTACT PLASMA PARATHYROID HORMONE IN PATIENTS WITH END STAGE RENAL FAILURE ON LONG-TERM HEMODIALYSIS. David Carmichael* & Barry Hume* (Introduced by Robert Berliner). Renal Unit, St. Mary's Hospital, London, UK.

Oral 1-alpha-hydroxycholecalciferol (1-alpha-VitD) has been used to suppress circulating levels of parathyroid hormone in patients with end stage renal failure (ESRF) on intermittent hemodialysis. We have administered 1-alpha-vitD intravenously to 10 patients after each twice-weekly dialysis for a total of 2-12 weeks. Three patients were withdrawn from the study at 2, 5 and 6 weeks for renal transplantation; one patient was withdrawn because of an odd taste in the mouth. There were no other adverse effects. The dose of 1-alpha-vitD was increased from 0.5 µg after each dialysis to maintain the serum calcium concentration near the top of the normal range (2.10-2.65 mmol/l); a maximum of 6 ug was given and the mean dose after each dialysis at week 12 was 3 µg (range 1-6 µg). The dose was reduced if hypercalcemia occurred. By week 3 there was a significant rise in serum calcium concentration (2.02±0.05 to 2.36±0.06 mmol/l; mean±SEM; $P<0.001$) and this was maintained at week 12 (2.47±0.09 mmol/l). Plasma concentrations of intact parathyroid hormone (iPTH) were significantly reduced by week 8 (55±22 to 27±21 pmol/l; $P<0.05$) and remained suppressed at week 12 (15±8 pmol/l).

We conclude that intermittent intravenous 1-alpha-vitD can suppress iPTH in patients with ESRF on hemodialysis.

EFFECTS OF CHRONIC METABOLIC ACIDOSIS ON $1,25(OH)_2D_3$ RESPONSE TO A LOW PHOSPHORUS DIET. D.A. Bushinsky, C. Nalbantian-Brandt* and M.J. Favus*. Univ. of Chicago, Chicago, IL.

During dietary calcium restriction chronic metabolic acidosis (CMA) increases arterial blood ionized calcium (Ca^{++}), which in turn reduces 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] production and serum levels. To determine whether the increase in Ca^{++} due to CMA would prevent stimulation of $1,25(OH)_2D_3$ by dietary phosphorus restriction, rats were fed either a normal (NPD, 0.65%) or low (LPD, 0.1%) phosphorus diet for 10 days. Ammonium chloride (NH_4Cl) was added (1.5%) to the drinking water of some rats (CMA) while others served as non-acidemic controls.

LPD increased serum $1,25(OH)_2D_3$ levels from 63 + 10 pg/ml to 193 + 10 (mean + SE, $p < 0.001$) in the absence of CMA. CMA did not affect the increase of $1,25(OH)_2D_3$ in response to LPD (LPD+CMA, 194 + 30, p = NS vs. LPD and $p < 0.001$ vs. NPD). LPD decreased serum phosphorus and increased Ca^{++}. Serum $1,25(OH)_2D_3$ levels were correlated inversely with serum phosphorus ($r = -0.657$, $n = 31$, $p < 0.001$) and directly with Ca^{++} ($r = 0.618$, $n = 27$, $p < 0.001$). Using stepwise linear regression the correlation between phosphorus and $1,25(OH)_2D_3$ accounted for the majority of the variance contributed by both phosphorus and Ca^{++} ($F = 12.30$, $p < 0.001$).

Thus, increased Ca^{++} during CMA does not inhibit the rise in serum $1,25(OH)_2D_3$ during LPD, indicating that phosphate restriction can overcome the inhibitory action of Ca^{++} on $1,25(OH)_2D_3$. Serum phosphorus appears to be a more potent regulator of $1,25(OH)_2D_3$ than Ca^{++} during CMA in the rat.

Mechanism of Hormonal Regulation of 25-Hydroxyvitamin D_3-1 alpha-Hydroxylase in Cultured Proximal Tubular Cells. Tai C. Chen, Norman P. Curthoys*, Christine A. Leone* and Jules B. Puschett. University of Pittsburgh, School of Medicine, Pittsburgh, PA.

The mechanism by which hormones stimulate the 25-hydroxyvitamin D_3-1 alpha-hydroxylase (1αH) activity was studied in cultured proximal tubular cells. The addition of parathyroid hormone (PTH), prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) caused a 2 to 6 - fold induction of 1αH activity. However, this stimulation occurs following a significant time lag of 3 to 6 hours. In contrast, the same concentrations of hormones have a rapid effect on intracellular cAMP concentration. The two prostaglandins produced very similar dose - dependent increases in cAMP accumulation as well as 1αH activity. Similarly, 8 - (4-chlorophenylthio) - cAMP elicited a 6 - fold induction of the enzyme. A time lag of 3 to 6 hours was also observed before the cAMP analogue initiated its effect. When cycloheximide, a translation inhibitor,or actinomycin D, a transcription inhibitor, was added to cultures at the time of hormone addition, the stimulatory effect of the hormone or the cAMP analogue was almost completely blocked. The results suggest that (1) cAMP may be the second messenger for the stimulatory effect of hormones on renal 1αH activity (2) PGE_1 and PGE_2 may act on the same receptor and (3) new protein synthesis may be required for the induction of 1αH by PGE_1 and PGE_2.

ORAL CALCITRIOL: AN EFFICIENT THERAPY FOR SECONDARY HYPERPARATHYROIDISM (HPT). G.A. Davidai*, B. Lobaugh*, S.J. Schwab, L.D. Quarles. Duke Univ. Med. Ctr., Depts. of Med. & Surg., Durham, N.C.

Attaining a normal serum calcitriol concentration without the occurrence of hypercalcemia may offer an effective means of treating secondary HPT in patients with end stage renal disease (ESRD). We examined the effect on parathyroid function of orally administered calcitriol in doses sufficient to normalize the serum $1,25(OH)_2D$ and compared these data to those obtained from similar patients subjected to parathyroidectomy (PTX). Eight subjects with ESRD and significant HPT were treated daily with calcitriol (0.64±0.06 ug, P.O.) and calcium carbonate (2.6±0.6 gm) with meals. This regimen normalized the serum calcitriol (21±1.4 vs 6.7± 1.7 pg/ml) and increased ionized calcium (1.27± 0.04 vs 1.10±0.27 mmol/L) concentrations compared to pretreatment values; normal serum phosphorus concentrations were maintained on reduced amounts aluminum hydroxide. After 15 months of therapy, a 71% reduction in the PTH concentration had occurred ($p<0.05$), a decrement similar to that reported with intravenously administered calcitriol (Slatopolsky et al, J Clin Invest 74:2136-2143, 1984). PTH concentrations were effectively normalized in 5 subjects (64±6.4 pg/ml). This degree of control compares favorably with that obtained following PTX (65±31 pg/ml). HPT may be effectively controlled in a subset of patients with ESRD without significant hypercalcemia by combined treatment with oral calcitriol and calcium carbonate. This salutary effect may result from direct actions of calcitriol on the parathyroid gland and/or gastrointestinal calcium absorption, or from a more generalized modulation of calcium homeostasis.

METABOLIC CLEARANCE RATE AND PRODUCTION RATE OF CALCITRIOL IN UREMIA. A. Dusso*, S. Lopez Hilker*, J. Lewis -Finch*, P. Grooms*, A. Brown*, K. J. Martin and E. Slatopolsky. Washington University Medical School. St. Louis MO.

We have previously shown that while both normal humans and dogs tightly control serum calcitriol levels after 25(OH)D administration, anephric humans and 5/6 nephrectomized dogs significantly increase circulating $1,25(OH)_2D$ when supraphysiological concentrations of 25(OH)D are reached in serum. Since plasma $1,25(OH)_2D$ levels are determined not only by its rate of production but also by its rate of degradation, we measured metabolic clearance rate (MCR) and production rate (PR) of $1,25(OH)_2D$ in normal dogs and in dogs with both moderate and severe renal failure, at normal and elevated concentrations of 25(OH)D. We used the single bolus injection method of Seeman et al, (J.C.I. 66:664,1980). Basal MCR in uremic dogs, either with moderate or with severe renal failure, did not differ significantly from normals. (6.7 ± 0.7 n=4; 6.8 ± 0.4 n=8; 6.8 ± 0.4 n=7 ml/min respectively). Oral 25(OH)D administration for two weeks decreased MCR of $1,25(OH)_2D$ in normal animals to 5.8 ± 0.3 ml/min. No significant differences in MCR were found in both groups of uremic dogs. 25(OH)D treatment did not alter production rates in normal dogs and in animals with moderate renal failure (basal $1,25(OH)_2D$ levels not significantly different from normal) but significantly increased $1,25(OH)_2D$ production from 0.13 ± 0.01 to 0.25 ± 0.04 μgm/day ($p<0.05$) in dogs with severe renal insufficiency. (Basal $1,25(OH)_2D$ levels significantly lower than normal.) These data show that in normal and uremic dogs 1) Serum $1,25(OH)_2D$ depends upon its production rate and 2) high levels of 25(OH)D stimulate PR of $1,25(OH)_2D$ only when serum $1,25(OH)_2D$ is below normal. This suggests that physiological levels of $1,25(OH)_2D$ regulate its own production.

HUMAN PROXIMAL RENAL CELLS IN CULTURE: HORMONAL EFFECTS ON PHOSPHATE TRANSPORT V. W. Dennis, D.A. Sens, J. P. Middleton, Duke Univ., Durham, NC, and Medical Univ. of South Carolina, Charleston, SC.

Sodium-dependent phosphate uptake was characterized in proximal renal cells derived from six individual human kidneys. More than 90% of phosphate uptake was sodium-dependent. Saturation kinetics revealed two transporters, one with a Vmax of 3.4 nmol/mg protein/3 min and an apparent Km for phosphate of 0.08 mM at 140 mM sodium, and one with a Vmax of 11.0 nmol/mg protein/3 min and a Km of 0.63 mM PO4. PTH, prostaglandin E2 and beta-selective adrenergic agonists increased cyclic AMP production in a dose-dependent manner but alpha-adrenergic agonists or calcitonin did not. PTH did not inhibit phosphate uptake at either 0.2 or 2.0 mM phosphate, concentrations designed to emphasize the higher or lower affinity transporter, or over a time course of 15-120 minutes in spite of activation of cyclic AMP-dependent protein kinase. Beta agonists and PGE2 also lacked effects on phosphate uptake. These data demonstrate that PTH inhibition is not a universal feature of sodium-dependent phosphate uptake in the proximal tubule, and that some factor in addition to activation of the cyclic AMP pathway is required.

THE PRESENCE OF CALCITONIN (CT) DECREASES THE CALCEMIC RESPONSE TO PARATHYROID HORMONE (PTH). A. Felsenfeld, M. Rodriguez, R. Dunlay*, L. Pederson*, and F. Llach. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, Okla.

A decreased calcemic response is present in renal failure. However, whether endogenous CT production modifies the magnitude of this response has not been studied. To evaluate this question, 2 groups of rats were treated identically except Group I had a total thyroparathyroidectomy (TPTX) and was given thyroxine, and Group II had a total PTX. In both groups, parathyroid glands were autotransplanted (Tx) at surgery. The adequacy of autoTx was verified by a serum calcium (Ca) > 9 mg/dl after 5 days of a zero Ca diet; then a 2-stage 5/6 nephrectomy was performed. Rats were then placed on a low Ca, high phosphate (P) diet for 12 days to produce 2° hyperparathyroidism. After a 24 hr fast, rat 1-34 PTH was infused via an Alzet pump at 1.5 U/hr for 48 hrs. During the infusion, rats were placed on a zero Ca, low P diet, and serum Ca and P (mg/dl) measured.

	0 hrs		24 hrs		48 hrs	
	Ca	P	Ca	P	Ca	P
I	8.3±.6	11.1±.8	15.2±.6	7.8±.3	16.7±.5	7.2±.3
II	7.9±.7	10.9±.1	10.4±.3*	5.2±.4*	13.0±.4*	6.5±.4

X±SE * P<.01 vs Group I. The serum creatinine was not different between the 2 groups at each time.

In summary, in rats with renal failure receiving exogenous PTH: 1) the magnitude of hypercalcemia was greater in the absence of CT; and 2) the serum P was higher in the absence of CT. In conclusion, 1) CT may be an important factor in the calcemic response to PTH; and 2) these results cannot be due to differences in serum P.

EXTRARENAL PRODUCTION OF CALCITRIOL IN NORMAL AND UREMIC HUMANS. J.Finch*, A.Dusso*, C.Ritter*, J.Delmez, G.Schreiner and E. Slatopolsky. Renal Div. Washington Univ. St.Louis. MO.

We have previously reported 1-alpha-hydroxylase activity in anephric humans. Since human alveolar macrophages are known to synthesize $1,25(OH)_2D$ when stimulated with γ-interferon or lipopolysaccharide, we measured 1-alpha-hydroxylase activity in adherent cells derived from peripheral blood mononuclear cells of 7 normal, 2 uremic and 5 anephric humans. Ficollpaque-purified mononuclear leukocytes were plated at a concentration of 10^7 cells/ml. After 18 h plates were washed to remove non adherent cells. Adherent cells were incubated with tritiated 25(OH)D (0.1 μCi) for 1 h at 37°C. Tritium coeluting with authentic $1,25(OH)_2D$ using HPLC was quantitated. (Values for each individual were measured in triplicate). In contrast to other reports, macrophages from normal humans were able produce a metabolite with the chromatographic properties of $1,25(OH)_2D$ in normal and reverse phase HPLC. The amount of tritiated $1,25(OH)_2D$ synthesized was 525 ± 50 cpm/h.well (Mean ± SEM). A six fold increase over normal was found in the two uremic patient studied (2915 ± 93 and 3967 ± 603 cpm/h.well respectively). The range for $1,25(OH)_2D$ production varied from 1308 ± 78 to 4849 ± 206 cpm/h.well in anephric individuals (2 to 8 fold over normals). These results demonstrate the capacity of macrophages from normal individuals to synthesize $1,25(OH)_2D$ and suggest that uremia acts as a stimulating factor to enhance this extrarenal site for 1α-hydroxylation. The mechanisms operating to determine the wide range of 1α-hydroxylase activity observed in anephric individuals and, therefore, a variable contribution to serum calcitriol levels should be further explored.

SUPPRESSION OF GROWTH HORMONE RELEASE ENHANCES THE PHOSPHATURIC RESPONSE TO PARATHYROID HORMONE IN IMMATURE RATS. A. Haramati, S.E. Mulroney*, and M.D. Lumpkin*. Dept. of Physiol., Georgetown Univ. Med. Sch., Washington, D.C.

Immature rats display a blunted rise in excretion of phosphate (Pi), but not cyclic AMP, in response to parathyroid hormone (PTH), perhaps as a consequence of the increased demand of the young rat for Pi during growth. To examine this issue, we tested whether chronic treatment with an antagonist to growth hormone (GH)-releasing factor (GRF-AN: [N-AC-TYR^1-D-ARG^2]-GRF-(1-29)-NH_2), would alter the renal response to PTH. We recently reported that GRF-AN suppresses the periodic surges of GH release and decreases somatic growth. Silastic catheters were placed in 10 immature (24-28 days old) Wistar rats and either GRF-AN (100 ug/kg) or saline alone was injected twice daily. After 2 days, clearance experiments were performed to determine the effect of increasing doses of synthetic rat PTH on the fractional excretion (FE) of Pi. * = $P<0.05$ compared to control rats.

	PTH dose (ug/100g.hr)			
FEPi%:	0	1.5	5	15
Controls	0.04 ±0.02	0.5 ±0.5	9.1 ±2.2	14.0 ±2.7
GRF-AN	0.04 ±0.01	5.3* ±2.2	25.7* ±1.0	39.1* ±4.5

FEPi was markedly enhanced in immature rats treated with GRF-AN, in which the release of GH and the rate of growth are suppressed. Accordingly, the attenuated phosphaturic response to PTH in developing rats is not due to immaturity, but rather may reflect an adaptation that facilitates the renal retention of Pi during growth.

REGULATION OF PTH MESSENGER RNA LEVELS IN PARATHYROID GLANDS OF CHRONIC RENAL FAILURE IN RATS. Masafumi Fukagawa*, Shinya Kaname*, Tetsuya Igarashi* and Kiyoshi Kurokawa. IVth Dept Int Med, Univ Tokyo Faculty Med, Tokyo.

Secondary hyperparathyroidism (2°HPT) is one of the major factors underlying bone diseases of chronic renal failure (CRF). In an effort to understand further the pathogenesis of 2°HPT of CRF, we studied the transcriptional regulation of PTH gene, the initial step of PTH production in CRF rats. CRF rats were prepared by 5/6 nephrectomy. Four weeks after 5/6 nephrectomy, parathyroid and thyroid glands were removed and total RNA was extracted by guanidium isothiocyanate method followed by CsCl centrifugation. The PTH mRNA levels were measured by primer extension using synthetic oligonucleotide complementary to the sequence between 50th and 79th nucleotides of rat PTH mRNA: actin mRNA was taken as a reference. Steady-state PTH mRNA levels were elevated at 4 wks following 5/6 nephrectomy. When 100 pmole or more 1,25-dihydroxyvitamin D_3 (1,25D) was given ip at 24 and 48 hrs before sacrifice, elevated PTH mRNA levels became suppressed in a dose-dependent manner. Interestingly, 24,25-dihydroxyvitamin D_3 (24,25D) given ip also suppressed PTH mRNA levels, but the dose of 24,25D required (500 pmole) was greater than that for 1,25D. These observations document elevated PTH mRNA levels in parathyroid glands in CRF and indicate the both 1,25D and 24,25D suppress elevated PTH mRNA in CRF. Data suggest the possibility that decreases in these vitamin D metabolites in CRF may play a critical role in the pathogenesis of secondary hyperparathyroidism of chronic renal failure.

Effect of the sympathetic nervous system on secretion of parathyroid hormone (PTH) in humans

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As earlier studies in cows have shown, acutely infused catecholamines (CA) induced an increased PTH secretion (J. Fischer et al., 1973). Therefore, we investigated the response of intact PTH (1-84) under various conditions of catecholamine excess and during infusion of atropine in 10 normal volunteers (age 20 to 29 years). In recumbent subjects following drugs in increasing doses were infused: norepinephrine (NE), epinephrine (E), and atropine in constant dosis, respectively. Equilibration period lasted 2 hr before infusion of next drug was started. On the following day subjects underwent aerobic and anaerobic physical exercise (tread mill). Blood was drawn for measurements of CA, PTH, and calcium (Ca) in plasma before and after each infusion period or during physical exercise.

NE-infusion had a decreasing effect on secretion of PTH (6.0 ± 1.5 vs 5.4 ± 1.8 pmol/l) and no changes of Ca (2.18 ± 0.16 vs 2.19 ± 0.6 mmol/l) were observed. During E-infusion PTH-values did not increase (3.3 ± 0.8 vs 3.4 ± 0.8 pmol/l) but 15 min after ending of infusion PTH rose up significantly (5.4 ± 1.3 pmol/l). Atropine did not influence PTH-secretion (4.1 ± 0.9 vs 5.1 ± 1.0 pmol/l).

In contrast, maximal physical exercise induced a slight but significant increase of PTH (4.3 ± 1.3 vs 5.6 ± 1.6 pmol/l) and of calcium (2.3 ± 0.1 vs 2.6 ± 0.1 mmol/l).

Our results showed a different response of PTH-secretion during exogenous and endogenous Ca-excess. Because of the slight increase of PTH under physical exercise we conclude that sympathetic nervous system plays only a minor role in secretion of PTH.

VITAMIN D METABOLITES INCREASE CYTOPLASMIC CALCIUM ION CONCENTRATION IN CULTURED PROXIMAL STRAIGHT TUBULE CELLS. Y Kawaguchi*, M Suzuki*, and T Miyahara* (Intr by K Kurokawa). 2nd Dept Int Med, Jikei Univ Sch Med, Tokyo.

Vitamin D metabolites exert acute and chronic influences on proximal tubule function. To further evaluate vitamin D action on the kidney, immediate effects of vitamin D metabolites on cytoplasmic calcium ion concentration ([Ca]c]) were examined in cultured proximal straight tubule cells of rabbit kidney. Fluorescence image of fura-2 was constracted for spacial analysis of [Ca]c. 1,25-dihydroxyvitamin D_3 (1,25D) and 25-hydroxyvitamin D (25D) evoked a transient rise in [Ca]c and 24,25-dihydroxyvitamin D (24,25D) caused a protracted rise in [Ca]c. These effects of vitamin D metabolites were dose-dependent. [Ca]c transient evoked by 1,25D, but not by 25D or 24,25D, was abolished in Ca-free media. Pretreatment of cells with caffeine (4 mM) to deplete Ca store of endoplamic reticulum (ER) or with TMB-8 (5 mM) to block Ca release from ER, both blunted the effect of 25D on [Ca]c, but not of 24,25D. 24,25D-induced protracted rise in [Ca]c was not diminished in the combination of Ca-free solution and pretreatment with TMB-8. In tubule cells pretreatment with mitochondrial uncouplers, which induced a rapid rise in [Ca]c, further rise in [Ca]c was not observed in response to both 25D and 24,25D. The data show that three vitamin D metabolites increased [Ca]c of proximal tubule cells and suggest that the effect is primarily via increased Ca influx with 1,25D, Ca release from ER with 25D, and possibly by Ca release from mitochondria by 24,25D.

CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN KINASE INHIBITOR (H-8) SUPPRESSES PARATHYROID HORMONE (PTH)-STIMULATED RISE OF CYTOSOLIC FREE CALCIUM CONCENTRATION ($[Ca^{2+}]_i$) IN SINGLE RABBIT CONNECTING TUBULES (CNT) Kai Lau and James E. Bourdeau. Michael Reese Hospital and University of Chicago, Chicago, Illinois.

We recently showed that 0.1-nM synthetic bovine PTH and 1-mM 8-Br-cAMP produce a sustained, reversible, and medium Ca-dependent increase in $[Ca^{2+}]_i$ in isolated rabbit CNT. Both PTH and dibutyryl-cAMP increase lumen-to-bath Ca flux in this nephron segment. These findings implicate cAMP as an intracellular messenger in these PTH-mediated effects. To investigate possible cellular mechanisms we measured the PTH-induced increase in $[Ca^{2+}]_i$ in the presence of H-8. Prior and/or concomitant exposure to 0.5-mM H-8 abolished the rise in $[Ca^{2+}]_i$ produced by 0.1-nM PTH in five CNT and suppressed it ~50% in another five. In the latter the rise in $[Ca^{2+}]_i$ was delayed. In contrast to the prompt and significant PTH-stimulated increase in $[Ca^{2+}]_i$ in controls, there was no increase in $[Ca^{2+}]_i$ during the 15 min of PTH administration in H-8-pretreated tubules as long as the inhibitor also was present. However, when H-8 was withdrawn, $[Ca^{2+}]_i$ rose immediately to reach a plateau value within a few minutes, indistinguishable from the maximal uninhibited, but earlier, response to PTH in controls. Thus, the inhibition by H-8 was rapidly reversible.

We conclude that a cyclic nucleotide-dependent protein kinase plays an important role in the PTH-stimulated rise in $[Ca^{2+}]_i$ in rabbit CNT. Since the increase in $[Ca^{2+}]_i$ is dependent on extracellular Ca and mimicked by 8-Br-cAMP, we propose that cAMP-dependent protein phosphorylation is an essential intermediate step in mediating PTH-stimulated Ca fluxes across plasma membranes of CNT cells.

UTILITY OF FREE $1,25(OH)_2D3$ (FD) MEASUREMENTS IN RENAL FAILURE (RF) PATIENTS (PT). K G Koenig*, J S Lindberg*, J E Zerwekh, P K Williams*, H M Cushner, J B Copley. Brooke Army Med Ctr, San Antonio TX, Southwestern Med Sch, Dallas TX, Ind Univ Med Sch, Indpls IN.

PT with various degrees of RF have low serum levels of total $1,25(OH)_2D3$ (TD); however it is unknown whether the true physiologic activity of the vitamin might better be reflected by measurement of the FD fraction. To investigate this issue we measured TD, Vitamin D Binding Protein (DBP), and FD levels in 3 groups of age, race and sex matched individuals: Grp 1 normal controls, Grp 2 PT with chronic RF (GFR 12 to 60 cc/min), and Grp 3 PT with ESRD treated with hemodialysis. None were pregnant, taking vitamin D supplements or estrogens, had liver dysfunction or nephrotic syndrome, or were malnourished. No PT had acidosis (HCO_3<18) or hypophosphatemia. Grp 2 and 3 PT received Ca^{++} and PO_4 binders in customary fashion.

Data (mean ± SEM) are below:

GRP	TD(pg/ml)	DBP(ng/ul)	%Free	FD(fg/ml)
1	29±8	787±76	0.63±0.03	193±0.05
2	13±3	929±61	0.58±0.04	63±0.01
3	4.6±2*	761±85	0.62±0.03	28±0.01*
N	5	6	10	5

*Significantly different from normal (P<0.05)

Both TD and FD decreased with decreasing renal function, while DBP and %Free did not. DBP levels were not altered in this PT population. TD and FD levels were highly correlated (r=0.968, P <0.0001). We conclude that in these PT, TD accurately reflects vitamin D status.

REMOVAL OF BIOLOGICALLY ACTIVE PARATHYROID HORMONE FROM BLOOD IN VIVO BY IMMUNOEXTRACTION. S. Lopez-Hilker*, K.J. Martin, N. Rapp*, G. Bruno* and E. Slatopolsky. Washington University School of Medicine, St. Louis, Missouri and Bio Sorb Inc. New Jersey.

Secondary hyperparathyroidism is a frequent complication of renal insufficiency. Multiple therapeutic approaches are required in order to prevent serious complications. In the present studies we have developed an immunoextraction procedure for the removal of large amounts of biologically active PTH from blood. Anti-PTH antibodies were purified from a goat antiserum by affinity chromatography on a human PTH 1-34 conjugated agarose column. The purified antibodies had a high affinity (10^{-10}M) for human PTH 1-34 by Scatchard analysis. The purified antibodies were then coupled to cyanogen bromide activated Sepharose. The Sepharose coupled antibodies retained their high affinity for human PTH. This solid phase reagent was incorporated into a perfusion cartridge for testing in vivo. Studies were performed in normal dogs under anesthesia, during a constant infusion of human PTH 1-34, which achieved a concentration of bioactive PTH of 0.6-1.2 ng/ml. The cartridge was perfused with blood from one jugular vein and returned to the other, at flow rates of 50-100 ml/minute. Blood samples were obtained prior to and after the antibody cartridge and assayed for immunoreactive PTH. Extraction of immunoreactive PTH across the cartridge ranged from 20 to 60% and was maintained for the duration of the experiment. These data demonstrate that immunoextraction of biologically active PTH from blood is feasable and effective. Such techniques may be useful adjuncts for the control of hyperparathyroidism in hemodialysis patients.

DECREASED SUPPRESSION OF PTH SECRETION BY LOW DOSE CALCIUM INFUSION IN CHRONIC RENAL FAILURE (CRF).

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Hyperparathyroidism in CRF may result from reduced suppressibility of the parathyroid gland by calcium but the stage at which this occurs is unclear. To address this question, the effect on serum PTH of physiologic increases in serum calcium (Ca) was determined by i.v. infusions of Ca (1.25 mg/Kg in 30 min) in 8 subjects with mild to moderate renal failure (MRF) [GFR by 51-Cr EDTA clearance: 53.5 ± 8.0 ml/min/1.73m^2 (mean ± SEM)], 7 on hemodialysis (HD), 5 with primary hyperparathyroidism (PH) and in 5 normal subjects (N). Intact PTH in pmol/L was determined by two-site immunochemiluminometric assay (R.C.Brown et. al. JCEM: 65, 407, 1987). During infusion, total Ca rose 0.22 ± 0.02; ionized Ca, 0.12 ± 0.02 mmol/L, with no difference between the groups. Hypercalcemia was observed in PH but in no other group. PTH fell in every subject during Ca infusion.

PTH (pM)	N	MRF	HD	PH
baseline	2.2±0.3	5.3±0.9[a]	27.1±7.4[b]	12.1±2.3[b]
+ calcium	<0.3±0.1	1.4±0.3[a]	14.6±6.2[b]	5.6±1.7[b]
% decrease	>85.2±5.6	74.2±3.5	55.6±7.8	55.7±6.2

a: $p<0.005$ vs N; b: $p<0.001$ vs MRF, $p<0.01$ vs N; (2 tailed Mann-Whitney U test). In conclusion, parathyroid tissue responded appropriately to i.v Ca in all groups but was completely suppressed only in 4 of the 5 normals. Fractional suppressibility decreased with increasing severity of CRF and in HD subjects was reduced to the degree found in PH. Although basal PTH levels were mostly within the normal range in MRF, basal and post-Ca PTH were significantly elevated compared with normal subjects indicating abnormal parathyroid gland function relatively early in CRF.

SUPPRESSION OF PARATHYROID HORMONE SECRETION BY INCREASED ENDOGENOUS DIACYLGLYCEROL IN BOVINE PARATHYROID CELLS.

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Enhancement of parathyroid diacylglycerol (DG) levels by increased extracellular calcium paradoxically does not mimic the action of phorbol esters to increase PTH secretion. To investigate the role of changes in cellular DG on PTH secretion independent from changes in extracellular calcium, we studied the effect of two inhibitors of DG metabolism (R59022, a DG kinase inhibitor and RHC 80267, a DG lipase inhibitor) on cellular DG content and PTH secretion. DG was measured using an enzymatic method that converts 1,2 DG to [^{32}P] phosphatidic acid. PTH was measured by radioimmunoassay. Normal bovine parathyroid cells were dispersed and incubated with low (0.5mMCa^{++}), normal (1.0mMCa^{++}) or high (2.0mMCa^{++}) calcium in the presence of R59022 and RHC 80267 or vehicle. In cells incubated with both inhibitors vs vehicle, the cellular DG levels (pM/μg protein; mean±SE) were 9.78±1.07 vs 6.67±0.83 at 0.5mMCa^{++} (P=.0166); 10.95±1.26 vs 7.22±0.48 at 1.0mMCa^{++} (P=.0095) and 14.68±1.48 vs 8.68±0.92 at 2.0mMCa^{++} (P=.003). PTH levels (pM/μg protein; mean±SE) secreted from cells treated with both inhibitors vs vehicle were 25.47±4.2 vs 52.69±6.08 cells at 0.5mMCa^{++} (P=.0001); 20.58±3.99 vs 34.77±3.08 at 1.0mMCa^{++} (P=0.0009) and 17.02±2.33 vs 29.7±2.7 at 2.0mMCa^{++} (P=.001). Thus in the presence of inhibitors of DG metabolism there is a significant increase in cellular DG levels and a significant suppression of PTH secretion regardless of the level of extracellular calcium. These data suggest that extracellular calcium may suppress PTH secretion by augmenting cellular DG which in turn suppresses PTH secretion.

PROTEIN KINASE-A ACTIVITY AND THE EFFECTS OF PARATHYROID HORMONE ON PHOSPHATE UPTAKE IN OPOSSUM KIDNEY CELLS.

KJ Martin, CL McConkey* and JC Garcia*, Renal Division, Washington University Medical School, St.Louis, MO.

Current evidence indicates that signal transduction following receptor binding of parathyroid hormone (PTH) involves the stimulation of adenylate cyclase as well as stimulation of phosphoinositide metabolism. Recent studies showing that PTH alters phosphate transport (PT) in opossum kidney cells at concentrations which do not increase cyclic AMP and that activators of protein kinase C also alter PT have led to the suggestion that there is a dual mechanism for the regulation of PT by PTH, protein kinase C at low levels of PTH and cyclic AMP at higher levels of PTH. The present studies were designed to evaluate the relationship between cyclic AMP dependent protein kinase (PK-A), a more sensitive indicator of alterations in cyclic AMP metabolism than measurements of total cellular cyclic AMP, and PT in OK cells in response to bPTH 1-34 and [Nle^8,Nle^{18},Tyr^{34}]bPTH 3-34 amide. bPTH 1-34 stimulated cyclic AMP with half maximal effect occuring at 2 nM. PTH 3-34 analog did not increase cyclic AMP. PT was inhibited by bPTH 1-34 in a dose dependent manner with half maximal effect occurring at 0.2 nM. bPTH 3-34 analog also altered PT although this peptide was 3 orders of magnitude less potent than bPTH 1-34. PK-A activity increased in response to bPTH 1-34 and correlated closely with the effects of PTH on PT. [Nle^8,Nle^{18},Tyr^{34}]bPTH 3-34 amide, which did not appear to increase cyclic AMP, caused a significant increase in the activity of PK-A. These data indicate that the effects of PTH peptides on PT are closely related to changes in the activity of PK-A and that alternate second messengers are not required to explain the effects of PTH on phosphate transport in OK cells.

INHIBITION OF Na^+-Pi CO-TRANSPORT BY PARATHYROID HORMONE (PTH) ONLY IN THE PRESENCE OF PHOSPHOLIPASE C ACTIVATION.

Akimitsu Miyauchi,* Judith Cole,* Roberto Civitelli,* Leonard Forte, and Keith A. Hruska. Renal Div., Jewish Hospital, St. Louis, MO. and University of Missouri and VA Hospital, Columbia, MO.

The OK cell line has been shown to possess PTH receptors coupled to adenylate cyclase and phospholipase C. Both cAMP-dependent protein kinase and protein kinase C have been shown to regulate Na^+-Pi co-transport in these cells. We developed several clonal cell lines from the parent cell line. All of these expressed stimulation of cAMP production in response to PTH stimulation. One cell line (OK-P), exhibited inhibition of Na^+-Pi co-transport in response to PTH (IC_{50}-10^{-11} M). The other cell line (OK-H) tested did not express PTH sensitive Na^+-Pi co-transport. In addition, only the OK-P clone exhibited stimulation of transient elevations of cytosolic Ca^{2+} (Ca^{2+}_i) by PTH. The PTH effect on Ca^{2+}_i in the OK-P clone was seen at 10^{10-11} M doses, similar in sensitivity to Pi transport inhibition. The PTH effect on Ca^{2+}_i was partially expressed in the absence of extracellular Ca^{2+}. We have previously shown that this is due to production of inositol trisphosphate and diacylglycerol. Thus, these data indicate a close association between activation of phospholipase C and inhibition of Na^+-Pi co-transport. They indicate a complex regulation of this transport function by PTH in which two pathways of signal transduction interact. They suggest an important cooperative role for the Ca^{2+}-protein kinase C message system in the regulation of Pi transport.

GTP-MEDIATED PHOSPHATIDYLINOSITOL BISPHOSPHATE HYDROLYSIS IN PARATHYROID CELLS.

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A GTP-binding protein appears to be an important regulator of the calcium supression of parathyroid hormone (PTH) secretion. In this study we focus on GTP-mediated phosphatidylinositol bisphosphate (PIP_2) hydrolysis in membranes prepared from bovine parathyroid cells. Cells were prelabeled with [^{32}P] phosphate and membranes were prepared by differential centrifugation. The membranes were incubated with various amounts of GTP, GDPβS and ionized calcium. Lipid extracts of the incubated membranes were separated by a TLC system optimized for visualizing PIP_2 which was located by radioautography. Increasing GTP concentration from 0 to 0.5mM caused a progressive increase in PIP_2 hydrolysis which was indicated as a decrease in the density of the autoradiograph. Ion exchange chromatography of water soluable products indicated an increase in inositol trisphosphate release with the addition of GTP. The hydrolysis of PIP_2 was inhibited by the addition of GDPβS. An ionized calcium concentration above 140 nM accelerates hydrolysis. Pretreatment of parathyroid cells with pertussis toxin increased cellular cyclic AMP levels and subsequently inhibited in vitro ADP-ribosylation of a 41kDa membrane protein consistent with modification of G_i. Membranes prepared from these cells still displayed GTP-mediated PIP_2 hydrolysis. In summary, bovine parathyroid cell membranes contain a GTP-mediated enzyme which hydrolizes PIP_2. This enzyme system is not affected by pertussis toxin treatment although other pertussis-sensitive processes are. In conclusion, these data are consistent with the existence of a G protein-mediated phospholipase C activity in parathyroid cells. The G-protein mediating PIP_2 hydrolysis is not pertussis toxin sensitive.

DETAILED INVESTIGATION OF REFRACTORY AUTONOMOUS HYPERPARATHYROIDISM AFTER PARATHYROID AUTOGRAFTING.

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Five hypercalcemic patients on renal replacement therapy who became normocalcemic after removal of four (4 cases) or 3 (1 case) parathyroid glands from the neck with forearm parathyroid autografting later redeveloped hypercalcemia. Investigations have included venous parathyroid hormone (PTH) measurements in each arm (two-site immunochemiluminescent method), neck vein catheterisation for PTH estimation, ultrasonography, CT scanning and Thallium-Technetium scanning, the last of which has proved especially valuable.

In three patients, hypercalcemia persisted despite attemps to remove all parathyroid tissue from the arm. In one, serum calcium returned to normal after removal of a retrosternal parathyroid tumour. One has evidence of parathyroid tissue in the neck. In the patient from whom only 3 glands were removed initially and who refuses surgery, investigation shows functioning parathyroid tissue in both neck and forearm. In a fourth patient serum calcium is normal after implant surgery but investigation shows functioning parathyroid tissue in the neck. In one patient investigation has failed to explain persistent hypercalcemia.

Hyperplasia of parathyroid cells spilled into the neck at operation or lying dormant in other sites occurs after "total" parathyroidectomy. Parathyroid autografting much complicates the assessment of patients in whom this has occurred.

EFFECTS OF ORAL (POD) AND INTRAVENOUS (IVD) 1,25 VITAMIN D THERAPY IN HEMODIALYSIS PATIENTS (HDP) TREATED WITH $CaCO_3$ AS SOLE PO_4 BINDER.

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$CaCO_3$ has been demonstrated to be an effective PO_4 binder in HDP. It is the purpose of this study to determine the responses to POD and IVD in combination with $CaCO_3$ as the sole PO_4 binder. 151 patients were initially treated with $CaCO_3$ without 1,25 Vit D for 6 months. A dialysate containing 2.5 mEq/L Ca was used to minimize↑ Ca. The HDP had a Ca x PO_4 product < 60 and for 6 months were treated with POD (n = 54) or IVD (n = 97). $CaCO_3$ therapy was continued as the sole PO_4 binder. Vit D dose was raised until serum Ca was ≤ 11 mg%. Alkaline phosphatase (AP), total Ca, ionized Ca (IC), and PO_4 were measured monthly. iPTH, 1,25 Vit D were measured every 3 months. Mean ± SD ($P < 0.05$* vs. control) were analyzed by ANOVA of transformed data over the study period.

IVD:	Total Ca	IC	PO_4	PTH	D_3	AP
Control	8.7 ± 1.5	2.10 ± 0.25	5.3 ± 1.70	62.5 ± 65	9.0 ± 5.1	186 ± 175
6 mon	9.7 ± 1.5*	2.40 ± 0.46*	6.6 ± 2.5*	34.3 ± 40*	17.0 ± 8.9*	138 ± 157*
POD:						
Control	8.9 ± 1.3	2.07 ± 0.26	5.7 ± 1.7	75.3 ± 60	7.8 ± 50	222 ± 175
6 mon	8.9 ± 1.3	2.19 ± 0.40*	6.2 ± 2.0	65.2 ± 4.6	13 ± 10.0*	197 ± 170

IVD had significantly lower ($P < 0.03$) AP, iPTH, and higher D_3 levels compared to POD at 6 months. The improvement in PTH in IVD was apparent after 3 months ($P < 0.05$).
POD did not suppress AP or iPTH levels despite increases in Vit D_3 and IC during 6 months of therapy. IVD is more effective in control of 2° HPT than POD.

THE MECHANISM OF DECREASED METABOLIC CLEARANCE OF CALCITRIOL IN EXPERIMENTAL RENAL FAILURE.

S Patel*, CH Hsu. Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

Previous studies from our laboratory have demonstrated that metabolic clearance rate (MCR) and synthesis of calcitriol is decreased in chronic renal failure (CRF) (Am J Physiol 253:F1015, 1987). In this study, we measured the MCR of calcitriol in thyroparathyroidectomized controls and rats with CRF produced by 5/6 nephrectomy. The MCR of rats with CRF (0.15 ± .01 ml/min/kg, n=7) was lower than that of the controls (0.19 ± .01, n=7, $p < 0.001$). Infusion of PTH, 0.8 U/h, for one week did not change the MCR in CRF (0.15 ± .01 vs controls, 0.24 ± .01, $p < 0.001$). Hyperparathyroidism is therefore, not the cause of the decreased MCR in CRF. Since calcitriol can induce its own degradation enzyme, rats with CRF were supplemented with calcitriol. Subcutaneous infusion of calcitriol (10 ng/kg/day) for one week significantly improved the MCR of rats with CRF (0.22 ± .01, n=9) when compared to those without calcitriol supplementation (0.17 ± .01, n=7, $p < 0.001$); however, it did not restore the MCR to the normal levels (0.25 ± .01, n=7, $p < 0.02$). Infusion of 24,25$(OH)_2D_3$ (1 ug/day) and 25$(OH)D_3$ (600 ng/day) for one week in rats with 3/4 nephrectomy also significantly improved the MCR in CRF (both 0.25 ± .01 vs 0.21 ± .01 ml/min/kg, both $p < 0.001$). Administration of 24,25$(OH)_2D_3$ significantly decreased the plasma concentration of calcitriol (52.3 ± 3.1 pg/ml vs 67.7 ± 6.0, $p < 0.05$). Treatment with 25$(OH)D_3$ also decreased the plasma levels of calcitriol (55.0 ± 4.3), but the decrease was not statistically significant. We conclude that supplementation of vitamin D metabolites significantly improved the MCR of calcitriol in CRF.

CALCITRIOL METABOLISM IS SUPPRESSED BY UREMIA IN RATS. S Patel*, CH Hsu. Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

We studied the effect of uremia on the metabolism of calcitriol in rats. The metabolic clearance rate (MCR) of calcitriol were decreased in renal failure rats measured four days after 5/6 nephrectomy (0.15 ± .01 ml/min/kg, n=6 $p < 0.001$); however, they remained normal in rats four days after ischemic clamp of bilateral renal arteries (ATN) (0.25 ± .01, n=6) when compared to the sham operated controls (0.24 ± .01, n=5). Serum creatinine was slightly, but significantly, lower in the rats with ATN (1.05 vs nephrectomy, 1.35 mg/dl). The effect of uremia on calcitriol metabolism was examined in normal rats by 24-hour infusion of phosphorus-free urine. The MCR and production rate (PR) of calcitriol were significantly suppressed when urines were infused to normal rats (MCR, 0.19 ± .01, PR, 16.2 ± 1.7 ng/kg/day, n=6 vs the controls infused with saline, MCR, 0.26 ± .01, PR, 33.6 ± 2.7, n=6). The MCR of calcitriol, however, were not suppressed when urines were infused into rats with ATN (0.25 ± .01, n=6) despite a marked elevation of creatinine. Infusion of ultrafiltrate of urine which contained substances with molecular weights (M.W.) approximately less than 1,200 D to normal rats also significantly suppressed both the PR and MCR of calcitriol. Infusion of known uremic toxins with M.W. less than 600, e.g., spermine, spermidine, methylguanidine and guanidinosuccinic acid, however, did not alter the MCR of calcitriol. We conclude that uremia suppresses calcitriol metabolism. The M.W. of the toxins were approximately less than 1,200 D. The suppression of calcitriol degradation by these toxins, however, requires intact renal tubules.

AMINO ACIDS (AA) INFUSION AMILIORATES UREMIC HYPERPARATHYROIDISM (HPT) IN HEMODIALYSIS (HD) PATIENTS. D. Sheikh-Hamad*, V. Gura, M. Roberts, P.A. Eide*, W. Paul*, S. Lustig*, N.N. Yoshimura*, and D.B.N. Lee. VA Med. Ctr., Sepulveda, Midway Dialysis Ctr., Los Angeles, Kendall McGaw Lab., Irvine, SmithKline Bio-Science, Los Angeles and UCLA Sch. of Med., Los Angeles.

Optimal and complication-free control of uremic HPT remains a clinical challenge. We have infused Vamine® (8.5% in 1L) during dialysis for 3 Mo to 4 malnourished and bed-ridden HD patients with severe HPT. This led to unassisted ambulation, increased bone density and reduction or disappearance of extensive soft tissue calcifications. We then prospectively studied the effect of 3 Mo intradialytic infusion of branch-chain AA (Freamine HBC®, 7.9% in 750 ml) with simultaneous oral carbohydrate supplementation on serum c-terminal PTH in 6 HD patients. PTH, in 1,000 pg/ml, rose from 24.9 ± 9.5 to 28.7 ± 10.3 ($P<0.05$, paired-t) during the first month and thereafter dropped progressively to 19.7 ± 7.4 ($P<0.05$) by the end of the third month. Serum ionized calcium, phosphorus and 1,25-dihydroxyvitamin D did not change. Serum AA profile, however, shifted to or towards normal. Thus, the reduction of serum PTH seems to be related to AA infusion rather than to changes in the traditional regulatory pathways. Sustained AA supplementation in HD patients as a safe method in the control of uremic HPT deserves further studies.

ASSOCIATION BETWEEN HYPERPARATHYROIDISM, HYPERTENSION AND PLATELET CYTOSOLIC CALCIUM IN CHRONIC RENAL FAILURE. AEG Raine*, L Bedford*, A Simpson*, CC Ashley*, JS Woodhead*, JGG Ledingham. Nuffield Dept. of Medicine, Univ. of Oxford, and Dept. of Medical Biochemistry, Univ. of Wales, Cardiff, UK.

Secondary hyperparathyroidism is common in chronic renal failure, but whether this results in abnormal cytosolic free calcium (Ca_i^{2+}) is unknown. To investigate relationships between serum PTH, platelet Ca_i^{2+} and blood pressure, 36 patients (23M, 13F), aged 18-77 years with impaired renal function were studied, 10 with normal PTH (mean 8.0±SEM 0.6 pmol/l), 17 with elevated PTH (35.0±7.2pmol/l) and 9 receiving nifedipine (PTH 36.2±5.9pmol/l). Renal function was similar in the 3 groups. Plasma total and ionised calcium, serum intact PTH (2-site immunometric assay) and platelet Ca_i^{2+} (fura-2 fluorescent indicator) was measured.

Platelet Ca_i^{2+} was increased in the high PTH group (138±16nmol/l) compared with normal PTH patients (83±7nmol/l; $p<0.02$), and in these 27 patients serum PTH and platelet Ca_i^{2+} were linearly related ($r=0.818$; $p<0.001$). However, platelet Ca_i^{2+} was normal (84±9nmol/l) in the nifedipine group, despite high PTH. A linear relation was present between platelet Ca_i^{2+} and mean blood pressure ($r=0.576$, $p<0.005$). Treatment of 9 patients with alfacalcidol for 2-11 months produced falls in both PTH (37 to 14pmol/l, $p<0.01$) and Ca_i^{2+} (157 to 78 nmol/l, $p<0.02$).

It is concluded that platelet Ca_i^{2+} is elevated in uraemia in association with hyperparathyroidism, is related to level of blood pressure and may be reduced by both Vitamin D analogues and calcium entry blockers. These findings may be relevant to the cardiovascular complications of renal failure.

THE STIMULATORY (Gs) AND INHIBITORY (Gi) GUANINE NUCLEOTIDE BINDING PROTEINS OF PTH-DEPENDENT ADENYLATE CYCLASE (AC) IN RENAL CORTICAL MEMBRANES FROM PHOSPHATE DEPLETED AND ACIDOTIC DOGS. Rebeca Starosta,* Carmen L. Milanes,* Nidia Pernalete,* Margarita Perez-Gonzalez,* Virgilio Paz-Martinez and Ezequiel Bellorin-Font. Centro Nacional de Dialisis y Trasplante. Hospital Universitario de Caracas. Venezuela.

Phosphate depletion (PD) and metabolic acidosis (MA) induce resistance of renal cortical AC to PTH. This resistance is also observed in the presence of other Gs activating ligands and increases when the two conditions occur simultaneously. We examined Gs and the functionally opposed Gi in renal cortical membranes from dogs with PD and PD + MA. The relative content of Gs as measured by cholera toxin-dependent ADP-ribosylation of the 42,000 Mr alpha subunit was markedly decreased in membranes from PD and PD + MA dogs (266.4 ± 13.6 arbitrary area units in controls vs. 174.0 ± 18 in PD, $p < 0.01$, and 97.3 ± 12.8 in PD + MA, $p < 0.01$). There was a positive correlation between Gs alpha content and maximal PTH-dependent AC activity in presence or absence of Gpp(NH)p ($r = 0.847$, $p < 0.001$ and $r = 0.753$, $p < 0.01$, respectively), or NaF ($r = 0.643$, $p < 0.05$). Basal activity with ATP-Mn as a substrate did not show correlation. The relative content of Gi determined by pertussis-dependent ADP-ribosylation was also lower, albeit not significant compared to controls. The functional state of Gs was examined by its property to protect the catalytic unit from inactivation by N-ethyl maleimide when preincubated with $GTP_\gamma S$. In all membrane preparations there was 45 to 50 % protection of basal activity at 30°C, but no difference was observed between groups, indicating a normal tight coupling of activated Gs and the catalytic unit. In conclusion, resistance of AC to PTH and other activating ligands under PD and PD + MA is associated with a decreased content of Gs alpha, suggesting that regulation of Gs may have a role in the renal adaptation to phosphate depletion and acidosis.

EFFECT OF HIGH CONCENTRATION OF GLUCOSE AND INSULIN ON PTH SECRETION IN CULTURED BOVINE PARATHYROID CELLS. T. Sugimoto*, C. Ritter*, J. Morrissey and E. Slatopolsky. Washington University, Dept. of Medicine, St. Louis, MO.

Lower levels of i-PTH, have been found in diabetic patients with or without renal failure. However, the exact mechanism remains unclear. In the present studies we examined the effect of high concentration of glucose and/or lack of insulin on PTH secretion from cultured bovine parathyroid cells. Increasing medium concentration of glucose or insulin removal caused a dose-dependent suppression of PTH secretion (31.7% inhibition for 50mM glucose). Time course and dose-response evaluation revealed that a significant suppression of PTH secretion was found within 48 hrs. of incubation; and occurred with as little as 15 mM glucose, respectively. The suppressive effect of high concentration of glucose and insulin removal was additive. The addition of choline chloride to the medium did not suppress PTH secretion, although the osmolarity was the same as that of the medium containing 50 mM glucose. When cells previously exposed to 50 mM glucose or lack of insulin were reincubated in the medium containing 5 mM glucose and 5 µg/ml insulin for another 48 hours, a complete recovery of PTH secretion was observed. In the cells exposed to 50 mM glucose or lack of insulin, the magnitude of the response of PTH secretion to 10^{-6}M isoproterenol was blunted. Gel electrophoresis revealed no change in the ratio of intact to fragment of hormone secreted from cells exposed to 5 or 50 mM glucose or minus insulin. The present studies demonstrate that high glucose and insulin removal modulated PTH secretion via different mechanisms, which are not due to an irreversible toxic effect.

25-HYDROXYLATED VITAMIN D_3 DERIVATIVES BLOCK PGE_2- and PTH-STIMULATED cAMP IN OK CELL LINE. H. Wald,* D. Rubinger,* P. Sherzer,* M.M. Friedlaender,* A. Moran,* and M.M. Popovtzer. Hadassah University Hospital, Jerusalem and Ben Gurion University, Beersheva, Israel.

To further examine the direct effect of PGE_2 on renal tubular cAMP system and to compare it with the tubular actions of PTH, experiments were performed with OK cell line. OK cells are known to exhibit the functional and enzymatic characteristics of proximal tubular cells. cAMP was assayed in OK cells that were treated with 1. PGE_2 (10^{-5}M) alone, 2. PGE_2+25(OH)D_3, 3. PGE_2+1,25(OH)$_2D_3$ and 4. PGE_2+24,25(OH)$_2D_3$; 1a. PTH (10^{-8}M) alone, 2a. PTH+25(OH)D_3, 3a. PTH+1,25(OH)$_2D_3$ and 4a. PTH+24,25(OH)$_2D_3$. Both PGE_2 and PTH stimulated cAMP in the OK cells in a dose dependent fashion as follows, PGE_2:10^{-6}M cAMP 76±16 (x±SE) pmoles/mg prot/5 min, PGE_2: 10^{-5}M cAMP 144±28 and PGE_2: 10^{-4}M cAMP 277±62, PTH:10^{-9}M cAMP 45±5, PTH: 10^{-8}M cAMP 364±20 and PTH: 10^{-7}M cAMP 543±62. cAMP levels were in 1. 139±9, in 2. 71±3 $p<0.005$ vs. 1., in 3. 102±5 $p<0.025$ vs. 1., and in 4. 106±6 $p<0.05$ vs. 1., in 1a. 210±8 in 2a. 149±8 $p<0.001$ vs. 1a., in 3a. 122±12 $p<0.001$ vs. 1a. and in 4a. 82±6 $p<0.001$ vs. 1a. These results demonstrate that PGE_2 similarly to PTH activates the cAMP system in the OK cell line. This dose dependent activation by both PGE_2 and PTH is shown to be inhibited by 25(OH)D_3, 1,25(OH)$_2D_3$ and 24,25(OH)$_2D_3$. These observations suggest that PGE_2 and PTH share similar mechanisms of action in the mammalian proximal tubule. This action appears to be mediated by the cAMP system and is modified by 25-hydroxylated metabolites of vitamin D_3.

PTH INHIBITS CALCIUM PUMP ACTIVITY OF BASOLATERAL MEMBRANE (BLM) OF RAT KIDNEY CORTEX IN THE ABSENCE OF 1,25(OH)$_2D_3$BUT NOT IN THE PRESENCE. Yusuke Tsukamoto. Dept. of Medicine, Kitasato Univ., Kanagawa, Japan.

The author reported in this previous meeting that Ca^{2+} pump catalyses an electroneutral Ca^{2+}/H^{+} antiport in BLM of rat kidney cortex. Since the hormonal regulation of this Ca^{2+} pump has remained unsolved, Ca^{2+} pump activity was studied in BLM vesicles (BLMV) of vitamin D deficient (D(-)) rat kidney cortex. Ca^{2+} pump activity (ATP-dependent $^{45}Ca^{2+}$ uptake) was expressed as Vmax (nmole $^{45}Ca^{2+}$/2 min/mg BLMV). Serum 1,25(OH)$_2D_3$ level was measured to assure D_3 deficiency. D(-) rats were fed either normal Ca diet (Ca 0.8%, P 0.6%; N diet) or high Ca diet (Ca 1.6%, P 0.6%; H diet). D repleted (D(+)) rats ($1,25D_3$: 25 pmol x 3 days, i.p.) showed higher Ca^{2+} pump activity (4.41 ± 0.57, m ± SD, n=8) than D(-) rats (N diet) (3.45 ± 0.65, n=6, $p < 0.05$). When D(-) rats were fed H diet for 6 days (serum Ca^{2+}: 2.35 ± 0.21 mEq/l), the activity (5.70 ± 1.08, n=6, $p < 0.01$) was much higher than D(-) rats fed N diet (serum Ca^{2+}: 1.38 ± 0.19). D(-) rats fed N diet showed higher serum m-PTH level (367 ± 28 pM, n=5) than D(-) rats fed H diet (243 ± 120 pM, n=6, $p < 0.05$). The pump activity of D(-) rats decreased to 4.39 ± 0.51 (n=6, $p < 0.05$) by the continuous infusion of bovine [1-34] PTH (2 u/hr) for 48 hours following 6 days feeding of H diet. When normal rats were parathyroidectomised, Ca^{2+} pump activities did not change significantly (sham: 2.81 ± 0.90 nmol/60 sec/mg, n=9 vs TPTX: 2.91 ± 1.07, n=12). In conclusion, 1,25(OH)$_2D_3$ stimulates Ca^{2+} pump activity. PTH inhibits the activity in the absence of $1,25D_3$ but not in the presence.

ALUMINUM (AL) UPTAKE AND TOXICITY IN FRIEND ERYTHROLEUKEMIA CELLS (FEC): A MODEL SYSTEM FOR STUDY OF ALUMINUM INDUCED ANEMIA. Kenneth Abreo, Jonathan Glass*, M'Liss Sella*. LSU Medical Center, Shreveport, LA.

Anemia is known to develop in Al overloaded dialysis patients and animals. The mechanisms of Al uptake and toxicity in erythrocytes are not currently understood. The effect of Al on cell growth and hemoglobin (Hb) synthesis was evaluated in cultured FEC. These cells can be induced with dimethylsufoxide (DMSO) treatment to undergo a coordinated program of iron uptake and Hb synthesis, resembling the final stages of erythroid differentiation.

Cell counts (10^6/ml), Hb production [µg/10^6 cells and % benzidine (B) positive cells] and cell Al (µg/L lysed cells) were measured in FEC grown in control (C) media (RPMI+10% FCS), and in media containing transferrin (Tf)(500 µg/ml), Al (340 µg/L) and Tf-Al (500 µg/ml Tf, 340 µg/L Al) at 96 hrs. following 1.5% DMSO induced differentiation. Results were as follows:

Group (n)	Cell Ct	%B	Hb/10^6	Cell Al
C (6)	5.7±0.7	76±8	11±0.5	6±1
Tf (6)	4.9±0.4	62±3	10±0.7	5±2
Al (6)	4.9±0.6	72±8	7±0.5	5±1
Tf-Al (6)	4.2±0.6	51±6	4±0.3	201±38
P	<0.001*	<0.001+	<0.001+	<0.001+

* Tf-Al vs C + Tf-Al vs C, Tf, and Al

These results indicate (1) Tf-Al inhibits FEC growth and Hb synthesis. (2) Binding of Al to Tf is necessary for Al uptake by FEC suggesting utilization of Tf pathway by Al. (3) FEC serve as an excellent model for study of Al induced anemia.

EFFECT OF RANITIDINE ON ALUMINUM ABSORPTION IN MAN. J.W. Coburn, J.A. Robertson, K.C. Norris, I.B. Salusky & W.G. Goodman. VA Med Ctrs, WLA & Sepulveda, & UCLA Sch Med, Los Angeles, Ca.

Toxicity due to aluminum (Al) absorption continues to affect renal patients treated with phosphate (PO4)-binding agents. The desire to find methods to reduce Al absorption prompted our evaluation of the effect of ranitidine (R). Because of higher solubility and presumed absorbability of many Al-salts at lower pH, we postulated that R might reduce Al absorption. After 2 control (C) days (D), 12 normals received either placebo (P) or R, 150 mg b.i.d. for 4 days in a double-blind study with Al(OH)-gel (Alternagel 5 ml q.i.d.) added on the last 3 days. Urinary (U) and plasma (P) Al and creatinine (Cr) were measured daily. One month later, all crossed over to the opposite agent (P-->R; R-->P) and the study was repeated. The results, in ug Al/g Cr/day, in

Day:	Control	Al-1	Al-2	Al-3
Al alone (P)	8±2	65±25	116±36	144±60
Al plus R	10±2	30±7	81±20	85±18#

R differs from P, $p < 0.05$

the table, show a significant decrease of U-Al with R by day 3 but no differences on days 1 & 2; paired comparisons showed no differences. P-Al rose from 5.8±0.5 to a peak of 12±4.4 ug/L in P and from 5.2±0.4 to 7.8±0.7 in R (R vs P, p = n.s.). Thus, ranitidine reduced aluminum absorption to 59% of C by day 3 as measured by its urinary excretion. These data suggest that ranitidine therapy may reduce the Al-load in renal patients receiving Al-gels for hyperphosphatemia that is unresponsive to other PO4-binding agents. However, further studies are needed to determine its long-term efficacy.

ATP STIMULATES CYTOSOLIC Ca^{2+} IN RENAL PROXIMAL TUBULES. Charles R. Filburn and Stephen Harrison. GRC, NIA, NIH, Baltimore, MD 21224 (Intro. by G. Kiebzak)

Adrenergic regulation through α_1-receptors of cytosolic Ca^{2+} ($[Ca^{2+}]i$) in renal proximal tubules has been shown. Since ATP is present in many adrenergic nerve terminals and appears to act as a co-transmitter with norepinephrine (NE), the effects of ATP and its derivatives on $[Ca^{2+}]i$ in Quin-2 loaded rat proximal tubules were assessed. Both ATP and ADP, but not AMP or adenosine, elicited a transient, 90% increase in basal $[Ca^{2+}]i$, with similar dose-response relationships ($EC_{50} \approx 5$ uM). Following the transient, $[Ca^{2+}]i$ fell to a sustained level 18% above basal; this effect depended on extracellular Ca^{2+} and was observed only at higher levels of ATP (2×10^{-4} M). Both ATP and ADP increase inositol phosphates in a dose-dependent manner. Pretreatment of tubules with a maximal dose of NE prevented a subsequent response to ATP, while PTH or lower doses of NE only slightly attenuated the response. Pretreatment with ATP had little or no effect on subsequent responses to PTH or NE. Various other nucleotides and analogs were less effective than ATP or had no effect. Only weak agonists attenuated stimulation by submaximal ATP; no simple antagonist was found. These data show that ATP and ADP, acting through a P-2 purinergic receptor, may play a role in regulation of $[Ca^{2+}]i$ in renal proximal tubules.

INTERORGAN PARTITIONING OF NITROGEN BY ORGANIC ACID. Dass, P.D.*, R. Martin*, J. Bayliss and I. Kurtz, UAMS, Little Rock, AR 72205.

Benzoate (B) has been clinically used to augment nitrogen (N_2) excretion in several disorders of N_2 metabolism. The rational for the use of B is that it conjugates with glycine in the liver to form hippurate (H) which is rapidly cleared by the kidney. The present study was designed to determine whether B effects N_2 metabolism in organs other than the liver. Fasted rats were given B (5.0 mmole/kg); the urine content and arteriovenous difference (A-V) for N_2 metabolites was quantitated across the liver, gut and kidney. B partitioned urea N_2 into H in the liver, and stimulated glutamine (gln) uptake 12 fold by the kidney. The A-V gln was 0.02±0.01 in controls (C) vs 0.12±0.01 mM in B. There was a concomitant 11 fold increase in urea degradation by the gut (A-V urea was -0.05±0.09 and +0.53±0.10 for C and B respectively). B enhanced urine excretion ($p<0.05$) of all nitrogenous products (μmole/100g/24 hr):

Experiment	NH_3	Urea	H	vol(ml)
C (n = 6)	297±27	1655±54	4±1	7±1
B (n = 6)	614±57	3152±177	304±40	16±2

Urine flow rate doubled while creatinine excretion was unchanged. In rat proximal tubule fragments B stimulated gln utilization 27% and NH_3 formation 51%. The stimulatory effect of B was blocked by acivicin, a selective inhibitor of γ-glutamyl-transferase (γ-GT).

Conclusion: B short circuits metabolic pathways not only across the liver but gut and kidney also. Enhanced renal ammoniagenesis may be due to activation of luminal γ-GT. This is a major departure from conventional view of intramitochondrial ammoniagenesis.

MECHANISM OF ENHANCED GASTROINTESTINAL (GI) ABSORPTION (ABS) OF ALUMINUM (AL) BY CITRATE (CITR). D.H. Froment,* B.A. Molitoris and A.C. Alfrey. VAMC, Denver, CO.

Citrate markedly enhances the GI abs of Al and can produce subacute Al neurological and skeletal toxicity in uremic patients. The mechanism by which Citr enhances Al abs was studied. After gavage of 0.89 mmol/kg of AlCitr plasma Al peaked at 74±13 as compared to 1.2±0.3 uM/L after a similar gavage with $AlCl_3$ which occurred within 40 minutes concomitant with peak glucose absorption. Using an everted duodenum, Al transfer (delta serosal fluid) and tissue uptake was determined at 4 and 37 °C. Although Al tissue uptake was markedly decreased by cold (91 vs 16 pmol/mg wet weight (ww) and Al transfer (6.63 vs 6.01 pmol/mg ww) unlike Citr (493 vs 38 pmol/mg ww) was unaffected by cold. In contrast, although tissue uptake was greater from Al lactate (2118 pmol/mg ww) there was no transfer to serosal fluid. To determine if Citr enhanced Al transferr by opening the tight junction (TJ) ruthenium red (RR) and Ussing chamber were employed. AlCitr was the only Al compound found to increase RR deposits in the paracellular spaces and produce a prolonged reduction in trans-intestinal resistance consistent with opening tight junctions. AlCitr was found to be 100 times more soluble at pH 5 to 8 than $Al(OH)_3$. In conclusion Al abs occurs passively through the paracellular pathway. Citr enhances Al abs by opening the TJ, because of this Al and Citr (compounds) should not be given together.

ALUMINUM INHIBITS SUPEROXIDE DISMUTASE (SOD)
R. Kestenbaum*, A. Adler, C. Caruso*, G. Berlyne. Nephrology Section, Brooklyn VA Medical Center and Health Science Center, SUNY, Brooklyn, NY.

Free oxygen radicals (FOR) are implicated in the pathogenesis of carcinogenesis, post-ischemic damage, and various nephropathies, and are generated in increased amounts in renal failure. SOD is an enzyme which catalyzes the dismutation of superoxide radicals, thereby removing free oxygen radicals which are continuously generated physiologically. We investigated the influence of aluminum on SOD activity in vitro, using prevention of oxidation of 6 hydroxydopamine hydrochloride to measure SOD. The results are shown in the table where ΔOD indicates SOD activity.

Aluminum concentration	No. of expts.	ΔO.D. at 1 minute (mean±SD)
0	14	0.071±0.047
$6x10^{-5}$M	14	0.037±0.032**
0	9	0.050±0.022
$6x10^{-6}$M	9	0.041±0.029

Statistical analysis using paired t-test, comparing each to its respective aluminum free control *p<0.05 **p<0.02.

The results show a significant depression of SOD activity by low concentrations of aluminum in vitro.

Conclusions: Aluminum inhibits SOD and thus may be responsible for excess of free oxygen radicals and resultant tissue damage and neoplasia in patients on hemodialysis.

PLASMA AND CELLUAR ZINC IN UREMIA. S. Mahajan, H. Wang, D. Abu-Hamdan, F. McDonald and A. Prasad V.A. Medical Center, Allen Park, Wayne State University School of Medicine, Detroit, Michigan

Changes in zinc (Zn) metabolism in dialyzed patients contribute to the presence of abnormal neurtophil or lymphocyte function, sexual and gonadal dysfunction, hyperprolactinemia, anemia, decreased taste and neuropathy. The criteria for the diagnosis of Zn deficiency in uremia are not established. The plasma Zn is low while tissue Zn is variable. Circulating cells represent easily accessible tissues for Zn analysis. To determine their usefulness in the assessment of Zn status of uremic patients, we measured Zn concentration in plasma (PZn), erythrocytes (EZn), neurtophils (NZn), lymphocytes (LZn) and platelets (PLT Zn) in 20 stable hemodialyzed patients and 15 normal controls. The data are mean ± S.D., * = p<.01 from controls. Cellular Zn is $\mu g/10^{10}$ cells. Patients in comparison to controls had low PZn (89±6* vs 116±10μg/dl), low NZn (39±4* vs 46±4), low LZn (40±11* vs 50±5) and low PLT Zn (2.9±0.3* vs 3.3±0.2). EZn was higher (52±10* vs 40±4μg/g/hb) and correlated with the degree of anemia (γ=0.53). These cellular Zn parameters correlated well with dietary Zn intake and were normal in 2 patients receiving Zn supplementation. These results suggest that measurement of Zn in circulating cells is helpful in the assessment of Zn status of otherwise stable, adequately dialyzed uremic patients.

NEUROTRANSMITTER REGULATION OF CYTOSOLIC Ca^{2+} IN OSTEOBLAST-LIKE BONE CELLS. Hiromichi Kumagai, Hisato Sakamoto, Sandra E. Guggino, Charles R. Filburn, and Bertram Sacktor. GRC, NIA, NIH, Baltimore, MD 21224

The effect of norepinephrine (NE), vasoactive intestinal peptide (VIP), and ATP on apparent $[Ca^{2+}]i$ were examined in osteoblast-like UMR-106 cells loaded with the fluorescent Ca^{2+} indicator Indo-1. NE at 10^{-4} M and VIP 10^{-6} M transiently increased $[Ca^{2+}]i$ from 135 nM up to 250 nM, while forskolin at 10^{-5} M caused a 34% increase. Stimulation by these agonists was partially attenuated by EGTA chelation of extracellular Ca^{2+} or pretreatment with verapamil, indicating the transients resulted from intracellular mobilization as well as increased influx of extracellular Ca^{2+}. The NE effect was partially inhibited by both propranorol and prazosin. ATP at 10^{-3} M elicited a very fast transient to 600 nM $[Ca^{2+}]i$ that was dose dependent, persisted in the presence of EGTA, and associated with a rapid increase in inositol phosphates (IPs). The time course of the response to ATP was multiphasic with two later phases dependent on extracellular Ca^{2+}: a secondary transient response, more discernible with 2-methylthio-ATP, and a later sustained increase. Neutrotransmitters, particularly ATP, serve as important regulators of $[Ca^{2+}]i$ in bone cells through both cAMP-dependent and IP_3-dependent mechanisms, and may contribute to bone metabolism.

CALCIUM CITRATE MARKEDLY AUGMENTS ALUMINUM ABSORPTION IN MAN. M.G. Mischel,* I.B. Salusky, W.G. Goodman, & J.W. Coburn. Med & Resch Svc., WLA VA Med Ctr & UCLA Sch Med, Los Angeles, Ca.

Aluminum (Al) toxicity occurs when Al excretion lags behind its absorption. Citric acid/Na citrate (Scholl's solution) markedly augments Al absorption, producing severe and even fatal Al-toxicity in uremic patients. Calcium citrate (CaCit), used as a phosphate-binder in uremic patients, might be given concurrently with Al-gels in patients refractory to either PO4-binder alone. Whether citrate when given as CaCit can also augment Al absorption is unknown. To study this, we gave Al(OH)3 (Alternagel 5ml q.i.d.) for 3 days to 9 normals, both with CaCit, 950 mg q.i.d., and without (control = C). In C, urinary (U) Al (ug/g creatinine/day) rose from 6.4±1.3 to 67±47, 114±50, and 117±48, respectively, on days 1, 2, and 3 of Al; with CaCit plus Al, U-Al rose from 9.4±2.1 to 212±48, 436±112, and 379±70, respectively, on days 1, 2, and 3 (P = 0.05-<.01, C vs Al + CaCit). With paired data, U-Al rose 10±3.6-fold, 4.7±1.1-fold and 5.4±1.0-fold more with CaCit plus Al than with Al alone over the 3 days ($p < 0.01$); 1 subject did not change. Plasma Al levels were 5.1±1.0 and 11.1±3.2 ug/L before and after Al and 5.9±0.9 and 10.3±0.8 before and after Al plus CaCit (CaCit vs C, n.s.). Thus, standard doses of CaCit can markedly enhance Al absorption from Al-gels. Because of the potential risk of acute and severe Al toxicity, we recommend that calcium citrate should be used very cautiously in renal patients who might inadvertently be given Al-gels, and its concomitant use with Al-gels is absolutely contraindicated in such patients.

RESPONSE TO EXOGENOUS 1,25(OH)2 D3 (1,25D) IN IDIOPATHIC RENAL STONE FORMERS. J. R. Weisinger, E. Bellorin-Font, L. Aveledo,* P. Durrego,* G. Gonzalez,* N. Pernalete,* C. Milanes,* R. Staroste,* I. Contreras,* Y. Paz-Martinez. Division of Nephrology, Hospital Universitario and Centro Nacional de Diálisis, MSAS. Caracas, Venezuela.

The pathogenesis of hypercalciuria could be the result of a primary increase in intestinal Calcium (Ca) absorption, increase sensitivity or production of 1,25D or a renal Ca leak. To evaluate the pathophysiology of hypercalciuria, we studied 72 stone formers and 21 controls (C) under a prospective protocol. On a normal Ca diet two consecutive 24h urine and blood samples were collected. After 7 days on a low Ca diet (2 mg/Kg) new 24 h urine and blood samples were obtained and a Ca load test performed. Oral 1,25D was then administered for 6 days (1 µg/ day), and 24 h urine, blood samples and a calcium load test repeated. The patients were subdivided according to their urinary Ca excretion (UCa) in hypercalciuric (H) (n=24) and normocalciuric (N) (n=48). After one week on low Ca diet, UCa decreased significantly in C ($p<0.001$) whereas no significant change was observed in N or H. Administration of 1,25D resulted in a significant increase in UCa in both C ($p<0.001$) and N ($p<0.01$). In contrast, in the H group UCa remained unchanged. Intestinal Ca absorption mediated by exogenous 1,25D, as assessed from the change in fractional Ca excretion, post Ca load minus fasting, was significantly increased in C and N ($p<0.001$). In the H group there was no further increase in Ca absorption after 1,25D. Serum 1,25D levels were similar in the three groups and increased in the same proportion after oral 1,25D.These results suggest that hypercalciuric stone formers do not have hypersensitivity to exogenous 1,25D and that factors other than 1,25D could be involved in the pathogenesis of hypercalciuria.

DIFFERENTIAL REGULATION OF ATP DYNAMICS IN RENAL NEPHRON SEGMENTS AFTER ISCHEMIA. Robert J. Anderson, Diane Lanese*, and Mark A. Dillingham. Denver VA and Univ. Colo. Hlth. Sciences Center, Denver, Colorado.

Electron microscopic studies suggest proximal nephron segments are more susceptible to ischemic injury than distal segments. To examine a biochemical basis for this differential sensitivity, a chemilumenesence technique was used to measure ATP content in individual microdissected rabbit S_3 segments of proximal tubules and cortical collecting tubules (CCT). Basal ATP content was comparable in S_3 and CCT. Ischemia induced by 15, 30 or 45 min. of renal artery occlusion results in progressive ATP depletion in S_3 of 50, 66 and 91% of control, respectively. Identical ischemia produces significantly (10-29%) less ATP depletion in CCT. To examine post-ischemic recovery of ATP generation, ATP was measured after 60 min. of reflow following 15 or 30 min. of ischemia. No recovery of ATP occurs in S_3 following 60 min. of reflow while a significant 70% ATP repletion occurs with reflow in CCT. To determine if post-ischemic ATP depletion can be reversed by substrate enhancement, tubules were incubated with adenosine. Adenosine increases ATP content in control S_3 and CCT and in post-ischemic S_3 but not in post-ischemic CCT. These studies demonstrate enhanced depletion of ATP with ischemia and impaired reflow recovery of ATP after ischemia in S_3 relative to CCT. These observations may underlie differential sensitivity of these tubular segments to ischemia. Also, our studies suggest a potential role for substrate enhancement to improve post-ischemic S_3 ATP content and function.

RENAL HANDLING OF MODIFIED HEMOGLOBIN. A. Amin,* B.K. Urbaitis, A. Razynska,* and E. Bucci*. Depts. of Physiol. (D.S.), Med. & Biochem. (M.S.) Baltimore, MD.

Free hemoglobin (Hb) dissociates into dimers which are filtered at the glomerulus. Methemoglobin, the possible initiator of Hb-induced renal failure may form in the acid environment of the distal nephron. Additionally, improperly prepared Hb may increase renal vascular resistance and decrease GFR. Chemical modification of the Hb molecule, to decrease the degree of dimer formation, and purification to remove endotoxin might prevent these events.

The present experiments were done to compare renal excretion and plasma-retention-time (T ½) of a chemically modified human hemoglobin (MHb), with a limited degree of dimerization, to that of endotoxin-free bovine Hb (HbB). The effects of these compounds on GFR were also assessed.

Standard clearance experiments were done on anesthetized (Inactin) rats. Clearance of Inulin was used as an estimate of GFR. Following two control clearance periods, 25mg/100gm body wt of HbB or MHb was given I.V. Data obtained are as follows:

	GFR µl/min	%Hb excreted/2hr	Plasma T ½ min
Cont	1417		
HbB	1783	30%	35
Cont	1384		
MHb	1213	4%	440

Histologic examination indicated the presence of ferric iron (Perl's stain) in one of 4 specimens treated with MHb. In conclusion: chemical modification of the Hb molecule, to decrease dimer formation, decreases Hb excretion independent of a decrease in GFR.

EXOGENOUS ADENINE NUCLEOTIDES REPLETE ENDOTHELIAL CELL ATP FOLLOWING OXIDANT INJURY VIA ADENOSINE UPTAKE. Sharon P. Andreoli, Department Pediatrics, IUMC, Indianapolis, IN.

Alterations in renal function from oxidant injury has been implicated in the pathogenesis of several animal and human renal diseases. When human umbilical vein endothelial cells (EC) are exposed to reactive oxygen molecules (O_2^-, H_2O_2, HOCL, and OH•) generated by glucose-glucose oxidase or hypoxanthine-xanthine oxidase, ATP levels fall to $<$ 20% of control. We studied recovery of EC ATP levels following oxidant injury by exposing EC to 5.5 mM glucose and 25 mU/ml glucose oxidase for 45 min. and then allowing the cells to recover for 3 hrs. After oxidant injury ATP levels declined from 4.96±.6 to 0.93±.15 pmoles/µg cell protein. After a 3 hr. recovery period, the ATP content of EC cells increased to 1.73±.21 pm/µg protein; when 25 µM of ATP, ADP, AMP or adenosine was added to the recovery media, regeneration of ATP was significantly ($P < .05$) enhanced to 4.47±.25, 4.64±.28, 4.83±.56 and 4.48±.24 pm/µg protein, respectively. Inosine, hypoxanthine, xanthine, or uric acid (25 µM) minimally improved recovery of ATP levels (1.89 - 2.78 pm/ug protein). Regeneration of ATP prevented ^{51}Cr release. When adenosine transport was inhibited with 50 µM dipyridamole or 50 µM NBMPR, repletion of ATP with exogenous ATP, ADP, AMP, and adenosine was attenuated to 1.08 to 2.30 pm/µg protein (20.8-40% of control) compared to 4.69 to 6.23 pm/µg protein (90-120% of control) without inhibitors. We conclude that ATP, ADP, AMP and adenosine augment repletion of EC ATP following oxidant injury via degredation to adenosine and subsequent adenosine uptake.

THE EFFECT OF GENTAMICIN (G) ON ORNITHINE DECARBOXYLASE (ODC) ACTIVITY IN OK CELLS. L. A. Arbeit, L. Ramsammy,*, R. Barnett and G.J. Kaloyanides. Dept. of Medicine, SUNY-Stony Brook, NY.

Polyamines (PA) have been implicated in the regulation of cell growth and proliferation. ODC is the first and rate limiting enzyme in the synthesis of PA. Since reversible cell injury elicits a significant increase in ODC, this enzyme is thought to play a critical role in the reparative response as well. Therefore, inhibition of ODC could be an important determinant of the injury induced by a toxic agent. In this study we examined the effect of G on ODC activity of OK_1 cells in vitro and in vivo. Addition of G directly to the assay reactants inhibited ODC activity in a dose-dependent manner.

G (M)	10^{-5}	10^{-4}	10^{-3}	10^{-2}
ODC (% of control)	95±7	56±8	24±4	8±3

Confluent OK_1 cells exposed to G (10^{-3}M) in the medium accumulated drug in a time-dependent manner. After 3 days of G exposure, ODC was markedly stimulated in OK_1 cells, but the degree of stimulation declined by day 4 and day 6.

	ODC (pmol CO_2/mg protein)		
Day	3	4	6
Control	173±36	99±4	70±8
G	877±13†	182±9†	147±15†

† p<.01

The marked rise of ODC at day 3 is consistent with a reparative response to G-induced cellular injury. The subsequent decline of ODC at days 4 and 6 suggests that ODC activity is being depressed possibly secondary to the progressive uptake of G by OK_1 cells. Inhibition of ODC activity may be an important determinant of G toxicity.

DELAYED LIPID PEROXIDATION IN RENAL CORTEX AFTER ADRIAMYCIN(ADR) ADMINISTRATION. JA Bertolatus and DA Bronsema*. Depts of Medicine, VA Medical Center and U of Iowa, Iowa City, IA.

Although doxorubicin (ADR) nephrosis is a convenient model of nephrotic syndrome in the rat, the mechanism of renal injury is uncertain. In in vitro studies of other tissues, ADR causes generation of reactive oxygen species(ROS), probably by redox cycling of a semiquinone metabolite of ADR. ROS generation in vitro is demonstrable within ≈1 hr. To determine whether ADR causes generation of ROS in tissues in vivo, we measured malondialdehyde(MDA) levels as an index of lipid peroxidation in homogenates of heart, lung, liver and renal cortex from rats given ADR, 5 mg/kg IV, or saline (CON). MDA was measured by a thiobarbituric acid method, which could quantitatively measure exogenous MDA added to normal renal homogenates, and expressed as nmoles MDA/mg protein(BioRad protein assay). At 1, 2, 3, or 12 hr after injections, tissue MDA levels were not different between ADR and CON (1-2 paired rats at each time). However, at 24 hr, ADR had mean (±1SD) renal cortical MDA of 1.00 ± 0.09 nmoles/mg protein, while CON had 0.79 ± 0.16 (n=5, both groups; p<0.03, t-test). Results were similar when expressed as MDA per unit tissue weight. Levels in other tissues were not different between groups at any time. The delay in appearance of lipid peroxidation in vivo can be explained by greater tissue defenses against oxidative stress, and suggests that ADR persists in renal cortex, leading to generation of ROS for at least 24 hr. Lipid peroxidation found specifically in kidney after ADR suggests a role for ROS in glomerular injury in this model.

PRODUCTION OF THE NEUROTOXIN 6-OH DOPAMINE (6OHD) BY DOPAMINE (D) INFUSIONS IN PATIENTS WITH SHOCK. T. Dalle Ave*, A. Sharma* and A.D. Baines. Dept. of Clinical Biochemistry, Univ. of Toronto, Toronto, Ontario, Canada.

The neurotoxin 6OHD can be produced in vitro by free radical attack on D. We examined the in vivo production of 6OHD by free radicals generated during cardiogenic, hypovolemic or septic shock in 50 acutely ill patients receiving therapeutic D infusions. Plasma catecholamines, including 6OHD, were measured by HPLC with coulometric detection. Identity of 6OHD was confirmed by gas chromatography with mass spectroscopy and by nuclear magnetic resonance. D infusion rates ranged from 1-27 ug/kg.min. 6OHD correlated with D infusion ($p=0.02$, $r=0.33$) and with plasma D concentration ($p=0.02$, $r=0.34$). The highest concentration of 6OHD was 52 uM. Concentrations greater than 1 uM were recorded in 33 patients. To measure the 6OHD neurotoxicity, rats were injected with a 6OHD bolus (10 mg/kg i.v.) with and without concomitant D infusion (10 ug/kg.min). Within 1 min of the injection plasma 6OHD was 1-22 uM and decreased with a half time of 1-2 min. Three days later renal NE stores were reduced by 49±15%. Thus, the concentrations of 6OHD observed in acutely ill patients during D infusion may have been high enough to damage sympathetic nerves. The unexpectedly low norepinephrine concentrations found in several patients were consistent with sympathectomy. If the blood-brain barrier is impaired during shock, 6OHD could produce irreversible central neuropathy as well as reversible peripheral neuropathy.

CYCLOSPORIN (CSA) INDUCED RENAL DYSFUNCTION AND ENZYMURIA IN THE RAT. R. Berty* and S. Adler. Dept. of Med., Montefiore Hosp. and Univ. of Pittsburgh Sch. Med., Pittsburgh, PA

As CSA causes changes in glomerular and tubular function, the effect of CSA on endogenous creatinine clearance (CCr), sodium excretion (NaEx) and enzymuria was studied. Rats (n=24) fed a special liquid diet received olive oil (CON) or 7.5 mg/kg CSA in oil (EXP) for 14 days. Body weight in CON rose in 14 days from 306 to 359 g and in EXP from 307 to 344 g. In 4 EXP rats, blood CSA 22 hours post dose ranged from 1384 to 1502 ng/ml. After 5 days of acclimation to the diet 24 hour sodium, citrate, gamma glutamyl transpeptidase (GGT), alpha-galactosidase (AG) and N-acetyl-B-glucosaminidase (NAG) excretions were identical in the two groups. Results on experimental day 14 are shown (mean ± SEM and CON versus EXP unpaired):

URINE	CON	EXP	p value
CCr(ml/min/100g)	0.46±.03	0.37±.01	< 0.001
NaEx(mEq/day)	0.95±.06	0.80±.06	> 0.05
CIT(umol/mgCr)	0.82±.13	1.25±.22	> 0.05
GGT(units/mg/Cr)	2.36±.18	2.63±.24	>0.20
AG(units/mgCr)	154±28	140±20	>0.50
NAG(units/mgCr)	1811±110	2393±164	<0.01

Daily NAG excretion rose at least 35% above baseline in each EXP rat (range 35-207%). In a separate study, a smaller CSA dose, 5 mg/kg, caused no changes compared to CON in any of the above values. The results show: (1) This is a stable reproducible animal model for CSA nephrotoxicity. (2) No tubular changes are noted in the absence of glomerular changes. (3) Isolated NAG enzymuria is the most sensitive indicator of CSA-induced renal tubular toxicity.

EFFECTS OF BENZOLAMIDE(B) AND ANTIOXIDANT THERAPY IN ISCHEMIC RENAL FAILURE(IRF) IN THE RAT. J.E. Bird, O.W. Peterson* and R.C. Blantz. Univ. of Calif. and VA Medical Center, San Diego, CA.

IRF results in a reduction in single nephron filtration rate (SNGFR) after 24 hrs reflow (45→15 nl/min) which is ameliorated by treatment with the antioxidant probucol (P) (28 nl/min), but the decrease in absolute proximal reabsorption (APR) to 6 nl/min was not prevented by P (JCI,1988). Current studies examined the effects of proximal tubular (PT) transport inhibition with B during the first 18 hrs of reflow after 1 hr ischemia. Glomerular filtration rate (GFR), late proximal (LP) and distal (D) SNGFR, APR, and loop reabsorption (ALR) were measured in uninephrectomized B treated control (CB), ischemic (IB) and P treated ischemic (IPB) rats. *=p < .05 vs CB. Mean ± SE are shown.

	GFR	SNGFR		APR	ALR
		LP	D		
CB	1.3±.1	62±2	50±2	21±1	24±2
IB	.3±.1*	37±6*	28±5*	16±3	13±3*
IPB	.3±.1*	47±6	36±7	19±4	16±3

B treatment in IB and IPB rats prevented the reduction in APR in IRF. LP SNGFR was improved in IB rats by B but remained below CB values. SNGFR in IPB rats was not different from CB, suggesting an additive beneficial effect of P and B. GFR in IB and IPB rats was lower than in CB, suggesting persistent tubular backleak. ALR and D SNGFR were significantly reduced in IB rats when compared to controls. Conclusions: 1. B treatment during reflow improved PT function indexed by APR in IRF, and produced a beneficial effect on SNGFR. 2. Treatment with an inhibitor of PT transport and an antioxidant produced major improvements in glomerular and tubular function in IRF.

THROMBOXANE AMELIORATES RENAL MEDULLARY HYPOXIA. M. Brezis, A. Shina*, K. Spokes*, F. H. Epstein, S. Rosen. Dept. of Med., Hadassah Univ. Hosp., Jerusalem, Israel & Depts. of Med. & Path., Beth Israel Hosp. & Harvard Med. Sch., Boston, MA

To test the effect of the renal vasoconstrictor thromboxane (TXA) on medullary oxygen balance in isolated perfused rat kidney, a stable TXA analog (U46619,10^{-7}M) was added to the perfusate, which was supplemented with amino acids. As expected, TXA decreased perfusion flow from 48±2 (SE) ml/min to 37±3 ($p<0.01$) and markedly reduced GFR from 0.7±0.1 ml/min to 0.1±0.1 ($p<0.001$). While the percent of filtered sodium reabsorbed was unchanged at 98±1%, the absolute flux of sodium reabsorbed along nephrons (T_{Na}) fell from 97±8 µEq/min to 33±13 ($p<0.001$). Necrosis of medullary thick ascending limbs (mTALS), regularly observed during isolated perfusion because of medullary hypoxia, was reduced by TXA from 20±5% of mTALS to 5±3 ($p<0.02$). TXA addition produced tubular collapse in 32±5% of nephrons (vs 2±1 in controls, $p<0.001$) - i.e., morphological expression of low distal urine delivery. Indeed, the extent of tubular collapse inversely correlated with GFR and T_{Na} ($r=0.7$, $p<0.001$). TXA-induced mTAL collapse was associated with protection from hypoxic injury ($r=0.7$, $p<0.001$). In a different set of experiments, an inhibitor of TXA synthesis (OKY 046) added to perfusate (3.3 mg/dl) augmented the proportion of mTALS with hypoxic damage ($p<0.005$). These results suggest that TXA ameliorates medullary hypoxia, possibly by decreasing GFR and tubular reabsorptive workload. Release of TXA in some forms of acute renal failure may serve to prevent hypoxic tubular necrosis.

EARLY MITOCHONDRIAL INJURY MEDIATES CISPLATIN (CPt) TOXICITY TO THE PROXIMAL TUBULE (PT): ATTENUATION BY GLUTATHIONE (GSH) AND SUPEROXIDE DISMUTASE (SOD). HR Brady*, BC Kone, ME Stromski*, G Giebisch, SR Gullans. Harvard Med. Sch., Boston, MA. and Yale Univ., New Haven CT.

To define the early subcellular events in CPt toxicity, we studied oxygen consumption (QO_2) and K^+ transport in rabbit PT suspensions using extracellular O_2 and K^+ electrodes. CPt caused delayed, progressive inhibition of QO_2 (V_{max} 90%, K_I 0.9 mM) and K^+ transport. At 40 minutes (see Table), CPt (1mM) markedly inhibited basal QO_2 (nmol O_2/min/mg prot.) as well as maximal ADP-coupled (nystatin) and -uncoupled (CCCP) QO_2. Na^+, K^+-ATPase activity (ouabain-induced K^+ efflux, nmol K^+/min/mg) and cytosolic K^+ content (nmol K^+/mg) were also significantly reduced. At earlier time points (10 min), however, when basal metabolism (QO_2) and K^+ transport were intact, maximal stimulation of cellular respiration with nystatin or CCCP uncovered significant mitochondrial dysfunction. (* $p < 0.05$)

	Control	CPt 10 min	CPt 40min
Basal QO_2	21±1	21±1	13±1*
Nystatin QO_2 (% basal)	163±3	120±9*	56±3*
CCCP QO_2 (% basal)	269±11	210±6*	69±12*
Na^+,K^+-ATPase activ.	154±6	159±2	46±2*
Cytosolic K^+ content	327±35	320±17	97±8*

CPt inhibition of basal QO_2 was significantly attenuated but not prevented by pretreatment with reduced GSH (2mM) or SOD (100 U/ml): control 26±1, CPt 10±1, CPt+GSH 15±1, CPt+SOD 14±1, n=5. Thus mitochondrial damage is the primary event in CPt toxicity to the PT. Oxygen free radicals appear to play a critical role in the evolution of CPt-mediated cell injury.

CYCLOSPORINE INHIBITS MESANGIAL CELL PROSTAGLANDIN PRODUCTION. Martin Bunke, Larry Wilder*. Div. of Nephrology, Univ of Louisville, Louisville VAMC, Louisville, KY.

Mesangial cells (MC) contract and increase their production of vasodilatory prostaglandins (PG) in response to vasoconstrictive compounds. This increase in MC vasodilatory PG production is postulated to minimize alterations in GFR in the presence of vasoconstrictive stimuli. Vasoactive agents which increase MC PG production increase the activity of protein kinase C. To test the hypothesis that cyclosporine (CyA) inhibits production of vasodilatory PG by MC in response to vasoactive agents and to protein kinase C activation, MC isolated from Sprague Dawley rats were incubated with CyA, 1 µg/ml or vehicle (V) for 24 hours. The MC were then incubated for 20 minutes with angiotensin II (AII), 0.2 µm, or the protein kinase C activator oleoylacetylglycerol (OAG), 10 µM. PG in the supernatant were determined by RIA. The data were analyzed by ANOVA. The data for MC PG production are below (pg/mg protein, mean±SD, N=7 AII, N=6 OAG).

	CyA-AII	V-AII	CyA-OAG	V-OAG
PGE_2	108±49	944±422	391±184	1002±257
$6KF_{1\alpha}$	68±51	209± 61	94± 59	234± 62
$PGF_{2\alpha}$	271±40	912±285	287± 61	850±162

The data show that CyA significantly inhibits the production of PG by MC exposed to either AII or OAG. We conclude that this decrease in MC vasodilatory PG production plays a role in the CyA-induced decrease in glomerular function, by removing a compensatory mechanism that maintains GFR in the presence of vasoconstrictive stimuli.

DIRECT RENAL EPITHELIAL PROTECTION FROM HYPOXIC INJURY WITH VERAPAMIL (Ver) PRETREATMENT. T.J. Burke, H. Singh,* P.E. Arnold,* and R.W. Schrier. Dept. Med., Univ. Colorado Sch. Med., Denver, CO 80262

It is unclear as to whether in vivo administration of Ver or other calcium channel blockers (CCB), which attenuate acute renal failure, exerts protection directly on renal epithelia or indirectly via improved renal hemodynamics, or both. In the present study, freshly isolated proximal tubules from the rat were harvested after percoll gradient centrifugation and gassed with 95% O_2/5% CO_2 for 15 min. Hypoxia (Hy) was then induced by gassing with 95% N_2/5% CO_2 for 30 min followed by reoxygenation (Reo) for 45 min. Ver (10^{-4} M) administered 15 min prior to Hy prevented the Hy-induced decrease in cellular K^+ (nmol/mg prot; 90±8 vs 158±16 nmol/mg, p<.05) and reduced LDH release from 48±3 to 41±5%, p<.05. The Hy-induced decrease in ATP was not affected. After Reo, Hy+Ver tubules exhibited higher K^+ levels than did Hy alone (238±15 vs 159±10 nmol/mg, p<.05) and ATP recovered to slightly higher levels as well (7.1±0.9 vs 5.9±0.6, NS). Control tubules treated with Ver for 15 min had increased K^+ compared to non-Ver treatment (335±11 vs 283±16 nmol/mg, p<.05) but % LDH release and ATP were identical to controls. 10^{-6} and 10^{-5} M Ver were less effective than 10^{-4} M Ver. Ver may, therefore, reduce K^+ efflux and/or K^+ conductance thereby increasing cellular K^+. The reduced LDH release in Ver-treated anoxic tubules suggests a role for CCB to attenuate membrane injury induced by O_2 deprivation.

SUBCELLULAR EVENTS IN SPERMINE (SPM) INDUCED ACUTE TUBULONECROSIS (ATN). R.A. Campbell, M.R. Nicolls, S. Nhoung, S. Williamson, J.B. Russi, K. McGrath. Oregon Health Sciences University, Department of Pediatrics, Portland, Oregon.

Progressive structural effects of natural endogenous amine (SPM) in producing ATN were studied. BalbC mice (M) P1 and P2 tubules (PT) responded to i.p. SPM, 75mg/kg, with enhanced apical endocytosis at 1 hr. P1 and P2 endosome (END) exocytosis by 3 hr was lytic to microvillae but not lytic in P3 despite massive fluid egress. Mitochondrial rupture and giant inclusion bodies were noted. Sprague-Dawley rats (R) treated with i.p. SPM, 23mg/kg/d, revealed lysosomal (LYSal) proliferation; 4.3 vrs 1.8 LYS/normal profile (p<0.01). LYSs were hypertrophic (p<0.05). Myeloid bodies (MBs) were seen in LYSs, P1 and P2 ENDs, basolateral folds and endothelial cells. LYSal MBs/cell profile increased from 0.42/PT cell to 2.2 with SPM (p<0.01). ENDal MB exocytosis was widespread. Control subapical ENDs were 2.3/PT cell; SPM profiles were 4.8 (p<0.05). Deep ENDs were 1.3 vrs 0.4 (p<0.05). SPM induced many clear END-END, some LYS-LYS, brushborder and bizarre cytosegresome fusions. Lysis of microvilli, cytoskeletal dissolution, basolateral fold loss, blebbing, deterioration of cytoplasmic matrix, compaction and rounding up of organelles and pre-mortem mitochondrial accumulations of dense calcium were noted. We conclude SPM with propensity for PT accumulation is a potent stimulus to numerous normal as well as pathological PT processes. Thus, repeated daily low SPM dose caused cell necrosis as did a single large dose. SPM toxicity to P1 and P2 was similar to that of the aminoglycosides.

ROLES OF THEOPHYLLINE (T) AND ADENOSINE (ADO) ON INTRACELLULAR ADENINE NUCLEOTIDES (AN) IN POST-ANOXIC CULTURED RENAL CELLS. P. Cadnapaphornchai, D. Kellner*, A. Golembieski* and F.D. McDonald. Wayne State Univ. Dept. of Medicine, Detroit, MI

Improvement of renal function in acute renal failure by T was thought to be due to its antagonistic effect on ADO, a renal vasoconstrictor. The role of T on cellular AN is less clear. When cultured cells from rabbit S3 segments were subjected to O_2 and substrate deprivation, ATP fell by 80%. Upon reoxygenation, ATP returned to 43 and 72% of non-anoxic cells at 3 and 24 hours respectively. ADO (0.25mM) alone increased ATP to 138% and 79% of control at 3 and 24 hours respectively. ATP was 36 and 186% of control at 3 hours with T and T+ADO respectively. The effects of T and combined T + ADO on AN at 24 hours were as follow (mean ±SE). *,+ significantly different from control and anoxia (A) respectively.

	control	anoxia	A+ADO	A+T	A+T+ADO
ATP	1.07 ±.07	.72* ±.03	.79* ±.07	.95+ ±.07	1.05 ±.06
ADP	.28 ±.05	.19* ±.05	.20* ±.04	.33+ ±.08	.31 ±.06
AMP	.09 ±.03	.09 ±.05	.08 ±.04	.10 ±.04	.09 ±.04
TAN	1.44 ±.09	.99* ±.05	1.08* ±.11	1.38+ ±.13	1.45 ±.05
EC	.84 ±.01	.81 ±.02	.83 ±.01	.81 ±.02	.83 ±.02

These findings suggest that ADO increases post-anoxic AN. The effect is short lived. T alone has no short term effect on AN but appears to raise AN at 24 hours. Combined T+ADO return AN to control at 24 hours. We conclude that combined T+ADO may be of benefit in acute renal failure.

MITOCHONDRIAL SUPEROXIDE DISMUTASE ACTIVITY AND RESPIRATION IN ISCHEMIC ACUTE RENAL FAILURE IN THE RAT. Maria E. Carvalho*, Vanda Yoshida*, Silvia Campos*, Marisa Medeiros*, Antonino Rocha*. (intr. by Roberto Zatz) Heart Institute - University of São Paulo, School of Medicine. São Paulo, SP - Brazil.

Changes in mitochondrial (Mito) O..2 consumption and activity of Superoxide Dismutase (SOD) in ischemic acute renal failure were studied by 1hr unilateral clamping of the renal artery in rats. Cortex and medulla were studied at the end of clamping, and 1hr and 18hr after reflow. Mito respiration was measured polarographically and SOD by NBT reduction. Results for the cortex after 1hr occlusion compared to contralateral control showed a decrease in State3 (ADP stimulated) respiration (202.0±12.7vs92.4±10.5 ngatoms O/mg/min p<.001), no significant change in State4 (succinate supported) and a decrease in Acceptor Control Ratio (ACR)[State3/State4](3.76±.12vs1.83±.08 p<.001). After 1hr reflow ACR rose from 1.83±.08 to 3,18±.06 (p<.001) but still below control, with similar results for the medulla. SOD (Units/mg) compared to control increased after 1hr clamp/1hr reflow [cortex: 1.24±.12vs1.65±.1 (p<.01); medulla: .76±.06vs1.3±.12 (p<.001)]. Mito respiration at 18hr was again decreased with reduced ACR (1.98±.11) and State3 (71.6±3.8). Thus reflow was associated with early improvement and late deterioration of Mito respiration. SOD behavior suggests a role for $O..2^-$ in such deterioration.

SUBSTRATE DEPRIVATION POTENTIATES CALCIUM INDUCED MITOCHONDRIA (M) INJURY. T.M. COIMBRA*, J.M. MESSANA*, D.A. CIESLINSKI*, AND H.D. HUMES, VAMC and University of Mich., Ann Arbor, MI.

Ischemia is characterized by substrate deprivation and hypoxia. Reperfusion is characterized by cellular and mitochondrial Ca^{++} overload. Since mitochondrial injury promoted by Ca^{++} overload during reperfusion is critical in ischemic cell injury, substrate deprivation during ischemia may add to the degree of mitochondrial injury induced by Ca^{++} during reperfusion. Accordingly, M were isolated from rabbit renal cortex and were incubated for 10-20 min with or without succinate (S) at which time $CaCl_2$ (75 nmoles/mg prot) was added with or without S. State 3 and 4 respiration were measured with succinate (5 mM) and expressed as natoms O/mg prot/min. n=4; *p< 0.05 vs S, + p< 0.05 vs S + Ca^{++}. Data expressed as means

Condition	ACR	State 3	State 4
S	2.80	106.43	37.97
S+Ca^{++}	2.03*	95.83	47.18*
No S	2.40*	109.72	45.79
No S+Ca^{++}	1.11*+	46.19*+	41.66

Thus, calcium induced mitochondrial injury is potentiated by prior substrate depletion. Furthermore, this potentiating injurious effect of substrate depletion was ameliorated by exogenous ATP suggesting a key role for adenine nucleotide depletion in this potentiation process.

NIFEDIPINE (N) AND CYCLOSPORINE (CsA) ON RENAL FUNCTION. Q. Corteza,* B. Urbaitis, S. Shen, M. Shu,*, A. Amin,* R. Ajemian,*, D.K. Klassen. Univ. of Maryland Hospital, Dept. of Medicine, Renal Division, Baltimore, MD.

CsA-induced nephrotoxicity is associated with marked increase in renal vascular resistance. Some evidence indicates that Ca++ antagonist N may be protective. This study was done to determine if N ameliorated the effect of CsA on renal function in rats. CsA was given at 20 mg/kg/d x7 days via an implanted feeding tube; N at 2.5 mg/kg/d infused constantly via an implanted intracorporeal osmotic pump. GFR, ERPF, RPF cortical (C) and non-cortical (NC) plasma flow were measured by inulin clearance, PAH clearance and arteriovenous PAH difference, respectively. Glomerular (G) production of prostaglandins (PG) were measured by RIA and expressed as ng/mg of G protein. Data obtained are as follows:

	Control (8)	CsA (4)	N (6)	CsA+N (8)
Vol	5.4±1	3.6±1	8.0±4	4.6±1
GFR	1478±204	886±312	1546±306	571±139
RPF	5125±781	3760±900	4799±967	3105±626
ERPF	3776±557	2791±1008	3671±727	2189±465
C	74%	77%	76%	70%
NC	26%	23%	24%	30%
PGE2	0.67	1.59	1.75	2.72
6-k PGF	0.47	0.78	0.98	1.38
TxB2	0.66	1.49	1.00	1.23

In conclusion, 1) with these doses and means of administration, N did not appear to offset the detrimental effects of CsA on renal function; 2) CsA and N each increase PG production; the combination of CsA and N seems to increase the production of the vasodilating PG more than each agent individually.

HYPERSENSITIVITY TO RENAL NERVE STIMULATION (RNS) IN ACUTE RENAL FAILURE (ARF) DUE TO THROMBOXANE (TX) AND ANGIOTENSIN (A). J.D. Conger and J.B. Robinette. UCHSC and VAMC, Denver, CO.

Aberrant renovascular reactivity in ischemic ARF is characterized by hypersensitivity to RNS. The mechanism of hypersensitivity is not understood. Based on studies showing that A and TX increase neurotransmitter release to RNS and A and TX activity are elevated in ischemic injury, the roles of intrarenal A and TX in hypersensitivity to RNS were examined in rats with norepinephrine (NE)-ARF. Renal vein: artery renin activity was 4-fold and urine TXB_2 2-fold higher in NE-ARF than control (C) rats (both $p<0.02$). Slopes (B) before infusion of A or TX inhibitors of renal blood flow (RBF) to RNS (0 to 10 Hz) for C and NE-ARF were 0.17±0.14 and 0.43±0.06 ml/Hz, respectively, (different at $p < 0.01$). Saralsin (S), 0.2 ug/kg/min, or OKY-046 (OKY), 0.3 ug/kg/min, failed to change B of RBF to RNS in C. In NE-ARF, however, B of RBF to RNS after S was 0.24±0.10 and after OKY 0.23±0.08 ml/Hz, similar to respective preinfusion values in C. Urine TBX_2 was reduced 3-fold after OKY in NE-ARF ($p< 0.01$) but only by one-third in C ($p< 0.05$). Prostacycline, $3x10^{-4}$ ug/kg/min, increased RBF by 50 percent in NE-ARF but did not change B of RBF to RNS (0.45±0.11 ml/Hz). Conclusions: Renal renin-angiotensin and thromboxane activity are elevated in ischemic ARF and responsible for the adrenergic hypersensitivity to RNS. S and OKY effects were not due to non-specific vasodilatation.

A RANDOMIZED TRIAL OF NICARDIPINE (N) IN THE PREVENTION OF CYCLOSPORINE A (CyA) NEPHROTOXICITY (NT) IN DIABETIC PA-TIENTS (D). A. Delgado, T. Hannedouche, A. Gnionsahe, C. Boitard, R. Assan, JF. Bach, JP. Grünfeld. Dept of Nephrol., Hôpital Necker, Paris, France (intr. by L. Bankir)

CyA can induce remission in very recent onset type 1 D but NT has long been a major concern. As calcium antagonists were found to exert a protective effect vis a vis CyA NT in animals, renal function was compared in D treated with either CyA alone 7.5mg/kg.d (n=15) or CyA+N 60mg tid (n=13). The following parameters were assessed before (M0) and after 3 mo of treatment (M3): glomerular filtration rate (GFR) as inulin clearance; mean arterial pressure (MAP); renal vascular resistance (RVR= MAP [1-Ht]÷Cpah); peak whole blood trough CyA (BlCyA). Results were:

	GFR ml/mn	MAP mmHg	RVR dyn.sec/cm5	BlCyA ng/ml
CyA alone				
M0	132 ±25	86 ±12	6555 ±1927	
M3	122 ±32	96 ±13**	7674 ±2300*	728 ±322
CyA+N				
M0	131±22	90 ±9	6295 ±1146	
M3	117 ±19**	96 ±9**	7984 ±1905**	1066 ±219§§

*p<.05,**p<.01 M3 vs M0; §§ p<.01 CyA vs CyA+N

At M0 all parameters were similar in both groups. At M3 MAP and RVR increased in both groups although slightly less in the CyA+N group. GFR decreased significantly only in the CyA+N group. BlCyA was significantly higher in the CyA+N group probably due to metabolic interferences between CyA and N. We concluded: 1) Low dose CyA increased RVR and MAP but did not alter GFR; 2) Chronic N administration was unable to prevent the CyA-induced changes in RVR and in GFR.

NUCLEAR MATRIX-BOUND DEOXYRIBONUCLEIC ACID (DNA) SYNTHESIS: "IN VITRO" STUDY IN ACUTE RENAL FAILURE. Bruno Di Paolo*, Paolo Cappelli*, Pio Palmieri*, Vincenzo Vocino*, Filomena Miscio* and Alberto Albertazzi* (intr. by J.F. Maher). Institute of Nephrology, Univ. of Chieti, Italy.

It is longtime known how the uremic liver is characterized by a loss of enzyme activity, derangement in affinity and by a decrease in ^{3}H-Thymidine incorporaction in DNA. Dialyzing the uremic toxin the ^{3}H-Thymidine incorporation is restored to normal values. The liver nuclei of 10 white rabbits were prepared from 5 normals and 5 nephrectomized animals 48 hours after the surgery, in order to understand the significance in terms of genome involvement and enzyme response to stimulation or induction. Matrix-bound DNA synthesis continued for at least 1 h with a decreased level of DNA synthesis in uremics as compared with nuclei from 5 controls (25.50±3.15% vs 105.40±6.80%, $p<.001$). Nuclear and matrix-bound alpha-polymerases for optimal DNA synthesis required an ATP consumption which in uremics was 6.80±1.90 mM greater than in controls (4.30±1.30 mM, $p<.05$). The nuclear and matrix-bound alpha-polymerases were stimulated 5-7 fold in controls vs uremics. In uremia the myriad features of overall syndrome seem to be responsible for the intranuclear, cytolosolic and extracellular derangement of metabolism. These findings have led to the proposal that the ubiquitous "uremic toxins", modifying the enzyme pool and the "uremic disease" even though relationships and effects still remain elusive.

PROTECTIVE EFFECT OF DEXAMETHASONE (DEX) ON GENTAMICIN NEPHROTOXICITY IN THE RAT. T. Eche*, R. Simonsen*, M. Griggs*, and R. Cronin. VAMC and UTX Southwestern Medical Center, Dallas, TX.

Interventions which increase renal cortical Na,K-ATPase activity (e.g. thyroid hormone, potassium loading) protect against gentamicin nephrotoxicity. We examined whether dexamethasone, a stimulator of proximal tubular Na,K-ATPase, protected against gentamicin nephrotoxicity in the rat. Dexamethasone was administered in two dosages, 0.25 mg/kg body weight or 5mg/kg body weight s.c. daily for three days alone or in combination with gentamicin, 80 mg/kg three times daily s.c. for a total of six doses beginning the day after the first dexamethasone dose. Clearance and enzymes studies were performed on the third experimental day. The results showed that inulin clearance (C_{In}) was significantly stimulated in the high dose dexamethasone group, HiDex, (1.8±0.1 vs 1.2±0.1 ml/min/100 g b.w. in controls (C), $p<0.05$, but not in the low dose dexamethasone group, LoDex, (1.3±0.1 ml/min/100 g b.w., NS). In rats receiving gentamicin alone, (G), C_{In} was lower than in animals receiving gentamicin plus low dose dexamethasone, LoDexG, (0.7±0.03 vs 1.1±0.1 ml/min/100 g b.w., $p<0.025$) or gentamicin plus high dose dexamethasone, HiDexG, (1.4±0.2 ml/min/100 g b.w., $p<0.005$). Compared to C rats, Na,K-ATPase activity was higher in LoDex (20.4±1.0 vs 17.5±0.8 µmol/mg protein/hr, $p<0.025$) and HiDex, 25.2±0.7 µmol/mg protein/hr, $p<0.001$). Enzyme activity was reduced in G compared to LoDexG (9.3±0.7 vs 13.2±1.1 µmol/mg protein/hr, $p<0.01$) and compared to HiDexG (14.7±0.7 µmol/mg protein/hr, $p<0.001$).

Thus, Dex protects against gentamicin nephrotoxicity independently of effects on GFR. The mechanism appears to be due to a biochemical effect, possibly related to stimulation of renal cortical Na,K-ATPase.

PLASMA ATRIAL NATRIURETIC FACTOR (ANF) LEVELS IN HUMAN ACUTE RENAL FAILURE. J.C. Dussaule, A. Kanfer, S. Czekalski, E. Rondeau and R. Ardaillou (Intr. by K.F. Badr), INSERM 64, Hôpital Tenon, Paris, France.

In order to investigate the physiopathological role of ANF in the course of acute renal failure (ARF), we measured endogenous plasma ANF, cyclic GMP, and renin activity (PRA) in 11 patients at the early stage of this disease (D_o ; plasma creatinine : 843 ± 93 µmol/l), during recovery (D_r ; plasma creatinine : 116 ± 12 µmol/l) and in the meantime. Plasma ANF (98 ± 26 pg/ml) and cyclic GMP (14 ± 3 pmol/ml) were initially elevated simultaneously with blood volume (75 ± 6 ml/kg). At the stage of recovery, plasma cyclic GMP (6 ± 1 pmol/ml) and blood volume (67 ± 4 ml/kg) returned to a normal range ($p<0.05$ vs. values at D_o) whereas plasma ANF, although lower than initially, was still elevated (70 ± 14 pg/ml). There was a peak of plasma ANF at the start of the polyuric phase associated with an increase of FE_{Na} in patients who had been neither dialyzed nor treated by diuretics. Differences between values at D_o and D_r for ANF were correlated ($p<0.05$) with those for cyclic GMP and blood volume. In contrast, the changes in PRA were not appropriate to those of blood volume : PRA was higher at D_o (4 ± 1.2 ng/ml/h) than at D_r (0.6 ±0.2 ng/ml/h ; $p<0.05$). These results suggest the following conclusions : 1- At the early phase of ARF, high plasma ANF and cyclic GMP levels are appropriate to hypervolemia ; 2- Increase of plasma ANF at the start of polyuria and persistence of high levels during recovery may play a role in the restoration of normal sodium and water balances in these patients.

RENAL CORTICAL MITOCHONDRIAL (RCM) INTEGRITY IN CYCLOSPORINE (CsA) NEPHROTOXICITY. Lawrence W. Elzinga, Leena Mela-Riker*, Linda Widener*, William M. Bennett. Oregon Hlth Sci Univ., Depts. Med., Biochem, & Surg., Portland, Oregon.

The nature of acute CsA nephrotoxicity is unclear. Therefore, CsA's effect on multiple parameters of RCM integrity was assessed.

Male Fischer rats (n=5) were given CsA 25 mg/kg i.p.x14 d. Pair-fed control rats received vehicle. On day 15, RCM were isolated and their respiratory activity (using 4 substrates), Ca^{2+} uptake and cytochrome concentrations were measured. Substrates were: glutamate-malate (G/M), palmatyl carnitine (PC), pyruvate (Pyr), and β-hydroxy-butyrate (βOHB). Inulin clearance (Cin), urine enzymes (NAG) and histology were determined in parallel. Data are mean ± SE.

	Cin (ml/min/100g)	NAG (IU/mgCr)	Ca^{2+} uptake (nmoles/min/mg protein)
CsA	.29±.23	.12±.04	354±13
Veh	.87±.10	.14±.03	416±16
p	<.005	NS	<0.025

State 3 Respirations (nmoles O_2/min/mg prot)

	G/M	PC	Pyr	β-OHB
Csa	40±3	34±1	27±3	21±2
Veh	46±3	38±2	36±2	26±2
p	NS	NS	<.05	NS

Respiratory control ratios and concentrations of cytochromes b, c, and aa_3 were unaffected.

Conclusion: Despite profound renal dysfunction and morphologic alterations, changes in RCM function due to CsA were minor and do not support a direct toxic effect of CsA on mitochondrial function as the primary mechanism of its acute toxicity.

GENTAMICIN-INDUCED INHIBITION OF PROTEIN SYNTHESIS IN CULTURED RENAL PROXIMAL TUBULAR CELLS. M.S.V. Enriquez* and J. Vadgama*. (intr. by W. Davidson). Harbor-UCLA Medical Center, Torrance, CA.

Nephrotoxicity is a major side effect of aminoglycosides. The use of whole animal models to study gentamicin (G) nephrotoxicity is complicated by the structural and functional heterogeneity of the kidney. We propose to eliminate that by utilizing a cultured cell model of monkey proximal tubular (PT) origin.

Our studies show that G causes a dose-dependent reduction in PT protein synthesis. A half-maximal inhibition was observed with 2.5mM G. The rate of ^{3}H-Leucine incorporation into TCA precipitable protein decreased more rapidly in 72-hr. old cells compared to 48-hr. old cells. The rate of DNA synthesis as measured with ^{3}H-Thymidine incorporation was decreased at 2.5mM G. In contrast, the rate of RNA synthesis as measured with ^{3}H-Uridine incorporation declined with as low as 0.5mM G. "Younger" cells showed a greater sensitivity to gentamicin in their RNA synthesis.

It has been suggested that dietary Ca^{++} loading could provide protection against G. PT cells exposed to 1-20mM Ca^{++} prior to the addition of G, PT cells simultaneously exposed to G and Ca^{++}, and PT cells that were given Ca^{++} after a 72-hr. G exposure did not exhibit significant reversal of G-induced growth inhibition. In addition, 2.5mM G decreased Na+dependent Ca^{++} influx. Inhibition was more significant with the "slower" pathway (5 min.) than with the more "rapid" pathway (30 sec.). G had no appreciable effect on Na+ independent Ca^{++} influx.

Ca^{++} channel blocking agents have been suggested to be beneficial for other types of acute renal failure. In our preliminary studies, there was no significant effect of nifedipine, diltiazem, or verapamil on G-exposed cells.

In conclusion, G causes a dose-dependent inhibition of total protein, DNA, and RNA synthesis in cultured PT cells. In this regard, additional studies are underway to define specific transport protein derangements caused by G. Ca^{++} and Ca^{++} channel blockers did not demostrate a significant reversal of gentamicin's negative effect on cell growth.

PROTECTIVE EFFECT OF GLYCINE (G) INFUSION IN A SINGLE NEPHRON MODEL OF ACUTE RENAL FAILURE (ARF). F.B.Gabbai,* O.W. Peterson,* and R.C. Blantz. Dept. of Medicine, UCSD and VAMC San Diego, CA.

Previous studies have shown both deleterious and beneficial effects of amino acid infusion in ARF. However, recent evidence demonstrates protection with G from hypoxia induced proximal tubule (PCT) injury in vitro. We have shown that intratubular administration of 0.5 ng uranyl nitrate (UN) reduces proximal reabsorption (APR) and decreases single nephron glomerular filtration rate (SNGFR) in the same nephron due to tubuloglomerular feedback (TGF) activation. We have examined effects of systemic G (1.5 cc/hr of 20g% solution) infusion in this single nephron model of ARF in 26 tubules (8 controls [C] and 18G) from 8 euvolemic M-W rats. UN (0.65 ng) was administered to early PCT over 3 minutes. Early distal SNGFR ($SNGFR_D$) was measured before UN and $SNGFR_D$ and late proximal $SNGFR_{LP}$ 5 minutes after intratubular perfusion UN (adjacent $SNGFR_{LP}$ as control). $SNGFR_D$, $SNGFR_{LP}$ and APR were pair-compared (*p<0.05 vs. pre-UN) (Mean ± SEM).

	$SNGFR_D$ (nl/min)		$SNGFR_{LP}$ (nl/min)		APR (nl/min)	
	Pre UN	Post UN	Pre UN	Post UN	Pre UN	Post UN
C	29±1	24±2*	37±2	26±2*	14±1	10±1*
G	40±2	38±3	44±3	44±3	16±2	15±2

UN perfusion decreased both SNGFR and APR in C. G increased baseline, pre-UN SNGFR and prevented the reduction in both APR and SNGFR after UN. G infusion clearly protects from UN induced ARF as noted in vitro. Beneficial G effects relate primarily to effects on APR and PCT injury and may also prevent SNGFR changes by decreasing TGF.

FUROSEMIDE (F) ACCELERATES GENTAMICIN (G) ACCUMULATION IN CULTURED RENAL CELLS. Y.Fukuhara*, H.Nakahama*, A.Yamauchi*, T.Takama*, Y.Orita* and T.Kamada*. Osaka Univ. Med. Sch., Osaka, Japan. (intr. by J.S.Handler)

F is known to potentiate G nephrotoxicity. The mechanism of potentiation is unclear. In our previous studies, we demonstrated that F enhanced G accumulation in rabbit renal tissues when injected as a bolus (Kidney Int. 33: 363, 1988) or repeatedly subcutaneously (Diuretics II, Elsevier, New York, p.693, 1987). In this study, we evaluated the effects of F on G accumulation in cultured renal cells (LLC-PK1) and hepatoma cells (H4IIE). Thus, we excluded effects secondary to F elicited hemodynamic changes. Seven days after seeding, the culture medium was exchanged for medium containing 1 mM G alone or 1 mM G + 1 mM F. Cell G concentration was measured by substrate labelled fluorescence immunoassay (TDA Gentamicin Kit). In LLC-PK1 cells, G concentration in G+F was significantly higher than that in G alone as indicated in the table, while G was not detected in H4IIE cells with or without F.

Medium	Cell Gentamicin Concentration nmol/mg protein	
	3 hours	6 hours
1 mM G alone	2.10±0.48	6.07±0.91
G + 1mM F	4.28±0.48*	8.25±1.60*

* p<0.01 vs G alone. Values are mean ± S.D.

We conclude that F can accelerate G accumulation in renal tissues and potentiate G nephrotoxicity.

HYPOALBUMINEMIA (HA) AS A RISK FACTOR FOR AMINOGLYCOSIDE (AG) NEPHROTOXICITY. G. Gamba*, A. Contreras*, J. Cortes*, Y. Santiago*, F. Nares*, A. Espinosa*, G. Jimenez*, J. Bobadilla*, G. López*, J.C. Pena. Instituto Nacional de la Nutrición Salvador Zubiran. Mexico City, Mexico.

We have recently informed that HA is a risk factor for AG nephrotoxicity (Rev. Invest. Clin 40:135,1988). Since AG do not bind to serum albumin (SA), the purpose of the present study was to find out if the higher risk of nephrotoxicity in pa - tients with HA was due to low SA per se or to malnutrition.

We have prospectively studied 113 ward patients who received amikacin I.V. >36hrs. All were evaluated for the following specific factors: age, sex, diagnosis, SCr, SA, peak and trought amikacin levels, and nutritional status. They were followed with SCr twice a week. Renal dysfunction was defined as an increase in SCr >0.5 mg/dl if the initial SCr was <2 mg/dl, or by an increase of >1 mg/dl if the initial SCr was >2 mg/dl. In 11 patients, amikacin pharmacokinetics were studied (6 with SA <2.5 g/dl and 5, >3 g/dl). There were no differences in age, sex, weight, diagnosis, and arterial pressure.

The overall incidence of toxicity was 11%. In patients with SA <3 g/dl it was 17% and in those with >3 g/dl it was 2.2%, X^2=4.6, p<0.05. There was no difference in the prevalence of malnutrition between patients with or without toxicity.

In the pharmacokinetic study, peak levels were higher in patients with SA <2.5 g/dl, compared with those with normal SA (12.7 ± 1.6 vs 9.3 ± 1.2 t = 3.53, p<0.01)

We have concluded that HA is a risk factor for AG nephrotoxicity, regardless of the nutritional status.

STRATEGIES TO INCREASE ATP GENERATION IN ANOXIC PROXIMAL TUBULES. R. Garza*, J. Ortega*, J. Stein, M. Venkatachalam. UTHSC, San Antonio, TX

Isolated rabbit proximal tubules were incubated in a standard medium (glucose 5 mM, lactate 4 mM, alanine 1 mM, butyrate 10 mM), with augmented alanine (5 mM; AA) or with AA plus equimolar α-ketoglutarate instead of lactate (AA-αKG). ATP (nmol/mg prot.) and LDH (% released) after 30' anoxia (anox), and with 60' recovery (rec) are shown. ($\bar{x}$ ± S.D.; n=6;* p < .005)

	ATP(anox)	LDH(anox)	ATP(rec)	LDH(rec)
Control	0.35±0.2	51.6±10	1.7±1.2	57.8±8.9
AA	0.6 ±0.2*	18.2±9*	7.6±2.6*	20.1±12*
AA-αKG	1.0 ±0.7*	8.5±2.2*	7.6±0.6*	8.3±3*

Capacity to reduce MTT tetrazolium, an index of mitochondrial function, was severely impaired both after anoxia and recovery in controls, but not in AA and AA-αKG. To assess ATP synthesis, ^{32}P(Pi) was included in the medium during anoxia. ^{32}P was markedly increased in ATP and membrane phospholipids in AA and AA-αKG relative to controls. Thus, AA and to a greater extent, AA-αKG, induced ATP synthesis during anoxia and protected the cells. Further, succinate levels during anoxia were elevated 4-6 fold in AA and AA-αKG relative to controls. αKG, by itself, was not protective (not shown). Extensions of glycolysis generating ATP with succinate as end product occur in anoxia resistant lower life forms (Science 219:1391, 1983). Such reactions are possible in mammalian kidneys (Am J Physiol 247: F618, 1984). How alanine might trigger these metabolic pathways in normally anoxia sensitive proximal tubules is not known.

CHARACTERIZATION OF THE INTERSTITIAL CELLULAR INFILTRATE IN CHRONIC CYCLOSPORINE NEPHROPATHY (CCN) D.M. Gillum, and L. Truong,Dept. of Medicine and Pathology, Baylor College of Medicine,Houston,Tx.

Features of CCN in male Sprague-Dawley (SD) rats include patchy areas of interstitial fibrosis, tubular atrophy and variable mononuclear cell infiltration. (Gillum et al,Transplantation 8/88). Phenotyping of these inflammatory cells with monoclonal antibodies (Ab) was accomplished using the avidin-biotin peroxidase technique to stain frozen sections of kidney tissue. Abs were directed against the following rat cell surface markers :OX1 (all hematopoietic cells),OX4 (B cells and macrophages), W3/13 (T cells),W3/35 (T helper cells) and OX8 (T non-helper cells). Cells were counted from multiple fields in involved and uninvolved cortex and corticomedullary junction. Kidneys were examined from CS treated animals as well as controls treated with olive oil vehicle and normal animals.

	OX1	OX4	W3/13	W3/35	OX8
CS	2.09	2.92	2.99	2.19	-
CONT	0.84	1.67	1.79	1.49	-
NORMAL	1	1	1	1	-

Relative score for each cell type in renal cortex

Examination of the corticomedullary junction revealed increased numbers of T and B cells in CS treated animals as well. We conclude that the cellular infiltrate of CCN is composed of relatively equivalent numbers of B and T cells, and the T cells are predominantly of the helper variety. Cytotoxic T cells could not be demonstrated.

INHIBITION OF 5'-NUCLEOTIDASE ENHANCES POST-ISCHEMIC RECOVERY OF METABOLIC FUNCTION. Karen M. Gandio, G. Thulin*, T. Ardito*, A. van Waarde*, M. Kashgarian and N.J. Siegel. Yale Un. Sch. of Med., Depts. Ped and Path, New Haven, CT.

We have shown diminished metabolic function and decreased cellular ATP levels in a proximal tubule suspension (PTS) obtained from ischemic kidneys. To study the effect of preservation of adenine nucleotides, rats were injected with AMPCP (4 mg/kg), the most potent inhibitor of 5'-nucleotidase which converts AMP to the permeable nucleoside adenosine. Two hrs later, rats underwent 45 min of ischemia. After 15 min of reflow, a PTS was harvested and O_2 consumption (O_2 nM/min/mg prot) was determined under basal (B), nystatin (N) and CCCP (C) stimulated conditions:

	Control	Untreated	AMPCP
B	34±0.8	22±0.6	30±1.1
N	55±2.2	32±1.5	58±3.0
C	100±5.8	57±2.2	88±8.0

The residual nucleotide pool at the end of ischemia was significantly increased in AMPCP rats (3.24±0.33 μmol/g vs 1.53±0.17 in untreated (UnT) rats, P<0.01). AMPCP rats had significantly better (P<0.01) metabolic function than UnT rats and were similar to nonischemic controls. Cellular ATP levels were significantly improved in AMPCP rats (3.8 ±0.6 nM/mg prot) as compared to UnT rats (2.9±0.1). Although histomorphometric analysis revealed evidence of ischemic damage in AMPCP rats, it was significantly less than UnT rats. Thus, inhibition of 5'-nucleotidase enhances the postischemic recovery of metabolic function and modifies the degree of cellular injury in the PT.

OXIDATIVE INSULT TO CULTURED PROXIMAL TUBULAR CELLS (PTC): A POSSIBLE ROLE IN REGENERATION. M.Goligorsky, V.Shen*, A.Laszlo*, T.Stokes, D.Menton*, D. Loftus*, M.Hirsch*, I.Karl*,and K.Hruska. SUNY at Stony Brook,NY 11794 and Jewish Hospital of St. Louis, MO 63110

Reoxygenation of PTC subjected to hypoxic perfusion is accompanied by the formation of free radicals. This is associated with an increase in Ca^{2+}_i and cell acidification. To examine the role of oxidative stress in cell physiology, we developed a model of graded H_2O_2 insult to PTC. 0.5-1.0 mM H_2O_2 resulted in reversible changes in cell topography, a 50% increase in soluble cGMP, reversible changes in Ca^{2+}_i and pH_i, and uncoupling of the communication-competent PTC monolayers. Higher concentrations of H_2O_2 resulted in similar changes which were irreversible within the experimental time frame. Mild oxidative insult to the quiescent PTC resulted in 26±6% increase in ^3H-thymidine incorporation (10% FBS resulted in a 53% increase), while higher concentrations of H_2O_2 suppressed it. The latter effect could be reversed with catalase pretreatment. Scratch wounding of the monolayers did not reproduce the effect of mild oxidative insult on ^3H-thymidine incorporation. Exposure of untreated PTC to the conditioned medium obtained from PTC treated with H_2O_2 resulted in a doubling of the stimulation of ^3H-thymidine incorporation. The data indicate that the relaxation of cell contacts as well as the secretion of a growth promotor(s) and/or destruction of growth inhibitor(s) can be responsible for the mitogenic effect of mild oxidative insult. The severity of oxidative insult determines whether toxic effects of free radicals counterbalance their effects on the regeneration.

Atrial natriuretic peptide and renal denervation - Effect on toxic acute renal failure - a comparative study

R. Herzog, K. Schafferhans, E. Heidbreder, A. Heidland, University of Würzburg, Department of Nephrology, FRG (Introduced by R.W. Schrier)

Objective of the study was to compare the protective action of ANP versus renal denervation (D) in urinanyl-nitrate (UN)-induced acute renal failure (ARF). We used female Sprague Dawley rats in the study. In group 1 (D) renal arteries were painted with 1 % phenol and animals were housed in metabolic cages over 2 days. On the day of the infusion studies UN (5 mg/kg b.w.) was injected intravenously as a bolus. In the second group ANP was infused at 10 µg/kg b.w./h prior to the toxic insult versus isotonic saline (NaCl). Inulin clearance (GFR), urinary volume (V), renal blood flow (RBF), electrolyte excretion and blood pressure was measured. UN induced a non-oliguric ARF in controls and in D-animals. In the latter group a significant increase of renal blood flow was found whereas GFR decreased comparable to controls following UN-administration. Remarkably UN had no effect on GFR of the ANP-group. ANP induced a slight and transient increase of RBF but prevented UN-ARF totally. We assume that other mechanisms than increased RBF, for example, dilating effect of ANF on glomerular cells antagonism against activated intrarenal renin-angiotensin-system and a decrement of intracellular calcium may be more significant in protecting kidneys from UN-ARF.

TIME COURSE AND LOCATION OF RENAL CELL (RC) REPLICATION IN THE RECOVERY PHASE OF ISCHEMIC ACUTE RENAL FAILURE (ARF). H.D. HUMES, J.M. MESSANA*, D.A. CIESLINSKI,* T.M. COIMBRA*, AND C.R. GALVAO*. VAMC AND UNIV. OF MI., ANN ARBOR, MI. The repair process of ARF is likely dependent upon tubule cell(TC) regeneration. To assess the time course and location of RC regeneration, rat kidneys were made ischemic with bilateral renal artery clamping for 30 min. After clamp release and varying hour time intervals, ^{3}H-thymidine (T) incorporation into renal tissue and BUN was measured. Data are presented as means for n=3-13.

Hour:	0	6	24	48	72	96	120
BUN(µg/dl)	21	53	126	158	115	113	61
T(10^3dpm/DNA)	17	93	77	236	255	117	--

Thus, RC regeneration peaked between 48 and 72 hours and correlated with the decline and recovery of renal function. To assess the location of RC regeneration, T labelled renal cells were counted in radioautographs of kidney tissue made ischemic and allowed to recover for 48 hours. Renal TC and interstitial cells (IC) were counted in 7-10 random fields in cortex (C), outer stripe (OS) and inner stripe (IS) of outer medulla. Data presented as average number of cells per field.

	C	OS	IS		C	OS	IS
TC:	59	34	18	IC:	0.6	1.2	0.5

Thus, TC replication in the proximal tubule and not the thick ascending limb predominates in the recovery phase of ischemic ARF. A very modest interstitial proliferation process also develops. RC replication is central in the repair process following ARF and is most predominant in proximal tubules.

A COMMON FINAL PATHWAY OF RENAL TUBULE CELL KILLING BY TOXINS AND ANOXIA (AN): ATTENUATION BY PROTEASE INHIBITION. D. Hreniuk, E. Guerra, and P.D. Wilson, Dept. Physiol, UMDNJ-RWJ Med. Sch., Piscataway, NJ.

Our previous studies have shown that the lethal effects of An and toxins in renal tubules, in vitro is dose and time dependent; tubule cell type-specific and can be attenuated by calcium restriction. To determine whether protease activation was a common mediator of cell killing, cultures of rabbit and human proximal straight tubules, (PST) subjected to 45 min An (95%N_2/5%CO_2); gentamicin (G, 1mg/ml) or cyclosporin (CsAlµg/ml), treatment were incubated with the Cys.-Asp.-, Ser.-and metalloprotease inhibitors: L-trans epoxy succinyl-leucyl amido (4-guanido) butane (E64), pepstatin; chymostatin and EDTA respectively. Cell death was measured by vital dye uptake; lactate dehydrogenase release; mitochondrial enzyme activity and SDS-PAGE of cell extracts metabolically labelled with [^{35}S]Met. Only E64 attenuated the amount of cell death induced by each agent. Percent of viable PST cells was:

	An	An+E64	G	G+E64	CsA	CsA+E64
PST	14+5	52+15	46+12	78+14	20+8	75+19

Full restoration of protein synthesis was achieved. Prior depletion of lysosomal enzymes by pretreatment with NH_4Cl offered no protection. Thus, cell death induced by An, G and CsA was mediated, via cytoplasmic, Ca^{2+}-dependent cysteine proteases and protease inhibitors may have chemotherapeutic potential in prevention of acute renal failure.

INTRARENAL PLATELET ACTIVATING FACTOR (PAF) IN THE EARLY PHASE OF ENDOTOXEMIA (E). K.H. Hwang,* J.J. Bourgoignie, D. Kikeri, G. Gavellas,* J.P. Pennell, S. Sabnis* and T. Antonovych. University of Miami, Miami, FL, and Armed Forces Institute of Pathology, Washington, DC.

The sequence leading from E to acute renal failure is poorly understood because E is associated with a cascade of biological events. PAF increases in E and PAF receptor antagonists have recently been shown to prevent, in-vivo, the nephrotoxic effects of E. The source of PAF in these studies remained undefined. We, therefore, evaluated the effects of E.Coli endotoxin on renal function in vitro using the rat isolated kidney preparation. Whereas glomerular filtration rate in time-control experiments (N=10) decreased from 0.99±0.06 (SE) to 0.85±0.05 ml/min ($p<0.05$), inulin clearance decreased from 0.95±0.04 to 0.45±0.03 ml/min ($p<0.001$) 60 min after exposure to 0.1, 1.0 or 20 mg endotoxin/100ml perfusate (N=24). The decrease in GFR was independent of dose, occurred without changes in perfusion flow rate or pressure, and was not associated with structural changes detectable on electronmicroscopy. PAF at 0.5-1mg/100ml perfusate reproduced the effects of E on GFR (N=11). Prior exposure of the isolated kidney to PAF receptor antagonists attenuated the effects of E on GFR ($p<0.005$ vs E alone) (N=9). The data indicate that 1) E has direct intrarenal effects independent of circulating humoral and/or cellular factors of extrarenal origin; 2) The renal effects of E appear to involve the intrarenal release of PAF.

EFFECT OF CYCLOSPORINE (CyA) ON ANGIOTENSIN II AND NOREPINEPHRINE STIMULATED GLOMERULAR PROSTAGLANDIN PRODUCTION. Alfred Jacobs, M Bunke, L Wilder*, Division of Nephrology, Univ. of Louisville and Louisville VAMC, Louisville, KY.

Isolated glomeruli (GL) contract and increase their production of vasodilatory prostaglandins (PG) in response to vasoconstrictive stimuli. This increase in GL vasodilatory PG production minimizes the decrease in GL surface area and alterations in GFR in the presence of vasoconstrictive stimuli. To test the hypothesis that CyA inhibits GL vasodilatory PG production in response to the vasoactive agents angiotensin II (A2) and norepinephrine (NE), we administered pair fed rats CyA, 10 mg/kg, subcutaneously daily for 7 days (Cy) or vehicle (V). Glomeruli were harvested by a sequential sieving technique and incubated for 45 minutes in Earles balanced salt solution with either A2 1 μM and NE 10 μM or saline vehicle. The data were analyzed by 2 way ANOVA and the Neuman-Keuls test. The data for glomerular PGs are below (pg/mg protein, mean±SD, N=7 each group, VB=veh+saline, VA2NE=veh+A2+NE, CB=Cy+saline, CA2NE=Cy+A2+NE).

	VB	CB	VA2NE	CA2NE
PGE_2	3285±359	1706±144	4421±467	2494±391
$6KF_1$	499±127	310± 56	683±148	449± 95
TxB_2	1268± 94	763±141	1602±313	892±162

CyA produced a significant decrease in the basal and stimulated production of PGE_2 and $6KF_{1\alpha}$. We conclude that CyA decreases baseline and A2, NE stimulated glomerular PGE_2 and $6KF_{1\alpha}$ production, this decrease in GL vasodilatory PG production in response to vasoconstrictive stimuli may play a role in the CyA-induced decrease in GFR.

Effect of Amphotericin B (AMB) on renal proximal tubular cells. Véronique Joly*, Claude Carbon and Patrick Yéni*. Inserm U.13, Fac. X. Bichat, Paris, France.

AMB nephrotoxicity is mainly mediated by an alteration in membrane permeability. We previously reported (ASN 1987) that AMB acute i.v. administration leading to peak serum levels of 5 mM alters proximal tubular functions in rabbits. This effect was investigated in vitro in primary cultures of rabbit proximal tubular cells grown to confluence in serum-free medium. Cells were incubated for one hour with fresh culture medium containing either 5 or 10 mM AMB and Na-dependent phosphate (Pi) uptake was measured. We compared the results obtained when AMB was present (1) or not (2) in uptake solution. The reversibility of toxicity was investigated in an additional study delayed one hour after the end of incubation at 10 mM (3). LDH release in the supernatant was measured.

V max (nanomoles /mg protein/10 min, mean ± SD)

	AMB treated cells	Control
AMB 5 mM (1)	26.3 ± 8.6*	45.3 ± 8.2
AMB 5 mM (2)	37.3 ± 13	49 ± 10.4
AMB 10 mM (1)	16 ± 5.7**	37.3 ± 4.2
AMB 10 mM (2)	22.3 ± 8.9*	37.2 ± 5.3
AMB 10 mM (3)	21.1 ± 0.1*	37.3 ± 4.2

Compared to control values: * p< 0.05, ** p<0.01

AMB was toxic on Na-dependent uptake through inhibition of Vmax without changing Km. This effect was observed without significant mortality as assessed by the absence of LDH release. Presence of AMB during the experiment reduced the threshold of toxicity, and abnormalities persisted after AMB removal. These results suggest that AMB could alter membrane permeability of tubular cells at therapeutic concentrations and induce irreversible damage. Primary cultures of proximal tubular cells provide a convenient method to elucidate the mechanisms of AMB toxicity.

LOW EXTRACELLULAR CALCIUM (CA^{++}) PROTECTS AGAINST THICK ASCENDING LIMB OF HENLE (TAL) DAMAGE IN THE ISOLATED PERFUSED RAT KIDNEY (IPK). G.C.Johnson*, T.J. Burke,, and P.F. Shanley. U. Colorado Hlth. Sci. Ctr., Depts. Path. and Med., Denver, CO.

Ca^{++} may be important in the pathogenesis of cell death. One test of this is to remove extracellular Ca^{++} during injury. In vivo this is impractical and in vitro the end points for cell death are often ambiguous. To overcome these obstacles, the effect of reducing perfusate Ca^{++} (8.1 mg/dl=Control; 0.6 mg/dl=LoCa) on the hypoxic TAL necrosis in the Krebs-Henseleit-albumin IPK was examined. Necrosis was present in 62% of TAL tubules in Controls and 9% in LoCa ($p<0.001$). Biopsies of the inner stripe of the outer medulla showed ATP (μM/g dry wt.) to be 5.86 ± 0.34 in vivo, and after 20 min of perfusion 2.48 ± 0.65 in Control and 1.78 ± 0.15 in LoCa. Compared to Control perfusions LoCa reduced GFR by 59% and TR_{Na} by 72% (both $p<0.01$). There was no effect on perfusate flow or oxygen consumption. Free water clearance, an estimate of TAL transport predictive of the extent of TAL necrosis in this model, was reduced by 96% with LoCa perfusion ($p<0.001$). Given the previously demonstrated transport dependence of this lesion, this functional depression by LoCa could perhaps account for the cytoprotection. However, since ATP depletion remains severe, it does not appear that LoCa reduces cellular hypoxia. This contrasts with the ATP-sparing effect of ouabain whose cytoprotection in IPK seems primarily the result of transport inhibition. The morphologic protection of TAL by low extracellular Ca^{++} may therefore support the idea that Ca^{++} entry plays a role in the mechanism of hypoxic necrosis.

DIFFERENTIAL EFFECT OF CISPLATIN ON LLC-PK1 AND MDCK CELLS. Deborah P. Jones.* Gregory Schonbaum,* Mark L. Johnson* (intr. by F. Bruder Stapleton). Dept. Pediatrics, and Center for Pharmacokinetics and Therapeutics, Univ. Tenn., Memphis and Dept. Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee.

Cisplatin (CP) induced nephrotoxicity has been attributed to inhibition of DNA synthesis, ATP synthesis and/or utilization but its precise mechanism remains unknown. To distinguish between these mechanisms and to examine the possibility of cell-type specific CP injury, we have measured ATP dependent reactions including cellular uptake (Up) and incorporation (Inc) of ^{3}H-Thymidine (THY), ^{3}H-Uridine (URI), and ^{32}S-Methionine (MET) by confluent cultures of LLC-PK1 and MDCK cells. The effect of CP on enzymes involved in glycolytic ATP synthesis or utilization (pyruvate kinase (PK), phosphofructokinase (PFK) and fructose 1,6 diphosphatase (FDPase)) were also examined. Cultures were incubated with 10 μM CP for 24 hours (h) and assayed 0 h, 24h and 48h later. THY results: mean±SEM

	LLC-PK1			MDCK		
THY Up	0h	24h	48h	0h	24h	48h
Con.	30.2±0.4	3.9±0.3	3.9±0.5	6.9±1.3	3.4±1.5	2.8±0.4
CP	9.7±1.1Δ	1.7±0.01*	2.5±0.2	9.9±1.0	3.2±1.8	2.8±0.4
THY Inc						
Con.	77.9±0.7	11.9±0.3	17.5±1.1	31.4±5.0	18.2±0.7	12.8±0.8
CP	43.4±0.1•	1.7±0.3•	2.8±1.2*	44.8±4.3	13.8±1.0*	10.3±1.1

*P<.05; ΔP<.01; •P<.005

URI Inc decreased to 30% of control (43.3±2.1 vs 13.1±1.6, P<.01) at O h but recovered to control levels by 24h and 48h and was unaffected in MDCK. URI Up, MET Inc and MET Up were not affected by CP in either cell line. Incubation with 10 μM/CP had no effect on activites of PK, FDPase and PFK. The significant reductions in THY Up and Inc observed in LLC-PK1 cells may reflect a selective sensitivity of this cell type to CP. The data do not support an effect of CP on ATP dependent processes common to these models of proximal and distal tubular cells.

EFFECTS OF HEAVY METALS ON ELECTRICAL PROPERTIES OF MADIN DARBY CANINE KIDNEY CELLS. A.Jungwirth, M.Paulmichl and F.Lang (intr. by G. Giebisch). Univ. of Innsbruck, Dept. of Physiology, Austria.

Renal damage is one of the major consequences of intoxication with heavy metals. To identify the effects of heavy metals on cultured renal epithelioid cells, the potential difference across the cell membrane (PD) has been recorded continuously during exposure of the cells to cadmium (Cd), mercury (Hg) or lead (Pb) ions. Cd (5 µmol/l) leads to a sustained, rapidly reversible hyperpolarization of the cell membrane from -53 ± 1 mV to -68 ±1 mV, decreases the cell membrane resistance (Rm) from 40 ± 2 MΩ to 27 ± 2 MΩ and increases the potassium selectivity of the cell membrane (tk) from 0.33 ± 0.02 to 0.64 ± 0.03. Half maximal effect is observed at 0.2 µmol/l. In the presence of potassium channel blocker barium, the effect of Cd is transient. Apparently, the potassium channels activated by Cd are subsequently blocked by barium with some delay. In the nominal absence of extracellular calcium, the effect of Cd is transient and can be elicited only once. Hg (1µmol/l) leads to a hyperpolarization of the cell membrane to -69 ± 2 mV, a decrease of Rm to 18 ± 5 MΩ and an increase of tk to 0.77 ± 0.02. Half maximal effect is observed at 0.3 µmol/l. The Hg induced hyperpolarization is not rapidly reversible upon removal of Hg and is followed by a gradual decline of PD during continued exposure to Hg. The Hg stimulated potassium conductance cannot be blocked by barium. 50 µmol/l Pb do not acutely alter PD. In conclusion, Cd and Hg, but not Pb, hyperpolarize the cell membrane of MDCK cells by activation of distinct potassium conductive pathways.

THE ROLE OF MICROFILAMENT DISRUPTION IN ISCHEMIC ACUTE RENAL FAILURE. P.S. Kellerman,* S. Linas, C. Hoilien*, R. Clark* and B.A. Molitoris. UCMC and VAMC, Denver, CO

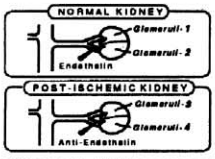
Ischemic injury results in rapid structural disintegration of the proximal tubule apical membrane. Since microfilaments, composed primarily of f-actin, are essential for the ultrastructure of both the microvilli and the underlying terminal web, we investigated the effects of ischemia on proximal tubule microfilaments utilizing a fluorescein-labeled anti-actin antibody. Control kidneys showed intact circumferential terminal webs and brush borders, while five minutes of ischemia resulted in gaps in the terminal web. Fifteen minutes of ischemia resulted in multiple large gaps in the terminal web and by 50 minutes there was diffuse redistribution of actin within the cytoplasm. To determine the cellular effects of microfilament disruption, microfilament specific doses of cytochalasin D (10 uM) were used in an isolated perfused kidney system. Cellular ultrastructure revealed patchy sloughing of microvilli, internalization of surface membrane and enlarged intercellular spaces, which were not seen in control kidneys. Cytochalasin D, which had no effect on GFR, decreased TR_{Na} from 97 ± 2% in the 15 minute control period to 91.7 ± 4, 83.7 ± 7, and 80.7 ± 2% during three succesive 15 minute periods. In summary, ischemic injury involves disruption of microfilaments, and a microfilament dissembler can induce cellular structural and functional changes similar to ischemia. We conclude that disruption of microfilaments plays an important role in the pathogenesis of ischemic injury.

ACUTE RENAL FAILURE DURING CHILDHOOD: FACTORS AFFECTING MORTALITY. Bruce Kaiser,* Stephen Lawless,* Coral Hanevold, Seth Schulman,* Sharon Bartosh,* Martin Polinsky,* H.J. Baluarte. Temple Univ. Sch. of Medicine, St. Christopher's Hospital for Children. Philadelphia, PA.

Between 1/85 and 12/87 a prospective analysis of all children (Ch) presenting with acute renal failure (ARF) was evaluated for factors affecting mortality (M). ARF was defined, in any child with no previous history of kidney disease, as a rise of creatinine > 2 times baseline and a fractional excretion of Na > 1. There was a total of 105 Ch; 55 (52%) had complete recovery, 11(11%) were left with renal impairment and 39 (37%) died. Factors not affecting M were: sex (male 57 Ch, M=32% female 48 Ch, M=44% p=ns) and age (0-1 year 29 Ch, M=38%; 1-3 yrs 26 Ch, M=35%; 4-10 yrs 26 Ch, M=38%; 11-18 yrs 24, M=42%, p=ns). Factors affecting M were urine output (oliguric 53 Ch, M=55%, non-oliguric 52 Ch, M=19% p <.001), location ARF developed (in hospital 41 Ch, M=54%, outside hospital 64 Ch, M=27%, p <.01), need of dialysis (dialysis 36 Ch, M=56%, no dialysis 69 Ch, M=28%, p <.005) and initiating disease. ARF following surgery 26 Ch (19 Ch were post op open heart surgery), M=62% and ARF following cardiac arrest/sepsis 21 Ch, M=67% had significantly higher M than ARF following: dehydration (9 Ch, M=0% p <.005); toxic acute tubular necrosis (9 Ch, M=22% p <.05); nephritis (9 Ch, M=11%, p <.01); hemolytic uremic syndrome (12 Ch, M=12%, p <.5); obstruction 7 Ch, M=0% p <.005). ARF remains a condition associated with high M in Ch especially in Ch presenting with ARF secondary to open heart surgery, cardiac arrest and sepsis, conditions usually associated with multiple organ system failure. In addition, the need for dialysis and urine output are important in predicting M.

ENDOTHELIN MAY BE THE VASOCONSTRICTOR CAUSING THE PERSISTENT GLOMERULAR HYPOPERFUSION IN POST-ISCHEMIC KIDNEYS. V Kon, T Yoshioka, A Fogo* and I Ichikawa. Vanderbilt University Medical Center, Nashville, TN.

Endothelin (E), recently isolated from cultured endothelial cells, is the most potent vascular smooth muscle constrictor released by mammalian endothelium yet described. Since vasoconstriction following hypoxia requires endothelium, the persistent hypoperfusion in post-ischemic kidneys may be due to the local actions of endothelin. We assessed glomerular response to endothelin in normal rats (n=7) and to rabbit anti-porcine endothelin serum (aE) (ID50 = 1.3 pmol/ml) in rats subjected to 25-min unilateral renal artery ligation 3 days prior to study (n=7). To achieve effectively high local concentrations, E (2 ng/kg BW/ min) and aE (13 nl/kg BW/ min) were continuously infused with a micropipette into a first-order branch of the renal artery, whereupon the hemodynamics of the infused and non-infused glomeruli within the same kidney were assessed by micropuncture.



[Mean; P<.05 between Glomeruli 1 vs 2 (†); 1 vs 3 (§); 3 vs 4(¶)]

	SNGFR	QA	PGC	RA	RE	Kf
	------nl/min------		mmHg	min·mmHg/nl		nl/min·mmHg
Glomeruli-1	36	153	52	0.21	0.15	≥3.4
Glomeruli-2	24†	96†	45†	0.35†	0.22	≥2.9
Glomeruli-3	17§	62§	49	0.59§	0.40§	1.6§
Glomeruli-4	27¶	129¶	55¶	0.24¶	0.21¶	1.4

In normal kidneys, glomeruli exposed to endothelin (Glomeruli-2) had lower SNGFR and glomerular plasma flow rate (QA) than non-infused glomeruli (Glomeruli-1) or glomeruli before any infusion in that kidney. These changes were associated with markedly elevated afferent arteriolar resistance (RA). In post-ischemic kidneys, glomeruli not infused with aE (Glomeruli-3) had depressed SNGFR, QA, glomerular pressure (PGC), ultrafiltration coefficient (Kf), elevated RA and efferent arteriolar resistance (RE) when compared with normals. By contrast, in glomeruli of post-ischemic kidneys infused with aE (but not non-immunized rabbit serum), this vasoconstricted pattern was largely ameliorated. We speculate that persistent hypoperfusion in post-ischemic kidneys is mediated largely by endothelin released from the injured endothelium.

PROTECTIVE EFFECT OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN PATIENTS WITH CHRONIC RENAL FAILURE (CRF) RECEIVING CONTRAST. BRC Kurnik, PB Kurnik*, I Cuttler, MC Stom, and LS Weisberg. UMDNJ/Robert Wood Johnson Medical School, Camden, NJ.

ANP preserves glomerular filtration rate (GFR) and/or renal blood flow (RBF) in several animal models of acute renal failure (ARF). Radiographic contrast is known to substantially decrease RBF and can induce ARF. This study was performed to examine the potential protective effect of ANP (8-33 Met-ANP) on RBF and contrast induced ARF in man. Patients with CRF (60% diabetic) were randomized to receive either ANP (50μg bolus, then 1μg/min) or standard therapy [15% mannitol (M) at 100 cc/hr] for two hours prior to and during cardiac catheterization using diatrizoate. RBF was measured directly using a thermodilution catheter placed in the left renal vein.

	Creatinine		RBF (%Baseline)	Serum ANP (pg/ml)	
	N	Baseline	24hr	65 min	Base | 65min
ANP	(10)	2.4+.6	2.6+.8	167+94*	105+64 294+195*
M	(10)	2.5+.8	2.7+.9	122+40	83+60 256+127*

X ± SD, *p<.05 paired t-test, 65 min vs. baseline

M stimulated ANP release, which may explain its renal protective effect. RBF increased at 65 min (signif for ANP and a trend for all pts) despite contrast administration, a known renal vasoconstrictor typically causing a 50% fall in RBF. Fractional excretion of sodium increased by 50% in both groups (p<.05).

In conclusion, both M (via ANP) and ANP maintain RBF and comparably preserve renal function in patients with CRF at risk for contrast induced ARF. Endogenous or exogenous ANP may be useful for prophylaxis against contrast induced nephropathy.

EARLY AND SELECTIVE EFFECTS OF GENTAMICIN (G) ON RAT RENAL BRUSH BORDER MEMBRANE (BBM) SODIUM-PHOSPHATE COTRANSPORT (Na-Pi) AND SODIUM-HYDROGEN EXCHANGE (Na/H). M. Levi, R. Cronin, B. Baird*, M. Griggs*, and P. Wilson*. VAMC and UT Southwestern Med. Ctr. Dallas, TX.

Proximal tubular dysfunction is characteristic of established G nephrotoxicity. The precise proximal tubular functional impairments in the early phases of G toxicity, however, are not known. We measured Na-Pi cotransport and $Na\text{-}H^+$ exchange in renal cortical BBM (rapid Millipore filtration, pmole/mg BBM protein/5sec) 2 and 4 days after G (30 mg/kg, sc, bid) in adult SD rats. 2 days of G caused a decrease in Na-Pi, 987 vs 1187 in control, p<.05, which decreased further to 613, p<.01 at 4 days. 2 days of G caused a decrease in Na/H exchange, 3503 vs 4236 in control, p<.05, which decreased further to 2212, p<.01 at 4 days. However, there were no significant changes at 2 days in Na-glucose (234 vs 210 in control, p=NS) or Na-proline (176 vs 160 in control, p=NS) cotransport activities. At 4 days both of these cotransporters were still normal. Therefore, G causes early and selective decreases in BBM Na-Pi cotransport and Na/H exchange activities. The resultant impairments in intracellular Pi and H^+ homeostasis may play an important role in the progression of G-induced tubular cell injury.

QUANTITATIVE MICROCHEMICAL IMAGING OF PROXIMAL TUBULES: LYSOSOMAL COMPARTMENTATION OF SULFUR AND CALCIUM DURING ANOXIA. Ann LeFurgey, L.J. Mandel, L.A. Hawkey* and Peter Ingram.* Duke University Medical Center, Durham, NC, and Research Triangle Institute, RTP, NC.

Microchemical cell imaging via quantitative electron probe x-ray microanalysis allows assessment of intracellular ion compartmentation; with this type of digital mapping, images of the distribution of physiologically important elements are obtained simultaneously at resolutions which permit identification of structural compartments.

Microchemical microscopy was performed on rapidly frozen, cryosectioned and freeze-dried samples of a suspension of rabbit proximal tubules incubated for 40' in the absence of oxygen. Values for S and Ca (mmol/kg dry wt) determined from each intracellular region were as follows:

	Control		Anoxia	
	S	Ca	S	Ca
Lysosomes	273±17	7.2±2.9	233±44	-1.2±4.9
Mitochondria	173±9*	2.7±1.0	90± 8*	3.2±1.3
Cytoplasm	181±17*	2.7±0.9	55± 5*	4.7±1.5
Nucleus	119±10*	1.7±2.1	30± 3*	-0.1±2.3

*p < 0.01 relative to lysosomes

In the cell S maps, one to twelve dense spherical regions were visible, each containing ~50% more S than adjacent cytoplasmic, mitochondrial or nuclear areas. The regions coincided with structures visible in correlative scanning transmission electron micrographs (STEM) which resembled lysosomal dense bodies. The high S content of these lysosomal compartments did not change during anoxia, although S in cytoplasm, mitochondria and nuclei decreased to <50% of control levels. Ca also occurred in lysosomal structures, at levels comparable to those in other cell areas.

In conclusion: 1) Lysosomes, which cannot be distinguished from mitochondria in STEM, were immediately discernible in cell maps of S. 2) After 40' of anoxia lysosomes maintained high S content; these data may suggest that sulfur-containing enzymes have not been released from this organelle. 3) Lysosomes should also be considered as a source/sink for intracellular Ca.

PROTECTIVE EFFECT OF ATRIAL NATRIURETIC FACTOR ADMINISTERED WITH MANNITOL FOLLOWING RENAL ISCHEMIA. Wilfred Lieberthal. Boston University Medical Center, Boston, Massachusetts.

Atrial natriuretic factor (ANF) reverses the vasoconstriction but does not improve GFR following 25 minutes of ischemia (I) in the isolated erythrocyte-perfused rat kidney (IEPK) (Clin. Res. 552 A, 1987). Since intratubular obstruction may play a role in this model of renal injury, the effect of mannitol (M) with and without ANF was studied. The IEPK was perfused at 100 mmHg with a perfusate containing erythrocytes (40% Hct). ANF(10ng/ml) and M (15mM) were added to the perfusate at the end of 25 minutes of ischemia. After reflow and a 20 minute equilibration period, perfusate flow rate and GFR were measured for one hour. Renal vascular resistance (RVR) was calculated.

	RVR (mmHg/ml/min/g)	GFR (ml/min/g)
Control (C)	10.1±0.4	0.59±0.06
C+ANF	11.1±0.4	0.89±0.06*
C+M+ANF	10.9±1.4	0.94±0.05*
I	15.9±0.9*	0.18±0.02*
I+M	17.1±0.5*	0.26±0.03*
I+ANF	11.1±0.4¶	0.25±0.07*
I+M+ANF	10.9±0.6¶	0.61±0.04¶

*p<0.05 vs C ¶p<0.05 vs I

Thus ANF counteracts post-ischemic vasoconstriction, whereas M has no effect. M and ANF given alone post-ischemia do not improve GFR but the combination of ANF and M results in marked protection of GFR. GFR in kidneys given both ANF and M post-ischemia is 3x higher than in ischemia and comparable to control kidneys.

Hypothesis: Hemodynamic factors and tubular obstruction both play a role in this model of ischemia. Simultaneous correction of both these abnormalities is necessary to substantially improve GFR.

ROLE OF XANTHINE OXIDASE (XO) IN ISCHEMIA/ REPERFUSION INJURY IN RAT KIDNEYS. SL Linas, D Whittenburg, JE Repine. University of Colorado School of Medicine, Denver, Colorado.

Oxygen metabolites (O_2*) formed during reperfusion of ischemic kidneys prevent recovery of renal function after short periods of renal ischemia. XO has been proposed as a source of O_2* during reperfusion. To determine whether the enzyme is converted from the non-O_2*-producing dehydrogenase (Type D) to the O_2*-producing oxidase (Type O) we measured Type D and Type O XO in renal cortical homogenates after 30 minutes of ischemia in vivo and 60 minutes of reperfusion by the isolated perfused kidney technique. Ischemia resulted in conversion of Type D to both reversible and irreversible Type O. Reperfusion resulted in further increases in Type O which was predominantly of the reversible type.

Type D and Type O XO (mU/gm)

	No Ischemia	Ischemia	Ischemia/Reperfusion
Type D	27.4	15.5	2.7
Type O_{TOTAL}	9.1	20.3	29.3
Type O_{REV}	2.8	10.8	18.4
Type O_{IRREV}	6.3	9.5	10.9

To determine the physiologic role of XO in renal ischemia we depleted rats of XO by feeding tungsten. After 4 weeks of tungsten, XO levels were reduced by 90%. Following ischemia/reperfusion renal function was markedly improved in XO depleted rats; glomerular filtration rate 457±55 vs 144±41 µl/min/gm; tubular sodium reabsorption 92±2 vs 71±4%, p<.001.

Conclusions: 1) XO is converted from Type D to Type O (predominantly reversible) during ischemia and reperfusion. 2) XO is an important source of O_2* during reperfusion of ischemic kidneys.

PROTECTION OF PATIENTS WITH CHRONIC RENAL INSUFFICIENCY (CRI) FROM THE TOXIC EFFECT OF RADIOCONTRAST MATERIAL (RCM). Bertin M. Louis, M.D., Henry I. Lipner, M.D., Namala L. Manohar, M.D., Brian S. Hoch, M.D., Alan Nissenbaum, M.D., & Philip C. Gorfien, B.A. Maimonides Medical Center, Bklyn.,NY.

Previous studies have reported a high incidence of renal failure (17.4%-41.7%) following use of RCM in patients with CRI. These studies used a criterion of a rise in creatinine of 1mg/DL. We have studied 54 patients, age: 70.12 ± 10.03 (34-84), with CRI undergoing various RCM procedures excluding IVP and oral tests. They were infused with a specially prepared solution (NSMF), measured osmolality 390mOsm/Kg, containing normal saline (NS) 1000cc, mannitol (M) 12.5Gms, furosemide (F) 200mg, at 100cc/hr from 1 hour before to 2 hours after the procedure. Urinary output was replaced with NS cc for cc. Serum creatinine, uric acid & phosphate did not rise significantly. In only 1 pt. did creat. rise by >1mg/DL (1.85%).

	pre	maximum post
creatinine	2.40mg/DL ± 0.1	2.50mg/DL ± 0.1
uric acid	8.2 mg/DL ± 0.3	8.2 mg/DL ± 0.3
phosphate	3.8 mg/DL ± 0.2	4.0 mg/DL ± 0.1

This solution produced a profuse diuresis and in no case precipitated overt congestive heart failure.

The infusion of a solution of NSMF, administered 1 hour before to 2 hours after a RCM procedure, protects patients with CRI from ARF.

COMPARATIVE NEPHROTOXICITY OF FREE AND LIPOSOMAL AMPHOTERICIN B (AMB AND L-AMB) DURING CHRONIC ADMINISTRATION IN RATS. Pascale Lonquet*, Véronique Joly*, Nathalie Séta*, Gerard Friedlander*, Claude Carbon and Patrick Yéni*. INSERM U.13, Fac. X. Bichat, Univ. Paris VII, France.

Incorporation of AMB into liposomes decreases AMB acute toxicity but the effects on renal functions during chronic i.v. administration have not been precisely evaluated. Nephrotoxicity of AMB and L-AMB were compared in male Sprague-Dawley rats infused daily over 2 hours for 5 days with one of the following treatments (Rx): 1) AMB 3.5 mg/kg, 2) L-AMB 3.5 mg/kg, 3) Saline. BUN, urinary osmolarity, enzymuria (NAG), and kaliuresis were studied at J0 ,i .e. before treatment , at J1, at J5 and at J7 i.e. 48h after the end of Rx. No death was observed during the experiment. Data are expressed as the ratio of experimental to pre-Rx values.

	Osm. U		NAG		BUN	
	AMB	L-AMB	AMB	L-AMB	AMB	L-AMB
J1	0.32**	0.38**	5.74**	3.40	1.93	1.37*
J5	0.37**	0.44**	5.22	2.66	2.35**	2.41**
J7	0.63**	0.80*	4.78	1.43	1.24	1.40*

* p<0.05; ** p<0.01 from paired t-test

Kaliuresis was unchanged after either Rx. AMB and L-AMB both increased BUN, and induced urinary concentration defect, AMB increase NAG excretion. These abnormalites were partly reversible at J7. We conclude from this study that 1) Henle's loop dysfunction preceded the decrease in GFR after AMB or L-AMB Rx, 2) AMB induced an early lysosomal injury, 3)Liposomes exerted limited protective effect on the renal toxicity secondary to AMB (3.5 mg/kg) chronic administration. Renal parameters should be closely monitored in patients receiving chronic L-AMB infusions for fungal infections.

EFFECT OF ANOXIA AND REOXYGENATION ON VASCULAR REACTIVITY. MODIFICATION BY DIETARY FISH OIL. Charles D. Malis, G.S. Varadarajan*, Thomas Force*, Peter C. Weber*, Alexander Leaf, and Joseph V. Bonventre. Medical Services, Mass. Gen. Hosp. and Harv. Med. Sch., Boston, MA

Post-ischemic vasospasm can impair tissue reperfusion, contributing to ischemic tissue damage. Experiments were designed to evaluate post-anoxic vasoconstriction in isolated aortic rings and to determine how dietary fish oil feeding may modulate post-anoxic vascular reactivity. We assessed vascular contractility in isolated endothelialized (E) and de-endothelialized (DE) aortas from fish oil (F) and beef tallow (B) fed rats prior and subsequent to anoxia and re-oxygenation (A). Vasoconstriction was induced with KCl, norepinephrine (NE), or vasopressin (VP), and relaxation with acetylcholine (ACH). Results are expressed for KCl as mg tension or for NE, VP, or ACH as fraction of KCl response. Pre A, E vessels from F fed rats showed greater relaxation to $5x10^{-9}$M ACH [2.6±0.7 vs. 0.4±0.2 (B), p<0.01]. In F DE aortas, there was less vasoconstriction with NE and VP. Post-A, E aortas from B fed rats showed increased vasoconstriction with KCL compared to pre-A. E aortas from F fed rats showed less response to KCl [40.8±6.9 vs. 125±34.3 mg (B), p<0.01] and greater relaxation with 10^{-9}M ACH [1.0±0.4 vs. 0.1±0.1 (B), p<0.01]. In experiments performed in vivo, with myocardial ischemia, reperfusion blood flow was greater in F hearts.

We conclude: 1) Post-A, E aortas from B fed rats showed enhanced contractility relative to pre-A; 2) Post-A, F reduces vasoconstriction and enhances relaxation; and 3) F promotes enhanced post-ischemic blood flow in vivo.

INTRACELLULAR GLUTATHIONE (GSH) IN THE PROTECTION FROM ANOXIC INJURY IN PROXIMAL RENAL TUBULES.
L.J. Mandel, R.G. Schnellmann* and W.R. Jacobs,* Dept. Cell Biol., Duke Univ. Med. Cen., Durham NC

Previous studies (Weinberg et al, J.Clin. Inv. 80:1446, 1987) have shown that GSH and glycine (GLY) are cytoprotective during anoxia when added extracellularly. The present studies investigate the role that intracellular GSH plays in this cytoprotection. Proximal rabbit tubules in suspension prepared with either high or low GSH contents were subjected to 40 min of anoxia and 40 min of reoxygenation. Low GSH tubules had $MgCl_2$ (control) GSH (1mM), or GLY (1mM) added during anoxia. High GSH tubules had either $MgCl_2$ or 2mM diethylmaleate (DEM) for 10 min prior to anoxia to rapidly deplete GSH content. Results:

Condition	Addition	preoxy GSH (nmol/mg)	anoxic %LDH release	%NYS-QO$_2$ recovery	%ATP recovery
low GSH	$MgCl_2$	5±1	42±7	30±8	36±5
	GSH	4±1	14±1*	117±20*	74±3*
	GLY	7±1	10±1*	96±8*	91±5*
high GSH	$MgCl_2$	11±1*	15±3*	80±15*	91±10*
	DEM	2±0.1*	17±5*	78±13*	63±9*

Conclusions: 1)GSH and GLY are cytoprotective when added during anoxia; 2) High GSH content is equally cytoprotective. Since this value is close to the normal in vivo GSH content, it could be an important protective mechanism in vivo; 3) Since addition of DEM did not alter protection, it is unlikely that GSH content itself is protective. Rather, the protection may come from the ability to produce GLY, which appears to be the common protective denominator in these experiments.

TUBULAR FUNCTION WITH SANDIMMUNE® (CYCLOSPORIN A).
June Mason*, P. Donatsch*, U. Rickenbacher*, (introduced by L.C. Moore)
Clinical and Preclinical Research, Sandoz, Basle.

To characterise alterations in tubular function caused by Sandimmune®, male Wistar rats were injected s.c. with 12.5, 25 or 50 mg/kg/d of the commercial drinking solution or its vehicle. Blood chemistry and renal clearances were determined before and 5 and 10 days after treatment.

At day 5, the plasma osmolality, uric acid and electrolyte levels were unchanged, except for Mg, that fell by 16%, 26% and 25% at the 3 doses. Creatinine clearances were reduced by 8%, 17% and 29% but urine volume and osmolality did not alter. Fractional excretion of all substances was unchanged, except for Mg, that fell by 9%, 14% and 14% at the 3 doses.

At day 10, the plasma osmolality, uric acid and electrolyte levels were still unchanged, except for PO_4, that fell by 9% at the highest dose and Mg, that fell by 31%, 33% and 33% at the 3 doses. Creatinine clearances were reduced by 14%, 18% and 23%. Urine volume was raised, and osmolality reduced by 16%, 57% and 60%. Fractional excretion of all substances was unchanged, except for PO_4, that fell by 12% at the highest dose and Mg, that was reduced by 11%, 11% and 10% at the 3 doses.

It seems that Sandimmune® does not disturb global tubular reabsorption and is not tubulotoxic. It does not clearly depress proximal nephron function, even at high doses, but does seem to affect distal nephron function, even at lower doses.

THE INFLUENCE OF ACUTE RENAL FAILURE (ARF) ON THE KINETICS OF INSULIN-STIMULATED (I-STIM) AMINO ACID TRANSPORT (AATX) IN SKELETAL MUSCLE.
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We found that 2-(methylamino)isobutyrate (MeAIB) is specific for the hormonally-responsive AATx System A in muscle; in ARF, the insulin-dose response curve revealed depressed maximal responsiveness with preserved sensitivity (AJP 251:F74, 1986). To determine why responsiveness is impaired, we measured the kinetics of maximal I-stim MeAIB uptake (10^4 uU/ml I) in epitrochlearis muscle from ARF and sham-operated (SO) rats. Transport rates were corrected for diffusion ($K_D[S]$) and kinetic constants determined from double-reciprocal plots.

	K_D(hr^{-1})	K_M(mM)	V_{max}(nMol/ul/hr)
SO	0.367 +/-0.017	0.372* +/-0.051	1.178* +/-0.103
ARF	0.315 +/-0.013	0.216 +/-0.047	0.778 +/-0.111

*P <0.05 SO vs ARF

K_D was not altered, but the K_M for MeAIB transport was reduced by ARF, consistent with increased System A transport affinity in response to I. In contrast, maximal transport velocity (V_{max}) was decreased by ~34%. Since protein synthesis (PS) is depressed by ARF we questioned whether the reduction in V_{max} by ARF was due to impaired I-stim synthesis of new transporters. Cycloheximide did not depress I-stim AATx in muscle from SO or ARF rats excluding this possibility.

In summary, the ability of I to stimulate System A AATx is impaired by ARF due to reduced transport capacity. This effect is independent of PS suggesting that uremia impairs recruitment of transporters and/or enhances their degradation when stimulated by insulin.

COMPARISON OF THE DIRECT NEPHROTOXICITY OF CLINICAL FORMULATIONS OF TWO NEWER LOW OSMOLALITY RADIOCONTRAST(RC) AGENTS.
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We have previously reported that RC-induced direct renal tubule cell damage is markedly potentiated by hypoxia (H) (Am. J. Physiol. 1987, J. Pharm. Exp. Ther. 1988). Using enriched rabbit renal proximal tubule segments (PTS), we now compare the direct toxicities of the non-ionic RC, iopamidol (I), and the ionic dimer RC, ioxaglic acid (Ix) (Na/Meglumine salt). PTS were exposed for 30' to 25 mM I or Ix with or without concomitant H. Cell viability parameters, including potassium content (K), calcium content (Ca), ATP content, (nmol/mg prot) and CCCLP-stimulated respiration (URR natoms O/min/mg prot) were measured 60' after reoxygenation. All values expressed as mean of 4-5 expts. *=p <.05 vs C; +=p <.05 vs I, **=p <.05 vs H; ++=p <.05 vs I+H

	K	Ca	ATP	URR
C	348	9.9	9.9	222
I	309	10.4	6.2	189
Ix	229*+	85.9*+	3.9	129*
H	196	6.4	2.3	42
H+I	107**	33.4**	1.5	39
H+Ix	66**++	55.9**	1.2	20**++

The cytotoxicity of both I and Ix is markedly potentiated by concomitant H. In addition, under oxygenated conditions, Ix, but not I, produces marked alterations in tubule cation homeostasis and respiration. Ix, as clinically formulated, is more toxic to PTS than I, under both oxygenated and hypoxic conditions.

ANALYSIS OF EFFECTS OF VASCULAR CONGESTION ON RENAL HEMODYNAMICS AFTER ISCHEMIA. Leon Moore and June Mason*. Dept. of Physiology, SUNY at Stony Brook, NY, and Sandoz Corp., Basel, Switzerland.

Ischemic acute renal failure (IARF) is associated with vascular congestion which is marked in the outer medullary inner stripe, less in the cortex, and minimal in the inner medulla. To investigate the impact of vascular congestion on kidney and intrarenal blood flows, a resistance network model of the renal vasculature was developed. It consists of six lumped elements representing 1) the arteriolar resistances of superficial and juxtamedullary glomeruli, 2) peritubular capillary resistances of the superficial and deep cortex, and 3) the resistances of the vasa recta and capillaries in the inner and outer medulla. Baseline resistances for normal kidneys were obtained from published data on renal blood flow (RBF), efferent arteriolar pressures, and intrarenal blood flows. Vascular congestion in IARF was then simulated by systematically increasing the individual resistances. The results show that significant cortical glomerular and peritubular congestion depresses RBF and perfusion of some regions, but it also may markedly raise the driving pressures across the inner medullary and deep cortical peritubular capillaries. As a result, blood flow to these regions may be well maintained despite partial congestion. It is concluded that capillary congestion lowers RBF but also acts to raise intravascular pressures. This is consistent with observations of normal or high glomerular and peritubular capillary pressures, and normal or high papillary vasa recta flows in IARF.

PREVENTION OF POSTISCHEMIC ACUTE RENAL FAILURE (ARF) IN CONSCIOUS DOGS BY CHRONIC DIETARY FISH OIL SUPPLEMENTATION. Hans.-H. Neumayer, Michael Heinrich, Martin Schmissas, Herrman Haller, Karl Wagner. Free Univ. of Berlin, Klinikum Steglitz, Dept. of Nephrology, FRG.

Omega-3 polyunsaturated fatty acids inhibit the synthesis of TxA_2 and other dienoic cyclooxygenase products of arachidonic acid and promote the formation of triene prostanoids, which may counterbalance renal vasoconstriction. We therefore studied the effect of dietary fish oil supplementation on the course of postischemic ARF in unilaterally nephrectomized, chronically instrumented conscious dogs, subjected to 120 min of ischemia. In contrast to control group A (n = 7), group B (n = 6) received an additional treatment of six capsules EICOSAPEN® daily, starting six weeks prior to ischemia (70 mg/kg/d EPA + 50 mg/kg/d DHA). Intracellular free Ca^{2+} on platelets decreased from 98±9 to 74±4 nmol (Quin-2, $p \leq 0.05$).

	Before ischemia		After ischemia day 1		day 3	
group	A	B	A	B	A	B
GFR (ml/min)	36±2	30±2	18±4	30±2++	20±4	31±2*
RBF (ml/min)	145±26	131±14	163±27	131±12	127±22	121±13
V_u(ml/min)	.8±.1	.9±.1	.4±.1	1.±.1++	.6±.1	.9±.1
P_{Cr}(µmol/l)	87±7	88±6	204±37	112±5	173±33	87±4**
C_{Cr}(ml/min)	23±3	26±2	10±3	21±1*	12±2	29±2++
$EPGI_2$(pg/min)	146±33	227±27	86±31	197±13**	144±77	192±13
$ETXB_2$(pg/min)	828±156	613±41	541±127	598±57	628±209	745±119

Mean±SEM, *$P \leq 0.05$, **$P \leq 0.01$, ++$P \leq 0.001$, $EPGI_2$=6-keto-$PGF_{1\alpha}$

Our data clearly demonstrate that in conscious dogs chronic dietary fish oil intake reduces the vulnerability of the kidney to ischemic damage, thus preventing postischemic ARF. The observed beneficial effect may in part be due to an inhibition of intracellular Ca^{2+} accumulation. Although not definitely proven in the present study, a modulation of eicosanoid formation by generating preferentially compounds with enhanced vasodilating capacity and reduced activity on platelet aggregation seems to be another possible mechanism of action.

SUSCEPTIBILITY TO OXIDANT STRESS: EFFECT OF DIETARY DEFICIENCY OF VITAMIN E (VIT E) AND SELENIUM (SE). KA. Nath, JP Tolins*, AJ Croatt* and MS Paller. Univ. of Minnesota, Dept. of Med., Minneapolis, Minnesota.

An ischemic insult triggers the generation of reactive oxygen species (ROS). That increased production of ROS exacerbates ischemic renal injury is supported by our finding that rats maintained on a diet deficient in antioxidants Vit E and SE (a cofactor for glutathione peroxidase) exhibit increased lipid peroxidation and serum creatinine following ischemia (Clin. Res. 36: 524A, 1988). To examine further the role of endogenous antioxidants, 3 wk old rats were maintained on a diet deficient (DEF) or replete (CON) in Vit E and SE for 6 wks. The right kidney was removed and the left renal artery occluded for 1 hr. Clearance studies under euvolemic conditions and histologic analyses were undertaken 24 hrs later: Results (mean ± SD; *p<0.05).

	GFR (ml/min)	RBF (ml/min)	TmPAH (ug/min)
CON (n=6)	0.55 ± 0.31	5.07 ± 2.50	208 ± 165
DEF (n=6)	0.06 ± 0.09*	0.77 ± 1.27*	3 ± 8*

Thus, DEF rats exhibited greater functional impairment as evidenced by markedly lower GFR, renal blood flow (RBF) and diminished transport maximum for p-aminohippurate (TmPAH). Mean arterial pressure (107 ± 5 vs 108 ± 7 mmHg) and hematocrit (43 ± 2 vs 43 ± 2 % vol) were not significantly different between the groups. In additional animals DEF rats also showed greater scores for tubulo-interstitial damage (6.1 ± 1.2 vs 4.1 ± 1.1*; 0-no injury, 10 maximum score), and higher serum creatinine (4.3 ± 1.3 vs 1.0 ± 0.3* mg/dl). Thus, dietary deficiency of scavengers of ROS exacerbates renal dysfunction and structural damage. These findings support the role of ROS in ischemic injury and emphasize the role of the diet in determining susceptibility to oxidant stress.

RENAL FAILURE FOLLOWING CARDIAC ANGIOGRAPHY: A PROSPECTIVE STUDY OF DIATRIZOATE AND IOPAMIDOL. Nancy Nora*, Arnold S. Berns. Northwestern University Med. Ctr. Chicago, Il., and St. Francis Hospital, Evanston, Il.

To evaluate the comparative nephrotoxicity of ionic (diatrizoate) and non-ionic (iopamidol) contrast media, a prospective study of two consecutive groups of patients was performed. All patients had pre-existing renal insufficiency, Cr ≥ 1.5mg/dl. Patients were hydrated prior to angiography with intravenous 0.45 NaCl. Demographics of the two groups were as follows (mean ± S.D.):

	Ionic	Non-Ionic	P
N	36	35	
M/F	26/10	22/13	
Age,yrs.	63.2 ± 11	67.9 ± 9	NS
Cr,mg/dl	1.56 ± .63	1.82 ± .66	NS
% diabetes	47	43	NS
contrast,ml	156 ± 56	139 ± 63	NS

Renal failure was defined as an increase in serum Cr ≥ 1.0mg/dl within 48 hours following contrast. Renal failure following diatrizoate was seen in 17% (6/36) of patients, compared to 26% (9/35) following iopamidol, p = .35. Only one patient required dialysis. Diabetes did not significantly increase the likelihood of renal failure in either group. These data indicate that in patients with renal disease, non-ionic contrast for cardiac angiography is as nephrotoxic as standard ionic media.

TOTAL PREVENTION OF GLYCEROL-INDUCED ACUTE RENAL FAILURE (ARF) WITH AN ADENOSINE-RECEPTOR BLOCKER. Donald E. Oken and Katherine M. Reilly*. Medical College of Virginia and VA Med. Center, Richmond, Virginia.

Various lines of evidence suggest a role for adenosine in the pathogenesis of ARF. Bidani and Churchill and Bowmer et al have shown that aminophylline or 8-phenyltheophylline decreased the severity of glycerol-induced ARF, but renal function still was notably depressed. The same agents have provided equally inconsistent and incomplete protection from ARF in this laboratory. Here, we have assessed the efficacy of the more potent adenosine-receptor blocker BW A1433U given at doses of 0.025 to 25 mg/Kg B.W. i.p. In 6 normal Sprague-Dawley rats, the highest (hyperpharmacologic) dose caused a small rise in Cin (2.3 ± SEM 0.1 vs 1.9 ± 0.1 ml/min, $p < 0.05$) without a significant change in MAP. BW A1433U was given i.p. 12h before and each 12h x 3 after i.m. injection of 10 ml/Kg B.W. glycerol. Rats given 0.025 or 0.25 mg/kg BW A1433U (N = 8 each) exhibited no change at all in serum creatinine concentration (Scr) 24h and 48h after glycerol (mean 48h ΔScr -0.02 ± 0.12 and +0.03 ± 0.07 mg/dl, N=8, respectively; control 48h ΔScr 4.1 ± 0.7 mg/dl). Cin at these doses is to be measured. 12 rats given 25 mg/Kg showed a mean rise in Scr of 0.9 ± 0.6 mg/dl at 48h ($p < 0.001$) and a reduction in Cin from 1.8 ± 0.2 to 1.3 ± 0.2 ml/min, a value far higher than that in volume replete, glycerol-injected controls (0.31 ± 0.2 ml/min, N = 12, $p < 0.001$). When first given 12 and 24h *after* the onset of ARF, the adenosine blocker offered no protection (48h Cin 0.34 ± 0.14, N = 18). To our knowledge, no other maneuver except long term salt loading has provided the degree of protection from glycerol-induced ARF exhibited by BW A1433U pretreatment. These results strengthen, but do not prove, the thesis that adenosine plays a key role in the pathogenesis of myohemoglobinuric ARF.

ORAL PROSTAGLANDIN E1 ANALOG MISOPROSTOL REDUCES RENAL ISCHEMIC INJURY. Mark S. Paller, University of Minnesota, Minneapolis, MN.

Prostaglandins (PG) may have a beneficial effect during renal ischemia. Studies employing PGE but not PGI_2 suggest that this protective effect may be a consequence of enhanced renal blood flow (RBF). We tested whether the orally active PGE_1 analog misoprostol protects against ischemic renal injury in the rat and whether any effect was hemodynamically mediated. Male Sprague-Dawley rats (n=6 per group) received 100 ug/kg misoprostol via an orogastric tube 30 min before right nephrectomy and occlussion of the left renal artery for 40 min. Control animals received 0.5 ml phosphate buffer. This dose of misoprostol caused no change in renal blood flow, GFR, or solute excretion in nonischemic kidneys. Renal function was measured 1 hr after ischemia (x ± SE; *p<0.05 vs control):

	MAP(mmHg)	C_{In}	RBF	RVR(mmHg/ml/min)
CONTROL	102 ± 7	0.12 ± 0.2	2.5 ± 0.3	43.2 ± 4.8
MISOPROSTOL	90 ± 8	0.34 ± 0.1*	3.5 ± 0.7	32.8 ± 8.6

24 hr after ischemia renal function was similarly improved by misoprostol:

	C_{In} (ml/min)	U/P creat	FE_{Na} (%)
CONTROL	0.11 ± 0.04	7.3 ± 1.4	11.8 ± 3
MISOPROSTOL	0.38 ± 0.09*	14.1 ± 2.9*	5.0 ± 1*

There was no difference in postischemic renal malondialdehyde content (an index of free radical injury) nor did misoprostol have any effect on preischemic renal glutathione content. Administration of an additional dose of misoprostol 24 hr prior to ischemia provided no greater protection than the single dose. In summary, PGE enhanced postischemic GFR and tubular function independent of any significant effect on renal blood flow. PGE may, therefore, have a "cytoprotective" effect during renal ischemia. Because it is active after oral administration misoprostol may be useful to prevent acute renal failure in clinical situations.

INDUCTION OF *c-fos* AND THE EARLY GROWTH RESPONSE GENE *Egr-1* IN MICE AFTER RENAL ISCHEMIA. Andre J. Ouellette*, Ronald A. Malt*, Vikas P. Sukhatme, and Joseph V. Bonventre. Surgical and Medical Services, Massachusetts General Hospital, Harvard Medical School and Howard Hughes Medical Institute, University of Chicago.

To identify specific genetic regulatory mechanisms associated with renal ischemia, renal accumulation of mRNAs coded by *c-fos* and the early growth response gene, *Egr-1*, has been measured in mice after unilateral nephrectomy with contralateral clamp-induced ischemia and reperfusion. RNA northern blot analyses show that *Egr-1* mRNA accumulates rapidly and transiently in post-ischemic mouse kidney and to a lower level in control kidneys after contralateral nephrectomy alone. After one hour of reperfusion subsequent to total arterial occlusion of the kidney for 10 or 30 min, *Egr-1* mRNA levels were approximately 5-fold greater in ischemic kidneys than in controls. *Egr-1* mRNA persists at diminished levels up to 24 hours after 10 or 30 min of ischemia, and *Egr-1* mRNA content was independent of whether ischemia occurred for 10 or 30 min. After uninephrectomy alone, *Egr-1* mRNA levels in the remaining kidney are maximal 30 min after surgery, diminished at 1 hour but not detectable at later times. Regulation of *c-fos* and *Egr-1* mRNA levels differs in that *c-fos* mRNA content varies with the duration of ischemia and is not detected 24 hours after injury.

In conclusion, differential expression of early growth response genes implicated in transcriptional activation may be an important determinant of functional tissue recovery after renal ischemia.

CHARACTERIZATION AND KINETICS OF THE INTERSTITIAL CELL INFILTRATE (ICI) ASSOCIATED WITH GENTAMICIN NEPHROTOXICITY (G-NTX). V. Pardo, B. Fernandez,* H. Alpert‡ R. Papendick‡ V. Esquenazi‡ and C. A. Vaamonde. VAMC, Dept. Pathology, Medicine and Surgery, Univ. Miami, Sch. of Medicine, Miami, FL.

The nature and functional significance of the ICI response associated with G-NTX was studied in female Sprague-Dawley rats receiving 60 mg/Kg BW/day (d) of G for 8 d. Controls (C) received Ringer's. Groups of G (ea.=8) and C (ea.=6) were sacrificed at d 2,3,4,8,14 & 28. Frozen sections were stained with a panel of 8 monoclonal antibodies to rat lymphohematopoietic antigens. Cells surrounding 100 cross sectioned tubules (TX) were quantitated by epifluorescence microscopy. Peripheral blood cells were estimated by flow cytometry.

Serum creatinine (Cr) was unchanged through d 4, peaked at d 8 (0.8±0.3 mg/dl;$p<0.02$ vs. C) and returned to baseline by d 14. Renal cortical G peaked between d 4 & 8 and decreased markedly by d 14. Focal tubular necrosis was first seen at d 8. By d 2-3, rats had immunohistochemical evidence of interstitial nephritis characterized by an increase of all the cell subsets ($p<0.05$-0.001) despite unchanged Cr and histology. The helper (W3/25)/suppressor-cytotoxic ratio (OX8) was inverted up to d 8. D 14 showed the highest cell counts in all subsets, however, monocytes (ED1)+ macrophages (ED2) (220/100TX) were prevalent, whereas B cells (OX33) were a minority (70/100TX). There were no changes in peripheral blood mononuclear cells. The inflammatory process was subsiding by d 28 but still unresolved.

These findings suggest a role of the early reactive cells on the pathogenesis of G-NTX.

INHIBITION OF TOBRAMYCIN REABSORPTION IN PROXIMAL AND DISTAL NEPHRON SEGMENTS BY ALKALINIZATION; A MICROPUNCTURE STUDY.

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Chronic acidification appears to potentiate aminoglycoside induced nephrotoxicity and renal cortical accumulation while some, but not all, studies show that alkalinization reduces nephrotoxicity. The purpose of the present study was to determine if reabsorption of Tobramycin (TB) in proximal and distal nephron segments in vivo is altered by pH. To assess this possibility on the single nephron level, 20 nl samples of ^{3}H TB were microinjected into proximal and distal tubules and its recovery compared to ^{14}C inulin in HCO_3-loaded (BL) or acid-loaded rats (AL). Results obtained in 66 tubules in 17 rats are summarized:

	PLASMA		^{3}H TB/^{14}C In X 100	
	pH	HCO_3 mM	Proximal	Distal
BL	7.61	43.9	92.5	99.6
	+.030	+2.28	+1.56	+1.59
	*	*	*=p<.001 *	*
AL	7.17	14.1	75.1	94.1
	+.014	+0.72	+1.88	+0.75

Late proximal flow rate (nl/min) was similar in BL and AL rats (BL=33+1.9 vs AL 30+2.1 nl/min,NS) however, associated with elevated pH and [HCO_3] of plasma and urine was a significant reduction in Proximal and Distal reabsorption of TB. These data provide evidence that alkalinization reduces TB reabsorption and may explain enhanced nephrotoxicity in acidosis and increased renal clearance when pH and/or [HCO_3] are elevated.

SHEDDING OF VIABLE TUBULAR CELLS (TC) INTO THE URINE IN EXPERIMENTAL "ACUTE TUBULAR NECROSIS" (ATN). L.C. Racusen, Y. Li,* B. Fivush, K. Solez. Johns Hopkins Univ. School of Medicine, Baltimore MD, Univ. of Alberta, Edmonton, Alberta, Canada.

We have reported the shedding of large numbers of viable TC in patients with ATN (Kidney Int. 33:364, 1988). To further study TC shedding, we used 4 groups of rabbits: 1)controls; 2)pedicle clamp (1 h), 3)glycerol-treated (15 ml/kg 50% solution) 4)$HgCl_2$-treated (15 mg/kg). Urine was collected over 6 hrs. TC viability was determined by Trypan blue exclusion. Histologic sections of kidney were ranked for tubular necrosis (TN). Results: (*p<.05 vs Gp.1)

Gp	Voided TC ($x10^6$)	Urine Vol (ml)	CTN (TC/ml $x10^4$)	% Viable	TN Rank
1	.3+.1	13+1	3+1	0	3.5+0
2	6+2	9+5	92+29*	38+11*	16.8+T.21*
3	19+5	46+7*	42+9	14+4	8.5+7*
4	31+18	35+11*	79+20*	59+8*	16.2+1.8*

Voiding of TC in ATN was positively correlated with Cr clearance (Ccr)(r=.78, p<.01, n=18) suggesting dependence on tubular fluid flow. Voided TC and Ccr were inversely correlated with histologic tubular necrosis (TN) (r=-.61 and -.56,p<.05). % viable TC was characteristic of individual models with % and CTN of viable TC in urine correlated with TN (r=+.7,p<.05) in groups 3 & 4. In transplant patients, the CTN of viable TC in urine correlated with serum Cr in the first 4 days after transplant (r=.48,p<.05,n=21). Shedding continued throughout the course of ATN. Analysis of TC shedding and viability may represent a non-invasive means of determining type and severity of tubular damage in ATN.

INHIBITION OF VASOCONSTRICTORS NORMALIZES GFR IN POST-OBSTRUCTED KIDNEYS. M.L. Purkerson and S. Klahr, Renal Division, Washington University Medical School, St. Louis, Missouri.

Previous studies designed to examine the role of increased synthesis of thromboxane and angiotensin II on renal function of the postobstructed kidney have utilized blockade of production of these two vasoconstrictors after release of obstruction of 24 hours duration. In the present studies we analyzed the potential contribution of angiotensin II and thromboxane A_2 to the marked decrease in GFR and renal plasma flow that occurs after unilateral release of bilateral ureteral obstruction (BUO) of 24 hours duration by pretreating four groups of rats with vehicle, an inhibitor of angiotensin-converting enzyme (enalapril), an inhibitor of thromboxane synthesis (OKY 046), or a combination of both inhibitors for 48 hours preceding the onset of obstruction. As compared to previous data in which synthesis of angiotensin II and thromboxane A_2 was blocked after release of obstruction of 24 hours duration, the results of the present experiments indicate that pretreatment with inhibitors of angiotensin II and/or thromboxane A_2 synthesis results in substantially greater increases in renal plasma flow and GFR than previously reported. Simultaneous inhibition of thromboxane and angiotensin II production normalized GFR in the post-obstructed kidney (Cin = 5.93 ± 0.53 vs 1.54 ± 0.15 ml/min Kg BW in rats receiving vehicle). Thus, the results of the present studies indicate an important quantitative contribution of thromboxane A_2 and angiotensin II to the decrease in GFR and renal plasma flow observed in obstruction. The effects of these vasoconstrictors during short-term obstruction account for at least 80 to 90 percent of the decrement in GFR and renal plasma flow observed after release of BUO in rats.

BULK SEPARATION OF RABBIT PCT AND PST: SUBSTRATE-DEPENDENT METABOLIC DIFFERENCES. Charles E. Ruegg* and Lazaro J. Mandel, Duke Univ. Med. Cen., Dept. of Cell Biol., Durham, NC.

Bulk isolation of PCT and PST was done to investigate mechanisms underlying selective injury to proximal tubular (PT) segments. Separate dissection of the outer cortex and outer medullary stripe was followed by Percoll gradient centrifugation. Bands were enriched in PT (> 95%) with an average tissue yield of 38 mg (73.7%) PCT and 13 mg (26.1%) PST (n=19) per rabbit. Both segments were enriched in the proximal marker leucine aminopeptidase (1.23 and 1.62) and de-enriched in the distal marker hexokinase (HEX: 0.22 and 0.21) for PCT and PST, respectively. Furthermore, HEX was 30% higher and lactate dehydrogenase (LDH) was 37% higher in PST versus PCT (n=9). PST and PCT suspensions (1 mg/ml) were incubated in DMEM for 1 hr at 37°C, followed by resuspension in either a buffer containing 5mM glucose as sole carbon source or DMEM. After 30 min, samples were analyzed and returned to DMEM for a 1 hr recovery. In PCT suspended in buffer, basal QO_2 decreased 67% from 40.7 to 13.3 nmol/min/mg, ATP decreased 52% from 8.9 to 4.3 nmol/mg, LDH release increased from 6 to 24%, and K content was unchanged. In contrast, PST suspended in buffer decreased basal QO_2 35% from 41.9 to 27.3, ATP levels decreased 33% from 10.0 to 6.8 while LDH release and K content were unchanged. The improved ability of PST to survive in the glucose buffer and their increased HEX and LDH activities suggest that the PST may have a greater capacity for glycolytic metabolism than the PCT and thus be able to endure longer periods of hypoxia.

RENAL EPIDERMAL GROWTH FACTOR (EGF) PRODUCTION DECREASES AND EGF RECEPTORS INCREASE FOLLOWING RENAL ISCHEMIA. R. Safirstein, P. Price* and R.C. Harris
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Urine is a rich source of EGF, and the kidney contains high concentrations of mRNA for the EGF precursor, preproEGF. We have recently reported reduced levels of preproEGF mRNA and urinary EGF excretion after renal artery clamping (Clin. Res. 36:597A) and now report the time course of changes of renal EGF production and changes of renal EGF receptor concentration following 50 minutes of vascular clamping. Measurement of urinary EGF excretion by radioreceptor assay revealed 90% decrease in EGF excretion within 1 hour post insult, and a return to only 5% of control by day 4. Poly (A^+) mRNAs were isolated by guanidinium isothiocyanate extraction and oligo-dT chromatography. Northern blot hybridization revealed a 60% decrease in preproEGF mRNA by 2 hours and virtual absence by 12 hours post insult, with only a 1% return towards normal by 4 days. In contrast, significant increases in ^{125}I-EGF binding were noted in membrane preparations prepared from both cortex and inner medulla, compared to the contralateral kidney. In inner medulla, EGF binding was increased 220% within 24 hours (n=4;p<0.05). At 4 days high affinity binding increased from 9.3 to 21.9 fmol/mg protein (n=2). In cortex, binding increased from1.2±0.3 to 2.6±0.3 fmol/mg protein (n=6; p<0.005). There were no differences in the basolateral marker, Na-K ATPase or the apical marker, alkaline phosphatase, between membrane preparations. There was no change in EGF binding affinity in cortex or inner medulla.

In summary, these studies demonstrate that ischemic renal insult reduces renal preproEGF mRNA levels and urinary EGF excretion, while at the same time the kidney expresses an increased number of EGF receptors. These data are consistent with regulation of renal EGF receptor density by intrarenal EGF production and suggest a possible functional role for urinary EGF. Increased EGF receptor number may also be a response to renal injury.

CONTRAST MEDIA INDUCED ACUTE RENAL FAILURE (CARF)-PROTECTIVE ACTION OF ATRIAL NATRIURETIC PEPTIDE (ANP)

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The present study was designed investigate the protective action of ANP in CARF. In this study we used female Sprague-Dawley rats. After a surgical preparation, removal of the right kidney and clamping of the left renal artery over a period of 20 minutes infusion studies were performed. Iopamidole (IOP) and ANP was infused intravenously at 2 g per hour (90 minutes) and 10 µg per kg body weight per hour (60 min), respectively. In group 1 rats received IOP solely (controls, CO), in group 2 and 3 ANP was infused after or before the toxic insult. GFR (inulin clearance), urinary flow (V), electrolyte excretion, renal blood flow and arterial blood pressure was measured. In controls, IOP induced a non-oliguric CARF. After the toxic insult ANP infusion induced an increase of glomerular filtration rate in group 2. In group 3 GFR exceeded basal values and remained elevated over the whole period of the experiment, in spite of IOP infusion. IOP has been cited to impaire renal function and decreases renal blood flow. This may be, at least in part, mediated by activation of intrarenal renin-angiotensin-system (RAS).ANP antagonizes the action of angiotensin II and norepinephrine on renal vascularature and glomerular cells, increases the glomerular surface area available for ultrafiltration and hence increases K_f. These actions may contribute to the protective effect of ANP in the early CARF.

INHIBITION OF MERCURIC CHRORIDE-INDUCED RENAL FAILURE IN MICE BY HERBAL MEDICINE. Nobuo Sakurai, Haruyuki Shirasawa, Koji Sugimoto, Kiyoshi Shibata, Yutaka Fujise, and Hisayoshi Sugihara. Depts. of Hygiene, Pathol., and Org. Chem., Hamamatsu Univ. Sch. of Med., Hamamatsu, and Dept. of Microbiol., Fac. of Pharm., Meijo Univ., Nagoya, Japan. (Intr. by K. Kurokawa.)

Inhibitin of acute renal failure by mercuric chloride was tested by Chinese herbal medicine. Mice were intraperitoneally injected with saline in the control and with a decoction of 13 different herbs in the treated twice prior to the subcutaneous inoculation with LD50 of mercury. The survival rate and serum urea nitrogen value after 1 week were 52.3% and 81.1 mg/dl, respectively, in the control and 86.8 and 41.4 in the treated. The mercury, demonstrated by gold toning method, was electron-microscopically seen in cytoplasm, lysosome, and in a small amount in nuclei and mitochondria after 3 hours, when the cytoplasmic matrix loosened in the control but not in the treated. After 6 hr, this cytoplasmic change progressed in the former but was usually inhibited in the latter. Coagulation necrosis became manifest after 12 hr in the control and showed karyolysis after 24 hr, whereas these changes were not observed in the treated so far. The herbal decoction seems to be efficacious angainst mercury poisoning. The hot water extracts of herbs were subjected to fractional separation. NMR spectroscopy may offer unique advantage in characterizing chemical constituents related to inhibitory factors in the decoctions. Biological assay of the acidic fraction containing phenolic materials are now in progress.

EVIDENCE FOR MITOCHONDRIAL (MITO) ELECTRON TRANSPORT DEPENDENT NECROSIS IN THE THICK ASCENDING LIMB OF HENLE (TAL). P.F.Shanley,& G.C.Johnson*. U. CO Hlth. Sci. Ctr., Dept. Path., Denver, CO.

Hypoxic cell injury is thought related to defective mito ATP production. Mito ATP production was inhibited by either electron transport blockade (10^{-5} rotenone=R) or by uncoupling oxidative phosphorylation with resultant electron transport stimulation (10^{-3} M Carbonyl Cyanide m-Chlorophenylhydrazone=CCCP) in the Krebs-Henseleit-hyperoncotic Albumin, nonfiltering (NF) isolated perfused rat kidney (IPK). Both agents reduced ATP compared to vehicle control (V) in the cortex (R=19%, CCCP=31%, both vs V p<0.001, R vs CCCP p<0.01) and in the inner stripe of the outer medulla (R=49%, CCCP=60%, both vs V p<0.05, R vs CCCP NS). Both also caused diffuse hypoxic-like proximal tubule (PT) injury. Their effects differed primarily with respect to O_2 consumption (QO_2) and TAL morphology. CCCP caused an initial burst of increased QO_2 (+46%) followed by a fall below baseline (-38% at 10 min). R caused a fall in QO_2 (-58% at 10 min) without the initial burst. TAL necrosis was 11% in V, 16% in R and 70% in CCCP (V vs R NS; CCCP vs V or R p<0.01). The morphologic response in TAL (but not PT) may thus depend more on the electron transport activity than on the ATP depletion accompanying disruption of mito function. The CCCP-induced TAL injury was similar to the transport-dependent hypoxic necrosis previously described in the IPK. CCCP can thus apparently bypass the protection against this lesion typically observed in the NF protocol suggesting that the role of solute transport in the usual hypoxic injury may be to stimulate mito electron transport.

REDUCED Na-K-ATPase ACTIVITY IN DISTAL NEPHRON IN GLYCEROL-INDUCED ACUTE RENAL FAILURE (ARF): RELATIONSHIP TO SEVERITY OF FUNCTIONAL IMPAIRMENT. P. Sherzer,* H. Wald,* and M.M. Popovtzer. Hadassah Univ. Hospital, Jerusalem, Israel.

To further characterize changes in tubular Na-K-ATPase in ARF, segmental analysis was performed in rat nephrons. Na-K-ATPase was assayed in the following segments: proximal convolution (PC), proximal straight (PS), outer medullary thick ascending limb (OMTAL), cortical thick ascending limb (CTAL), distal convolution (DC) and cortical collecting duct (CCD) in 3 groups of rats: 1. intact, 2. moderate non-oliguric ARF and 3. severe oliguric ARF. GFR and $C_{Na}/GFR \times 100$ were in 1. 0.80 ±0.05 ml/min and 0.68±0.06, in 2. 0.14±0.02 and 1.46±0.35, and in 3. 0.04±0.01 and 0.46±0.15 respectively. Na-K-ATPase in PC and PS were similar in all 3 groups. Na-K-ATPase levels were in OMTAL: in 1. $37\pm2\times10^{-11}$ mole/min/mm, in 2. $20\pm1\times10^{-11}$ $p<0.001$ vs 1., and in 3. $24\pm2\times10^{-11}$ $p<0.001$ vs 1.; in CTAL: in 1. $40\pm2\times10^{-11}$, in 2. $33\pm1\times10^{-11}$ $p<0.001$ vs 1., and in 3. $27\pm2\times10^{-11}$ $p<0.001$ vs 1. and vs 2.; in DC: in 1. $43\pm2\times10^{-11}$, in 2. $33\pm1\times10^{-11}$ $p<0.001$ vs 1. and in 3. $22\pm1\times10^{-11}$ $p<0.001$ vs 1 and vs 2.; in CCD: in 1. $20\pm1\times10^{-11}$, in 2. $25\pm1\times10^{-11}$ $p<0.001$ vs 1., in 3. 24±2 $p<0.05$ vs 1. No differences were noticed in Mg-ATPase suggesting specificity of the changes in Na-K-ATPase. These results show that Na-K-ATPase remains unchanged in PC and PS, and is elevated in CCD in moderate and severe ARF. Na-K-ATPase is reduced in OMTAL, CTAL and DC and there is a direct relationship between this reduction and the severity of ARF. The present findings suggest that TAL and DC are the tubular sites of impaired Na transport in ARF.

DISSOCIATION OF HYPOXANTHINE PRODUCTION AND POSTISCHEMIC RENAL FUNCTION. **Norman J. Siegel, K.M. Gaudio, A. van Waarde*, M. Stromski*, G. Thulin*, M. Kashgarian, and R.G. Shulman*. Yale Univ. Sch. of Med., Depts. of Pediat., Pathol., and Mol. Biophys. and Biochem., New Haven, CT.**

We have evaluated the relationship between renal function (C_{in}) 24 hrs after ischemia and a) the accumulation of hypoxanthine (HYPX) during ischemia and b) the regeneration of ATP at 2 hrs of reflow determined by ^{31}P NMR in vivo. These observations have been made in rats subjected to: a) ischemia of 15, 30, 45 or 60 min (N=27), b) postischemic infusions of ATP-$MgCl_2$, AMP-$MgCl_2$, T_4, or dopamine (N=30), c) inhibition of adenine nucleotide catabolism by 2'-desoxycoformicin or AMPCP (N=15).

There was no relationship between the concentration of HYPX prior to reflow and subsequent C_{in} (R=0.22, 400 to 950 µl/min/100 g BW (non-ischemic valve 950-1050) and when HYPX was significantly elevated (0.5-0.7), C_{in} raised from 200 to 750. This discordance between HYPX and C_{in} suggests that free radical formation from xanthine oxidase activity is not a primary determinant (Paller, JCI 1984) of post-ischemic renal damage.

In contrast, there is a consistent, highly significant correspondence between renal ATP levels after 2 hrs of reflow and C_{in} 24 hrs later (R=0.945, P<0.001). The remarkable consistency of this relationship under varied experimental conditions, allows one to suggest that salvage and restoration of high energy metabolites is a fundamental determinant of the ability to recover from renal ischemia.

ANALYSIS OF ^{67}Ga ACCUMULATION IN EXPERIMENTAL TUBULO-INTERSTITIAL CHANGES.
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^{67}Ga scientigram is suggested for one of the useful methods for diagnosis of the tubulo-interstitial changes instead of renal biopsy. We investigated the mechanisms of accumulation of ^{67}Ga and the proteins binding with ^{67}Ga in the renal interstitium using animal models.

Autoimmune tubulo-interstitial nephritis (TIN) or $HgCl_2$-induced glomerulonephritis (GN) were induced in male Brown Norway rats and ^{67}Ga imaging was compared with their renal histological changes. Intensity of ^{67}Ga accumulation to the kidneys was expressed as a ratio against their accumulation to the lumbar vertebra in the same rats. The imaging and the accumulation intensity of ^{67}Ga to the kidneys were greater in GN (ratio 1.42±0.1) than TIN (0.53±0.19) at the fourth week after the respective treatments, suggesting that ^{67}Ga accumulation depends on plasma exudation rather than cell infiltration in the renal interstitium. Urinary protein excretion was in accordance with the renal intensity of ^{67}Ga accumulation in GN. In next experiments, the affinity of ^{67}Ga to the exudate of renal interstitium were examined by means of γ-scanner and thin layer chromatography. The major proteins producing ^{67}Ga accumulation in the interstitium seems to be transferrin and some other acute phase reactants.

According to above facts, ^{67}Ga scientigram is the useful examination method for detecting tubulo-interstitial changes by binding of ^{67}Ga to the acute phase reactants.

MILD ISCHEMIA PREDISPOSES THE S_3 SEGMENT OF THE PROXIMAL TUBULE TO ACUTE GENTAMICIN TOXICITY. IM Spiegel*, PF Shanley, R Dahl*, BA Molitoris. Michael Reese Hospital & Medical Center, University of Chicago, Chicago, IL., UCHSC. VAMC, Denver, CO.

To determine if previous mild ischemia predisposes to gentamicin(G) nephrotoxicity a unilateral nephrectomy plus renal pedicle clamp model (15 min) followed by 4 hr of reperfusion was studied. At the time of G administration (100 mg/kg, ip) inulin clearance (0.47 ± 0.03 vs 0.46 ± 0.05 ml/min, n=5) had normalized. G toxicity, which normally effects S_1 & S_2 after 6-10 days, occurred rapidly and was limited to the S_3 segment (table, 24 hr post treatment, *$P<0.01$).

		S_3 Segment Injury			
Treatment	Plasma Cr	Absent	1+	2+	Total
Sham only	0.7 ± 0.1	5	0	0	0/5
Is only	0.7 ± 0.1	5	0	0	0/5
Sham + G	0.8 ± 0.1	7	1	0	1/8
Is + G	1.1 ± 0.2*	1	5	2	7/8*

Since mild ischemic injury effects primarily the S_3 segment and increases apical acidic phospholipids to which G binds before undergoing endocytosis we evaluated the uptake and intracellular processing of G by studying the endocytic-lysosomal pathway. Intravenously injected horseradish peroxidase (HRP), a marker for fluid phase endocytosis, was taken up and confined to subapical endosomes at 15 minutes in control proximal tubule cells but appeared throughout the cytoplasm in post-ischemic kidneys. At 2 hr post-injection, HRP was present in large abnormal vacuoles in S_3 cells from post-ischemic kidneys whereas it was confined to secondary lysosomes and endosomes in control kidneys. In summary, minor ischemia predisposes the S_3 segment of the proximal tubule to acute G injury. We postulate the mechanism involves abnormal trafficking and compartmentalization of endocytosed G in previously ischemic cells.

NICKEL INDUCED INHIBITION OF CELL VOLUME REGULATION
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Nickel Nephropathy is characterized in the acute phase by aminoaciduria and proteinuria, and in the chronic stage by scarring and tumorogenesis. These effects are thought to be secundary to Ni(II) enhancement of lipid peroxidation. We therefore tested the possibility that nickel could alter electrolyte transport across the cellular membranes of renal proximal tubule cells. Isolated proximal tubules from C. Auratus, incubated in isotonic Ringer's solution, were exposed to 0.05, 0.10, 0.5 and 1 mM NiCl for 3 min, followed by exposure to a hypotonic, NaCl poor, Ringer's solution (110mOsm). In our experimental conditions (non-perfused tubules) the tubule's lumen is closed and does not open during hypotonic stimulation. Changes in tubular diameters were studied at 24 C in an inverted microscope equiped with video recording capabilities. The tubular diameters were measured at 10 sec intervals using the freeze-frame mode of the video system. Nickel did not induce significant changes in cell volume in isotonic solutions, or in the initial degree of cell swelling after exposure to hypotonic Ringer's. However, in a dose related manner, Ni(II) inhibited the cellular ability to osmoregulate in hypotonic solutions. These observations indicate that nickel disrupts the ability of proximal renal cells to acomplish the necessary translocation of osmotic particles to attain volume regulation. We postulate that this effect may be secundary to alteration of lipid-protein interactions within the plasma membrane, perhaps related to Ni(II) enhanced lipid peroxidation.

ADVERSE SYSTEMIC AND RENAL HEMODYNAMIC EFFECTS OF AMPHOTERICIN B (AMPHO): ROLE OF ENDOGENOUS PLATELET ACTIVATING FACTOR (PAF). JP Tolins, B Ha*, L Raij. U. of MN. and VAMC, Minneapolis, MN.

Use of AMPHO is complicated by severe systemic and renal toxicity. Because of similarities between AMPHO toxicity and the spectrum of bioactivity associated with PAF, we hypothesized that the toxic effects of AMPHO may be mediated by stimulation of endogenous PAF release. Male Sprague-Dawley rats (275-350g) were pre-treated with the specific PAF-receptor antagonist, SRI 63-675 (SRI, 15 mg/kg iv load followed by 0.23 mg/kg/min), or vehicle, before infusion of high-dose AMPHO (1.75 mg/kg iv over 1h). In SRI treated rats (n=7) no hypotension was observed and all rats survived. However, AMPHO induced systemic hypotension (nadir BP 50-60 mmHg) or death in 5/8 vehicle pre-treated rats (63% shock or death, $P < 0.05$ vs SRI). In rats given a lower dose of AMPHO (1.2 mg/kg iv over 60 min) systemic hypotension was not observed during AMPHO infusion, allowing determination of GFR ([3H]-inulin clearance) and renal blood flow (RBF, flow probe). Pre-treatment with SRI resulted in significant preservation of final RBF (SRI vs Vehicle, -31±9 vs -65±11 % change from baseline, $P < 0.04$) and final GFR (SRI vs Vehicle, 1.08±0.16 vs 0.68±0.08 ml/min, $P < 0.05$). Thus, pre-treatment with a specific PAF-receptor antagonist prevented hypotension and death after high-dose AMPHO infusion, while blunting renal vasoconstriction and preserving GFR after low-dose AMPHO. We conclude that endogenous PAF may mediate, at least in part, the adverse systemic and renal hemodynamic effects of AMPHO.

ACUTE RENAL FAILURE (ARF) FOLLOWING BONE MARROW TRANSPLANTATION (BMT). M. Thornton*, L. Gamelin*, and R.A. Zager, Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA.

To assess the incidence, risk factors, and outcome of ARF following BMT, the hospital courses of 272 patients transplanted at FHCRC during 1986 were retrospectively reviewed. 53% of patients at least doubled their initial serum creatinine (Scr x2) within 21 days post BMT and 24% required dialysis (D). The degree of renal functional impairment dramatically impacted on patient mortality (D, 84%; Scr x2, 37%; no Scr x2, 17%). Post BMT jaundice due to hepato-venoocclusive disease (VOD), weight gain ($\geq$2.0 kg), amphotericin B use, and a pre-transplant Scr of $\geq$0.7 mg/dl were each independently associated with the subsequent development of D-requiring ARF ($p<0.001$). Aminoglycoside, vancomycin, cyclosporine A use, and acute graft versus host disease were not associated with ARF. A mismatched graft was a significant risk factor for ARF but only by univariate, not multivariate, analysis. During the 48 hrs prior to doubling the Scr, 63% of D patients had positive blood cultures, 39% were hypotensive (systolic BP $\leq$90) and 95% had a temperature of $\geq 38^{o}C$. Urine Na concentrations were $\leq$40 m eq/l in 85% of D patients tested. Autopsy kidney specimens revealed no histologic explanation for ARF in virtually any of the patients. Conclusion: ARF can be an extremely common complication following BMT. Its development is typically preceded by VOD, weight gain, amphotericin use, septicemia, and hypotension. The available data suggest that the ARF is hemodynamic rather than structural in nature and it augers a grave prognosis.

ISOLATION, CULTURE AND CHARACTERIZATION OF HUMAN RENAL COLLECTING DUCT CELLS. Anna L. Trifillis* (intr. by Barbara Urbaitis). Dept. of Pathology, University of Maryland School of Medicine and Maryland Institute for Emergency Medical Services Systems, Baltimore, Maryland

The value of isolated nephron segments for in vitro nephrotoxicity studies is well recognized. We have previously developed an in vitro model of human proximal tubule cells isolated from cadaver kidneys (Trifillis, et al., J. Urology 133: 324-329, 1985). Using similar methods, we have isolated epithelial cells from the papillary region of human kidneys. Papillae were dissected from 10 cadaver kidneys and digested in Minimum Essential Medium (MEM) containing 300 U/ml collagenase. Cells were plated at a density of 10^4 live cells/ml in MEM supplemented with insulin and 10% fetal calf serum. Confluent monolayers with a cobblestone appearance were observed at 10-15 days. Cells of primary isolates and first passages after storage at $-135^{o}C$ exhibited epithelial cell ultrastructure including cell junctions, intermediate cytoplasmic filaments and microvilli. Histochemically, these cells were negative for Factor-VIII (a marker for endothelial cells) and γ-glutamyl-transferase (a marker for proximal tubule cells). However, when stained by the immunoperoxidase method with peroxidase-labelled peanut lectin (Arachis hypogea), which binds specifically to human distal tubule and collecting duct cells, the cells uniformly exhibited dark brown reaction product. The results indicate that human renal collecting duct cells can be isolated and cultured for in vitro studies.

EPIDERMAL GROWTH FACTOR (EGF) ENHANCES RENAL REGENERATION AND ACCELERATES RECOVERY FROM ISCHEMIC ACUTE RENAL FAILURE (ATN). Y.K. Tsau*, J.T. Norman and L.G. Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

While measures aimed at preventing or ameliorating the course of ATN may have value if initiated prior to onset of the disease, there is no information about how to shorten the course of ATN once it is established. To address this issue, we examined the effect of EGF in vivo, since we had previously shown EGF to be a potent mitogen for renal tubular cells. Bilateral renal ischemia was induced in Sprague-Dawley rats by renal artery occlusion for 90 min. Following reperfusion of the kidneys, EGF (0.1 μg/hour) (n=10) was administered over 7 days via an intra-aortic catheter with its tip proximal to the renal arteries using an osmotic minipump. Controls received NaCl (n=10). In control rats serum creatine [Cr] increased from baseline of 0.6 ± 0.1 ml/min. to a maximum of 5.1 ± 2.4 at day 2 and declined to 1.9 ± 0.3 at day 7. In EGF-treated rats maximum [Cr] at day 2 was 3.8 ± 2.0. Between days 4 and 7 [Cr] was significantly lower than control with more complete recovery at day 7 ([Cr] = 1.09). Autoradiography of EGF binding to control ATN kidneys at 15, 24 and 48 hours showed a 2.6 fold increase at 48 hours. Conclusion: 1) EGF enhances renal regeneration after ATN and hastens the rate and degree of recovery from ischemic acute renal failure. 2) Up-regulation of EGF receptor binding contributes to the regenerative response in ATN.

HEPARAN SULFATE CHARGE DENSITY IN ADRIAMYCIN AND PUROMYCIN-AMINONUCLEOSIDE NEPHROSIS. **C. Whiteside, K. Prutis, Membrane Biology Group, Department of Medicine, University of Toronto, Toronto, Canada.**

Temporal correlation between proteinuria and heparan sulfate (HS) depletion in Adriamycin (ADR) and Puromycin-aminonucleoside (PAN) rats was observed using polyethyleneimine (PEI) staining of the glomerular basement membrane (GBM) and a microassay for HS core protein. Sprague-Dawley rats (N=5-8) received a tail-vein injection of ADR (7.5 mg/kg), PAN (150 mg/kg) or saline. On days 2,5,10 and 15, glomeruli were stained with PEI and sites/μm GBM visualized on E/M. In ADR the HS sites (N=25/rat) were reduced by day 5 (control 20.1± 1.8sites/μm; ADR 18.6 ± 1.8sites/μm, $\bar{X} \pm SD$, p<.05) and persisted to day 15 (control 19.7± 1.8sites/μm; ADR 17.4 ± 1.3sites/μm, p<.05) preceding the increased proteinuria on days 5-10. In PAN the HS sites were reduced by day 1 (control 20.9±1.8sites/μm; PAN14.9 ± 1.5sites/μm, p<.05) persisting to day 15 (control 19.7± 1.8sites/μm; PAN 13.0 ± 1.6sites/μm, p<.01) preceding the increased proteinuria between days 2-5. HS content in glomeruli isolated from ADR rats had no persistent change:day 2 (88 ±4% control, p<.05), day 5 (84±7% control, p<.05), day 10 (103±8 % control, NS). PAN had no persistent HS change: day 2 (98±6% control, NS), day 5 (84±5% control, p<.05), day 10 (100± 10% control, NS). Reduced HS density is not due to reduction in core protein and does not correlate temporally with increasing glomerular permeability.

RECOVERY OF METABOLIC INHIBITOR-TREATED TUBULES PROTECTED BY GLYCINE (GLY) OR GSH. J.M. Weinberg, J.A. Davis* and M. Abarzua*. V.A. Med. Ctr. and Univ. of Michigan, Ann Arbor, MI.

To assess the protection against metabolic inhibitor-induced injury to isolated rabbit proximal tubules (PT) provided by GLY and GSH, PT were exposed to either sodium cyanide (CYN) (2 mM), antimycin (ANTI) (10 μM), oligomycin (OLIGO) (15 μM) or carbonylcyanide m-chlorophenyl-hydrazone (CCCLP) (10 μM) for 15' then were washed and incubated in medium containing 5 mg/ml BSA (to facilitate removal of hydrophobic agents) for 45' more prior to measurement of structural and metabolic parameters. Otherwise untreated PT were compared to PT treated with either 2 mM GLY or 2 mM GSH throughout all phases of the experiment (N=4-7). All inhibitors at 15' increased free LDH from 5.6±0.7 to 30-40% ($p < 0.01$) and caused severe depletion of cell ATP and inhibition of respiration (RR). The LDH release (LR) but not the inhibition of respiration or depletion of ATP was prevented by GLY or GSH. After washing (AW), CYN-treated tubules showed only moderate additional LR, and had partial recovery of ATP and RR. All parameters were improved by GLY and GSH. OLIGO and ANTI-treated PT had continuing high LR AW and no recovery of ATP or RR. GLY and GSH substantially ameliorated the LR but did not improve ATP or RR. CCCLP-treated PT also had high LR AW which was prevented by GLY or GSH. GSH but not GLY produced major improvement of RR and ATP after CCCLP. These data show: a) ability to remove inhibitors varies, b) sustained protection by GLY and GSH AW even when ATP and RR do not improve and c) a unique effect of GSH to improve mitochondria after CCCLP.

ATRIAL NATRIURETIC FACTOR VERSUS DOPAMINE IN THE PREVENTION OF NONSTEROIDAL-ANTIINFLAMMATORY AGENT INDUCED ACUTE RENAL DYSFUNCTION. David Yasmineh*, M. M. Redfield*, T. R. Schwab, B. S. Edwards*, and J. C. Burnett, Jr., Mayo Clinic, Rochester, MN

Hypotensive hemorrhage (HH) followed by retransfusion (RT) in dogs pre-treated with the nonsteroidal anti-inflammatory agent (NSAID) indomethacin (10 mg/kg, iv) produces acute renal dysfunction (ARD) with increased renal vascular resistance (RVR) and decreased glomerular filtration rate (GFR). Atrial natriuretic factor (ANF) producing renal vasodilation may prevent ARD produced by HH+NSAID. This study addressed renal vasodilation as the mechanism for ANF protection by comparing intrarenal ANF to the renal dilator dopamine (dop) and measured renal hemodynamics prior to, immediately (Imm-RT) and 2 hours after RT (2hr-RT). †p<.05 compared to baseline.

	Baseline	IMM-RT	2hr-RT
Group I (HH, n=8)			
GFR, ml/mn	27±3	27±5	14±4†
RVR, RU	0.70±.07	0.70±.06	1.22±.25†
Group II (HH+ANF, 0.3 μg/kg/min, n=7)			
GFR, ml/min	29±4	33±4	33±4
RVR, RU	0.78±.09	0.63±.09	0.76±.07
Group III (HH+dop, 1 μg/kg/min, n=5)			
GFR, ml/min	38+3	25±6	12±5†
RVR, RU	0.87±.14	0.63±.11	0.94±.21

This study demonstrates that ANF may prevent ARD produced by HH+SNAID. Further, the mechanism of protection may be independent of renal vasodilation as ANF, in contrast to dop, preserves GFR despite similar actions on RVR.

MITOCHONDRIAL RESPIRATION AND NaKATPase ACTIVITY: DIFFERENT BEHAVIOR OF RENAL CORTEX AND MEDULLA IN ISCHEMIC ACUTE RENAL FAILURE. Vanda Yoshida*, Maria E. Carvalho*, Antonio Seguro*, Silvia Campos*, Antonino Rocha*. (intr. by Roberto Zatz) Heart Institute, São Paulo Univ. Med. Sch. SP - Brazil.

Changes in mitochondrial (Mito) respiration and NaKATPase activity in cortex and medulla were studied with a 15min renal artery clamping and 1hr and 18hr after reflow in rats. Mito respiration was assessed polarographically and NaKATPase by inorganic phosphate released from ATP. In the cortex, (vs contralateral control), with a 15min ischemia, there was a decrease in State3 (S3, ADP stimulated) respiration (246.4±13.9vs175.9±8.3 ngatoms O/mg/min $p<.05$), no significant change in State4 (S4, succinate supported) and decrease in Acceptor Control Ratio (ACR, S3/S4)(4.06±.06vs3.42 ±.09 $p<.001$). In contrast, the medulla results for S3, S4 and ACR were unchanged. After 1hr reflow, Mito respiration was still impared in the cortex. However, in the medulla, there was a decrease in S3 (286.7±19.4vs224.9±22.7 $p<.05$) and ACR (4.09±.18vs3.35±.12 $p<.05$); S4 remained unchanged. After 18hr reflow Mito respiration in cortex and medulla returned to basal values. NaKATPase with ischemia was unchanged in cortex (25.51±3.12vs25.58±4.51 umoles Pi/mg/hr), but was decreased in medulla (56.28±6.48vs34.1±5.44 $p<.001$). Thus, with ischemia the cortex showed relatively more Mito respiration depression but more preserved NaKATPase than the medulla. Reflow worsened Mito respiration in the medulla (but not the cortex). These data suggest different mechanisms of injury for cortex and medulla in ischemic acute renal failure.

MECHANISMS IN HEMOGLOBIN (H) INDUCED ACUTE RENAL FAILURE (ARF). R.A. Zager and L.M. Gamelin*, Univ. of Washington, Dept. of Medicine, Seattle, WA.

To evaluate pathogenetic mechanisms in H-ARF, rats were infused with stroma free human H under aciduric or alkalinuric conditions. Aciduric rats developed azotemia (24 hr BUN 88 ± 8mg/dl), distal nephron heme pigment casts, and foci of proximal tubular (PT) necrosis. The latter were most prominent in PT segments with greatest heme uptake, denoted by heme stained endolysosome (EL) formation. Ex vivo acidification (pH 5.2) of urine from alkalinuric rats resulted in conversion of H to met-H (not hematin) with subsequent precipitation, suggesting that met-H is the constituent forming casts in aciduric rats. A link between cast formation/tubular obstruction and PT necrosis was indicated by showing that transient tubular obstruction induced by 5h of ureteral ligation increased PT heme uptake, PT necrosis, and exacerbated the severity of ARF. This resulted under aciduric or alkalinuric conditions indicating that H can be toxic irrespective of urine pH, assuming sufficient PT uptake. H-emia decreased renal blood flow (RBF) 70% and a direct H-ischemic synergy was identified: a non toxic H dose drastically exacerbated renal artery occlusion (30 min) induced ARF by increasing PT necrosis and casts, effects not lessened by antioxidants (DFO, DMTU, Na benzoate). Conclusion: Human H has a direct nephrotoxic effect. Aciduria potentiates it by forming met-H casts, thereby facilitating PT heme uptake. H-emia can contribute to ischemic ARF both by decreasing RBF and by increasing both cast formation and the extent of PT necrosis, presumably by a non oxidant action.

POST-ISCHEMIC GLOMERULI ACQUIRE IMMUNITY AGAINST THE TOXIC EFFECTS OF REACTIVE OXYGEN SPECIES (ROS) THROUGH ENHANCED LOCAL LEVELS OF ANTIOXIDANT ENZYME (AOE). T. Yoshioka, T. Bills*, H. L. Greene* and I. Ichikawa. Dept. of Pediatrics, Vanderbilt Univ. Med. Ctr., Nashville, TN.

Kidneys exposed to certain toxic substances, e.g., puromycin, gentamicin, become resistant to subsequent exposures to these toxins. Since ROS have been suggested to be important intermediaries in the glomerulopathy induced by these substances, AOE may have a role in developing this resistance. We therefore examined if an increase in the level of ROS affects local AOE activities. Three (Day-3, n=6) or 6 days (Day-6, n=8) following renal ischemia (20 min), the activities of catalase (CAT), glutathione peroxidase (G-Px) and superoxide dismutase (SOD) were assessed in isolated glomeruli of Munich-Wistar rats.

[CAT: Uk/mg prot; G-Px, SOD, XO; U/mg prot, GFR: ml/min; Values are mean; $p<.05$ vs C (*) or Day-3(†)]

	CAT	G-Px	SOD	GFR	GFR-p	XO-p
C	0.20	23	56	1.0	0.3	2.5
Day-3	0.18	12	49	0.5	0.2	3.1
Day-6	0.46*†	44*†	101*†	0.9†	0.7*†	2.1

Although AOE activities at Day-3 were comparable to normal rats without previous ischemia (C, n=6), AOE activities were elevated at Day-6. GFR was measured in a separate set of C (n=6), Day-3 (n=4) and Day-6 (n=6) rats. At Day-3, GFR was ~50% of C, but returned to near C levels at Day-6. To evaluate the functional significance of heightened AOE activities at Day-6, these same rats were restudied after a challenge with ROS by ischemia (30 min) + reperfusion (60 min). Repeat GFR measurement (GFR-p) showed a marked depression in C and Day-3, however, only mild hypofiltration in Day-6. Since xanthine oxidase levels in post-ischemic glomeruli (XO-p) were comparable among the groups, the contrasting pattern in post-ischemic hypofiltration between C and Day-3 vs Day-6 is due to altered degradation and not generation of ROS in Day-6 glomeruli.

The results indicate that the local level of endogenous AOE is an important determinant for the ROS-induced glomerular injury. This data also demonstrates for the first time that glomerular AOE activities are augmented following exposure to high level of ROS, and suggestes that enhanced AOE activities constitute effective glomerular defense mechanism against recurrent ROS-mediated injuries.

SALICYCLIC ACID (SA) DOES NOT CAUSE RENAL VASOCONSTRICTION BUT DOES INDUCE A DIURESIS AND NATRIURESIS IN COMMON-BILE-DUCT-LIGATED (CBDL) CIRRHOTIC MINIATURE SWINE (MS). E. Zambraski, S. Guidotti*, L. Schwarz*, J. Diamond*, D. Atkinson*. Rutgers Univ., New Brunswick, NJ.

In patients with liver disease, or normal subjects who are sodium depleted, the administration of a non-steroidal antiinflammatory drug, or acetyl-salicylate, adversely affects the kidneys; vasoconstriction and sodium retention occur. We recently observed that SA did not cause renal vasoconstriction but did induce a diuresis and natriuresis in the sodium depleted dog. To determine if SA would similarly influence the kidneys in a cirrhotic subject, the effects of SA (40 mg/kg) were evaluated in 5 normal and 6 CBDL MS. Four of the 6 CBDL MS were ascitic. Plasma SA in the two groups ranged from 150–200 µg/ml. SA significantly decreased PGE_2 excretion in the normal and CBDL MS by 56 and 27%, respectively. In normal MS SA did not alter renal blood flow (RBF) or GFR but did significantly increase urine volume (UV) and sodium excretion (UnaV). In CBDL MS the effects of SA are listed below. (Mean ± SEM; *$P<0.05$).

	RBF (ml/min)	GFR (ml/min)	UV (ml/min)	UnaV (µEq/min)
Control	258±86	58±14	.27±.03*	13±2*
SA	242±80	46±6	.46±.04*	59±10*

In 5 additional CBDL MS meclofenamate reduced RBF (659±107 to 418±38 ml/min; $P<.05$) and decreased GFR, UV, and UnaV by 39,30 and 59%, respectively. These data demonstrate that SA does not adversely affect renal function in a cirrhotic subject. Also, SA causes a diuresis and natriuresis in this sodium retaining state.

CHARACTERIZATION OF UREMIC LEFT VENTRICULAR HYPERTROPHY (LVH). G. Abu-Jawdeh*, S. Mujais, A. Abu-Alfa*, M. LaPointe*, N. Istefan*. Northwestern U and VA Lakeside, Chicago IL.

To determine whether uremic LVH is distinct from LVH of other hypertensive states, we biochemically profiled the myocardium in sham and severely uremic Sprague-Dawley rats. Fractional protein synthetic rate (FPSR) was measured by [^{3}H]-leucine incorporation in vivo, LV norepinephrine content (NE) by HPLC, DNA by bisbenzimidazole fluorescence, and protein composition by 2-d electrophoresis.

		Sham	p	Uremic
BUN	(mg/dl)	19±1	0.01	164±31
LV	(mg/g)	2.1 ±.05	0.01	2.8 ±.05
LV DNA	(mg/g)	1.7 ±.03		1.9 ±.11
LV protein	(mg/g)	144 ±6		147 ±4
LV NE	(ng/g)	391 ±24		471 ±46
FPSR	(%)	4.96 ±.9		4.77 ±.8

Except for an increase in a minor protein family, proportional protein composition was similar in both groups. We conclude that in uremic LVH an increase in all biochemical parameters suggests a uniform adaptive process similar to LVH of the SHR model, but distinct from renovascular hypertension LVH.

EXCESS PARATHYROID HORMONE (PTH) ADVERSELY AFFECTS LIPID METABOLISM IN CHRONIC RENAL FAILURE (CRF). Mohammad Akmal, Kasim Sadika*, Amin R. Soliman*, Shaul G. Massry, Div. Nephrol., Univ. So. Calif., Los Angeles, CA.

Hyperlipidemia is common in CRF but its mechanisms are not defined. Excess PTH has been implicated in its pathogenesis. We examined the effects of PTH on lipid metabolism utilizing intravenous fat tolerance test (IVFTT) and post heparin lipolytic activity (PHLA) in normal dogs and in 2 groups of animals with similar degree and duration of CRF. One group had intact parathyroid glands and secondary hyperparathyroidism (CRF-NPX) and the other was parathyroidectomized and maintained normocalcemic (CRF-PTX). Results are given in Table:

Normal	CRF-NPX	CRF-PTX
Fasting triglycerides (mg/dl)		
49±2.7	82±6	42±6
IVFTT (Area under curve mg.60 min)		
4637±693	8673±949*	5442±277
PHLA (after 30 min, fatty acids		mole/ml/min)
275±14	151±10*	317±19

*p<0.01 vs normal or CRF-PTX

The data show that CRF-NPX dogs had Hypertriglyceridemia, diminished PHLA and fat intolerance. These abnormalities were prevented by prior parathyroidectomy. Thus, excess PTH was responsible for impaired PHLA in CRF resulting in impaired fat removal. This action of PTH may be related to the previously reported inhibitory effect of PTH on insulin release from pancreas causing relative insulin deficiency in CRF. The data assign a new dimension for PTH toxicity in CRF.

SEX VULNERABILITY TO GLOMERULOSCLEROSIS AFTER SUBTOTAL NEPHRECTOMY (Nx) IS NOT DUE TO DIFFERENCES IN GLOMERULAR TUFT VOLUME. S Adler, J Lombet,* C Nast, P Anderson,* R Glassock. Depts. of Med, & Path. Harbor-UCLA Med Ctr, Torrance, CA.

Previous work from our laboratory (Kidney Intl 33:370, 1988) showed that male rats develop earlier glomerulosclerosis, more proteinuria and higher procollagen α1 (IV) mRNA levels than female rats after subtotal Nx despite similar degrees of azotemia and systemic arterial hypertension. We calculated glomerular tuft volume (GTV=1.25 glomerular area $^{3/2}$) morphometrically in saline-perfused Bouin's-fixed kidneys from 1 2/3 Nx and sham Nx age-matched rats of opposite sex. These studies were performed to determine whether larger GTV are implicated in the increased severity of glomerular functional and histological changes observed in males. GTV was measured in 14 male rats undergoing 1 2/3 Nx (n=6, NxM) or sham Nx (n=8, SNxM) and 15 females undergoing 1 2/3 Nx (n=7, NxF) or sham Nx (n=8, SNxF) 7 weeks after either the sham procedure or a 2 step reduction in nephron mass. Initial body weights were higher in male (254±17gm) than female (216±18gm) rats, as were final body weights (NxM 302±43 gm; SNxM 333±14 gm; NxF 241±36 gm; SNxF 258±17 gm) ($p \leq 0.05$). Despite differences between sexes in initial and final body weights, mean GTV were similar in male and female sham Nx groups (SNxM 0.78±0.06 x $10^6\mu^3$, SNxF 0.77±0.06x$10^6\mu^3$, $p \geq 0.05$). Glomerular hypertrophy occurred equally in NxM and NxF ($p<0.01$ compared to sham operated rats of the same sex). GTV did not differ in NxM and NxF rats (NxM 1.6±0.5 x $10^6\mu^3$, NxF 1.5±0.3x$10^6\mu^3$, $p>0.05$). These findings do not support the notion that differences in wall stress due to dissimilar glomerular volume accounts for the relatively poorer outcome observed in male rats.

SUPERIORITY OF CAPTOPRIL (CAP) OVER COMBINATION TRIPLE THERAPY (TRX) IN ARRESTING DIABETIC GLOMERULOPATHY IN THE RAT. S. Anderson, S.L. Riley,* H.G. Rennke, D.L. Garcia, and B.M. Brenner. Brigham & Women's Hosp., Boston, MA.

To compare the effects of CAP and TRX (reserpine, hydralazine and hydrochlorothiazide), we performed a 16-month study in nondiabetic control (C) rats and in insulin-treated moderately hyperglycemic diabetic rats receiving no other therapy (DM); captopril (DM/CAP); or TRX (DM/TRX). At 6-10 wks: (Means ± SEM)

Group (n)	$\overline{AP}$	$\overline{P}_{GC}$	SNGFR	Q_A
	---mmHg---		---- nl/min----	
C (8)	114±3	48±1	42±3	146±12
DM (8)	117±3	64±2*	66±5*	219±19*
DM/CAP (8)	98±3*+	47±1+	61±5*	233±19*
DM/TRX (11)	104±3+	49±1+	63±4*	229±14*

*p<.05 vs. C, +p<.05 vs. DM, ‡p<.05 vs. DM/CAP.

CAP and TRX lowered mean arterial pressure ($\overline{AP}$) and glomerular capillary pressure ($\overline{P}_{GC}$) without affecting the single nephron (SN) GFR or plasma flow rate (Q_A) in early diabetes. At 16 months, despite comparable glycemic and systolic blood pressure (SBP) control in the treated groups, albuminuria (UalbV) and focal glomerular sclerosis (FGS) values differed substantially:

Group (n)	SBP	$\overline{P}_{GC}$	UalbV	FGS
	---- mmHg---		mg/d	%
C (14)	126±3	--	14±3	1.4±0.5
DM (11)	145±4*	--	59±8*	12.0±0.6*
DM/CAP (8)	124±5+	46±1	3±.4*+	0.4±0.1*+
DM/TRX (10)	115±4+	56±2‡	36±11+‡	4.0±1.3*+‡

The late studies revealed continued control of $\overline{P}_{GC}$ with CAP but not TRX (each n=4). Thus, TRX appears to delay disease and modestly protect, while CAP affords superior long-term protection.

ISOLATED PROXIMAL TUBULES (PT) AND PROXIMAL TUBULAR CYSTS (PT-CY) OF CPK MICE HAVE INCREASED NA-K ATPase ACTIVITY. E.D. Avner, W.E. Sweeney*, D. Ellis. Childs Hosp of Pgh., Pgh. Pa.

In renal cystic diseases, the progressive enlargement of a subpopulation of nephrons destroys adjacent tissue. Recent data demonstrate that alterations in Na^+pump driven transtubular transport in discrete segments of cystic nephrons may have pathogenic import in such progressive tubular enlargement. We therefore determined Na-K ATPase activity in isolated PT and PT-CY from CPK mice, an experimental model of autosomal recessive cystic disease in which we have previously demonstrated a positive relationship between whole kidney Na-K ATPase and PT-CY formation (Ped Nephrol 2:210,1988). Enzyme digestion and double Percoll density gradient separations of newborn and 5 day old control (CON) and CPK kidneys yielded distinct fractions which were >95% enriched for PT and PT-CY respectively. Na-K ATPase ($nmol \cdot min^{-1} \cdot \mu gDNA^{-1}$)was determined by enzyme-linked kinetic microassay:

	Newborn		Day 5	
	PT	PT-CY	PT	PT-CY
CON	.36±.02	-	.51±.02	-
CPK	.49±.03ϕ	.62±.02ϕϕ	.60±.03ϕ	.71±.04ϕϕ

At both stages studied, morphologically normal PT from CPK kidneys had ↑ Na-K ATPase activity relative to CON (ϕ $p<.05$) while PT-CY from CPK kidneys had ↑ Na-K ATPase activity relative to morphologically normal CPK PT as well as CON (ϕϕ $p<.05$ vs CPK PT; $p<.01$ vs CON).

We conclude that↑ PT Na-K ATPase is a marker of CPK gene expression, and speculate that ↑ Na pump mediated PT fluid secretion contributes to PT-CY formation and progressive enlargement.

RENAL SODIUM HANDLING AND THE RENIN-ANGIOTENSIN SYSTEM (RAS) IN CHRONIC RENAL FAILURE (CRF) DURING INFUSION OF ATRIAL NATRIURETIC PEPTIDE (ANP). T.Bayer,* H.Meyer-Lehnert, K.Glänzer,* H.J.Kramer. Med. Poliklinik, Univ. of Bonn, West Germany.

We examined the effect of 60 min i.v. α-hANP (24 ng/min/kg) in 8 patients with CRF (51.9±3.4 yrs, C_{In} 34.4±6.8 ml/min) and in 8 control (C) subjects (54.1±4.1 yrs, C_{In} 117.1±3.6 ml/min). During ANP, C_{In} increased by 16.2±1.4% in C and by 70.7±4.2% in CRF ($p<.001$). Urine volume increased from 4.9±1.2 to 8.6±1.3 ml/min in C ($p<.01$) and from 3.6±0.5 to 8.1±1.0 ml/min in CRF ($p<.01$). Basal urinary Na excretion was 21.6±5.5 mmol/h in C and 16.9±2.5 mmol/h in CRF (NS). The increase of Na excretion during ANP was not different in C (+126.7±28.5 %) and in CRF (+142.3 ± 22.3 %). However, increase of fractional Na excretion during ANP was greater in C than in CRF (180.9±67.0 vs 68.4±24.7 %, $p<.01$). Basal aldosterone (A) was higher in CRF than in C (103.6±9.3 vs 181.8±23.0 ng/l, $p<.05$). A decreased during ANP in both CRF and C (-26.0±7.5 % and -41.3±5.9%, $p<.05$ and $p<.01$ vs basal, resp.), but in CRF, A was still higher than in C during and after ANP (71.7±4.4 vs 97.0±5.6 ng/l during ANP, $p<.01$; 72.9±4.5 vs 111.6±7.5 ng/ml 60 min after ANP, $p<.01$). Plasma renin activity was elevated in CRF (0.50±0.08 vs 1.22±0.23 ng AI/ml/h, $p<.01$), decreased during ANP and reached lowest levels 60 min after ANP (0.71±0.23 vs 2.32±0.57 ng/ml, C vs CRF, $p<.01$). Plasma vasopressin levels were not different in C and CRF and were not affected by ANP. Thus, in CRF, ANP stimulates urinary electrolyte excretion to a lesser extend than in C. This may at least partially be due to sustained increase of activity of the RAS in CRF.

IN DIABETIC RATS, POLYUNSATURATED FATTY ACIDS (PUFA) INFLUENCE PLASMA LIPIDS, EICOSANOID PRODUCTION AND RENAL DISEASE. U.O. Barcelli, D. Beach,* A. Motz,* J. Deddens,* B. Thompson,* M.A. Weiss, Depts. of Medicine and Pathology, Univ. of Cincinnati Medical Center, Cincinnati, Ohio.

Dietary PUFA influence renal disease in animal models. We compared diets containing 10% evening primrose oil (EPO), safflower oil (SO) or fish oil (FO) versus 10% beef tallow (BT) in diabetes. Female Sprague-Dawley rats received intravenous streptozotocin 65 mg/kg. Insulin prevented ketoacidosis. Percent survival (SRV), plasma triglycerides (TG) and total cholesterol (TC) in mg/dl, platelet thromboxane B_2 (TXB_2) in ng/ml and urinary protein (UP) in mg/16h were measured:

Number	BT	EPO	SO	FO
wk 0	(n=20)	(n=23)	(n=21)	(n=22)
SRV	40	50	62	39
wk 37				
TG	169	131	198	79[b]
wk 30	(71,407)	(60,288)	(91,432)	(50,107)
TC	150	129	127	92[a]
wk 30	(112,202)	(110,153)	(101,160)	(65,132)
TXB_2	243	118[b]	174	30[a]
wk 25	(147,402)	(58,239)	(105,287)	(17,51)
UP	53	18[c]	20[c]	36
wk 35	(11,242)	(4,83)	(6,72)	(7,105)

Values are geometric means (-SD, +SD).
[a] = $p<0.01$, [b] = $p<0.05$, vs BT by ANOVA.
[c] = p 0.06 vs BT by ANOVA in ranked values.

Dietary PUFA also affected renal fatty acid composition and eicosanoid production. FO markedly lowered plasma lipids and TXB_2. EPO and SO may favorably alter the evolution of diabetic renal disease. Various PUFA have diverse beneficial effects in this model.

HEMODYNAMIC FACTORS MAY CONTRIBUTE TO THE GREATER TENDENCY TOWARDS GLOMERULAR SCLEROSIS IN MALE (M) VS FEMALE (F) RATS. Chris Baylis. Univ of West Virginia, Dept. of Physiol., Morgantown, WV.

Compared to F, the M kidney exhibits a greater tendancy to develop glomerular sclerosis with, eg. ageing or renal ablation. These studies, performed in M and F rats, uninephrectomized (UNX) at weanling age and maintained on 40% protein for 9 months, investigated whether glomerular hemodynamic differences eventually occur between the sexes. Measurements made were kidney weight (KW), single nephron (SN)GFR, glomerular plasma flow (Q_A), glomerular pressure gradient (ΔP), glomerular ultrafiltration coefficient (K_f) and 24h protein excretion (UpV). Data; mean±SE. ¶$p<0.05$; M vs F, unpaired t-test.

	KW g	SNGFR nl/g.KW/min	Q_A	ΔP mm Hg	U_pV mg/g. KW/24h	K_f nl/g.KW/(s.mmHg)
M	2.5±0.1¶	31±2¶	133±23	46±1¶	22±4	0.041±0.007
F	1.3±0.1	39±1	166±10	40±1	16±7	0.044±0.004

Both M and F kidneys hypertrophied by similar %s due to UNX but absolute KW was greater in M vs F due to greater body size. When factored for KW, SNGFR was significantly and Q_A non-significantly lower in M vs F. U_pV and K_f were similar between the sexes. ΔP was higher in M vs F, due to elevated glomerular blood pressure (P_{GC}). Thus, 9 months after UNX, the M exhibited the beginning of a functional decline compared to F. The fact that P_{GC} is higher in M vs F at this stage is predicted to lead to further worsening of glomerular injury in M vs F with time, as does indeed occur. The greater KW might also contribute to the susceptibility of M to glomerular injury.

RECONSTRUCTION OF A NEPHROTIC GLOMERULAR CAPILLARY NETWORK: LOCALIZATION OF EPITHELIAL CELL DAMAGE AND SCLEROTIC REGIONS. T. Bertani*, G. Caruso*, F. Inzoli* and A. Remuzzi*. Mario Negri Institute for pharmacological Research, Bergamo, and Politecnico di Milano, Milano, Italy. (Intr. by M.J. Dunn).

Adriamycin (ADR) nephrosis is an experimental model of glomerular epithelial cell (EpC) damage and focal glomerulosclerosis. To localize glomerular structural damages along the capillary network we related the pathological changes with the network pathway. In a SD rat, treated with ADR (7.5 mg/Kg, 10 weeks after ADR) kidney were fixed by perfusion and the tissue embedded in Epon. Semithin sections (1 µm) of one glomerulus were cut and stained with toluidine blue. Each section was photographed under oil-immersion and printed (final magnification x 1200). The outlines of the capillary lumina were traced on transparent sheets and numbered. The outline numbers were stored in a data base and linked to the number of the outlines of the same capillary lumen in the previous and in the following section. A computer program was developed to read the data base and to draw a capillary network pathway. Capillary profiles showing EpC damage (blebs and cytoplasmatic swelling) and sclerosis were evaluated in each microphotograph and related to the corresponding capillaries in the graphed network. The network is characterized by the presence of three main lobes. The percentages of capillaries presenting EpC damage and sclerosis, for the three lobes, are as follows:

	Lobe A	Lobe B	Lobe C
EpC swelling	49% (41/83)	43% (34/79)	54% (42/77)
EpC blebs	13% (11/83)	18% (14/79)	27% (21/77)
Sclerosis	2% (2/83)	29% (23/79)	36% (28/77)

Glomerular capillaries affected by EpC damage were uniformely distributed from the afferent to the efferent end of the capillary network. Most of capillaries affected by sclerosis were in the middle part of the network. We conclude that: 1) EpC damage, due to the toxic effect of ADR, is diffused and uniformely distributed along the capillary network; 2) on the contrary, the sclerotic process developed mainly in two lobes leaving the third one only minimally affected.

RENAL AND GLOMERULAR HEMODYNAMICS IN 3/4 NEPHRECTOMIZED (NX) DOGS. S.A. Brown*, D.R. Finco, L.G. Navar. Univ of AL at Birmingham, Birmingham, AL and Univ of GA College of Veterinary Medicine, Athens, GA.

Previous studies have suggested that the course of compensatory response following partial reduction of renal mass in the dog differs from that observed in rats. We investigated whole kidney and single nephron hemodynamics in 3/4 NX (n=12) and sham operated (n=10) dogs 4-6 weeks following reduction in renal mass. Renal blood flow (NX: 5.07±0.23; sham: 4.06±0.23 ml/min/gm k wt, P<0.01) and GFR (NX: 0.99±0.05; sham: 0.71±0.02 ml/min/gm k wt, P<0.01) were elevated in dogs with 3/4 NX compared to shams. There was no difference in mean arterial pressure (AP) (NX: 122±8 vs sham: 118±12 mm Hg, NS). In 3/4 NX dogs, there was impairment of autoregulation of both RBF (NX:0.65% vs sham: 0.90% change RBF/mm Hg change in AP, P<0.05) and GFR (NX: 0.74% vs sham: 0.25% change GFR/mm Hg change in AP, P<0.05). Micropuncture studies demonstrated increases in SNGFR (128.5±7.9 vs 69.5±6.9 nl/min, P<0.01), estimated glomerular pressure (74.1±3.4 vs 62.9±3.0 mmHg, P<0.05), proximal tubule free flow pressure (28.4±2.0 vs 19.5±1.1 mmHg, P<0.05), and peritubular capillary pressure (14.2±015 vs 10.9±0.9 mmHg, P<0.05) in dogs with 3/4 NX. The comparatively small increase in transcapillary hydrostatic pressure gradient (45.6±3.8 vs 43.3±3.6 mmHg) and lack of overt systemic hypertension may be important in limiting progressive nephron loss in 3/4 NX dogs.

EFFECTS OF 8 VS 25 PERCENT PROTEIN DIET ON RENAL FUNCTION OF BABOONS WITH A REMNANT KIDNEY. J. J. Bourgoignie, G. Gavellas,* K. H. Hwang,* S. Sabnis,* and T. Antonovych. University of Miami, Miami, FL, and Armed Forces Institute of Pathology, Washington, DC.

To assess progression of renal disease and the effects of protein intake in a species phylogenically close to man, 10 young adult baboons (Papio hamadryas) were subjected to 20-30% infarction of the left kidney and, 2 mos later, to right nephrectomy. They were then randomized to a synthetic diet containing either 8% or 25% protein. Hemodynamic and metabolic measurements were obtained in awake animals every 4 mos. Baseline mean blood pressure, inulin clearance, protein and urea nitrogen excretion in intact animals on 15% protein averaged 75.5±3.5 (SE) mmHg, 42.9±2.7 ml/min, 52±4.3 mg/24h, and 3.8± 0.4 g/24h, respectively. At 12 mos, values in the same baboons with a remnant kidney on 8% vs 25% protein averaged 100.6 vs 96.7 mmHg, 29.2 vs 54.9 ml/min (p<0.01), 111 vs 108 mg/24h, 3.4 vs 11.0 g/24h (p<0.001), respectively. Electron microscopic examination of renal biopsies obtained 8 mos after nephrectomy was normal but for slightly increased mesangial matrix in 2 animals of each group. Thus, blood pressure increased (p<0.01), proteinuria doubled (p<0.01) and adaptations in GFR developed within 4 mos of renal mass reduction, without significant changes occurring between 4 and 12 mos. The adaptations in GFR were markedly attenuated by low protein intake. Further follow-up is necessary to assess progression of renal disease and the impact of different protein diets.

OUTCOME OF GLOMERULAR INJURY IN LUPUS NEPHRITIS. A. Chagnac,* R.K. Sibley, S. Strober,* and B.D. Myers. Depts. Med. & Path. Stanford Univ., CA.

Treatment with lymphoid irradiation (TLI) and prednisone abolished clinical and serologic activity of SLE for 3 yrs in 10 nephrotic patients with proliferative lupus nephritis (PLN). Physiologic and morphometric techniques were used serially, before and again 12 and 36 mo after TLI. Judged by a progressive reduction in fractional clearance of albumin, IgG and uncharged dextrans of r_s 48-60Å, there was sustained improvement of barrier size-selectivity. Other findings from time 0 to 12 and 36 mo (a=p<0.05 vs 0 mo, b=p<0.05 36 vs 12 mo) included a transient improvement only in GFR, 42±6 to 54±7[a] and 45±9 ml/min[b]; corresponding computed K_f was: 2.3±0.4 to 4.9±1.0[a] and 2.9±0.6 ml/(min·mmHg)[b]. Regardless of a negative (N=6) or positive (N=4) GFR slope vs time, serial biopses revealed glomeruli (G) to exhibit a trend to bimodality of G tuft area (GA). The % of G with GA<6000μ^2 increased from 23 to 33[a] and 51%[a,b], reflecting progressive global sclerosis. Among patent, remnant G, the % hypertrophied with GA>27000μ^2 increased from 0 to 1.7 and 10.7%[a,b]. Remnant G cell density per 1000μ^2 GA (5.1±0.4 to 4.1±0.3 and 3.1±0.4[a]) and electron dense deposit density as a percent of GBM area (10.2±1.6 to 6.2±2.0[a] and 3.2±1.2%[a]) declined progressively. We conclude that despite subsiding immune G inflammation and reduced transmural protein traffic, treated PLN is complicated by progressive glomerulosclerosis. The prevailing GFR depends on the balance between non-filtering, (sclerotic) and hyperfiltering, (remnant) glomeruli.

LONG TERM EFFECTS OF GALACTOSE (G) ON RENAL HEMODYNAMICS AND HISTOLOGY. M.Coco, H.Aynedjian,* B.Mutz,* L.Sablay,* N.Bank. Dept.of Medicine & Pathology, Montefiore Med.Ctr., Bronx, NY.

In order to study the long term effects of high G feeding on renal function and pathology, we fed normal SD rats 50% G diet for 8 months. A control group received the same diet plus Sorbinil (G+S), an aldose reductase (AR) inhibitor of the polyol pathway. Additional controls consisted of rats fed a normal (N) diet and N+S diet. Each rat consumed 20g food/day. Two days prior to micropuncture studies, 24h urine was collected for albumin (alb) excretion. Following micropuncture, kidneys were perfusion-fixed in situ at a pressure equal to the blood pressure. Kidneys were examined under light microscopy (LM) and EM. Results follow:

Diet	SNGFR nl/min	PG mmHg	QA nl/min	Kf nl/m/mmHg	Alb mg/24h
G	34.3*	46.5	106*	3.5*	29.6*
G+S	25.4+	49.5	71+	1.8+	12.2
N	27.6	47.7	89	2.5	11.3
N+S	31.8	46.8	96	2.9	12.2

*p<.05 vs N +p <.05 vs G

Results show that G rats had increased QA, SNGFR, Kf and urinary alb excretion as compared with both N and G+S. On LM there was increased mesangial expansion in G and G+S groups. On EM, G demonstrated increased mesangial matrix. G+S had a similar response. These findings indicate that long term G feeding results in renal hyperperfusion, hyperfiltration and albuminuria. Sorbinil corrected the hyperfiltration and albuminuria but had no effect on glomerular pathology.

HIGH PROTEIN FEEDING STIMULATES RENAL THROMBOXANE PRODUCTION IN THE DIABETIC RAT. DM Collins*, TM Coffman, P Ruiz*, P. Klotman. In diabetic rats, the development of glomerulosclerosis is accelerated by high protein feeding. In other models of renal disease, protein induced renal injury is associated with stimulated renal production of the vasoconstrictor eicosanoid thromboxane (Tx). We hypothesized that high protein feeding may worsen glomerulosclerosis in diabetes by increasing renal thromboxane production. We pair fed rats with streptozotocin induced diabetes diets containing 60% or 8% casein for two months. Inulin clearance and renal blood flow (RBF) were then measured, and the right kidney examined by light microscopy. The left kidney was perfused in situ with a cell free Kreb's Henseleit solution. Thromboxane, 6ketoPGF1a, and PGE2 were measured in the venous effluent by RIA.

Dietary Protein (%)	60%	8%	p value
GFR (ml/min/kg)	11.79±0.81	9.28±0.93	<0.025
RBF (ml/min/kg)	75.02±7.15	51.47±5.88	<0.025
Tx (pg/min)	551±126	390±87	<0.05

Inulin clearance and RBF were higher in rats fed 60% protein. Renal thromboxane production was significantly greater in rats fed the high protein diet; production of 6ketoPGF1a and PGE2 was not different between dietary groups. Hypercellularity occured more frequently in high protein (HP) than low protein (LP) fed rats (6 of 10 HP vs 3 of 9 LP), as did tubular hypertrophy (10 of 10 HP vs 4 of 9 LP), and arteriolar thickening (4 of 10 HP vs 1 of 9 LP). We conclude that protein feeding stimulates renal thromboxane production and exacerbates renal morphologic injury in the diabetic rat. These effects occur despite the presence of relative hyperfiltration in high protein fed animals. Our findings suggest that thromboxane may play a role in the pathologic response to protein feeding in the diabetic model through a mechanism unrelated to its vasoconstrictive properties.

GLOMERULAR HYPERFILTRATION OCCURS IN STREPTOZOTOCIN-DIABETIC (STZ-D) BUT NOT IN SPONTANEOUSLY DIABETIC RATS. Andrew J Cohen, Ronald G Rossetti,* and Jeffrey S Stoff, Univ of Massachusetts Medical Center, Worcester, MA.

Studies of the spontaneously diabetic (BB/W-D) rat have revealed glomerular basement membrane thickening without the mesangial expansion observed in STZ-D. To examine whether these histologic differences are echoed in hemodynamic dissimilarities we performed ^{3}H-PAH and ^{14}C-inulin clearances in chronically catheterized, unanesthetized rats for determination of renal plasma flow (RPF, ml/min/100 g) and GFR (ml/min/100 g), respectively. Three groups of diabetic (D) and nondiabetic (N) animals were studied: STZ-D in Wistar rats were compared with vehicle-injected control (STZ-N), BB/W-D were compared with diabetes-resistant (BB/W-N), and STZ-diabetes was induced in diabetes-resistant BB/W rats (STZ-BB-D) and compared with vehicle-injected diabetes resistant (STZ-BB-N) rats. All animals were of equivalent age and duration of diabetes. All diabetic animals received protamine-zinc insulin to maintain a blood sugar of 250-450 mg/dl. (n) = 6 animals in each diabetic and non-diabetic group.

	RPF			GFR		
	STZ	BB/W	STZ-BB	STZ	BB/W	STZ-BB
D	3.95±.69	3.06±.27	2.97±.54	1.18±.18	1.10±.17	1.17±.15
N	2.60±.08	2.30±.14	1.51±.13	0.76±.03	0.93±.09	0.56±.08
p	<.01	<.05	<.02	<.02	NS	<.01

All diabetic groups displayed increased RPF. However, while STZ-induced diabetes caused glomerular hyperfiltration, this was not observed in the BB/W-D. Hence, the morphologic differences between the STZ-D and the BB-D are reflected in the presence or absence of glomerular hyperfiltration, respectively. Increased GFR in STZ-BB-D but not in BB/W-D suggests STZ may induce hyperfiltration.

DEFECTIVE CHYLOMICRON CLEARANCE RESULTS FROM PROTEINURIA AND NOT HYPOALBUMINEMIA IN NEPHROSIS R.W.Davies*, I.Staprans*, G.A. Kaysen Martinez and San Francisco VAMC., U.C. Davis, UCSF. Martinez and San Francisco CA.

Hyperlipemia of nephrosis results from both increased synthesis and decreased removal of blood lipids. It was unknown whether these derangements resulted from reduced serum oncotic pressure (π), hypoalbuminemia, or from proteinuria. We have reported that hyperlipemia correlates with altered glomerular permeability, paralleling the urinary loss of albumin and α_1 acid glycoprotein (αAG). Serum triglycerides (TG - mg/dl), π (mm HG), αAG (mg/ml), renal clearance of αAG (αAG_{Cl}- mcl/min) and serum half life of chylomicrons (CM $T^{1/2}$ - min^{-1}) were measured in normal Sprague Dawley rats (SD-C) and rats with hereditary analbuminemia - HA (HA-C) and in HA with passive Heymann Nephritis (HA-N)

Group	TG	CM $T^{1/2}$	π	αAG	αAG_{Cl}
SD-C	133±21	9.1±.6	22.6±2	3.3±.1	0
HA-C	264±56*	8.4±.7	15.3±2*	2.7±.3	0
HA-N	1012±229*	46.5±24*	9.7±2*	0.7±.1*	36±12*

* P < 0.05 compared to SD-C. Although TG was greater in HA-C than in SD-C, CM $T^{1/2}$ was the same in both groups, despite reduced π, and analbuminemia in HA-C. CM $T^{1/2}$ and TG both increased in HA-N. CM $T^{1/2}$ correlated with αAG_{Cl} ($P<0.05$). Decreased π, and analbuminemia may cause increased TG without urinary protein loss, but not increased CM $T^{1/2}$. Altered glomerular permeability, even in the absence of albumin synthesis, causes an increase both in CM $T^{1/2}$ and in TG, suggesting that the urinary loss of a liporegulatory substance, and not hypoalbuminemia, causes reduced chylomicron clearance in nephrosis.

AGGRAVATING EFFECTS OF HYPERLIPIDEMIA ON MACROPHAGE FUNCTION IN NEPHROSIS. Jonathan R. Diamond and Morris J. Karnovsky, Department of Pathology, Harvard Medical School, Boston, MA

Dietary hypercholesterolemia aggravates puromycin aminonucleoside (PA) nephrosis. Because acute nephrosis is accompanied by both hyperlipidemia and increased glomerular macrophage (GM) number, we sought to explore whether these processes are related. We studied the effects of a 4% cholesterol/1% cholic acid dietary supplement in normal rats (HC); nephrosis (PA) following a single jugular venous bolus injection of PA, 5 mg/100gm BW; and a combination of these (PA/HC). During peak nephrosis (14 days after PA), GM number *in situ*; peritoneal macrophage phagocytic index (PI) and thromboxane (TXB_2) production were evaluated (mean ±SE; significance by ANOVA):

Group(n)	GM#	TXB_2 (pmol/mg cell prot.)	PI(%)
Control(7)	1.6±0.1	7±2	25±7
PA(6)	2.6±0.2*+	6±3++	59±7*+
HC(7)	3.7±0.6*+	9±3+++	72±1*+
PA/HC(6)	6.8±0.6*	19±3*	88±2*

*p<.02 vs. control; +p<.01 vs.PA/HC; ++p<.02 vs. PA/HC; +++p<.05 vs. PA/HC

The HC group had significantly greater fasting total cholesterol (FTC) values only in contrast to the control and PA rats, whereas the PA/HC group had both significantly greater FTC and triglycerides when compared to the other 3 groups.

GM number, TXB_2 production and PI were all significantly increased in the PA/HC group in contrast to the other three. Hyperlipidemia may aggravate PA nephrosis via enhanced monocyte/macrophage function.

HYPERPHOSPHATEMIA AND THE CALCEMIC RESPONSE TO PTH IN VARIOUS STAGES OF RENAL INSUFFICIENCY. R. Dunlay*, M. Rodriguez, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, OK.

A decreased calcemic response is an important factor in the hypocalcemia and secondary hyperparathyroidism of chronic renal failure (CRF). Hyperphosphatemia may be an important factor in the reduced calcemic response. The present study evaluates the calcemic response to PTH in rats with varying degrees of CRF and phosphate (P) intake. Three groups of rats were studied: 1) normal; 2) moderate CRF (MRF); and 3) advanced CRF (ACRF). Rat PTH (1-34).21 ng/hr was infused via Alzet pump for 48 hours. During the infusion, rats were placed on a diet containing P, 1.2% or 0%. Changes in serum (s) Ca and sP during PTH infusion at 24 and 48 hrs were:

Time, 24 hrs	sP (mg/dl)		sCa (mg/dl)	
Diet	P,1.2%	P,0%	P,1.2%	P,0%
N	7.5±.4	5.2±.3	11.0±.3	12.1±.2
MRF	11.0±1.8*	6.2±0.7	10.7±.3	11.2±.3*
ACRF	14.0±2.6*	5.7±.8	10.1±.4*	10.3±.2**
Time, 48 hrs				
N	7.4±.4	5.5±.2	14.4±.7	17.0±.6
MRF	9.5±1.7*	5.6±0.3	11.9±.5*	14.3±1*
ACRF	12.4±2**	5.8±.3	9.7±.6**	12.3±.6**

*P<.05, **P<.01 compared with N.

In the 3 groups, baseline PTH levels were 46±.4, 87±20, and 170±25 pg/ml and were inversely correlated with the calcemic response.

In summary, the calcemic response in CRF: 1) was decreased; 2) was further decreased with hyperphosphatemia; and 3) at normal serum P levels, was still decreased and was related to the degree of CRF.

DEFECTS OF MYO-INOSITOL TRANSPORT IN THE DIABETIC RAT PROXIMAL TUBULE. J. Dominguez, R. Bloom,* and J Rothrock*. Dept. of Med., Indiana University School of Med. and V.A. Med. Ctr., Indianapolis, IN.

In diabetes mellitus (DM) urinary excretion of myo-inositol (MI) is enhanced. DM produces MI depletion in tissues which do not use glucose for their metabolism, such as proximal tubules (PT). We tested the hypothesis that MI depletion in rat PT may occur in DM due to defects of MI influx, which in normal PT is activated by extracellular sodium (Na_o). First, we determined the effect of 25 mM glucose (G) on MI transport in normal PT in 150 mM Na_o. At 30 seconds, in the presence of G, MI influx was 29 ± 21 pmoles/mg protein. When G was replaced by 25 mM mannitol (M), MI influx increased to 51± 18, $p < 0.05$ (mean ± S.D., $n = 9$). At 10 minutes, MI influx in the presence of M was 595 ± 71, which decreased to 320 ± 80 when G was present, $p < 0.01$. The inhibition of MI influx by G was also observed at 1, 2, 3, 5, and 8 min., $p < 0.02$. Similarly, when Na_o was 15 mM, G inhibited MI influx from 0.5 to 10 minutes, $p < 0.05$, $n = 9$. We then examined MI influx in PT from streptozotocin-diabetic rats (DPT) in the presence of 150 and 15 mM Na_o, with M or G in the medium. We found that 150 mM Na_o did not evoke a higher influx than 15 mM Na_o: at 30 seconds, 150 mM Na_o = 112 ± 50 and 15 mM Na_o = 164 ± 52, $p > 0.4$; at 10 minutes, 150 mM Na_o = 1167 ± 603 and 15 mM Na_o =1390 ± 456, $p > 0.9$, $n = 5$. We also determined that G did not inhibit MI influx in DPT at 15 or 150 mM Na_o: In 150 mM Na_o, 30 second MI influx was 112 ± 50 in M and 73 ± 24 in G, $p > 0.4$, and at 10 minutes 1167 ± 603 in M, and 755 ± 265 in G, $p > 0.4$, $n = 5$. We conclude: first, G inhibits MI influx in normal PT but not in DPT; second, in DPT, activation of MI influx by 150 mM Na_o is not detected, which may be caused by true inhibition of MI uptake or reduced net influx (influx − efflux) secondary to enhanced MI efflux. We suggest that G may not be the sole determinant of MI depletion in DPT, as sodium activated uptake is also disrupted.

USE OF P-450 OXIDASE INHIBITOR, ETHANOL, TO DETECT AND MODULATE ENDOGENOUS CARCINOGENIC NITROSO-DIMETHYLAMINE (NDMA) PRODUCTION IN CHRONIC RENAL FAILURE (CRF). S.R. Dunn, P.S. Lele, S. Goyal, M.L. Simenhoff. Deptartment of Medicine, Jefferson Medical College, Phila. PA.

Previously, we have demonstrated raised levels of NDMA in the stomach and duodenum of patients with CRF secondary to increased dimethylamine and intestinal bacterial overgrowth. The mechanism of production in each location is not the same, as differentiated by ascorbate ingestion. The studies utilized the differences, modulating synthesis by ethanol with & without ascorbate administration; ascorbate preferentially binds to nitrite in the acidic stomach preventing nitrosation. Ethanol, a P-450 oxidase inhibitor, prevents 1st pass hepatic metabolism of NDMA absorbed from the bowel in rats, unmasking endogenous G-I synthesis. In the current study, 11 patients were given 25g ethanol load, and blood drawn at varying intervals. NDMA was measured in the blood using gas chromatography and Thermal Energy Analysis. Mean baseline blood NDMA in the 11 patients increased significantly ($p<0.01$) from 192±40 ng/kg to 389±35 at 5 or 10 minutes after ethanol, representing a 203% rise. No change was seen in 3 healthy control subjects. In a subset of 4 patients given 4 grams of ascorbate for 5 days prior to ethanol load, the NDMA rise at 5 or 10 minutes decreased to 55% of the non ascorbate treated group (120±11 to 215±39 ng/kg) suggesting significant inhibition of NDMA production. We conclude that P-450 oxidase inhibition unmasks the intestinal NDMA production in man. The uremic intestine acts as a unique model to test NDMA production in a population with increased cancer incidence.

NIFEDIPINE DECREASES GLOMERULAR INJURY IN RATS WITH REMNANT KIDNEYS BY INHIBITING GLOMERULAR HYPERTROPHY. Lance D. Dworkin, Miriam Parker,* and Helen D. Feiner., Depts. of Med. & Path., New York Univ. Med. Ctr., N.Y., N.Y.

Increased capillary wall tension - due to elevated glomerular pressure (P_{GC}) and/or increased glomerular capillary radius (R_{GC}) - may be an important, shared pathophysiologic mechanism of glomerular injury after ablation of renal mass. If so, then agents which reduce either P_{GC} or R_{GC} ought to prevent damage. We therefore determined whether antihypertensive therapy could prevent increases in R_{GC} and inhibit injury in rats after 1 and 2/3 nephrectomy. Groups were untreated (CON), or given nifedipine (NIF), or enalapril (ENP). Awake systolic pressure (SBP) and protein excretion rate ($U_{prot}V$) were monitored. After 8 weeks, kidneys were perfusion fixed, weighed (KID WT), and examined for glomerular sclerosis (SCLER). Glomerular volume (G_V) and R_{GC} were determined morphometrically. SBP was elevated in CON (227±8 mmHg, P<0.05) but not in NIF (128±2) or ENP (128±5) (Mean±S.E.). Although body weight did not differ among groups, KID WT was lower in NIF (1.2±0.1 g, P<0.05)) than in CON (1.7±0.1) or ENP (1.7±0.1).

GROUP	N	$U_{prot}V$ mg/24h	SCLER %	G_V $\mu m^3 \times 10^6$	R_{GC} μm
CON	8	73±9	18±5	2.0±0.1	3.8±0.1
NIF	5	19±4*	3±1†	1.2±0.1*§	3.1±0.1*§
ENP	6	24±3*	9±5	2.0±0.2	3.7±0.1

*P<0.05 vs CON; †P<0.06 vs CON; §P<0.05 vs ENP

While nifedipine and enalapril reduce SBP and lessen glomerular injury in rats with remnant kidneys, nifedipine alone inhibits the hypertrophic response in remnant glomeruli. Thus, due to the markedly lower value for R_{GC}, nifedipine causes a structurally based decline in vessel tension of similar magnitude to that produced by agents which prevent glomerular injury by reducing P_{GC}.

CORRECTION OF GLUCOSE INTOLERANCE (GINT) AND THE IMPAIRED INSULIN RELEASE OF CHRONIC RENAL FAILURE (CRF) BY VERAPAMIL. George Z. Fadda*, Mohammad Akmal, Amin R. Soliman*, Loren G. Lipson* and Shaul G. Massry, Div. Nephrol and Geriatric Med., Univ. So. Calif., Los Angeles, CA.

GINT in CRF is due to resistance to insulin action and to impaired insulin release from pancreas. The latter is due to secondary hyperparathyroidism of CRF and is caused by PTH-induced calcium accumulation in pancreas. Thus, a calcium channel blocker may antagonize the effect of PTH, and normalize GINT in CRF. We examined this postulate by studying intra venous glucose tolerance and insulin release from pancreatic islets in normal and CRF rats and in CRF rats treated with verapamil (VER). Rats with 42 days of CRF displayed impaired GINT, significant reduction in insulin release by islets (p<0.01), and doubling of calcium content of pancreas (p<0.01) as compared to normal rats. Simultaneous treatment of CRF rats with VER for 42 days reversed these abnormalities. Verapamil treatment of normal rats did not affect any of parameters studied. The results show that VER by preventing calcium accumulation in the pancreas reversed the abnormalities in insulin release that occur in CRF. The latter effect resulted in higher blood levels of insulin during glucose infusion which overcome the peripheral resistance to insulin and corrected GINT of CRF.

EFFECT OF DIETARY PROTEIN INTAKE (DPI) ON COMPENSATORY RENAL GROWTH (CRG) IN DWARF RATS. A M El Nahas, J E Le Carpentier. Clinical Sciences Research Centre, Northern General Hospital, Sheffield, UK.

The precise contribution of growth hormone (GH) to the development of CRG and in the mediation of the trophic effects of a high protein diet (HPD) on the kidney remain unknown. We have studied the effects of dietary protein manipulation on CRG in a new mutant strain of dwarf rat known to be selectively deficient in GH. Ten such rats were fed a medium protein diet (MPD - 18% casein) and another 10 a HPD (78%). One month later, 6 rats in each group underwent a right uni-nephrectomy (UNx) and 4 a sham operation. A week later the left kidney was removed. Comparable groups of adult male Wistar rats were studied. At the time of UNx and sacrifice, kidneys were weighed and the DNA and protein content measured. CRG was estimated by comparing right and left kidney weight (KW). In sham operated Wistar as well as dwarf rats, kidney weight increased on HPD; Wistar - MPD: 0.84±0.07gm (M±SD), HPD: 1.07±0.10gm, p<0.005, dwarf - MPD: 0.58±0.03gm, HPD: 0.79±0.03gm, p<0.001. CRG (Δ%) was comparable in Wistar (18±3%) and dwarf rats (14±5%) rats. However, the stimulatory effect of HPD on CRG was only observed in Wistar rats (27±4%). In dwarf rats, CRG was identical on M and HPD (15±2%). From these data we postulate that GH is not required for the expression of kidney growth on HPD or after UNx in rats. However, it might contribute to the HPD enhancement of CRG.

EFFECTS OF A PHOSPHATE BINDER IN UREMIC RATS. D.A. Feinfeld, J. Russell,* L. Sherwood,* and T. Shimamura. Dept. of Med., Albert Einstein Coll. of Med., Bronx, NY, and Dept. of Path., UMDNJ-Robert Wood Johnson Med. School, Piscataway, NJ.

To examine the role of phosphate (P) in the pathophysiology of chronic renal failure, we studied the effect of dietary P binding in uremic rats. A week prior to 7/8 nephrectomy, one group (AL) was given $Al(OH)_2$-aminoacetate with food. A second nephrectomized group (No AL) was pair-fed the same diet (23.4% protein, 0.86% P) without the P binder. Plasma chemistries and renal function were measured after one week of uremia. Results:

		AL	No AL
Inulin clearance	(ml/min)	0.36±0.05	0.43±0.06
PAH clearance	(ml/min)	0.79±0.10	1.03±0.14
Creat. clearance	(ml/min)	0.40±0.05	0.40±0.06
Plasma creatinine	(mg/dl)	1.1±0.1	1.5±0.1[b]
urea nitrogen		67±6	77±4
calcium		12.9±0.6	8.7±0.7[b]
phosphate		4.2±0.4	8.1±0.3[c]
Urine creatinine	(mg/day)	6.3±0.5	8.8±1.0[a]
protein		80±8	109±8[a]

(a: p<.05; b: p<.01; c: p<.001 between groups)

Despite similar inulin and creatinine clearances in both groups, mean plasma creatinine was 24% lower in the AL rats compared to the No AL rats. There was also less proteinuria in the AL group.

We conclude that P binding in uremic rats is associated with decreased proteinuria. The significantly lower 24-hour urine creatinine in AL rats suggests that their lower plasma creatinine was due to decreased creatinine production. Decreased P, increased calcium, or differences in parathyroid hormone levels may alter muscle metabolism in uremia.

ARTERIAL RESPONSES TO NOREPINEPHRINE (NE) IN ACIDOSIS AND UREMIA: COUPLING OF INOSITOL PHOSPHATE (IP) AND CONTRACTION. A.W. Fox*, R. C. May, and W.E. Mitch, Renal Div., Emory U. Atlanta, GA.

Abnormal blood pressure regulation in chronic uremia (CRF) and metabolic acidosis has been linked to defects in a-adrenergic function. In cultured vascular cells, transduction of NE to contraction involves IP turnover. To determine if changes in IP metabolism are involved in abnormal NE responses, IP accumulation and contractile responses were measured in the same arterial segments from non-acidotic CRF (SUN 105 vs 20 mg/dl control; pH 7.35 vs 7.41 control) rats or acidotic rats with normal kidneys (blood pH 7.1 vs 7.4, control) and compared to those from pair-fed, control rats. To check for specificity of the results, responses to vasopressin (AVP) were also measured. In control arteries, NE and AVP produced similar maximal responses of [3H]-IP accumulation and contraction. However, both acidosis and CRF caused a similar (2- to 3-fold) desensitization of IP accumulation and contraction to NE without altering maximal responses of either. In contrast, IP and contractile responses to AVP were unaffected indicating that desensitization to NE was not simply a reflection of impaired metabolism. Studies with specific agonists and antagonists revealed that the NE responses involved alpha-1 receptors. We conclude that both uremia and metabolic acidosis cause abnormal receptor activation in arteries. This yields parallel shifts in the IP accumulation and contraction response curves to Ne and emphasizes the intimate relationship between IP production and contraction in arteries.

EFFECTS OF HYPERGLYCEMIA ON PROLIFERATION IN RENAL PROXIMAL TUBULAR CELLS IN CULTURE — ROLE OF ALTERED CELLULAR MYO-INOSITOL (MI) METABOLISM. S. Goldfarb, F. Ziyadeh, and T. Haverty, Renal Electrolyte Section, U. of Penna., Philadelphia, PA

Moderate hyperglycemia results in renal enlargement, predominantly involving proximal tubular structures. To study this phenomenon, we examined the proliferation and hypertrophy of cells derived from mouse proximal tubules (MCT) maintained in continuous cell culture.

Cells were synchronized in serum free media (100 mg/dl glucose, G-100) and proliferation was assayed by ^{3}H-thymidine incorporation at 24, 48 and 72 hours after incubation in G-100 or 450 mg/dl glucose (G-450). G-450 produced a progressive reduction in ^{3}H-thymidine incorporation which was 130% below baseline at 72h. This was confirmed by cell counts of confluent cultures. Cellular hypertrophy (mg protein per cell) was 8% higher in cells in G-450 than G-100 medium. Insulin, 2 μg/ml produced a 150% increase in proliferation of G-450 cells but only produced a 110% increase in G-100 cells. High ambient glucose reduces MCT cell proliferation, induces cellular hypertrophy and increases cell proliferative response to insulin, a prototypic growth factor.

To test for altered cellular MI metabolism induced by high G we incubated cells in G-450 and 800 μM MI. MI incubation normalized the ^{3}H-thymidine uptake to values seen with G-100 cells. Altered MI metabolism, probably resulting from polyol pathway activation underlies the abnormalities in cell proliferation and hypertrophy produced by G-450 medium. Increased cell hypertrophy and proliferative response to growth factors like insulin could contribute to diabetic nephromegaly.

GLOMERULAR HYPERTENSION, RATHER THAN HIGH CHOLESTEROL (Ch) LEVELS, PREDOMINATE IN THE GENESIS OF GLOMERULAR SCLEROSIS (GS) IN ANALBUMINEMIC (ANALB) RATS WITH RENAL ABLATION. CK Fujihara*, EBA Prado*, MJBA Prado*, MM Santos*, NFT Sanfelice*, RM Padilha*, MC Pires* and R Zatz. Univ. of São Paulo Med. School, São Paulo, Brazil.

Recent studies in our laboratory showed that, despite persistently elevated Ch levels, ANALB rats, unlike Sprague-Dawley (SD) controls, fail to develop aging GS. We then verified whether ANALB rats would develop GS after 5/6 nephrectomy (NX). Three groups of NX rats were used: SD, ANALB and enalapril-treated ANALB+E. After 2 weeks of NX, glomerular hydraulic pressure (Pgc) was 57±3 mmHg in SD, 67±2 in ANALB and 56±1 in ANALB+E, $p<0.05$ ANALB vs. SD or ANALB+E (normal 52 in SD, 45 in ANALB). Moreover, extensive glomerular fibrin deposits were seen in ANALB rats. Unlike SD, ANALB rats failed to lower plasma renin activity below normal. Results after 8 weeks of NX (TCP=tail-cuff pressure, mmHg, GFR in ml/min, Prot = proteinuria, mg/24h, GS expressed as glomerular score, Ch in mg/dl, *=$p<0.05$ vs. SD, +=$p<0.05$ vs. ANALB, n=8 rats/group):

	TCP	GFR	Ch	Prot	GS
SD	145±8	0,9±0,1	60±4	104±22	9±1
ANALB	198±21*	0,5±0,2*	142±11*	158±68	148±24*
ANALB+E	97±4*+	0,9±0,1+	138±8*	23±3*+	26±10+

Renal functional and structural impairment was much more severe after NX in ANALB than in SD rats. Enalapril lowered Pgc and limited GS without altering Ch in ANALB rats. These results suggest that marked glomerular hypertension, rather than high Ch, is key to the development of severe GS in this model. High Ch and intracapillary coagulation, however, may also have contributed to the rapid progression of GS verified in this study.

THE EFFECTS OF DIETARY PROTEIN CONTENT ON GLOMERULAR CAPILLARY WALL TENSION IN REMNANT KIDNEY (RK) MODEL. K Griffin*, A Bidani, M Schwartz, E Lewis. Rush Medical College, Chicago, IL.

We have previously demonstrated that a low protein (LP) diet preserves renal autoregulation thereby protecting glomerular capillaries (GC) against transmission of systemic hypertension in the RK model. According to Laplace law, the tension (T) across the GC wall is a product of transcapillary hydraulic pressures (ΔP) and capillary radii (T=PxR). Autoregulatory (transonic flow probe) and morphometric studies were performed 3-4 weeks after 5/6 ablation in male Sprague-Dawley (b.w. 200-300g) rats fed isocaloric 22% standard protein (SP) or 8% (LP) diets. BP and Scr after ablation were similarly elevated in SP (159±6, 0.8±.08) and LP (147±8, 0.9±.06) rats but 24° protein excretion was significantly greater in SP (22±3 vs 8.5±2, $p<.05$, mean ± SEM). Autoregulation of RBF was impaired in SP but not LP rats.

	Glom Vol($\times10^{-6}$)	GC Vol($\times10^{-3}$)	GC Radii(um)
LP(n=5)	1.43±0.23	302 ± 36	3.2 ± 0.1
SP(n=5)	2.57±0.17*	575 ± 90*	4.1 ± 0.2*

The GC wall tension for a range of ΔP was calculated using mean GC radii of LP and SP rats.

	GC wall tension dynes/cm	
	LP	SP
Δ P 30 mmHg	12.8	16.4
Δ P 45 mmHg	19.2	24.6
Δ P 60 mmHg	25.6	32.8

These data demonstrate that both of the determinants of GC wall tension may be significantly affected by dietary protein content and consequently may determine the eventual extent and severity of glomerular injury.

OSMOTIC FRAGILITY OF RED BLOOD CELLS (RBC) IN HEMODIALYSIS (HD) PATIENTS WITH ERYTHROPOIETIN (rHu EPO) THERAPY

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Free Univ. of Berlin, Dept. of Nephrol.+, Inst. of Biochem.++, Boehringer, Mannheim Comp.+++

The aim of our study was to find out whether or not the induction of erythropoiesis by rHu EPO application affects function and age dependent distribution of RBC's in HD patients (n=14). RBC's were separated by density gradient centrifugation (Percoll) into different groups of cell age, aspartataminotransferase (GOT) activities were measured as a RBC age reflecting enzyme, and the median osmotic fragility (MOF) of younger (YF) and elder RBC fractions were estimated. rHu EPO corrects the anemia in all treated patients: Hb 61.6± 14.8 to 94.4± 18.9 g/l; p<0.01. After 2 weeks of rHu EPO therapy the YF of RBC's increased (38.2± 9.6 to 68.5± 12.4%; p<0.05) and reached a steady state after 12 weeks of therapy (53.1± 6.8%; p<0.20). GOT activities of YF of RBC's increased from 6.44 to 8.18 after 2 weeks resp. 7.63 U/g Hb after 12 weeks of rHu EPO therapy. The MOF of RBC's, which is lowered in HD patients compared with healthy persons, decreased further correspondingly to the increase of GOT activities indicating a better osmotic resistance of RBC's after rHu EPO therapy in HD patients.

RETINOIC ACID STIMULATES COLLAGEN BIOSYNTHESIS BY CULTURED RAT KIDNEY MESANGIAL CELLS.

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The increased synthesis and excess deposition of extracellular matrix components are hallmarks of glomerular sclerosis. Presumably these components arise, at least in part, from altered matrix production by mesangial cells. Yet, to date, little information is available regarding the factors which influence matrix biosynthesis by mesangial cells. Plasma levels of Vitamin A are elevated in chronic renal disease, and evidence indicates that this molecule can alter matrix production by cells in other tissues. Therefore, studies were undertaken to evaluate the effects of retinoic acid (the acid form of Vitamin A) on collagen production by cultured rat kidney mesangial (RKM) cells. When RKM cells were grown in medium containing retinoic acid (1 μM), neither the growth nor protein synthesis rates were significantly affected. Analysis of the collagens synthesized by RKM cells grown in the absence or presence of retinoic acid indicated that in both cases >90% of the total collagen synthesized was type I. The remaining collagen types produced were types III, IV and V in both control and treated cultures. However, growth of RKM cells in the presence of retinoic acid resulted in a greater than 3-fold increase in the synthesis of each genetic type of collagen chain - α1(I), α2(I), α1(III), α1(IV), α1(V) and α2(V). This enhanced collagen production did not reflect changes in either the distribution (secreted vs. cell-associated) or in the ratio of type I homotrimer to heterotrimer collagen. Thus, because retinoic acid *in vitro* enhances collagen synthesis by mesangial cells, these findings suggest that it may have a role in abnormal extracellular matrix production *in vivo*.

FEEDBACK MEDIATED REDUCTION OF HYPERFILTRATION DURING ACETAZOLAMIDE (ACZ) INFUSION IN DIABETIC PATIENTS (D).

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Hyperfiltration in D is associated with increased proximal reabsorption rate and decreased distal delivery of sodium which could desactivate tubuloglomerular feedback (TGF) To test this hypothesis renal function was assessed in 15 recent onset D, before (C) and after ACZ infusion (5mg/kgBW). In 9 additional D a vasodilatory effect on TGF response was investigated before and after ACZ+ IV enalaprilat (EA) 1.25mg. The following parameters were assessed: glomerular filtration rate (GFR) as inulin clearance; renal vascular resistance (RVR) as MAP.(1-Ht)÷Cpah where MAP is mean arterial pressure, Ht hematocrit, and Cpah PAH clearance; fractional distal delivery (FDD) as CLi/GFR where CLi is lithium clearance. Results were:

	GFR (ml/min)	RVR (dyn.sec/cm5)	FDD
C (n=15)	138±24	6106±1609	0.23±0.05
ACZ	110±17***	6557±1836**	0.35±0.07***
C (n=9)	131±22	6662±2050	0.22±0.03
ACZ+EA	120	5468±1747*	0.32±0.05***

*p<.05;**p<.01;***p<.001 ACZ or ACZ+EA vs C

Decrease in GFR during ACZ infusion was strongly correlated with initial GFR (r= .72,p<.002), and with increase in FDD (r=.77,p<.001). We concluded: 1) ACZ decreases GFR in hyperfiltering D through increase in FDD and in RVR. 2) EA could offset ACZ-induced changes in RVR and in GFR suggesting a modulatory effect of the renin-angio-tensin system upon TGF response mechanisms.

LOWERING CHOLESTEROL AMELIORATES RENAL DISEASE IN EXPERIMENTAL NEPHROTIC SYNDROME.

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To examine the role of hypercholesterolemia on the progression of renal disease, the nephrotic syndrome was induced in uninephrectomized Sprague-Dawley rats using repeated injections of Puromycin and Protamine sulfate. Two groups of rats were studied. GpI (n=8) received Lovastatin (4mg/kgBW s.c.), GpII (n=8) vehicle daily. Blood and urine collections were made at days 23 and 60. Clearance studies and renal histology were obtained in 4 animals of each group at day 60. Lovastatin treated rats had significantly lower cholesterol at day 23 and 60 than vehicle treated rats (207.5±17.5 vs 300.4±32.4 and 131.9±17.5 vs 242.8±54.9 mg/dl p<0.05). The proteinuria and hypoalbuminemia were unaffected. At day 60 the Lovastatin treated rats had a lower BUN (66.5±18.7 vs 180.8±35.5 mg/dl p<0.05) and greater Cin (1.92±0.49 vs 0.2±0.06 ml/min/kgBW p<0.02) than the vehicle treated rats. Blood pressure was similar in both groups. The percentage of severely affected glomeruli was significantly greater in the untreated group (92.8±3.8 vs 55.5±8.4 % p<0.01). In addition there was a correlation between the cholesterol level at day 23 and the BUN at day 60. Thus reducing the hypercholesterolemia of chronic PAN nephrosis with Lovastatin lessens the renal impairment and glomerulosclerosis that occur in this model independent of changes in proteinuria or blood pressure. This suggests hypercholesterolemia plays an important role in the pathophysiology of the progression of renal disease in this model possibly through increased lipoprotein traffic in the mesangium.

REGULATION OF SODIUM/POTASSIUM ADENOSINE TRIPHOSPHATASE, PROTEIN KINASE C (PKC), AND PROSTAGLANDIN (PG) PRODUCTION IN DIABETIC RAT GLOMERULI.
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Prior studies of peripheral nerve have suggested that diminished Na+ K+ ATPase (NKA) activity secondary to reduced PKC mediates diabetic injury. The purpose of this study was to examine the activity of NKA, PKC, AND PG production in diabetic rat glomeruli (GLO).

Diabetes was induced by the iv tail vein injection of streptozotocin 50 mg/kg. A sensitive fluorescence assay was developed that allowed the accurate measurement of as little as 1 nmol of ADP generated by NKA. PKC was studied after GLO were homogenized and centrifuged at 100,000g for 1 hr at 4 °C. PKC activity was studied using a histone assay. PGE_2 and 6-keto-$PGF_{1\alpha}$ were determined by RIA.

Twenty-eight days following the induction of diabetes, NKA activity averaged 330 ± 29 nmol/mg/min in GLO and 235 nmol/mg/min in vehicle injected (VI) animals (n=5, p <.02). Cytosolic PKC activity averaged 28.7 ± 5.1 pmol/mg/min in VI animals at day 9 and was not diminished in diabetic GLO. Activity in VI GLO membranes was undetectable; activity in diabetic membranes averaged 13.2 ± 1.8 pmol/mg/min (n=5, p <.01). One uM phorbol 12-myristate 13-acetate, a stimulator of PKC, caused an increase in PGE_2 and 6-keto-$PGF_{1\alpha}$ in both VI and diabetic GLO.

These data suggest that diminished Na+ K+ ATPase activity is an unlikely mediator of glomerular injury in diabetes and that PKC may play a role in the increased GFR.

PERINATAL DIETARY SALT AND PROTEIN MODULATE THE HYPERTENSIVE AND PROTEINURIC RESPONSES TO RENAL ABLATION OF THE MATURE OFFSPRING.
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Populations assumed to have high salt-low protein diets show high incidence of strokes and/or renal failure. Using the renal ablation model, we wanted to define the role of "intrinsic" renal changes induced by maternal dietary protein and salt on the offspring variability of BP and renal susceptibility to injury (proteinuria). Lewis Wistar inbred females (7-9/group) received one of 4 salt-protein diets: I) L-L, low salt (0.3% NaCl), low protein (12%); II) H-H, high salt (4%), high protein (12%), III) L-H, low salt-high protein, and IV) H-L, high salt-low protein. Diets were given for 4 weeks prior to pregnancy, and during pregnancy and lactation. Pups had normal chow after 19 days. When 8 weeks old, half of the pups had 1 1/2 nephrectomies (Nx), the others had sham operations. At 8 weeks post Nx we measured protein excretion (PROT) in all pups (12-14 pups/group) and a subset of 5-6 pups/group had BP measurements every 4 h for 24 h. A 3-way ANOVA of the 24 h average BP values showed a dominant effect of Nx, F=90.76 ($p<10^{-9}$). A 3-way interaction (F=10, p<.01) showed that the salt effect upon BP, was dependent on Nx and on the protein level of the maternal diet; H-H decreased (137 ± 2) while H-L increased BP (157 ± 3). Prot, mg/24 h, was higher in H-H (65 ± 8) than all other groups. Sham pups had normal BP and Prot. Thus, Nx unmasked an "intrinsic" renal defect induced by H-L that resulted in more severe hypertension and proteinuria in the mature offspring fed normal diet. These studies suggest that the variability in renal disease severity could be modulated by maternal nutrition.

JUXTAMEDULLARY GLOMERULI OF YOUNG KIDNEYS ARE MOST SUSCEPTIBLE TO SEVERE FOCAL GLOMERULAR SCLEROSIS (FGS) IN THE RAT.
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It has been puzzling investigators for some time that whereas FGS appears most frequently in the juxtamedullary region (JM) in human, studies in rat models of FGS have thus far failed to demonstrate this pattern. The internephron heterogeneity of glomerular size, JM glomeruli larger than superficial region (SF), characteristic of human kidneys, is present only in young rats. We examined the severity of FGS of JM and SF glomeruli in remnant kidneys of young (Y, ~30 day) and adult (A, >90 day) Munich-Wistar rats 6 weeks after 1 + 2/3 nephrectomy (NPX). The planar area (PA, in 10-3mm2) and sclerosis index (SI, 0-4 scale) were determined on thin-section specimen in 60-80 consecutive glomeruli in JM and SF areas in Y and A rats with (n=6 and 6) and without NPX (n=5 and 4). Results (mean; § P<0.05 vs JM of the same age group):

	ADULT				YOUNG			
	JM		SF		JM		SF	
	PA	SI	PA	SI	PA	SI	PA	SI
non-NPX	7.0	0.02	6.8	0.00	5.2	0.00	4.5§	0.00
NPX	11.2	0.26	10.0§	0.26	10.9	0.75	9.6§	0.67

Thus, in normal non-NPX Y, but not A, PA of JM was significantly greater than that of SF. This pattern was duplicated in remnant kidneys after NPX, while proportionally greater hypertrophy was achieved in both JM and SF of Y than those of A. For both JM and SF, SI was significantly greater in Y than in A. Although not shown, the standard deviation of SI among glomeruli within a given kidney was ~2-fold greater in Y than A, indicating that the sclerosis of Y is more focal than that of A. Within a given kidney, JM was characterized by having greater number of severely sclerotic glomeruli than SF in Y: Thus, in Y, a significantly greater percentage of glomeruli had SI ≥3 in JM (14.3±4.1 %) than in SF (8.5±2.5 %) whereas, in A, similar percentages of JM (3.2±1.2 %) and SF (3.7±2.8 %) glomeruli had SI of ≥3. 1) Thus, in the rat, the glomerular sclerosis is more severe and focal in rapidly growing young kidneys than in those of adults. 2) In young kidneys, which have an internephron heterogeneity of glomerular size similar to human, JM glomeruli were shown to be more susceptible to FGS than those of SF.

EFFECTS OF REDUCED RENAL MASS ON TISSUE LIPIDS AND RENAL INJURY IN HYPERLIPIDEMIC ZUCKER RATS.
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Evidence from experimental models of chronic renal failure suggests that lipid abnormalities may contribute to progressive renal injury. Hyperlipemic obese and normolipemic lean Zucker rats were subjected to unilateral nephrectomy (Nx) or sham (S) surgery at 8 weeks of age. After 32 weeks, the extent of focal glomerulosclerosis (FGS) and tubulointerstitial injury (TI) was assessed (mean ± SD, two-way ANOVA):

	Obese		Lean		P-Value	
	Nx	S	Nx	S	Nx	Obesity
Body Wt. (g)	523 ±43	529 ±73	323 ±39	313 ±46	N.S.	<.01
FGS (%)	13.4 ±14.3	6.7 ±7.3	4.1 ±0.2	0.1 ±0.2	<.01	<.01

TI was closely correlated to FGS (r=.95, p<.01). Major lipid classes and fatty acids (FA) were also measured in renal cortical tissue. The degree of cholesterol esterification (cholesteryl esters/cholesterol) was positively correlated with FGS (r=.51, p<.05) and TI (r=.56, p<.05). Among phospholipid FA, the ratio of oleic to linoleic acids was strongly linked to both FGS (r=.83, p<.01) and TI (r=.80, p<.01), suggesting a possible role for a relative essential FA deficiency in renal injury. These changes, analogous to those found in atherosclerosis, suggest that specific abnormalities in lipid metabolism may be important in chronic, progressive renal injury.

HIGH PROTEIN DIETS STIMULATE ALBUMIN SYNTHESIS IN NEPHROTIC RATS AT THE SITE OF ALBUMIN mRNA TRANSCRIPTION G.A. Kaysen, H. Jones Jr.*, F.N Hutchison, Department of Medicine Martinez V.A.M.C., U.C. Davis, Martinez CA.

High dietary protein intake (DPI) directly stimulates albumin synthesis (AlbSyn mg/100g/h) and albuminuria (UalbV mg/100g/h) in rats with Heymann nephritis (HN). Increased AlbSyn might be due to increased amino acids available for protein synthesis, or instead to altered albumin gene expression. AlbSyn, hepatic albumin mRNA content (AlbmRNA), and AlbmRNA relative to ß actin mRNA (as an internal control) (Alb/ßAct), were therefore measured in rats with HN fed either 8.5% protein (LP), or after DPI was increased to 40% for 4 days (HP). Enalapril (E) was used to modulate the proteinuric effect of HP.

Group	N	SAlb	UalbV	AlbSyn	Alb/ßAct
HP	6	11.5±1	12±1*	17.7±2*	23±4*
HPE	7	17.8±2*	6.5±1*	15.6±2*	14±2*
LP	8	12.0±1	3.2±.3	9.6±1	8.6±1
LPE	5	12.7±2	3.0±.6	8.2±.5	9.3±1

*P<0.05 compared to LP. High DPI increased AlbSyn, AlbmRNA and Alb/ßAct in HN. All three were increased significantly in HPE compared to either LP or LPE, even though serum albumin (Salb mg/ml) was greater in HPE. Reduction of UalbV with E allowed increased AlbSyn caused by HP to increase Salb. Both AlbmRNA and Alb/ßAct correlated with AlbSyn. (r = 0.531 P<0.05, and 0.553 P<0.01 respectively). The rate of transcription of AlbmRNA relative to that of 28s ribosomal RNA was .071+.007 in LPN vs .201+.025 in HPN P<.01 in isolated hepatic nuclei, using a nuclear run-on assay. HP directly stimulates AlbSyn in nephrosis at the transcriptional level.

COLLECTING DUCT Na:K: PUMP RESPONSE TO OBSTRUCTION. H. Kimura* and S. K. Mujais. Northwestern U and VA Lakeside, Chicago IL.

We tested the hypothesis that disturbed Na:K pump function in the cortical collecting tubule (CCT), the major regulatory site of K secretion, contributes to impaired K secretion after obstructive injury. Na:K pump function was evaluated by measuring ouabain-sensitive ^{86}Rb uptake (pmol/mm/min) in CCT isolated from rat kidneys at various times after ureteral ligation. (#p<0.001 vs Sham; + p<0.001 vs 3 h).

	Sham	3 h	24 h	96 h
N	11	4	9	6
Mean	20.6	13.5#	12.1#	10.5#+
SE	±0.7	±0.4	±0.8	±0.4

Recovery was complete 24 h after release of 3 h obstruction, but partial and delayed after 24 h obstruction. We conclude that a rapid and progressive decline in CCT Na:K pump occurs with obstructive injury and likely contributes to the derangements in CCT function, notably potassium secretion. Early relief of obstruction leads to complete recovery of Na:K pump function, but with prolonged obstruction irreversible damage to the CCT may supervene.

MEDIUM myo-INOSITOL (MI) DEPRIVATION INHIBITS RAT GLOMERULAR NA/K ATPASE ACTIVITY. E. Kern and S. Goldfarb, Renal Sections, Univ of Iowa, Iowa City, IA and Univ of PA, Philadelphia, PA

Increased GFR in diabetic rats is temporally associated with lowered glomerular Na/K ATPase activity: we have previously shown that the high GFR is ameliorated by aldose reductase inhibition or by raising plasma MI levels despite ongoing hyperglycemia (Clin Res 34:725a, 1986). Hyperglycemia impairs normal MI uptake in some tissues by increasing polyol pathway activity. To determine if a component of glomerular Na/K ATPase activity (assessed by ouabain-inhibitable O_2 uptake) is peculiarly dependent on uptake of external MI, pooled glomeruli from 3-4 rats were prepared and washed in chilled buffer lacking MI, then incubated 15 minutes at 37° C in media lacking, or containing 0.5 mM MI. The O_2 uptake of each sample was determined before and after the addition of 1.0 mM ouabain.

nM O2/mg protein/min	-MI	+MI(0.5 mM)	
Total O_2 uptake	2.17 + .12	2.55 + .10	**
Ouab-insens O_2 uptake	1.75 + .10	1.84 + .07	NS
Ouab-sens O_2 uptake	.42 + .09	.71 + .12	*

n=7 *p<.05 **p<.02

Addition of medium MI caused a 68% increase in ouabain-sensitive O_2 uptake in glomeruli prepared in media lacking MI. No effect of medium MI was seen in a separate prep of proximal tubule. We conclude that a large component of glomerular Na pump activity requires external MI for maintenance, and is inhibited when MI uptake is prevented by the lack of medium MI. Loss of this component of Na pump activity may contribute to the altered glomerular function of early diabetes.

GLOMERULAR EPITHELIAL CELLS (EC) AND MESANGIAL CELLS (MC) SEGREGATE IMMUNOGLOBULINS AND FIBRIN (F) IN SEPARATE "COMPARTMENTS" WITHIN HYALINE DROPLETS (HD) - AN IMMUNOGOLD ULTRASTRUCTURAL STUDY. Priscilla Kincaid-Smith, Mitsuru Nakajima* and Douglas Mathews*. Dept of Nephrol., Royal Melbourne Hosp., Melbourne, Vic., Australia.

HD appear in EC and MC in active glomerular lesions in association with F. HD were labelled with immunogold in pre-eclampsia (PET), Anti GBM glomerulonephritis (GN), IgA GN, mesangiocapillary Type 1 GN, focal sclerosing GN and polyarteritis. In MC in PET and in all EC, labelling for F was restricted to one or more electron dense (ED) central areas in HD. In MC in PET, IgA, IgG and IgM labelling was confined to a semi translucent peripheral zone (STPZ). Double labelling confirmed central F and peripheral IgM in the same HD. In 3/3 EC in GN with positive IgM labelling IgM was present only in STPZ. In GN, IgA IgG and C_3, unlike IgM, showed labelling across ED and STPZ areas in EC. In EC in PET albumin IgM IgA IgG and C_3 labelling was present across both ED and STPZ areas. Positive labelling in HDs corresponded to labelling for individual immunoglobulins, F and C_3 in adjacent subendothelial or mesangial deposits in 15/18. In 3 labelling was negative in both HD and glomerular deposits. In 2 labelling in HD was positive but negative in deposit. In one labelling in deposit was positive but negative in HD. This suggests: (1) HD in EC and MC are protein reabsorption droplets of proteins passing through damaged basement membrane from intraglomerular deposits; (2) segregation of F, C_3 and immunoglobulins in different "compartments" of HD suggest different intracellular processing of these proteins.

TUBULAR ALBUMIN (A) BLUNTS THE RESPONSE TO FUROSEMIDE (F) - A MECHANISM FOR DIURETIC RESISTANCE IN NEPHROTIC SYNDROME (N.S.).
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As loop diuretics are highly bound to albumin in serum and the protein bound fraction of a drug is considered inactive, F binding to A within the nephron may be a mechanism for F's attenuated response in N.S. To test this hypothesis, in vivo loop segment microperfusion was performed in non proteinuric rats (n=7/group) with artificial tubule fluid (TF), with TF containing 6×10^{-6} M furosemide (TF + F) and with TF + F to which 3.8µm A (fat free fraction V) was added. NaCl concentration in perfusates was identical. Fractional loop segment chloride reabsorption (FLCl) was 56±2 in TF perfused loops but only 29±2% in TF + F perfused loops (P<.001). Addition of A to TF had no effect on FLCl in the absence of F. In contradistinction, addition of A to TF + F blunted (P<.001) the inhibitory effect of F on FLCl (A+TF+F: 45±1%) but did not abolish it completely (TF vs A + TF + F; P<.001). Absolute loop chloride reabsorption demonstrated a similar pattern. In vivo perfusion rates and collectate fluid to plasma inulin ratios were not different between groups. The percent of F bound to A in the perfusate (determined by HPLC) correlated with the reduction in F response. We conclude: 1) A in tubule fluid blunts F's inhibitory effect on FLCl; 2) as A has no effect on FLCl in the absence of F, the attenuated F response presumably occurs through A to F binding; 3) A to F binding in the tubule may contribute to diuretic resistance in N.S.

cAMP REGULATES CHLORIDE CONDUCTANCE IN HUMAN RED CELLS.
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We have previously reported that red blood cell (RBC) membranes of uremic patients have a significantly greater chloride conductance relative to potassium conductance (GCl/GK) than normals and that this defect is corrected by dialytic therapy. Because cAMP mediated hormones (e.g. PTH, norepinephrine) are increased in uremia, and cAMP can regulate conductive pathways in epithelia, the effect of cAMP on membrane conductance was evaluated in normal human RBC ghosts. Paired, washed RBC's were incubated 30' at 37°C without or with 10uM forskolin or 1mM 8-(4chlorophenylthio)-cAMP (8CPT-cAMP) and 1 mM IBMX (a phosphodiesterase inhibitor). Ghosts were then prepared without additives. [KCl]in and conductances relative to K+ were determined using the potential sensitive fluorescent probe DiS-C3-(5).

	[KCL]in	GCl/GK	GNa/GK	n
Untreated	95.2±3.84	0.17±0.03*	0.57±0.06	7
Forskolin	92.1±2.02	0.33±0.02*	0.64±0.05	
Untreated	92.8±1.88	0.22±0.01*	0.53±0.03	7
8CPT-cAMP	90.5±1.14	0.38±0.02*	0.61±0.03	

* = significant difference from untreated RBC.

These findings indicate that cAMP can regulate relative chloride conductance in human RBC's. Thus, as cAMP levels are increased in uremia, the previously observed increase in GCl/GK in uremic RBC may be due, in part, to increased levels of this messenger. Furthermore, it is suggested that similar cAMP effects in cells in which Cl is at or above electrochemical equilibrium (e.g. muscle,nerve and many epithelia) could depolarize the membrane potential and induce some of the diverse alterations in cell function seen in uremic subjects.

MAGNETIC RESONANCE IMAGING (MRI) OF DIABETIC NEPHROPATHY IN RATS.
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The potential of MRI in monitoring the development of kidney disease in diabetes mellitus (DM) was evaluated. DM was induced in Sprague Dawley rats with 60mg/kg STZ ip and one rat imaged before and 3 and 12 days after injection. Corresponding blood glucose levels were 153, 539 and 554 mg/dl. Multiplanar whole-body MR images were obtained with an eight inch diameter extremity receiver coil and a 0.35 Tesla Diasonics scanner. Moderately weighted T_1 images (TE-50ms, TR-500ms) and T_2 weighted images (TE-50ms, TR-2000ms) indicated that dramatic reproducible changes were detectable. A significant increase in the size of the kidneys occurred by day 3 and was greater by day 12 as measured by the longitudinal axis (20mm to 25mm). Expansion of the vasculature leading to and surrounding the kidney was apparent on day 3 and increased by day 12. A most notable observation was the dramatic loss of the cortico-medullary junction. The following changes in "in vitro" T_1 and T_2 measurements for the dissected kidney zones of cortex (C), outer medulla (OM) and inner medulla (IM) in five other STZ treated rats also occurred.

Relaxation time (mec)

	Control			STZ-treated		
	C	OM	IM	C	OM	IM
T_1	374	499	594	313	395	548
T_2	30.4	35.9	38.1	34.2	28.8	32.2

INFLUENCE OF TRANSFORMING GROWTH FACTOR-β (TGF-β) AND GLOMERULAR ENDOTHELIAL CELL (EN) CONDITIONED MEDIUM (CM) ON MESANGIAL CELL (MC) MATRIX SYNTHESIS.
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The platelet product TGF-β stimulates fibroblast matrix synthesis. Because platelet deposition can accompany glomerular diseases, the effect of TGF-β on MC matrix synthesis was studied. Since EN can influence the MC phenotype, the effect of EN-CM on MC matrix synthesis was also examined.

Total and collagenase-sensitive (Cs) ^{3}H-proline (^{3}H-P) as well as ^{3}H-thymidine (^{3}H-T) incorporation were measured. Values represent the % change from control. Data for rat and bovine MC did not differ and were combined (mean ± SEM, *p <0.05, n = 5-6).

TGF-β (ng/ml)	0.1	0.3	1.0	3.0
^{3}H-T (%)	-29±9	-27±14	-36±12	-53±3*
^{3}H-P (%)	+ 1±5	+ 2±5	+11±3	+38±5*
Cs ^{3}H-P (%)	- 4±5	+ 5±7	+ 7±7	+31±5*

EN-CM enhanced total and Cs ^{3}H-P incorporation in MC by 31 ± 8 and 23 ± 7%, respectively, and increased MC number by 43 ± 7% (p <.05, n=3).

Specific TGF-β receptors on MC were demonstrated by competitive inhibition of ^{125}I-TGF-β binding with unlabeled TGF-β (EC_{50} =19±2 pg/ml, n=3). EN-CM did not compete for these receptors.

Thus, TGF-β inhibits MC DNA synthesis and enhances MC matrix production. Stimulation of matrix synthesis by EN-CM likely reflects an increase in cell number rather than a TGF-β effect since EN-CM did not compete for TGF-β receptors.

INCREASED SINGLE NEPHRON PROTEINURIA IS ASSOCIATED WITH DENUDATION OF EPITHELIAL CELLS FROM THE PERIPHERAL CAPILLARY WALL AFTER REDUCTION OF NEPHRON NUMBER IN NEPHROTIC RATS. PL Miller*, HG Rennke and TW Meyer, Stanford University and VAMC, Palo Alto, CA, and Harvard University, Boston, MA.

Single nephron proteinuria (SNpro) increases markedly when nephron number is reduced in rats with adriamycin nephrosis (Clin Res 34:699A,1986). We sought to relate this increase in SNpro to a change in glomerular structure. Groups of rats with established adriamycin nephrosis and similar baseline proteinuria were subjected to 4/5 renal ablation (ANx) or sham operation (A). Morphometric and micropuncture studies 10 days after operation showed: (mean±SEM, *p<0.05 ANx vs. A)

	UproV	GFR	SNpro	SNGFR	AreaDnd	AreaPCW
	mg/d	ml/min	ng/d	nl/min	$10^3\ \mu^2$	$10^3\ \mu^2$
ANx	671*	0.56*	73*	56*	6.5*	154.1
n=8	±27	±.05	±10	±3	±1.8	±7.1
A	791	1.68	17	37	1.7	171.9
n=9	±44	±.12	±2	±2	±0.5	±9.0

Nephron loss resulted in a four-fold increase in estimated SNpro so that total proteinuria (UproV) was only slightly reduced in ANx rats despite 4/5 reduction in nephron number. Increased SNpro in ANx rats was associated with a proportional increase in the glomerular peripheral capillary wall area denuded of epithelial cells (AreaDnd) while total peripheral capillary wall area (AreaPCW) remained constant. These findings support the view that denudation of epithelial cells from the peripheral capillary wall is a structural equivalent of impaired permselectivity in adriamycin nephrosis and show that this defect is markedly exacerbated following reduction in nephron number.

INJURY TO RAT MESANGIAL CELLS IN CULTURE BY LOW DENSITY LIPOPROTEINS.
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Low density lipoproteins (LDL) play a key role in the development of atherosclerotic plaques and may also contribute to glomerulosclerosis. Previous studies of ^3H thymidine incorporation have shown that mesangial cells, like smooth muscle cells which they resemble, proliferate faster in culture if LDL was added to the medium. In these experiments, LDL (apoprotein B) concentrations in excess of 250ug/ml (roughly twice normal rat plasma levels) resulted in morphological changes and detachment of cells from the culture vessel. This apparent injurious effect was further investigated using a ^{51}Cr release assay. Rat mesangial cells, preincubated for 24 hrs with $Na[^{51}Cr]O_4$, were grown in modified medium enriched with human LDL. After a further 24 hrs, medium was collected, cells dissolved in 0.5 M NaOH and ^{51}Cr activity in both fractions was measured. Comparing cells grown in media containing 50 and 500 ug/ml LDL, ^3H thymidine incorporation was 50% less and ^{51}Cr release 18% greater at the higher LDL concentration. At an LDL concentration of 800umol/l extensive cell damage was observed under phase contrast microscopy and 81% of incorporated ^{51}Cr was released by cells. These results show that high physiological concentrations of LDL cause mesangial cell injury in vitro and support the role of lipids in the pathogenesis of glomerular injury.

CHRONIC UREMIA CHANGES BOTH INSULIN RECEPTOR NUMBER AND FUNCTION IN SKELETAL MUSCLE OF RATS. W. Mitch, R. May, W. Hardman*. Emory Univ. Sch. of Med., Renal Div., Atlanta, Ga.

We have previously shown that insulin-stimulated protein synthesis is suppressed in muscles from chronically uremic rats (J.C.I. 79:1099, 1987). To study how uremia confers insulin resistance, rats were subjected to subtotal renal ablation (CRF) and pair-fed a high protein diet with sham-operated controls (SO) for 2-4 weeks. CRF rats were moderately azotemic (SUN 122± 17, CRF vs 14± 3 mg/dL, SO) and despite identical food intake gained less weight (2.05±0.34, vs CRF 3.62 ± 0.26 g body wt/day, SO). Hindlimb muscles from each rat were pulverized under liquid nitrogen, homogenized, and insulin receptors were partially purified by wheat germ agarose (WGA) affinity chromatography. Despite greater plasma insulin levels, WGA eluates from muscles of 8 of 9 CRF rats specifically bound more insulin than controls (CRF 37% > controls, P <0.05). The only CRF rat with depressed muscle insulin binding had the least weight gain (0.35 g/day) despite 15 g/day food intake. In a subset of 4 pairs of CRF and SO rats, insulin dose-response curves were generated for autophosphorylation of the insulin receptor. The insulin receptor subunits were separated under reducing conditions by SDS-PAGE, localized by autoradiography, and ^{32}P incorporation into the beta-subunit determined. Maximal insulin-stimulated autophosphorylation was inhibited 44% by CRF even though we used an equal number of receptors for each comparison. The degree to which autophosphorylation was suppressed varied inversely with the greater insulin binding capacity (r=0.9). Conclusion: insulin receptor function is impaired by chronic uremia. This may be mitigated in part by an increase in insulin receptor number. High plasma insulin did not down-regulate insulin receptor number, suggesting that insulin receptor function regulates receptor number. Defects in insulin receptor function may contribute to poor nitrogen utilization in uremia and if receptor number cannot increase in CRF, then growth will be especially poor.

SORBINIL PREVENTS GLOMERULAR HYPER-PERFUSION IN DIABETIC RATS. P. Mower, H.Aynedjiam, S.Silverman, B.Wilkes, N. Bank. Montifiore Med.Ctr., Bronx NY and North Shore Univ. Hosp. Manhasset, NY.

Studies were undertaken to clarify the mechanism by which aldose reductase inhibition prevents the abnormal hemodynamics seen in early insulin dependent diabetes mellitus. Streptozotocin diabetic rats(D) receiving 2U NPH insulin daily and control rats(N) were pair fed with one half of each group receiving 8mgm sorbinil daily mixed in their food.(DS&NS). At 8-15 days, micropuncture studies were carried out to examine glomerular hemodynamics. An additional group of animals identically prepared, was sacrificed to determine glomerular angiotensin II receptor(AII R) binding. Results:

GR	SNGFR	SNFF	Q_A nl/min	R_A dyne.,S.cm.$^{-5}$
N	31.6±.8	0.31±.01	103±4	3.25±.10
N+S	29.3±1.1+	0.35±.01*	84±4+	4.22±.22+
D	47.3±1.5*	0.27±.01	173±7*	1.65±.06*
D+S	32.9±4.3+	0.35±.01*	95±11+	3.49±.38+

(*p 0.05 v N + p 0.05 v D).

The administration of S completely prevented the increased SNGFR, and afferent plasma flow; and caused an increase in single nephron filtration fraction and afferent arteriolar resistance. As has been previously reported, the diabetic animals had decreased glomerular AII R binding. This was not corrected by the administration of S. The normal animals fed S were unchanged from their controls, except SNFF increased. We conclude that the abnormal hemodynamics of diabetes mellitus can be completely prevented by the administration of S by increased vascular tone at both pre and post glomerular sites. This correction is unrelated to the normalization of AII R binding.

RENAL GROWTH AND INJURY ARE INDUCED IN THE INTACT KIDNEY BY DIETARY DEFICIENCY OF ANTIOXIDANTS. KA Nath, TH Hostetter. Univ. of Minnesota, Dept. of Med. Minneapolis, MN.

Reactive oxygen species (ROS) cause renal injury in several models of renal disease. We studied the effect of augmentation of ROS induced by dietary deficiency of antioxidants on the intact kidney. Rats, weaned at 3 wks, were maintained for a further 9 wks on a diet deficient (DEF) or replete (CON) in vitamin E and selenium (a cofactor for glutathione peroxidase). Diets contained ~15% protein, were isocaloric, and had identical electrolyte and lipid content. Results: means ± SE, *p<0.02.

	$U_{PROT}V$ (mg/24 h)		Kidney Wt (g) at 9 WK		Body Wt (g) at 9 WK
	3 WK	8 WK	Wet	Dry	
CON	3.0	10.9	1.05	0.27	324
(n=6)	±0.4	±1.4	±0.02	±0.01	±6
DEF	2.4	22.3*	1.29*	0.32*	277*
(n=9)	±0.2	±3.5	±0.08	±0.01	±7

DEF rats thus exhibit increased rates of urinary protein excretion ($U_{prot}V$). The wet and dry kidney weights were increased in DEF rats inspite of lower body weights. The kidneys of the DEF rats contained more protein (150.2 ± 5.1 vs 128.2 ± 1.4 mg*). Other organs such as the liver were comparable in weight while the heart weight was lower in the DEF rats. There were no significant differences in systolic blood pressure and creatinine clearances between the two groups. Muscle potassium was similar in DEF and CON rats: renal growth in DEF rats was not due to potassium deficiency. Thus, dietary deficiency of vitamin E and selenium promote renal injury in the setting of enhanced renal growth. We suggest that increased cellular action of ROS, occasioned by impaired scavenging mechanisms, is a stimulus to renal growth and injury in the intact kidney.

ENALAPRIL (E) REDUCES GLOMERULAR INJURY IN OBESE ZUCKER (OZ) RATS. M.P. O'Donnell, B.L. Kasiske, S.A. Katz*, P.G. Schmitz*, W.F. Keane, Hennepin Co. Med. Ctr., Univ. of Minnesota, Mpls, MN.

Studies have suggested that angiotensin converting enzyme inhibitors may ameliorate focal glomerulosclerosis (FGS) independent of effects on glomerular hemodynamics. We investigated the effects of E therapy in OZ rats, a model in which the initiation of glomerular injury is not dependent on elevated glomerular capillary pressure (P_{GC}). Male OZ rats received either tap water or E (50 mg/L) beginning at 8 weeks of age. Male lean Zucker (LZ) rats served as controls. Blood pressure (BP), serum cholesterol (CHOL), and urine albumin excretion ($U_{alb}V$) were measured every 6 weeks. Results (mean ± SE) at 38 weeks (different superscripts indicate p<0.05):

Group	PRA	BP (mmHg)	CHOL (mg/dL)	$U_{alb}V$ (mg/24h)	KW (g)	FGS (%)
OZ	9.0^a	144^a	149^a	98^a	2.92^a	8.3^a
(n=9)	±1.9	18	29	29	0.06	2.5
OZ+E	19.2^b	125^b	105^b	25^b	2.50^b	1.4^b
(n=7)	±4.5	9	9	8	0.07	0.8
LZ	22.8^b	124^b	75^b	3^c	2.47^b	0.5^b
(n=8)	±3.0	4	5	1	0.04	0.5

Plasma renin activity (PRA; $ngAI \cdot ml^{-1} \cdot hr^{-1}$) was lowest in untreated OZ rats. PRA was also reduced in untreated OZ compared to LZ at 9 and 20 weeks of age. Thus, E normalized BP, CHOL, and kidney weight (KW), and prevented FGS, in a model of glomerular injury characterized by reduced PRA and normal P_{GC}.

EFFECT OF DIALYTIC INTERVENTION ON THE FUNCTION AND MORPHOLOGY OF GLOMERULI IN RATS AT AN EARLY STAGE OF PROGRESSIVE RENAL DISEASE. F. Nishijima*, M. Ikoma*, J. Lipman*, P. Teschan, A. Fogo* and I. Ichikawa. Depts. of Pediatrics, Pathology and Medicine. Vanderbilt University Med. Ctr., Nashville, TN.

Initiation of dialysis in patients with failing kidneys often results in depression of residual renal filtration function. In the long run, however, dialysis may be beneficial for preservation of the renal structure and function by reducing the hemodynamic load to damaged nephrons, or by preventing nephron hypertrophy, a phenomenon closely linked to the final scarring processes. One week after implantation of a peritoneal dialysis (PD) catheter and subtotal nephrectomy, rats were paired by the level of BUN. Group 1 rats were subjected to 1 hr/cycle, 8 cycles/day PD daily with 1.5% dextrose dialysate. At the onset of PD, creatinine clearance of PD was calculated to be 0.35 ml/min. Group 2 rats were subjected to an identical procedure except for zero dialysate indwelling time. During the subsequent two to 3 weeks before sacrifice, BUN of Group 1 rats remained at ~35 mg/dl, whereas Group 2 BUN increased to 71±18. At sacrifice, function and structure were assessed in 5 pairs of Group 1 and 2 animals to evaluate the effect of PD on the function and morphology of early remnant glomeruli. Whole kidney GFR averaged 0.23±0.04 ml/min in Group 1, a value substantially lower than that of Group 2 (0.44±0.11). Likewise, glomerular capillary pressure averaged 56±1 mmHg in Group 1, a level significantly lower than that in Group 2 (65±2). A similar pattern was detected in mean systemic arterial pressure (120±11 mmHg vs 129±4). The degree of glomerular hypertrophy was assessed on thin section specimens by measurement of mean planar area (PA mean), and sclerosis index (SI, 0-4 scale) was evaluated in 60 randomly selected glomeruli of the remnant kidney at sacrifice. Despite the tendencies indicating that the pressure and flow imposed upon the glomerular capillary were dampened by PD, no discernible difference was present between Group 1 and Group 2 in the degree of hypertrophy (PA mean of 7.2±1.0 vs 6.7±0.7, $x10^{-3}$ mm^2) or in SI (0.14±0.09 vs 0.08±0.06). The observed lack of beneficial effect of PD on the early stage of morphology warrants a further study for the effect of PD in the late stage where the efficiency of PD relative to the residual renal function is magnified.

ACQUIRED RENAL CYSTS IN 5/6 NEPHRECTOMISED RATS: THE ROLES OF OXALATE DEPOSITS IN RENAL TUBULES & A RENOTROPIC FACTOR. Keiji Ono & Kazuhiko Kikawa* Ono Geka Clinic & Fukuoka Tokushukai Hospital, Fukuoka & Kasuga, Japan

The mechanisms leading to acquired renal cyst (ARC) formation in uremic patients remains uncertain. The present study was undertaken to try and clarify the importance of oxalate(OX) crystals in tubular obstruction or stenosis in 5/6 nephrectomised(NRT) rats & in healthy controls. Three experiments(EXP) were performed:in EXP I, 30 5/6 NRT rats were allowed free access to water containing 8 mg/ml vitamin C(VC)(OX precursor) & other 20 5/6 NRT rats were given tap water without VC. In EXP II, 6 5/6 NRT rats were injected s.c. daily with 5 mg of Na-OX & 6 other untreated 5/6 NRT rats served as controls. In EXP III, 6 healthy rats were given s.c. injections of 10 mg Na-OX daily. The histological appearance of the renal remnant in both VC treated & untreated rats was the same except one difference; OX deposits were found in the renal tubules of VC treated rats in the 11th & 12th post operative months. However, ARC were noted well before the occurrence of OX deposition. In EXP II, although renal function was the same in both OX treated & untreated rats for 25 days after 5/6 NRT, cysts appeared earlier & in larger number in the OX treated rats than in untreated rats. In EXP III, OX deposits were noted in renal tubules in healthy rats treated with Na-OX for more than 2 weeks & renal cysts were also noted. In conclusion, the present study suggests that the pathogenesis of ARC is multifactorial. However, OX deposit in renal tubules is an important causative factor in the formation of ARC.

FOCAL AND SEGMENTAL SCLEROSIS IN A RABBIT UNINEPHRECTOMY MODEL. AGGRAVATION BY REPEATED PREGNANCY. David Packham*, Tim Hewitson*, Judith A.Whitworth and Priscilla Kincaid-Smith. Dept of Nephrol., Royal Melbourne Hosp., Melbourne, Vic., Australia.

Four of 5 uninephrectomized virgin (UNx/V) and and 4 of 5 uninephrectomized repeatedly pregnant (UNx/Preg) rabbits developed focal and segmental hyalinosis and sclerosis (FSHS) in glomeruli. This differed significantly from 2/10 FSHS lesions in non nephrectomized pregnant (NN/P) and 2/10 FSHS in non nephrectomized virgin (NN/V) rabbits at 15 months ($p=0.0026$). Focal sclerosis was increased (median 3.6%) in NN/P compared with NN/V (0.4%).

After left nephrectomy median right kidney weights per Kg body weight were significantly greater in nephrectomized rabbits than right kidney from non nephrectomized rabbits (3.9gm/Kg cf 2.6gm/Kg $p<0.001$). Glomerular area of largest 10% glomeruli divided by kidney weight was less in previously uninephrectomized animals than in nephrectomized ($p<0.001$), indicating glomeruli had hypertrophied less than tubules.

Mean plasma urea was higher at sacrifice (15 months) in UNx/Preg (11.6±0.4mmol/l) than in UNx/V (7.7±0.7mmol/l). Mean urine protein rose from 0.35±0.4gm/l to 0.85±0.9gm/l during pregnancy in UNx/Preg ($p<0.001$) while NN/P remained constant (0.38±0.07gm/l).

Mean BP of 10 UNx rabbits increased from 81±3mmHg to 92±1mmHg over 15 months ($p=0.022$).

This is the first description of focal and segmental hyalinosis and sclerosis (FSHS) in rabbits.

REDUCED RENAL VASOCONSTRICTIVE RESPONSE TO ADENOSINE IN ACUTE EXPERIMENTAL DIABETES. J. Perlmutter*, D. Senesky, D.A.Simmons, E.F.O.Kern, and S.Goldfarb. Dept. of Med., Univ. of Pa., Phila., Pa.

Acute diabetes is characterized by increased renal blood flow and this effect has been assigned a pathogenetic role in the late tissue injury seen in diabetes. The mechanism of the phenomenon of increased tissue blood flow is not understood. Recent in vitro studies have suggested that hyperglycemia through activation of the Polyol Pathway reduces Na/K ATPase activity in vascular smooth muscle and that reduced adenosine (adeno)responsiveness contributes to this defect. Since adeno is normally a renal vasoconstrictive agent and since reduced coronary artery responsiveness to adeno in diabetes has been observed, we tested the hypothesis that reduced renal responsiveness to adeno may underlie the high GFR seen in acute Streptozotocin (STZ)-induced diabetes. We therefore examined the acute renal hemodynamic response to adeno in normal and STZ-diabetic rats (blood glucose >300, <450 mg/dl).

In normal rats (n=10), suprarenal arterial infusion of Adeno (33 µgm/kg./min) reduced GFR from 9.61 ±.30 to 8.42 ± 21 ml/min/kg ($\Delta = -12.4\%$) while a sham infused group (n=11) was unchanged (GFR:9.95 ± .6 to 9.82± .4 ml/min/Kg ($\Delta=-1.3\%$). In 8-10 day STZ rats, adeno had an effect identical to the sham infused, hyperglycemic control group: (Diabetic control, sham infused (n=17) GFR :11.37±.21 to 10.43± .32, $\Delta=-8.3\%$; Diabetic adeno (n=12) GFR:10.9± .19 to 9.8±.14 ,$\Delta=-10.1\%$). While adeno effect to significantly reduce GFR was seen in normoglycemic rats, there was no difference between sham infused and adeno infused diabetic rats.

These data suggest that acute STZ diabetes is characterized by a reduced or absent response to the vasoconstrictive actions of adeno. Since adeno acts primarily through constriction of the afferent arteriole, the high nephron plasma flow and GFR of acute STZ-induced diabetes may be the result of deficient adeno response.

INTESTINAL ABSORPTION OF VITAMINS C AND E IN EXPERIMENTAL AZOTEMIA. M.V. Pahl,* N.D. Vaziri, G. Khamiseh,* F. Oveisi,* Division of Nephrology, University of California, Irvine, Irvine, California.

Renal failure (RF) has been shown to alter intestinal transport of a number of vitamins (vits). We studied jejunal transport of vits C and E in animals with subtotal nephrectomy (RF group) and compared the results with those of sham-operated (S) and pair-fed (PF) groups. *In vivo* recirculating perfusion and *in vitro* everted sac techniques were employed. The *in vitro* experiments were repeated using sacs containing either uremic or normal sera. *In vivo* absorption of both vitamins was significantly reduced in the RF group compared to the S and PF groups, which showed comparable rates. In contrast, the *in vitro* transport and uptake of both vitamins was increased in the RF and PF groups when compared with the S group suggesting increased permeability. This appears to be mediated by reduced nutrient intake, common to both groups. The disparity between *in vivo* and *in vitro* results in the RF animals points to an inhibitory influence present in the intact animal. However, the rates of transport by sacs containing uremic and normal sera were similar, thereby excluding the effect of uremic chemical environment. Serum vit C levels in RF and PF groups were reduced, thereby excluding its elevated serum level as an inhibitory factor. In conclusion, *in vivo* jejunal absorption of vits C and E is impaired in rats with RF despite the *in vitro* evidence of hyperpermeability.

FATTY ACIDS (FA) OXIDATION BY MYOCARDIUM (MYCR) IN CHRONIC RENAL FAILURE (CRF): ROLE OF PARATHYROID HORMONE (PTH) AND MYOCARDIAL CALCIUM. Alessandra F. Perna, Miroslaw Smogorzewski and Shaul G. Massry, Div. Nephrol., Univ. So. Calif., Los Angeles, CA.

Secondary hyperparathyroidism and myocardiopathy occur in CRF, and heart is a target organ for PTH which causes a rise in MYCR calcium. FA are major sources of energy for MYCR and a defect in FA oxidation may contribute to MYCR dysfunction. We therefore studied the interaction between CRF, PTH and MYCR calcium on FA oxidation in normal rats, 21 days CRF rats with and without treatment with verapamil, CRF-parathyroidectomized (PTX) rats maintained normocalcemic, and rats receiving 4 days of 1-84 PTH with and without verapamil treatment. CRF and PTH-treated rats had significant ($p<0.01$) increase in MYCR ^{45}Ca uptake and calcium content, impaired oxidation of long and short chain FA, and reduced activity of palmitoyl carnitine transferase (CPT) but no change in carnitine content. Both PTX in CRF rats and verapamil administration to CRF or PTH-treated rats reversed all these abnormalities. Verapamil given to normal rats had no effect on all parameters studied. The results show that 1) CRF causes impaired oxidation of FA due to reduced activity of CPT 2) this is due to excess PTH of CRF, 3) the action of PTH is mediated by its ionophoric property causing a rise in MYCR calcium, and 4) a calcium antagonist corrects the action of PTH. The data provide for another pathway through which excess PTH in CRF exerts an adverse effect on MYCR metabolism and for a therapeutic approach.

REGULATION OF MESANGIAL CELL PROLIFERATION AND COLLAGEN SYNTHESIS BY RENAL GLOMERULAR AND CORTICAL CONDITIONED MEDIA. Sem H. Phan, Bridget M. McGarry, and Roger Wiggins*. Univ. Michigan Med. Sch., Depts. Pathology & Medicine, Ann Arbor, MI.

To examine the mechanism of renal fibrosis in nephrotoxic nephritis (NTN), glomeruli (Gl) and renal cortical tissue (Co) from normal and diseased animals were analysed for the secretion of substances capable of regulating mesangial cell (MC) proliferation and collagen synthesis. NTN was induced in rabbits using guinea pig anti-GBM IgG. Animals were sacrificed and samples of Co and Gl were incubated in serum free media. Conditioned media (CM) were tested for growth factor (GF) and collagen synthesis stimulatory activities on rabbit MCs isolated from normal Gl. Gl CM from control animals either had no effect or inhibited MC proliferation and protein synthesis as assessed by ^{3}H-thymidine and ^{3}H-proline incorporation, respectively. Control Co CM stimulated MC proliferation without affecting protein synthesis significantly. Both Gl and Co CM from diseased animals stimulated MC proliferation and protein synthesis above that of control CM, but with different kinetics. Increased GF secretion in both Gl and Co CM occurred rapidly, peaking at day 4 and decreasing gradually toward control levels at day 14. In contrast, protein synthetic stimulatory activity secretion was maximal at day 14 with only minimal increases on day 4. The effect was greater on collagen than non-collagenous protein synthesis. These results suggest that in NTN early increased secretion of GF may result in proliferation of MCs which could then synthesize collagen at a higher rate in response to mediator(s) secreted by cells in cortical tissue.

Comparative effects of cholestyramine and the HMG Co-A reductase inhibitor simvastatin on lipoprotein profile in the hyperlipidemia of nephrotic syndrome (NS). A.J. Rabelink*, R. Hené*, D. Erkelens**, E. Dorhout Mees. Depts. of Nephrology and Internal medicine** University Hospital Utrecht. The Netherlands.

Hyperlipidemia is a common feature of NS. In prolonged unremitting NS, hyperlipidemia may be present for many years. Lipoprotein profile and autopsy studies make it likely that this hyperlipidemia accelerates atherosclerosis. In a cross-over study we compared the safety, tolerability and efficacy of six weeks of simvastatin (20 mg b.i.d.) and six weeks of cholestyramine (8 g b.i.d.) in eight patients with hyperlipidemia due to unremitting nephrotic syndrome.

Results	placebo	simvastatin	
Tot.chol. (mmol/l)	10.0 ± 0.7	6.4 ± 0.6**	a
LDL-chol. (mmol/l)	7.0 ± 0.6	4.3 ± 0.6**	a
HDL-chol. (mmol/l)	1.0 ± 0.1	1.2 ± 0.1	
Triglycer. (mmol/l)	4.5 ± 0.6	2.7 ± 0.4*	b
Apo-A-I (g/l)	1.97 ± 0.11	2.17 ± 0.12*	
Apo-B (g/l)	1.60 ± 0.15	1.12 ± 0.18**	a
	placebo	cholestyramine	
Tot.chol. (mmol/l)	10.5 ± 1.2	9.4 ± 0.7	
LDL-chol. (mmol/l)	7.6 ± 1.2	5.9 ± 0.8**	
HDL-chol. (mmol/l)	0.9 ± 0.1	1.1 ± 0.2	
Triglycer. (mmol/l)	4.3 ± 0.6	5.2 ± 1.4	
Apo-A-I (g/l)	1.98 ± 0.10	2.06 ± 0.10	
Apo-B (g/l)	1.67 ± 0.13	1.60 ± 0.13	

(=p<0.05, ** =p<0.01 vs placebo; a=p<0.01, b=p<0.05 simvastatin vs cholestyramine.)*

Conclusions:1) Simvastatin is effective in reducing cholesterol and triglycerides, and improved the LDL-to-HDL ratio in NS. 2) Simvastatin is more effective in the treatment of nephrotic hyperlipidemia than cholestyramine. 3) Simvastatin was well tolerated and no side effects were noticed in this short term study.

INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGF BPS) ARE INCREASED IN SERUM OF CHILDREN WITH CHRONIC RENAL FAILURE (CRF) AND INHIBIT CARTILAGE GROWTH *IN VITRO*. David Powell*, Phillip Lee*†, Robert Baxter*††, Javier Correa*‡ and Warner Burch*‡ (intr. by Leighton Hill). Baylor Col. Med., Houston, Tx.; †Children's Hospital, Denver, Colo.; ††Royald Prince Alfred Hospital, Camperdown, Australia; ‡Duke Univ., Durham, N.C.

Linear growth is poor in CRF children despite normal serum levels of growth mediators, including IGFs. Serum IGFs are bound by 25 and 53 kD BPs (BP-25 and BP-53); these BPs may inhibit IGF-mediated growth of many tissues. We studied IGF BPs in CRF children by using a charcoal assay to compare IGF-I binding, and specific RIAs for BP-25 and BP-53 to compare BP levels, in serum from 15 prepubertal CRF children vs. 15 age- and sex-matched normal (NL) children. Our results:

	age(mean) +/-S.D.)	serum IGF-I binding (%)	BP-25 (µg/ml)	BP-53 (µg/ml)
CRF	6.8±4.4	17.4±3.5*	2.3±2.2*	4.7±1.1*
NL	6.6±3.9	12.5±3.2	0.5±0.4	2.6±0.7

*CRF≠NL, p <0.05, by Wilcoxon paired-sample test.

We also studied the effect of pure BP-25 on chick embryo pelvic cartilage growth *in vitro*. In serum-free medium, this cartilage produces IGF-like peptides which stimulate basal growth. We found that basal increases in cartilage wet weight, and further increases stimulated by 0.6 nM IGF-I, were inhibited by BP-25 in a dose dependent manner and by as little as 0.4 nM BP-25.

We conclude: 1) serum IGF BP levels and binding activity are increased in CRF children 2) BP-25 inhibits cartilage growth *in vitro*; 3) IGF BPs should be evaluated for their effects on growth and differentiation of chondrocytes *in vivo*.

QUANTIFICATION OF FOCAL GLOMERULOSCLEROSIS IN THE RAT BY TRIDIMENSIONAL MORPHOMETRICAL ANALYSIS. A. Remuzzi*, R. Pergolizzi and T. Bertani. Mario Negri Institute for Pharmacological Research, Bergamo, Italy. (intr. by R.J. Glassock).

Focal segmental glomerulosclerosis (FSG) is the lesion that usually follows a condition of persistent proteinuria in experimental animals and humans. Whether focal glomerulosclerosis initiates in few glomeruli, the remaining being intact, or it involves the entire glomerular population 'ab initio' is an open issue. We address this with a tridimensional morphometrical analysis in ADR nephrosis, a model of glomerular damage which evolves to FSG. From a control rat and an ADR treated rat with heavy proteinuria we cut 300 consecutive serial kidney sections 4 µm thick; each section stained with PAS, was examined under light microscopy at 25 x magnification. In 15 control and in 50 ADR glomeruli glomerular tuft, Bowman's capsule and sclerotic regions were manually outlined using a drawing tube. Surface area of this regions were then measured using stereology technique. Calculated capillary tuft, Bowman's capsule and sclerosis volumes ($\mu m^3 \times 10^{-6}$), together with the percentage of glomerular volume affected by sclerosis are as follows:

	Cap. tuft Vol.	Bowman's cap.Vol.	Scl. Vol.	%Vol.Scl.
Contr	1.14±0.16	1.48±0.25	-	-
ADR	1.11±0.23	1.55±0.29	0.16 (.02-.60)	3.53±4.15

In the control rat none of the glomeruli showed sclerotic changes; by contrast in the ADR animal 94% of glomeruli (47 out of 50) were affected by sclerotic changes which however involved only 2.99% of the total glomerular volume (range for individual glomeruli 0.1-18.6%). The high number of glomeruli affected by the sclerotic process measured using this technique contrasts with findings derived from the analysis of single sections in which the percentage of glomeruli with sclerosis ranged from 25.9 to 58.1%.

We conclude that: 1) ADR nephrosis, even in a relatively early phase, induces diffuse sclerotic changes affecting almost all the glomeruli; 2) conventional analysis of FSG greatly underestimates the number of glomeruli affected by sclerosis and is not predictive of the percentage of glomerular volume affected by sclerosis. This technique can be useful for future studies on the progressive nature of glomerular diseases.

CORRECTION OF AMINO ACID (AA) METABOLISM BY RECOMBINANT HUMAN ERYTHROPOIETIN (rHu EPO) IN HEMODIALIZED PATIENTS (HD). *E.Riedel[+], *H.Hampl[++], *M.Nündel[+], *P.Scigalla[+++],*M.Kessel[++] (intr. by J. Bosch) Free Univ.of Berlin, Dept.of Biochem.[+], Nephrol.[++], Boehringer Mannheim Comp.[+++]

Many efforts to compensate the impaired AA metabolism in HD patients failed so far. Thus, we should try an endogenous approach to influence this by correction of anaemia wiht rHu EPO. The plasma AA pattern (estimated by HPLC) of 18 healthy persons were determined and compared with 14 HD patients for changes in plasma AA before and up to 25 weeks after rHu EPO therapy:

Control (µmol/l)	group(c) x̄±s	before(b) x̄±s	Pat. p(c/b)	after(a) EPO x̄±s	p(b/a)
ser	95±18	67± 16;	p<0.01	86±22;	p<0.05
gly	196±52	317±113;	p<0.01	228±56;	p<0.05
HOpro	11± 5	25± 15;	p<0.01	15±12;	p<0.10
gln	555±115	470±127;	p<0.10	563±115;	p<0.10
arg	89±18	95± 20;	p<0.10	72±23;	p<0.05
orn	56±19	78± 16;	p<0.01	52±18;	p<0.05
val	187±45	137± 32;	p<0.01	155±25;	p<0.10
tyr/phe	1,33±0,31	0,78±0,17;	p<0.01	0,98±0,21;	p<0.05

Outstanding is the correction of serine values (liver metabolism) correspondingly with a decrease of the serine precursors glycine (metabolic marker) and hydroxyproline. The lowered essential AA valine and leucine were corrected. The elevated values of ornithine, arginine (KREBS-cycle) were decreased; diminished glutamine (NH_4-detoxication) and decreased tyrosine/phenylalanine quotient were elevated. The administration of rHu EPO does not only affect erythropoiesis, but also corrects AA pattern in the tendency towards the normalization by a better 0_2-availability.

CHOLESTEROL BIOSYNTHESIS IS ALTERED IN UREMIC GUINEA PIGS. R. Jean Shapiro, Dept. Medicine, University of British Columbia, Vancouver, Canada.

In a remnant model of uremia in the guinea pig we have shown that significant hyperlipidemia ensues, with elevations in plasma cholesterol and triglyceride. Turnover studies examining plasma clearance of radiolabelled low density lipoproteins (LDL) in this model indicated that the hyperlipidemia was due to both an increase in LDL synthesis as well as decreased LDL receptor-mediated catabolism. To assess hepatic cholesterol synthesis in uremia, we examined the activity of hydroxymethylglutaryl coenzyme A reductase (HMGCoAR), the rate-limiting enzyme of cholesterol biosynthesis in hepatic microsomal fractions isolated from normal (N) and uremic (UR) guinea pigs. N and UR guinea pigs were maintained on normal guinea pig chow and sacrificed at mid-light cycle. 100000Xg hepatic microsomal fractions were assayed in the presence of NaF for determination of the active form of HMGCoAR. HMGCoAR activity was significantly higher in the UR group compared to the N group of guinea pigs: 18.3±8.8 vs 8.4±3.2 pmol/min/mg (x̄±S.D.; p<0.01). Plasma cholesterol levels were also significantly higher in UR 2.9±0.8 vs 0.8±0.2 mmol/L (x̄±S.D; p<0.001). The paradoxical increase in HMGCoAR activity in the face of suppressed LDL receptor activity suggests that uremia may be associated with a specific deficit in LDL receptor expression, leading to a depletion of a regulatory pool of cholesterol in the hepatocyte, with consequent stimulation of cholesterol synthesis.

CONVERTING ENZYME INHIBITOR (CEI) TREATMENT REVERSES BROADENING OF EPITHELIAL CELL FOOT PROCESSES IN RATS WITH REDUCED NEPHRON NUMBER. JW Scholey*, PL Miller*, HG Rennke, and TW Meyer, Stanford University and VAMC, Palo Alto, CA, and Harvard University, Boston, MA.

CEI treatment increases the glomerular ultrafiltration coefficient (Kf) in rats with reduced nephron number. We sought to relate this increase in Kf to changes in glomerular epithelial cell structure. Micropuncture and morphometric studies were performed in normal rats (Con) and in rats 6 wks after 5/6 renal ablation. One group of ablated rats received no treatment (Rem) while a second group (RS) received the CEI RS10085 for 2 wks prior to study. Results: (mean±SEM, *p<.05 vs Con, ‡p<.05 vs Rem)

	SNGFR nl/min	AP ---mmHg---	PGC	Kf nl/s/mmHg	Wa nm	VG $10^6\mu^3$
Con	49	108	51	0.052	731	1.51
n=6	±3	±2	±1	±.005	±10	±.05
Rem	112*	140*	65*	0.061	868*	2.64*
n=6	±8	±8	±4	±.007	±50	±.09
RS	111*	98‡	51‡	0.104*‡	751‡	2.66*
n=6	±6	±5	±1	±.007	±27	±.11

Arterial pressure (AP), glomerular capillary pressure (PGC), and single nephron GFR (SNGFR) were elevated in Rem rats. Kf remained normal in Rem rats despite an increase in glomerular volume (VG) while mean epithelial cell foot process width (Wa) increased. Maintenance of elevated SNGFR despite reduction of PGC in RS rats reflected an increase in Kf. This increase in Kf was associated with a reduction in Wa while VG remained elevated. These findings suggest that CEI treatment increases Kf in remnant glomeruli by restoring foot process width toward normal and thus increasing the filtration slit length available for ultrafiltration.

PHLORIZIN PREVENTS THE RENAL HYPERTROPHY OF DIABETES MELLITUS. A. Spitzer, M. Barac-Nieto. A. Einstein Coll. of Med., Depts. of Pediatrics, and Physiology and Biophysics, Bronx, NY.

There is reason to propose that renal hypertrophy of diabetes mellitus, a possible precursor of the nephropathy, is causally related to hyperglycemia and/or the associated increase in renal glucose-Na^+ cotransport (A. Kumar, et al. Kidney Int. 33:792-797, 1988). To test this hypothesis diabetes was induced in Sprague-Dawley rats by the intravenous injection of 45 mg/kg BW of streptozotocin. Starting 24 hrs later one group of diabetic animals received subcutaneously 400 mg/kg day phlorizin, dissolved in propylene glycol; another group received the vehicle alone. Normal animals served as controls. Measurements were done 96 hrs after induction of diabetes.

	Body Wt. Initial g	Body Wt. Final g	Kidney Wt. Wet g	Kidney Wt. Dry g	Glucose conc. Blood mg/dl	Glucose conc. Urine g/dl
Diabetic (4)	333.9 ±1.2	309.9 ±3.9	2.97* ±.03	0.68* ±.02	385* ±22	1.75* ±.14
Phloriz (3)	329.7 ±5.5	303.0 ±9.4	2.56 ±.05	0.59 ±.02	102 ±7	2.50 ±.15
Control (4)	312.2 ±12.5	319.0 ±14.0	2.44 ±.08	0.54 ±.01	96 ±5	0 -

Means ± 1SE *=p<0.05 diabetic vs phlorizin treated diabetic and vs control.

Phlorizin, which inhibits renal glucose cotransport and minimizes hyperglycemia, prevented the development of renal hypertrophy of diabetes. Thus, hypoinsulinemia is not sufficient to induce renal growth. Hyperglycemia and/or the increase in glucose-Na^+ cotransport are required for diabetic renal hypertrophy to occur.

DIFFERING ANTIPROLIFERATIVE EFFECTS OF HEPARIN AND NON-ANTICOAGULANT HEPARIN ON RAT MESANGIAL CELLS IN CULTURE. TJ Stokes, E Tetens*, EA Wurst*, S Klahr. Renal Division, Washington University Medical School, St. Louis, MO.

Studies in the rat remnant kidney model of chronic renal failure (JCI 81: 69-74, 1988) demonstrated a protective effect of a non-anticoagulant heparin analog, N-desulfated heparin (NDSH), on progressive glomerular sclerosis. To determine if NDSH's protective effect was due to its antiproliferative properties, we compared the influence of unmodified heparin and NDSH on the growth of rat mesangial cells (MC) in culture. MC proliferation was assessed by ^{3}H-thymidine incorporation. Sensitivity of the growth inhibition assays was enhanced by reducing the fetal bovine serum (FBS) concentration in the medium to 2%. MC in the first passage were incubated in 2% FBS for one day, followed by one day with heparin or NDSH. The following day, ^{3}H-thymidine was added to the medium and cellular incorporation of ^{3}H-thymidine was measured 48 hours later. Heparin at 10 U/ml did not inhibit ^{3}H-thymidine incorporation, while 50 U/ml suppressed growth by 71.1 ± 3.2% ($p<0.01$). A concentration of NDSH equivalent to 50 U/ml produced only 10.5 ± 7.3% (p=NS) growth inhibition. Five-fold higher concentrations of NDSH, however, did inhibit ^{3}H-thymidine incorporation by 25.7% (n=3).

We conclude that the non-anticoagulant heparin analog, NDSH, has much less antiproliferative activity on MC in culture than unmodified heparin. The antiproliferative properties of NDSH may not fully account for its in vivo protective effect in the remnant kidney.

MUSCLE METABOLISM IN END STAGE RENAL DISEASE (ESRD): EFFECTS OF DIALYSIS AND TRANSPLANTATION. H. Szerlip, K. McCulley*, B. Chance*. Depts. of Med. and Biochem/Biophys., U of PA, Phila. PA.

ESRD patients have abnormal muscle metabolism as evaluated by ^{31}P Magnetic Resonance Spectroscopy. In this study, we examined muscle metabolic parameters before and at 1 hour after dialysis as well as 2-5 months after transplantation. Forearm muscles were studied during rest, increasing levels of exercise, and recovery. Measurements were made of work, free phosphate (Pi), phosphocreatine (PCr), and intracellular pH. We compared recovery of PCr/Pi·min, end-exercise intracellular pH, maximum work, and amount of work accomplished at the end of exercise as a % of maximum.

In 5 pts, dialysis had no effect upon the measured metabolic parameters although it was associated with more rapid fatigue (74% and 65% maximum work achieved pre- and post-dialysis, respectively, $p<0.05$). In contrast, in 4 of 5 pts, transplantation was associated with more rapid recovery of PCr/Pi·min ($\bar{X}$ + SE: 0.86±0.8 pretransplant vs 3.7±1.7 post transplant). In conclusion, transplantation (but not dialysis) improves muscle bioenergetics in most ESRD patients.

PATHOGENETIC ROLE OF INCREASED TUBULE CYTOSOLIC FREE Ca CONCENTRATION ($[Ca^{2+}]_i$) IN PROGRESSIVE RENAL INSUFFICIENCY: AMELIORATION BY A LOW PO_4 DIET. S. Tan,* M. Pesigan,* E. Lorr,* R. Crockett,* T. Ondrizek,* and K. Lau. Michael Reese Hospital & University of Chicago, Chicago, IL.

Previous studies suggest improved functional preservation by a low PO_4 diet in several models of progressive renal failure. The mechanism for this salutary effect is not clear. We tested the hypothesis that PO_4 restriction decreases tubule $[Ca^{2+}]_i$, which reduces toxicity and attenuates the decline in renal function. Normotensive rats were either left intact or nephrectomized and after recovery for 2 weeks, they were randomized to a low (0.02%) or a normal (0.6%) P diet for 5½ weeks. In rats fed a normal P diet, nephrectomy increased renal tubule $[Ca^{2+}]_i$ (257 ± 10 vs 171 ± 12 nM), as measured by the fura 2 fluorescence technique with dual wavelength excitation. These changes were also associated with an increase in serum creatinine from 0.9 ± 0.1 after 5/6 nephrectomy to 1.9 ± 0.2 mg/dl 5 weeks later. In contrast, the low P diet decreased serum creatinine (1.2 ± 0.1 mg/dl) in rats with 5/6 nephrectomy despite comparable baseline and serial body weights. This was confirmed by a higher inulin clearance (2.58 vs 1.75 ml/min). The low P diet also reduced renal tubule $[Ca^{2+}]_i$ (225 ± 10 nM) and arterial blood pressure in nephrectomized rats (systolic = 161 ± 8 vs 210 ± 9; diastolic = 131 ± 9 vs 154 ± 8 mmHg).

These results support the hypothesis that cytosolic free Ca concentration plays a pathogenetic role in progressive renal insufficiency since interventions which blunt its elevation in renal tubules retard the functional deterioration. Low P diet affords additional protection by attenuating the degree of hypertension in renal failure.

HIGH AMBIENT GLUCOSE STIMULATES SORBITOL ACCUMULATION IN CULTURED MESANGIAL CELLS. Shunya Uchida* and Kiyoshi Kurokawa. IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo, Japan.

Increased activity of polyol pathway has been implicated in the pathogenesis of certain complications of diabetes mellitus. Although the presence of aldose reductase (AR), a key enzyme to convert glucose to sorbitol, is recently shown in mesangial cells in culture (Kikkawa et al, Diabetes 36:240, 1987), a role of polyol pathway in the genesis of diabetic nephropathy remains unclear. We thus investigated the regulation of sorbitol (S) accumulation in cultured rat mesangial cells. Mesangial cells of passage 2 through 17 which contract to 10^{-7}M angiotensin II were used. After the cells reached confluent, the concentration of fetal calf serum (FCS) in the medium was changed from 20 to 0.5%. This resulted in a reduction in cell S from 60±3.5 (SD) to 43±4.2 nmol/mg prot, while AR activity remained virtually constant (1.5 nmol/min/mg prot). Cell S increased in response to increasing glucose in the medium in a dose-dependent manner: cell S increased by 60-100% in 4 hrs by raising medium glucose from 5 to 55 mM. By contrast, 50 mM mannitol had no effect. Moreover, S accumulation was enhanced by 20-30% by 5 µg/ml insulin and the effects of insulin and glucose was additive. Our data confirm the presence of polyol pathway in mesangial cells and show that both high ambient glucose and insulin stimulate sorbitol accumulation with their effects being additive. These findings may provide a clue to understand the mechanism underlying the development of diabetic glomerulopathy.

URINARY GLUCOCORTICOIDS CORRELATE WITH PROGRESSION OF RENAL FAILURE. M. Walser, L. Ward*, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Progression of chronic renal failure during 35 treatment periods in 27 patients was measured as the rate of change of bimonthly radioisotope GFR for an average of 15 months. Treatments comprised (1) mild protein restriction (2) more severe restriction plus amino acids, or (3) the same diet plus ketoacids. Progression was significantly ($p<0.025$) correlated with urinary 17-hydroxy-corticosteroid excretion (17OHCS) in all 3 treatment groups; overall r was 0.78 ($p<0.0001$). Multiple regression analysis showed that the following factors were not additional significant determinations of progression: urea N excretion, phosphate excretion, protein excretion, serum Ca x P product, alk. p'tase, uric acid, triglycerides, cholesterol, etiology, mean arterial pressure, or enalapril treatment. However, when 17OHCS excretion was factored by GFR (with which it was correlated), additional significant regressors appeared: serum triglycerides and polycystic kidney disease, which tended be associated with more rapid progression, and ketoacid treatment, which tended to be associated with slower progression. Mean 17OHCS excretion differed significantly between the three treatment groups, in the order (1)>(2)>(3). Changing from amino acids to ketoacids (or vice versa) without change in diet was associated with lower 17OHCS excretion on ketoacids. Thus progression is related to glucocorticoid production (and to triglyceridemia, which is correlated with it). Ketoacids appear to slow progression by suppressing production of glucocorticoids.

RESISTANCE TO FOCAL GLOMERULOSCLEROSIS (FGS) AFTER UNILATERAL NEPHRECTOMY (UN) IN WKY RATS IS COMPATIBLE WITH GLOMERULAR HYPERTROPHY. Pieter J. Westenend*, Yvonne Nooyen*, and Jan J. Weening* Univ. Leiden and Groningen, Depts of Pathology, The Netherlands (intr. by Ph.J. Hoedemaeker).

Male rats (n=10 per group) of two normotensive rat strains, Wistar Zeist (W) and Wistar Kyoto (WKY), were studied for the development of FGS, proteinuria (UP) and glomerular hypertrophy at 8 months (mo.) after sham surgery or UN, performed at age 3 mo. Four separate groups (n=5-7) were studied for GFR and RPF determined by inulin clearance and extraction at 1 mo. after surgery. Results (see table) show that sham W and UN W developed FGS and increased UP, whereas WKY did not. Glomerular tuft volumes (GV) increased in both strains at 1 mo. (table) and at 8 mo. after UN (not shown). At 1 mo. GV expansion was associated with a significant increase of GFR in both strains but filtration fraction increased in UN W only.

group	FGS	UP	GV	GFR	FF
	%	mg/24h	$\mu m^3 x 10^6$	ml/min	%
sham W	3±3	130±101	1.2±0.3	0.9±0.4	18±6
UN W	15±13*	255±145*	1.8±0.4*	1.5±0.3*	29±7*
sham WKY	0	50±11	1.0±0.2	0.9±0.2	21±6
UN WKY	0	57±15	1.4±0.2*	1.5±0.3*	20±7

values Mean±SD;*$p<0.05$, Mann Whitney U.

In summary, FGS resistance in WKY was found to be compatible with marked glomerular hypertrophy in the presence of a relatively low filtration fraction after UN.

INTERACTIONS OF LOW DENSITY LIPOPROTEIN WITH RAT MESANGIAL CELLS. J.Wasserman*, A.Santiago*, H. Holthofer*, M.Epstein*, D.Schlondorff. Albert Einstein College of Medicine, Bronx, N.Y.

Hyperlipidemia may contribute to the pathogenesis of glomerular sclerosis. We therefore studied binding and uptake of human low density lipoprotein (LDL) by cultured rat mesangial cells. In addition, effects of LDL on cell proliferation and PGE_2 synthesis were determined. At 4°C mesangial cells bound [^{125}I] LDL in a time-and concentration-dependent manner with half-maximal binding observed at 5ug/ml of LDL-protein. Binding was blocked by excess unlabeled LDL and by heparin. Binding and uptake of LDL at 37°C markedly exceeded that at 4°C, continued to increase even with longer periods of incubation and showed no saturability, consistent with uptake of LDL by mesangial cells. Furthermore mesangial cells showed positive fluorescence after incubation with DiI - LDL, a probe that becomes fluorescent after uptake only. LDL had a biphasic effect on mesangial cell proliferation as determined by [3H] thymidine incorporation: LDL at 10 ug/ml enhanced [3H] thy-midine uptake modestly, but significantly, whereas a progressive and marked inhibition occurred at LDL concentrations from 100 to 500 ug/ml. Although LDL at 10 and 100ug/ml significantly stimulated PGE_2 production, inhibition of PGE_2 synthesis by meclofenamate did not influence the effects of LDL on [3H] thymidine incorporation. We conclude that mesangial cells show specific binding and uptake of LDL and that high concentrations of LDL are toxic to mesangial cells. These findings may pertain to the pathogenesis of glomerular lesions in hyperlipidemia of renal disease.

RECEPTOR MEDIATED BINDING OF HUMAN LOW DENSITY LIPOPROTEIN (LDL) TO RAT MESANGIAL CELLS IN VITRO.
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Lipid deposition within mesangial cells is a feature of focal glomerulosclerosis and appears to be analogous to foam cell formation in atherosclerotic plaques. Mesangial cells share characteristics with smooth muscle cells and macrophages, the two cell types which give rise to vascular intimal foam cells by receptor mediated uptake and accumulation of LDL. In order to determine whether mesangial cells also possess lipoprotein receptors, cultured rat mesangial cells were exposed to I-125 labelled human LDL. Experiments were performed at 4°C to prevent ligand internalisation. Initial results demonstrated that mesangial cells bound human LDL and further studies have confirmed that binding is saturable and specific. Scatchard analysis suggested a single binding site (Kd 22.7 ug/ml). Under the same conditions, human fibroblasts bound rat LDL with considerably higher affinity (Kd 3.0 ug/ml). Binding to both cell types was competitively inhibited by heparin. Mesangial cells, unlike peritoneal macrophages, did not bind fluorescently labelled acetyl-LDL. These results suggest that mesangial cells possess LDL binding sites with features in common with the apo B/E receptor demonstrated on fibroblasts. Mesangial cell lipid accumulation may therefore be mediated by specific cell surface LDL receptors.

GLOMERULAR ANGIOTENSIN II (AII) RECEPTOR DEFICIT IN DIABETES: SPECIFICITY AND ROLE OF GUANOSINE NUCLEOTIDES. Barry Wilkes, Ira Pion* and Sabrina Silverman*. Div. of Nephrology/ Hypertension, Dept. of Medicine, North Shore Univ. Hospital and Cornell Univ. Medical College, Manhasset, NY 11030

The binding of ^{125}I-AII to glomeruli is reduced in hyperfiltering diabetic (DM) rats. Hyperfiltration is reversed when plasma AII is increased to levels which result in a normal number of occupied receptors. Several abnormalities in GTP metabolism have been reported in DM which may result in increased glomerular GTP. GTP is known to inhibit AII binding, but not binding by AII antagonists. We measured angiotensin binding sites in glomeruli isolated from rats with 1 wk untreated streptozotocin DM using the AII antagonist, ^{125}I-sar^{1}ile^{5}-AII (SAR). SAR binding sites which are independent of GTP were reduced by 34% in glomeruli from DM rats (control, 88.5±11.1x10^{6} vs DM, 58.1±8.1x10^{6} receptors/ glomerulus, P<.005). 24h urinary cGMP, and basal and ANF-stimulated cGMP production in isolated glomeruli were not different in control vs DM rats. The specificity of the AII receptor deficit for glomeruli was studied in separate experiments by comparing ^{125}I-AII binding to hepatic membranes in normal and DM rats. In contrast with DM glomeruli which had 27% fewer AII receptors (P<0.01), hepatic AII receptors were unaltered. We conclude that the AII receptor deficit in diabetes occurs in glomeruli but not hepatic membranes, and can not be explained by changes in guanosine nucleotides.

SHORT TERM EFFECT OF NIFEDIPINE IN STABLE CHRONIC RENAL FAILURE (CRF). J P Wight, A M El Nahas. Northern General Hospital, Sheffield, UK.

Calcium channel antagonists have been shown to reduce intraglomerular pressure and glomerulosclerosis in rats with CRF. We designed this study to explore the effect of Nifedipine (N) on renal haemodynamics in 5 patients with stable GFR (26±12ml/min, M±SD). GFR (51Cr-EDTA) and ERPF (131I-Hippuran) were measured by continuous infusion before and after a 100gm protein meal. Studies were done at time 0, after 1 month on N: 10mg thrice daily and after a further month without the drug. GFR and ERPF were not affected by the protein meal challenge on or off N. Nifedipine had no effect on GFR or ERPF. Mean blood pressure fell on N: pre: 115±13mmHg (M±SD), post: 103±20, p<0.01. However renal vascular resistance did not change significantly. These results confirm the loss of renal reserve in patients with CRF. They also show that in patients, as in experimental animals with CRF, calcium antagonists have no effect on GFR or ERPF. Clearly an effect on intraglomerular or tubular pressure cannot be excluded. Further studies are required to assess the potential of N in the management of patients with progressive CRF.

PROTEIN LOADING (P) AND EXERCISE INDUCE MICROALBUMINURIA (MA) IN TYPE I DIABETICS (DM) WITHOUT OVERT NEPHROPATHY (NE). James F Winchester, AB Chapman*, AS Boyle*, SE Mulroney*, L Rizell*, K Dawn*, R Eastman*. Georgetown University Medical Center, Washington, D.C.

Bicycle exercise (EX) has been used to induce MA (MA >15 and <180 ug/min predictive of overt NE) in DM without overt NE. Since P increases renal blood flow, we compared the effect of P and EX on albumin excretion rates (AER) in DM. AER determined by radioimmunoassay, were measured in 5 DM subjects (3m, 2f; age 37.5 ± 8.5 yrs; duration DM 17.5 ± 4.5 yrs) on 2 occasions, after a water load of 800 ml, followed by EX (20 min at 4.5 metabolic equivalents (METS), and a further 20 min at 6.0 METS), and oral P (80g mostly red meat), at timed (T, min) intervals from baseline (BL) were compared. Blood glucose was maintained at >100 and <350 mg/dl in both studies, and AER was normal (24 hr urine collection = 6 ± 3.9 ug/min) within 6 months prior to study in all subjects.

AER (ug/min)[a]

BL1	BL2	T120	T150	T180	T240	Protein load
9.1	7.4	34*	35.5*	36*	17.5	
x/÷	x/÷	x/÷	x/÷	x/÷	x/÷	
1.9	1.6	1.8	1.5	2.0	1.6	
BL1	**BL2**	**T40**	**T60**	**T80**	**T100**	**Exercise**
3.2	2.2	3.9	18.7*	5.7	3.1	
x/÷	x/÷	x/÷	x/-	x/÷	x/÷	
1.5	1.4	1.6	1.6	1.6	1.4	

BL1 & BL2 = 1st and 2nd void after water load, * significantly greater than BL by Student's T test and derived p values calculated after log transformation. [a] AER= geometric mean x/÷ confidence factor.

Significant increase from BL was found in both groups (2p = 0.02). Correlation was found between both BL conditions (r >0.99, p <0.002) and continued within studies from BL to peak AER (r >0.966, p < 0.007). P may be an alternative provocative method for eliciting MA in DM, an early predictor of NE, and of use in DM at risk from EX.

INCREASED RELEASE OF ATRIAL NATRIURETIC PEPTIDE (ANP) BY THE ATRIA OF RATS WITH EXPERIMENTAL RENAL FAILURE. Norman L.M. Wong, Deja Huang,* and Eric F.C. Wong.* Dept. of Medicine, University of British Columbia, Vancouver, Canada.

Plasma ANP levels are raised in chronic renal failure (CRF). We have studied the spontaneous release of ANP from the isolated atria in rats with (n=11) and without CRF (n=8) (5/6 nephrectomy 3 weeks before study) with a modified Langendorff preparation. 24 hour clearances were performed prior to the perfusion studies. Plasma BUN was elevated in CRF rats (BUN 65±7 vs 16±1 mg %). Overall glomerular filtration rate was 2.36±0.06 ml/min in normal as opposed to 0.85±0.11 ml/min in CRF rats ($p<0.01$ compared to normal). Fractional excretion of sodium was significantly higher in CRF rats (FE Na 0.52±0.01 vs. 1.68±0.21% $p<0.01$). Plasma ANP was 96±4 pg/ml in normal rats and 108±4 pg/ml in rats with CRF ($p<0.05$ compared to normal). The isolated right atrium was perfused with Tyrode's solution for 100 min at 0.5 ml/min. The effluent was collected with a fraction collector every five minutes for the determination of IR-ANP. The results showed that ANP secretion by the CRF rat's atrium was significantly higher (ANP release 18±3 vs. 10±1 pg/min/mg tissue $p<0.01$). A significant positive correlation was found between ANP release by CRF atria and plasma BUN ($y=0.33X-5.0$ $r=0.79$ $p<0.01$). A correlation was also observed between ANP release and fractional excretion of sodium ($y=0.74X+0.5$ $r=0.83$ $p<0.01$). These results suggest that ANP played an important role in sodium homeostasis in rats with reduced renal mass.

ROLE FOR PLATELET ACTIVATING FACTOR IN THE HETEROLOGOUS PHASE OF NEPHROTOXIC SERUM NEPHRITIS. J Yoo*, D Schlondorff, J Neugarten. Montefiore Med Ctr and AECOM, Bronx, NY.

We have previously demonstrated in the rat that intrarenal administration of platelet activating factor (PAF), in a dose that does not alter systemic hemodynamics, reduces glomerular filtration rate (GFR) and effective renal plasma flow rate (ERPF) and increases the fractional clearance of protein. Increased glomerular synthesis of PAF has been demonstrated in nephrotoxic serum nephritis (NSN). The present studies were undertaken to evaluate the hypothesis that enhanced endogenous production of PAF plays a role in the impaired glomerular permselectivity and renal hemodynamic alterations of the heterologous phase of NSN in the rat. Three groups of rats were studied: IV infusion of nephrotoxic serum (NTS), IV infusion of NTS in rats pretreated with the PAF receptor antagonist WEB 2086, and IV WEB 2086 alone. Baseline measurements of GFR, ERPF and proteinuria were carried out during a control period and repeated 1 hour after the experimental maneuvers outlined.

	NTS (n=8)	WEB+NTS (n=7)	WEB (n=5)
GFR (ml/min)	1.46±0.43*	2.52±0.2	2.69±0.15
RPF (ml/min)	3.80±0.68*	5.12±1.04*	6.51±1.37
Proteinuria	0.56±0.13*	0.24±0.09	0.11±0.01

*P<0.05 vs. pre-infusion values.

Pretreatment with WEB ameliorated NTS-induced renal functional impairment and proteinuria. These data suggest that enhanced endogenous renal production of PAF plays a major role in the heterologous phase response of the kidney to nephrotoxic serum.

ONCOTIC PRESSURE REGULATES THE LEVELS OF ALBUMIN (Alb) mRNA AND APOLIPOPROTEIN B (ApoB) mRNA IN CULTURED RAT HEPATOMA CELLS (H4IIE). A.Yamauchi*, S.Yamamoto*, Y.Fukuhara*, Y.Orita*, T.Kamada*, T.Noguchi*, and T.Tanaka*. Osaka Univ.Med.Sch., Osaka, Japan. (intr. by J.S. Handler).

We recently reported that albumin gene transcription is enhanced in liver of nephrotic rats (Am.J.Physiol. 254:E676-E679, 1988). To gain further understanding of the regulation of hepatic protein synthesis in nephrosis, we examined the effect of changes in medium oncotic pressure on Alb mRNA and ApoB mRNA levels in H4IIE cells. The addition of albumin (A.A.) or dextran (Dex. MW 6 X 10^4)(A.D.) to the medium reduced Alb mRNA and ApoB mRNA levels in a concentration-dependent manner, while the level of β-Actin mRNA was not changed.

Conc.	(A.A)	mRNA levels		(A.D)		
g/dl	Alb	ApoB	β-Act	Alb	ApoB	β-Act
0	100±3	100±2	100±7	100±6	100±7	100±5
2	80±4	88±8	95±5	85±1	84±6	96±3
4	69±7*	82±5*	102±8	82±2*	75±14**	102±9
8	61±9*	75±4*	110±9	71±3*	68±10*	110±11

* $p<0.01$ vs 0 g/dl, ** $p<0.05$ vs 0 g/dl

When cells were first cultured in medium with 8 g/dl of Dex, then switched to medium with lower concentrations of Dex, Alb mRNA and ApoB mRNA levels were increased in inverse proportion to the final concentration of Dex. These increases were diminished in the presence of actinomycin D. We conclude that oncotic pressure is an important factor in the regulation of Alb and ApoB gene transcription.

MODULATION OF MESANGIAL CELL PROLIFERATION AND HEPARIN-INDUCED GROWTH INHIBITION BY MESANGIAL EXTRACELLULAR MATRIX. M. Yoshimoto*, T. Homma*, I. Ichikawa, R. L. Hoover*. Departments of Pediatrics and Pathology, Vanderbilt University Medical Center, Nashville, TN.

It is currently believed that the inhibitory effect of heparin on progression of glomerular sclerosis is related to its antiproliferative effect on mesangial cells (MC) and that MC proliferation is modulated by various extracellular matrix (ECM) constituents. We examined the effects of ECM produced by MC (MCM) on MC proliferation. MCM coating of 24 well plastic culture dishes was obtained by treati confluent MC with either 20mM NH_4OH (A-MCM) o 0.5% Triton X-100 (T-MCM). MC were then seeded on the MCM at a density of 10^4/ml/well. Plating efficiency, assessed as MC counts after overnight incubation, did not differ between non-treated (C) and MCM-coated dishes. MC proliferation, however, was increased by MCM, i.e., 139% and 141% of C on A-MCM and T-MCM, respectively, after 2 days in culture. Saturation density was also higher on MCM, i.e., 122% and 142% of C on A- and T-MCM, respectively (n=4). In contrast, gelatin, fibronectin and collagen type I coating of dishes had no effect on MC proliferation, although more cells attached initially [133%, 135% and 145% of C, respectively (n=4)]. The antiproliferative effect of heparin (5 μg/ml) was attenuated from 14.5% inhibition on C to -0.9% and -6.0% on A- and T-MCM, respectively, following 2 day incubation with heparin (n=4). By the end of 6 day, percent growth inhibition was 13.1% on C while it was 5.0% and 4.9% on A- and T-MCM, respectively (n=4). ED_{50} increased from 8±3 on C to 20±4 μg/ml on T-MCM (n=4).

These observations indicate that the extracellular matrix of mesangial cell origin promotes mesangial cell proliferation and attenuates the effect of heparin, and may thus contribute to the deterioration of glomerular architecture.

AGING ANALBUMINEMIC RATS FAIL TO DEVELOP FOCAL GLOMERULAR SCLEROSIS (FGS). R Zatz, EBA Prado*, MJBA Prado*, MM Santos* and CK Fujihara* - Univ. of São Paulo Med. School, São Paulo, Brazil.

In the Analbuminemic Rat (ANALB), a mutant Sprague-Dawley (SD) rat with no serum albumin, cholesterol (Ch) and triglyceride levels are persistently elevated, reaching over twice those seen in SD rats. Glomerular filtration of proteins seems to be greatly enhanced in these animals in comparison with SD. Since both hyperlipidemia and mesangial overload have been implicated in the genesis of FGS, we investigated whether ANALB rats are proner than SD to the development of FGS. Glomerular hydraulic pressure (Pgc) and Ch were determined in 6 SD and 6 ANALB rats at 3 months of age. Proteinuria (Prot) was measured at 6, 12 and 18 months in 8 SD and 9 ANALB rats, while histologic examination of the kidneys was performed at 18 months. Results (Mean±SE,*=p<0.05 Pgc in mmHg, Prot in mg/24h, Ch in mg/dl):

	Pgc	Ch	Prot	%FGS
Age	3m	3m	18m	18m
SD	53±1	51±1	134±15	10±1
ANALB	45±1*	120±3*	27±8*	1±0.6*

In addition, filtration of proteins, while remaining unaltered with aging in ANALB rats, appeared greatly increased in 18-month SD rats. Thus, despite persistent hypercholesterolemia and increased filtration of macromolecules, ANALB rats fail to develop even aging FGS. These results indicate that hypercholesterolemia and mesangial overload are incapable of initiating per se, at least in this model, the process of FGS. Other factors, possibly including glomerular hemodynamic alterations, must be involved in the genesis of this process.

REGULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR DURING RENAL GROWTH. M.T. Behrens,* A.L. Corbin* and M.K. Hise. Univ. of Maryland Medical School, Div. of Nephrology, Baltimore, MD.

Previous studies (KI 33:387, 1988) have demonstrated that circulating EGF plays a critical role in the growth response of the kidney following uninephrectomy (UNx). The purpose of this study was to examine the regulation of EGF receptors following two growth stimuli-UNx and folic acid (FA) administration. Brush border (BBM) and basolateral (BLM) membranes were prepared from rat kidney cortex 24 hrs following sham nephrectomy (SNx), UNx or 250 mg/kg FA intraperitoneal. Binding of 0.2 nM ^{125}I-EGF was determined in the presence and absence of 400 nM unlabeled peptide.

Binding of EGF was present on BLM but not on BBM. Affinity labeling documented the presence of the receptor. Other growth factors did not displace activity indicating ligand specificity. High affinity binding witha K_D of 6.2×10^{-11} M and a 10 fold lower K_D were evident from Scatchard analysis. Binding of EGF to control, vehicle injected and SNx animals did not differ at 24 hrs. Binding of 5 nM EGF to BLM of SNx animals at 24 hrs averaged 228 ± 13 cpm/150 ug protein and did not differ in UNx animals; binding to FA BLM averaged 410 ± 41 cpm/150 ug protein (n=4, p <.005).

These data demonstrate: 1) there are high affinity EGF binding sites on BLM prepared from rat kidney cortex; 2) following a stimulus for cell hypertrophy in the kidney, receptors are not down regulated; and 3) during mitogenesis in the kidney, high affinity EGF receptors are up regulated on BLM.

EFFECT OF ATP AND PHOSPHORYLATION POTENTIAL ON THE FUNCTION OF THE SODIUM PUMP IN DOG CORTICAL TUBULES. Hélène Ammann, Josette Noël, Yvan Boulanger, Patrick Vinay, André Gougoux. Nephrology Service, Notre-Dame Hospital and Groupe de recherches en transport membranaire, Université de Montréal, Canada.

In order to examine the potential effect of the cell [ATP] and phosphorylation potential on the function of the sodium pump in intact renal cells, the [adenylates] of cortical tubules was first modified by a 20 min incubation with effectors as described below (mM)

	ROTENONE 1 µM	FRUCTOSE 10mM	NIL	AMP 2.5 mM	ADENOSINE 2.5 mM
ATP	1.8	3.0	3.7	5.6	5.7
Pi Potential	.19	.63	.57	1.2	1.1

The tubules were then incubated in Krebs Henseleit saline using two different [Pi] (5 and 10 mM), and 10 mM glutamine with either 10 mM Lactate (low ATP) or Pyruvate (high ATP) as substrates. Tubular respiration was measured before and after application of a sub maximal dose of nystatin, added in order to impose an identical increment of work to the Na^+K^+ ATPase in all conditions. Ouabain was then applied to inhibit the pump. No significant effect of [ATP] or Pi potential on cell repiration was noted above normal [ATP]. In a second group of experiments, tubules were permeabilized to ATP with small amount of digitonin. The respiration was measured before and after introduction of increasing [Mg-ATP]. A fixed quantity of Na^+ was then introduced and ouabain was again applied. A fixed ouabain-sensitive respiration was observed at all [ATP] . These results demonstrate 1) that the Na^+K^+ ATPase is not regulated by [ATP] in intact tubules when the [ATP] is at or above the normal concentration; 2) that the stoichiometry Na^+/ATP is probably constant when the ATP/ADP ratio, the Pi potential and/or the free energy released from ATP hydrolysis are altered.

DISSOCIATION OF AMMONIUM EXCRETION AND TUBULAR GLUTAMINE OXIDATION IN OBSTRUCTIVE NEPHROPATHY. C Bennett* and S. K. Mujais Northwestern U and VA Lakeside, Chicago IL.

The contribution of impaired intrinsic tubular glutamine (Gln) utilization to the decreased urinary NH_4 excretion (UNH_4V) of obstructive nephropathy is not clear. We examined, in parallel, UNH_4V and proximal convoluted tubule $^{14}CO_2$ production from U-^{14}C-Gln by SD rats at various times after ureteral obstruction (Obs). An early and major decrease in UNH_4V (Obs: 34±12 vs Sham: 144±40 nEq/min, p<0.001) was observed 24 h after obs in proportion to the diminution in GFR (Obs: 162±24 vs Sham: 1182±122 µL/min, p<0.001). In contrast, no change in $^{14}CO_2$ production was observed after 24 h (135±23 vs 123±15 pmol/mm/min) or 48 h (127±12 vs 124±21) of obs, while a moderate decrease (69±4 vs 142±11, p<0.001) occured with prolonged obs (4 days). We conclude that the early decrease in UNH_4V after ureteral obstruction is not due to impaired intrinsic tubular Gln utilization, but to other determinants of NH4 excretion. In chronic obstruction, decreased NH_4 excretion is related to impaired tubular Gln utilization.

ROLE OF KIDNEY FATTY ACID BINDING PROTEIN (kFABP) IN PROXIMAL TUBULAR FATTY ACID (FA) METABOLISM. S.C. Borkan*, K.T. Lam*, P. Brecher* and J.H. Schwartz. Boston University Medical Center and Boston City Hospital, Boston, MA.

The function and intra-cortical location of a recently identified, 15.5 kDa kFABP are unknown. To localize kFABP, proximal tubules (PT) were separated from glomeruli and small vessels with a magnet after *in-situ* renal perfusion with $FeSO_4$. Western blot analysis was then performed on the cytosolic protein fractions of tissue homogenates (n=3) and showed kFABP to be predominantly in PT. To study the role of kFABP in tubular FA oxidation, a model of kFABP depletion was developed (uninephrectomized (Unx) rats treated with DOC and 0.9% NaCl drinking water (EXP, n=6) resulted in a >90% decrease in kFABP content) and compared with kFABP replete, Unx controls (CTL, n=3). Oxygen consumption (QO_2) was measured in isolated PT segments under four conditions: no substrate, 0.5 mM oleate alone and/or 4 mM lactate. Data are expressed as % of QO_2 without substrate. Oleate increased QO_2 by 33±3% in CTL and 9±4% in EXP. Lactate produced identical increments in QO_2 (30±2 vs 31±6%). Oleate+lactate produced a 70±10% increase in QO_2 in CTL, but only a 52±8% rise in EXP. Nystatin, which maximally stimulates Na^+ transport, resulted in an 80±10% increase in QO_2 in oleate-exposed CTL vs 11±7% in EXP. These results suggest that: (1) kFABP might facilitate tubular transport and mitochondrial oxidation of FA; (2) carbohydrate supports greater metabolic rates than FA in kFABP-depleted PT; (3) mitochondrial FA oxidation, not Na^+ delivery, is the rate limiting step for Na^+ transport only in the kFABP-depleted PT.

ANGIOTENSIN II STIMULATES AMMONIAGENESIS IN CANINE RENAL PROXIMAL TUBULAR SEGMENTS. M.C. Chobanian, C.M. Julin,* T.L. Goodfriend.* Univ. of Wisconsin Sch. of Med., Dept. of Peds. and Int. Med., Madison, WI.

Angiotensin II (ANG II) is known to regulate metabolic and transport processes in the proximal tubule, including stimulation of gluconeogenesis and increasing HCO_3 transport (Liu FY and Cogan MG, J. Clin. Invest. 80:272, 1987). To determine whether ANG II affects ammoniagenesis in the proximal tubule, we measured ammonia production in suspensions of canine renal proximal tubular segments incubated with 10 mM L-glutamine ± 10^{-6}M ANG II. Ammonia production was a linear function of time for 120 min and averaged 511.1 ± 33.5 and 590.2 ± 36.6 µmol/gram protein/120 min in the absence and presence of ANG II respectively ($p < .005$). Half-maximal stimulation of ammonia production occurred between 10^{-7}M and 10^{-8}M ANG II. The inactive ANG II analogue, DES-1,2-ANG II (10^{-6}M), had no effect on NH_3 production. The ANG II antagonist (10^{-6}M), SAR-1-ILEU-8-ANG II, blocked the effect of ANG II on ammonia production. Amiloride (5 x 10^{-4}M), H-7 (PKC inhibitor, 4 x 10^{-5}M), and HA1004 (cAMP protein kinase inhibitor, 4 x 10^{-5}M) had no effect on the action of ANG II on ammoniagenesis. Decreasing the pH of the incubation media from 7.4 to 7.0 had no effect on ANG II stimulated ammonia production, whereas at pH 7.6 the stimulatory effect of ANG II on ammoniagenesis was lost. We conclude that ANG II stimulates ammonia production in canine renal proximal tubular segments. This effect appears to be mediated via the ANG II receptor. The requirement for high concentrations of ANG II may be related to in vitro degradation of the peptide. In vitro ANG II stimulated ammoniagenesis in proximal tubular segments, under normal and acidotic conditions, could reflect a role in vivo for ANG II in the regulation of renal acid-base metabolism.

EFFECT OF AMILORIDE ON CITRATE TRANSPORT IN RABBIT PROXIMAL CONVOLUTED TUBULES AND BRUSH BORDER MEMBRANE VESICLES. Steve Brennan, J.Michael Freiberg, and Wadi N. Suki. Baylor College of Medicine, Renal Section, Houston, Texas.

The relative importance of intracellular vs lumenal pH in regulation of citrate transport (Jcit) in the proximal convoluted tubule (PCT) is controversial. The purpose of the present studies was to use amiloride (Amil) to clarify the role of lumenal pH on Jcit in rabbit PCT and in renal brush border membrane vesicles (BBMV). Isolated PCT were perfused and bathed with defined solutions using standard techniques, and disappearance of ^{14}C-citrate (1mM) was used to measure Jcit. In the absence of Amil, a slight time-dependent decrease in Jcit was seen (ΔJcit=-0.58+0.30 pmol/mm/min). The addition of 1mM Amil caused a significant decrease in Jcit (ΔJcit=-2.34+0.36 pmol/mm/min, $p<0.005$ vs time control). Since Amil inhibition of Jcit may have been due either to the relative alkalinization of the lumen resulting from inhibition of Na-H exchange, or to a direct, nonspecific inhibition of that Na-dependent cotransporter, further studies of Jcit were performed using BBMV. Na dependent Jcit by the BBMV exhibited a 10-fold overshoot above equilibrium levels. Compared to the 5 sec Jcit in the absence of Amil (661+44 pmols/mg protein), there was a tendency to a rise in the presence of 1mM Amil (776+ 75 pmol/mg protein, n=5, $0.05 > p > 0.1$). Based on these findings, we conclude that the failure of Amil to inhibit Jcit in BBMV suggests that the observations in the perfused PCT result from the effect of Amil to alkalinize lumenal pH, this in turn causing Jcit to decline, as previously shown.

CULTURED KIDNEY CELLS POSSESS A RETROENDOCYTIC PATHWAY FOR INSULIN. D. Dahl*, T. Tsao* and R. Rabkin. Stanford University and VA Medical Center, Palo Alto, CA.

Filtered insulin is absorbed in the proximal tubule by receptor-mediated endocytosis. Following endosomal acidification, insulin dissociates from its receptor and is transported to its site of degradation. The receptor recycles to the cell membrane. Additional pathways for endocytosed proteins have been described in other cell types. One of these pathways is retroendocytosis (endocytosis followed by exocytosis of intact protein). To confirm that this pathway exists in renal epithelium we studied insulin processing in the proximal epithelium-like opossum kidney cell line. Monolayers were exposed to ^{125}I-insulin (4 x 10^{-10}M) for 30 mins, then surface bound insulin was removed by acid washing, and intracellular radioactivity released into fresh media was analyzed by gel filtration chromatography. At 60 min, 18±3% of the radioactivity in the control cell media eluted with intact insulin and 80±3% represented insulin degradation products. In the presence of 0.1 mM chloroquine (CQ), which raises endosomal and lysosomal pH, 39±2% of the radioactivity represented intact insulin and 54±2% degradation products. The CQ effect is likely due to endosomal alkalinization which impairs uncoupling of insulin from receptors destined to recycle to the cell surface. Further experiments were conducted with 2 mM bacitracin (BAC), a proteolytic inhibitor which blocks intracellular insulin degradation. Controls released 12±2% of internalized radioactivity as trichloroacetic acid precipitable ^{125}I-insulin and 64±3% as degradation products. In the presence of BAC release of intact insulin increased to 27±2% and release of degradation products fell to 51±4%. We conclude that cultured renal epithelial cells possess both retroendocytic and degradative pathways for insulin, and inhibition of the degradative pathway with CQ or BAC diverts insulin to the retroendocytic pathway.

SIMILAR EFFECTS OF ARGININE VASOPRESSIN (AVP) AND GLUCAGON (GLU) ON Na^+, Cl^-, K^+, Mg^{++}, Ca^{++} TRANSPORT IN CORTICAL (cTAL) AND MEDULLARY (mTAL) THICK ASCENDING LIMBS OF MOUSE NEPHRON. C. de Rouffignac, M. Wittner, A. Di Stefano, N. Roinel, C. Bailly, C. Amiel, P. Wangemann, R. Nitschke, R. Greger (intr. by T.Anagnostopoulos). CEA, DB/SBCe Saclay (FRANCE) ; INSERM U251, Paris (FRANCE) ; Inst. Physiologie, Freiburg (R.F.A).

The effects of AVP and GLU (10^{-10} and $1.2.10^{-8}$ $mol.l^{-1}$) on transepithelial net fluxes (J_{Na^+}, J_{Cl^-}, J_{K^+}, $J_{Ca^{++}}$, $J_{Mg^{++}}$) were investigated in isolated perfused cTAL and mTAL. The net fluxes were determined by electron probe analysis of the perfused and collected fluids. Transepithelial potential difference (PD_{te}) and resistance (R_{te}) were recorded. In cTAL, AVP (n = 11 tubules) and GLU (n = 8) significantly increased $J_{Mg^{++}}$ (AVP: 0.39 ± 0.08 to 0.58 ± 0.10 ; GLU:0.51 ± 0.08 to 0.84 ± 0.08 $pmol.min^{-1}.mm^{-1}$) and $J_{Ca^{++}}$ (AVP: 0.86 ± 0.13 to 1.19 ± 0.15 ; GLU:0.52 ± 0.13 to 1.34 ± 0.30 $pmol.min^{-1}.mm^{-1}$). J_{Na^+} and J_{Cl^-} were not significantly altered by AVP, whereas they were slightly increased by GLU. Neither PD_{te} nor R_{te} were changed by either AVP or GLU. In the post-experimental period, however, a significant decrease in J_{Na^+}, J_{Cl^-} and PD_{te} was noted with both hormones. In mTAL, Mg^{++} and Ca^{++} transports were close to zero ; AVP (n = 8) and GLU (n = 9) elicited no effect on these transports ; both hormones significantly increased J_{Na^+}, J_{Cl^-} and PD_{te}, and decreased R_{te}. No significant effect of either AVP or GLU on J_{K^+} in cTAL and mTAL was noted. In conclusion , in the mouse cTAL: AVP and GLU strongly stimulated Mg^{++} and Ca^{++} transports ; an effect of these hormones on Na^+ and Cl^- transports could not be excluded. In the mouse mTAL: no significant Mg^{++} and Ca^{++} transports were detected ; AVP and GLU did not modify this situation whereas they strongly stimulated Na^+ and Cl^- transports.

REGULATION OF ATP CONTENT IN MICRODISSECTED RAT CORTICAL COLLECTING TUBULES (CCT). Mark A. Dillingham and Carolyn Burke*. Univ. of Colo. Hlth. Sci. Ctr., Denver, Colorado.

Active transport presumably fueled by ATP occurs within the mammalian CCT. However, factors regulating ATP content of the CCT remain incompletely characterized. We used a chemiluminescence technique to measure ATP content in individual microdissected rat CCT. Rotenone, an inhibitor of oxidative phosphorylation, exerts a significant dose dependent depletion of ATP with an ID_{50} OF 10^{-7}M. Iodoacetate, an inhibitor of glyceraldehyde 3-P dehydrogenase and glycolysis, also results in a dose dependent decrease in ATP in CCT (ID_{50} $5x10^{-5}$M). Addition of pyruvate, a substrate which does not require glyceraldehyde 3-P dehydrogenase for metabolism, restores ATP levels to baseline in iodoacetate treated tubules. To determine if substrate enhancement affects ATP levels, CCT's were exposed to adenosine (10^{-3}-10^{-7}M) and significant increases in ATP occurred at doses less than 10^{-4}M. To see if alterations in ATP utilization change ATP content, CCT's were exposed to a Na-K ATPase inhibitor, ouabain (10^{-3}-10^{-7}M) which decreased ATP levels at 10^{-3} and 10^{-4}M and increased them at 10^{-6} and 10^{-7}M. To assess cell calcium regulation of ATP, tubules were exposed to ionomycin (10^{-6} and 10^{-7}M) which significantly decreases ATP by 20-40%. Together, these results suggest that in CCT both oxidative phosphorylation and glycolysis generate ATP. However, oxidative phosphorylation is the more important process since oxidation of pyruvate without glycolysis maintains ATP in CCT while glycolysis without oxidative phosphorylation does not. Further, substrate augmentation with adenosine and blocking Na/K ATPase with low dose ouabain increases ATP levels while higher doses of ouabain and increasing cell calcium with ionomycin decreases ATP in the CCT.

REGULATION OF RENAL PROXIMAL TUBULE (RPT) METABOLISM BY BICARBONATE/CO_2. Kathleen G. Dickman* and Lazaro J. Mandel. Duke Univ. Med. Ctr., Dept. of Cell Biology, Durham, North Carolina.

Bicarbonate is an important component of physiological salines due to its unique buffering properties and role in renal transport processes. The present study reports yet a third role for bicarbonate/CO_2 - that as a regulator of renal metabolism. Rabbit RPT were incubated in HAM/DMEM supplemented with either 15 mM HEPES (-bicarb) in air (pH 7.4) or 15 mM HEPES and 15 mM $NaHCO_3$ (+bicarb) with 5% CO_2 (pH 7.4). The effects of these 2 treatments on O_2 consumption (QO_2, nmol/min·mg), lactate production (nmol/min·mg) and ATP and K+ contents (nmol/mg) were examined. (-)bicarb RPT exhibited a 50% reduction in basal QO_2 (16±1 vs 31±1) which was due to selective inhibition of ouabain-sensitive QO_2 (5±1 vs 16±1). Nystatin addition stimulated basal QO_2 in (+)bicarb RPT but had no effect in (-)bicarb RPT, indicating that the loss of ouabain-sensitive QO_2 was not due to decreased NA entry. Rather, inhibition appears to be localized to the mitochondria since FCCP-uncoupled QO_2 was severely reduced (27±3 vs 79±13) in (-)bicarb RPT. Consistent with inhibition of QO_2, lactate production was stimulated in (-)bicarb RPT (9±1 vs 0.5±0.3), while ATP content was reduced (6±1 vs 12±1). Despite large decreases in both ATP content and QO_2, K+ content was only slightly reduced in (-)bicarb RPT (314±14 vs 343±9) suggesting that K+ permeability was decreased in the absence of bicarbonate. We conclude that incubation of RPT in the absence of bicarbonate/CO_2 1) inhibits oxidative metabolism; 2) stimulates glycolysis; and 3) educes K+ permeability.

EFFECT OF CYSTINE LOADING ON RENAL MEMBRANE TRANSPORT AND METABOLISM. John Foreman and Linda Benson, Medical College of Virginia, Department of Pediatrics, Richmond, VA.

The pathophysiology of the Fanconi Syndrome associated with cystinosis remains enigmatic. We have previously shown that renal tubule cells incubated with cystine dimethylester (CDM) accumulate large amounts of cystine which results in impaired uptake of solute, an in vitro equivalent of cystinosis. This model was used to examine rat renal brushborder membrane transport to determine if this was the site of the impairment in solute transport. Cortical tubule fragments were incubated with 2 mM CDM for 10 min. Intracellular cystine rose from undetectable levels to 42 μmoles/mg protein. This preincubation had no significant effect on 0.06 mM proline uptake at .25, 1 and 20 min by brushborders isolated from the treated tubules. Directly incubating brushborder membranes with CDM also had no significant effect on solute transport.

To test whether raised intracellular cystine levels affect metabolism, isolated tubules were preincubated with 2 mM CDM for 10 min and resuspended in fresh buffer containing ^{14}C labelled substrates. Cystine loading markedly reduced CO_2 generation from lactate, butyrate, and succinate compared to control tubules preincubated in buffer alone. To study O_2 consumption, tubules were preincubated in 2 mM CDM for 10 min and resuspended in fresh buffer for O_2 measurements. O_2 consumption was also markedly reduced by raising intracellular cystine. The results indicate that raising intracellular cystine impairs cell metabolism. It appears that this, and not an alteration in the luminal membrane itself, is responsible for the decreased solute transport.

EFFECTS OF ALPHA ADRENERGIC AGONISTS ON INTRACELLULAR AND INTRAMITOCHONDRIAL pH IN RAT PROXIMAL NEPHRONS. Frank A. Gesek* and Anton C. Schoolwerth. Dept. of Medicine and Physiology, Medical College of Virginia, Richmond, VA.

Alpha adrenoceptors have been shown to stimulate ^{22}Na uptake by the luminal Na/H antiporter. Rat proximal nephron segments were used to examine α-adrenoceptor alterations in Na/H exchange by monitoring intracellular (pH_i) and intramitochondrial pH (pH_m). To obtain pH_i, tubules were loaded with the fluorescent probe, BCECF-AM (10 μM) in a HCO_3-free Na buffer. The intracellular distribution of the weak acid [2-^{14}C]DMO was used to calculate pH_m, using values of medium pH, pH_i, cell volume and matrix content. The α_2-adrenoceptor agonists clonidine (CLON) and B-HT 933 (BHT), the α_1-agonist cirazoline (CIR) and the mixed agonist norepinephrine (NE) all produced a dose-related increase in pH_i. A maximum increase was observed at 1 μM, with changes occurring in <1 min. Compared to control, CIR increased pH_i 0.17$\pm$.01, CLON 0.16$\pm$.01, BHT 0.18$\pm$.01 and NE 0.15$\pm$.01 ($p<.05$). Pretreatment with the Na/H inhibitor ethylisopropyl amiloride (EIPA, 10 μM) or 0.1 μM of the adrenoceptor antagonists (prazosin, yohimbine and idazoxan) blocked agonists alterations in pH_i. The agonists produced no effect on pH_m. In summary, both α_1 and α_2 adrenergic agonists increased pH_i in a dose-dependent manner in proximal nephron segments, with no effect upon pH_m. Agonists responses were blocked by receptor antagonists and EIPA, and reflect changes in pH_i due to α-adrenoceptor stimulation of the Na/H antiporter.

THE PATHWAY OF AMMONIAGENESIS IN LLC-PK1 CELLS. G.Gstraunthaler and W.Pfaller (intr. by Joseph S. Handler) University of Innsbruck, Austria.

The LLC-PK1 porcine renal epithelial cell line, which retained several properties of the proximal tubule, has been used to study ammoniagenesis and its regulation by metabolic acidosis in vitro. As previously shown (Pflügers Arch.411:R90,1988), LLC-PK1 epithelia, grown in conventional monolayer technique, clearly responded to extracellular pH-changes with an adaptive increase in both L-glutamine (GLN) uptake and NH3 production by 60%.

Under control conditions (pH 7.6) as well as in metabolic acidosis (pH 7.0), rates of GLN uptake and NH3 generation displayed a ratio of 1:1. In addition, the adaptive increase in GLN uptake and NH3 generation was paralleled by an increased equimolar production of L-alanine (ALA). Thus transamination appears to be the main ammoniagenic pathway in these cells, resulting in the formation of ALA by glutamate-pyruvate transaminase (GPT) instead of a second NH3, formed by glutamate dehydrogenase (GLDH). Analysis of the key enzymes in renal ammoniagenesis, phosphate-dependent glutaminase (PDG) and GLDH, revealed no changes in enzyme activities up to 72 h of pH-adaptation. GPT activity even slightly decreased during the adaptation period as compared to controls.

The LLC-PK1 ammoniagenic response thus reflects the situation in the dog, where, in contrast to the rat, PDG and GLDH remain unchanged and the metabolic flux from glutamate to α-ketoglutarate is performed via transamination by GPT rather than deamination by GLDH. The possible activation of α-ketoglutarate dehydrogenase and its role in the stimulation of ammoniagenesis in metabolic acidosis in LLC-PK1 cells remains to be elucidated.

OXYGEN CONSUMPTION (QO_2) IN THE ISOLATED PERFUSED KIDNEY (IPK). J. Girone,* A. Besarab, J. Caro,* A. De Guzman.* Thomas Jefferson Univ., Divsions of Nephrology & Hematology, Philadelphia, PA.

Most studies of QO_2 in the IPK have used cell-free media. Such kidneys do not produce erythropoietin (EPO). Red cell rat IPK does. We measured QO_2, tubular Na reabsorption (TNa), & EPO in red cell IPK at varying oxygen tensions (pO_2), since either pO_2 or QO_2 may be determinants for EPO synthesis as well as TNa.

We perfused at high (>75) or low (<75 torr) pO_2 and with or without filtration (BSA 10.5-11 g/dl) at hct of 12-17 vol%. GFR, RBF & O_2 delivery (DO_2) were also measured.

In the initial experiments, kidneys were perfused at a pO_2<75 with (N=11) or without (N=9) filtration for 5 hrs. QO_2 did not change, 5.4 vs. 4.6 uM/min/g. TNa decreased from 79 to 0. EPO production was equal 3.1 $\pm$ 0.3 vs 2.5 $\pm$ 0.3.

In the second set of experiments 37 kidneys were perfused at pO_2<75 or >75, and 177 clearance periods from 60 to 240 min were analyzed. Stepwise linear regression was performed. For pO_2>75, QO_2 depended on TNa only ($y = 2.87 + .023$ TNa, $R^2 = .22$, $p<0.01$). For pO_2<75, QO_2 was independent of TNa but varied inversely with pO_2 ($y = 5.69 + .15$ RBF/g $- .06pO_2$, $R^2 = .41$, $p<0.01$). Intercepts of the 2 lines were significantly different ($p<0.05$).

We conclude: 1) TNa is an important determinant of QO_2 at high pO_2 but not under low pO_2 where EPO synthesis occurs; 2) QO_2 processes are likely to be different under high and low pO_2; 3) QO_2 increases with decreasing pO_2 under low pO_2, but the metabolic step remains to be identified.

EFFECTS OF GLUCOSE ON ANGIOTENSIN II (AII) AND ARGININE VASOPRESSIN (AVP)-STIMULATED INOSITOL PHOSPHATE RELEASE IN CULTURED RAT MESANGIAL CELLS (MC). N.J. Guzman, F.T. Crews and C.C. Tisher. Dept. of Pharmacology & Div. of Nephrology, Univ. of Florida, Gainesville, Florida

Activation of the polyol pathway leading to intracellular accumulation of sorbitol and depletion of myo-inositol has been described in glomeruli of diabetic rats and in rat MC exposed to high levels of glucose. To determine if these changes have any effects on receptor-mediated phosphoinositide (PI) hydrolysis, cultured rat MC were incubated in media containing 5.5 (control) or 28mM glucose, and both AII (100nM) and AVP (10nM)-stimulated release of ^3H-inositol phosphate (IP) was measured using anion exchange chromatography. After one week in media containing 28mM glucose, maximal AII and AVP stimulation of IP release was decreased by 19±5% ($p<0.005$) and 18±5% ($p<0.01$) of controls, respectively. When MC were exposed to 50mM glucose, maximal AII stimulation of IP release was decreased by 55±3% ($p<0.001$). Moreover, addition of myo-inositol (490µM) to the culture media prevented the decrease in agonist stimulated IP release seen after incubation in high glucose media. In summary, rat MC exposed to high glucose concentrations for a period of one week show decreased PI hydrolysis and second messenger IP release in response to the known mesangial cell constrictor agonists, AII and AVP. We conclude that this abnormal response most likely reflects the effects of depletion of intracellular myo-inositol levels due to decreased uptake from the media. This decreased response to AII and AVP could also lead to impaired MC contractility and might help to explain the altered glomerular hemodynamics observed early in diabetes mellitus.

GLUCOCORTICOIDS (GC) STIMULATE RENAL AMMONIAGENESIS BY INHIBITING PROSTAGLANDINS(PG). M.Heifets, M.Carley, M.M. Marrero,* C.P. Bastl and R.G. Narins. Section of Nephrology, Temple Univ., Phila., PA.

GCs stimulate while PGs depress total renal ammoniagenesis (TRA). Since GCs also inhibit PG synthesis we evaluated whether dexamethasone(DEX) in physiologic doses stimulates TRA by this mechanism. Accordingly, PG depleted (1mg/kg meclofenamate, I.V.) or intact adrenalectomized rats were supplemented with 1,5,10mcg/0.1kg DEX for 24 hr. Clearance studies were done and TRA calculated as the sum of urinary NH_3 excretion and the product of renal a-v difference and renal plasma flow (C_{PAH}). Data are mean ± SEM.

	PG INTACT			PG DEPLETE		
DEX dose: (mcg/24^0)	1	5	10	1	5	10
n=	(6)	(9)	(10)	(8)	(8)	(8)
TRA(nmol/mLGFR)	777[a] ±139	1099 ±127	1398[b] ±171	1225[b] ±188	1438[b] ±213	1220[b] ±192
PGE_2(urine) pg/min	1070[c] ±85	399[d] ±147	182[e] ±38	156[f] ±45	47[f] ±18	52[f] ±22

"a" significantly less than "b" p<0.05 (ANOVA); "c-e" significantly different from each other; "f" significantly less than "c,d,e".

As the dose of DEX increased within the physiologic range, PG excretion decreased and TRA increased. Arterial pH, plasma K+ and GFR (C_{in}) were similar in all groups. PG depletion increased TRA to a uniform level and abolished DEX stimulatory effect. We conclude that glucocorticoids stimulate TRA via PG-dependent mechanism.

INFLUENCE OF CHRONIC RENAL FAILURE ON HEPATIC DRUG METABOLISM INSUFFICIENCY. Pavel Martasek*, Karim Solangi, Michal L. Schwartzman*, Leonard G. Meggs, Alvin I. Goodman, Richard D. Levere* and Nader G. Abraham*. New York Medical College, Depts. of Medicine and Pharmacology, Valhalla, New York.

Several lines of evidence have implicated impaired hepatic drug metabolism as a potential cause of drug toxicity in end stage renal disease (ESRD). We have employed the 5/6 subtotal nephrectomized rat to examine the effect of chronic uremia (10 weeks) on the hepatic drug metabolizing system by measuring P450 content (P450) and the activities of heme oxygenase and several drug metabolizing enzymes; aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin (7ER). Nephrectomized rats and sham-operated controls were sacrificed; serum BUN concentration rose to 85 ± 19 in uremic rats as compared to 13 ± 8 in controls. The levels of hepatic AHH and P450 were decreased in uremic rats, 35% and 42%, respectively, but 7ER was increased by 58%. Hepatic heme oxygenase level was increased to 1.71 ± 0.23 nmole bilirubin/mg/hr as compared to 0.96 ± 0.14 nmole bilirubin/mg/hr in sham-operated controls ($P < 0.05$).

In summary, we observed an increased expression of heme oxygenase in uremic rats, possibly explained by hormonal mediators or biochemical changes associated with ESRD. Decreased levels of hepatic P450 and drug metabolizing enzymes may be a consequence of the increase in expression of heme oxygenase, and play a role in clinical drug toxicity.

HEPARIN INHIBITS RAT RENAL CORTICAL ADENYLATE CYCLASE ACTIVITY IN VITRO. Ze'ev Katzir,* Hanna Wald,* Dvora I. Rubinger,* Mordecai M. Popovtzer Hadassah Univ. Hospital, Jerusalem, Israel.

Previous studies demonstrated inhibitory effects of heparin on adenylate cyclase system in vitro in pancreas, ovary, liver and platelets. The present study was designed to evaluate the effects of heparin, of its non-antithrombotic analog N-acetylated-N-disulfated heparin (N-N-heparin) and of its structural analog dextran sulfate on basal and on PTH- and glucagon-stimulated adenylate cyclase activity in rat renal cortex in vitro. Both heparin and N-N-heparin inhibited basal and PTH- and glucagon-stimulated adenylate cyclase, whereas dextran sulfate did not have an effect. The concentrations of heparin and N-N-heparin causing 50% inhibition (I_{50}) for the baseline activity were 45ug/ml and 500ug/ml, respectively. The I_{50} of heparin for the PTH- and glucagon-stimulated adenylate cyclase were 33ug/ml and 85ug/ml, respectively. The I_{50} of N-N-heparin for the PTH- and glucagon-stimulated adenylate cyclase were 50ug/ml and 120ug/ml, respectively. Forskolin- and Mn^{++}-stimulated adenylate cyclase activities were also inhibited by heparin while NaF-stimulated activity was not affected by it. Increasing Mg^{++} concentrations did not change the inhibition of baseline and PTH stimulated adenylate cyclase activities by heparin. These results show that heparin may alter CAMP/adenylate cyclase system in the kidney and therefore it should be considered as a possible interfering factor when changes in CAMP/adenylate cyclase are encountered. Furthermore, our data point to the catalytic unit of the adenylate cyclase system as the site of heparin interaction.

AN IN VITRO MODEL FOR THE STUDY OF PROTEIN TURNOVER OF PROXIMAL TUBULES. R. May, W. Hardman*, W. Mitch. Emory Univ. Sch. of Med., Renal Div., Atlanta, GA.

Compensatory renal growth is often due to increased tissue protein rather than hyperplasia. Yet the determinants of protein turnover, protein synthesis (PS) and degradation (PD) in renal epithelia are undefined. To examine PS and PD, kidneys from rats weighing 400-600 grams were perfused in vitro with collagenase, and proximal tubule (PT) segments separated by Percoll gradient density centrifugation and incubated in minimal essential medium containing L-(U-^{14}C) phenylalanine (Phe), but without protein or lysine. PS was measured as the incorporation of phenylalanine (Phe) into protein and was linear over a 90 min. incubation period (729±61 pmole Phe/mg protein/hr, N=6). Less than 3% of the intracellular lysine (Lys) pool is metabolized by PT per hr (5.5 pmole Lys oxidized/mg tubule wt); vs 307 pmole leucine were oxidized/mg tubule wt/hr. No transmembrane gradient for Lys was found after a 30' preincubation period, and there was no measurable intracellular Lys accumulation during incubation. Thus, the release of lysine into the incubation media must be from breakdown of intracellular proteins. Release was linear with time (4973±832 pmole Lys/mg protein/hr). 1 mM cycloheximide resulted in an increase in Lys release (2.7 pmole Lys increase/mg protein for each pmole inhibition Phe incorporation/mg protein). When PT were incubated with 77 uM chloroquine to inhibit lysosomal proteases PS was unaffected (535 chloroquine vs 582 pmole Phe/mg protein/hr, control) but PD fell 30% (3767 chloroquine vs 5379 pmole Lys/mg protein/hr, control). Incubation of PT with 10 mU/ml insulin did not affect PS (602 insulin vs 668 pmole Phe/mg protein/hr, control), but inhibited PD by 39%. Conclusions: This model permits simultaneous measurement of PS and PD in freshly-harvested, well-differentiated kidney tubules and should yield insights into the control of renal growth.

RENAL TCA-CYCLE METABOLISM AND AMMONIA-GENESIS. Itzhak Nissim, Marc Yudkoff* and Ilana Nissim*
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Our aim was to elucidate the mechanism(s) by which adaptations of renal TCA-cycle metabolism affects ammoniagenesis in various acid-base states. To this end, renal tubules isolated from control, chronically acidotic or alkalotic rats, were incubated at pH 7.4 with either 1 mM [5-^{15}N, 3-^{13}C] glutamine or [3-^{13}C] pyruvate in the presence and absence of bicarbonate (BC) , 3-mercaptopicolinic acid (3-MPA) or fluorocitrate (FC). GC-MS and/or ^{13}C-NMR were utilized to monitor the flux of ^{13}C through TCA-cycle intermediates, amino acids and glucose as well as the flux of ^{15}N toward $^{15}NH_3$. In acidosis, experiments with glutamine showed a significantly higher production of $^{15}NH_3$, ^{13}C labelled succinate, malate and glucose and diminished formation of ^{13}C α-ketoglutarate (α-Kg), glutamate and aspartate compared with control. Alkalosis was associated with lesser changes in ^{13}C flux from glutamine to TCA-cycle intermediates but the production of $^{15}NH_3$ was reduced. In control, tissues absence of the BC significantly decreased the levels and increased ^{13}C-enrichment of succinate, malate and α-kg, whereas the production of ^{13}C glucose and aspartate was elevated. Either 3- MPA or FC had little effect on ^{13}C succinate or malate production from glutamine. Experiments with [3-^{13}C] pyruvate showed a remarkable formation of ^{13}C citrate, malate, alanine, aspartate and glutamate in alkalosis compared with control, whereas the opposite occurred in the absence of BC or in acidosis. However, formation of ^{13}C glucose, lactate and ^{13}C-acetyl-CoA were significantly higher in acidosis. The data indicate that chronic acidosis (a) inhibits flux through citrate synthetase and NADP-linked malate dehydrogenase and (b) accelerates flux through αKg and succinate dehydrogenases and thereby glutamate dehydrogenase. Thus, alterations of acid-base states are associated with multi-focal changes of flux through the various steps of the TCA-cycle, cytosolic malate metabolism and possibly, pyruvate carboxylase.

EFFECT OF CYCLOSPORIN A ON INTRACELLULAR PROTEIN CATABOLISM IN RAT PROXIMAL TUBULE SEGMENTS. C.J.Olbricht, C.Bossaller*, E.Gutjahr*, and K.Burgwitz*. Med. School, Hannover, FRG

Cell proteins are catabolized by intracellular proteolytic enzymes. To evaluate the effect of cyclosporin A on cellular protein catabolism in the kidney the activities of the lysosomal proteolytic enzymes Cathepsin B and L (CAT B+L) were measured by ultramicroassay in the microdissected segments S1, S2, and S3 of rat proximal tubule (Olbricht et al,Am J Physiol 250: F1055,1986). Pairfed rats received during 6 weeks 30 mg/kg/day of Cyclosporin (CYC) or solvent only (CON) by gavage. Enzyme activities are expressed in pmol of substrate released per min incubation time per mm tubule length. Kidney weight (KW) and creatinine clearance(Ccr) were measured. Values are mean±SEM.

	n	KW	Ccr	Cathepsin B and L		
				S1	S2	S3
		g	ml/min/100g	pmol/mm/min		
CON	8	0.80±0.03	0.62±0.03	20±2	9±1	5±1
CYC	8	0.72±0.02*	0.49±0.03*	47±6*	16±6*	6±2

Asterisks indicate $p < 0.05$ vs control.

Kidney weight and creatinine clearance are decreased by CYC, indicating renal damage. The activities of CAT B+L are increased in S1 and S2 segments. In other organ systems increased activities of CAT B+L are accompanied by elevated cellular protein breakdown (DeMartino and Goldberg, Proc Natl Acad Sci USA,75:1396,1978). Hence, the results suggest increased cellular protein breakdown in proximal tubule cells induced by CYC. Stimulation of cellular protein catabolism may be relevant to CYC nephrotoxicity.

RESPIRATORY CONSEQUENCES OF GLUCOSE TRANSPORT IN DOG CORTICAL TUBULES. Josette Noël, Alberto Tejedor, Patrick Vinay, André Gougoux. Nephrology service, Notre-Dame Hospital and Groupe de Recherches en transport membranaire, Université de Montréal, Montréal, Canada.

Proximal tubules brush-border membrane transport various substrates including glucose by sodium-driven cotransport processes. Therefore an increment of cell respiration due to the secondary activation of the sodium pump occurs following application of D-Glucose, or of its non-metabolizable analog, a-methyl-D-glucoside, to rabbit proximal tubules (Gullans S et al. J. Memb Biol 78:257,1984). We showed that in dog cortical tubules, the application of these sugars does not stimulate the overall cell respiration. However, the ouabain-sensitive respiration increased. Phlorizin (0.2 mM), an inhibitor of the BBM glucose transporter, abolished this effect. Furthermore using rabbit or dog tubules, the stimulation determined by glucose differed from that of a-methyl-D-glucoside, suggesting that the pattern of cell efflux influences the transepithelial sugar transport. The application of both sugars individually or together simultaneously decreased the activity of the DCCD-sensitive H^+ATPase, but had no effect on the overall non-phosphorylative oligomycin-insensitive mitochondrial respiration . In contrast, the addition of lactate + glutamine increased the ouabain-sensitive, the DCCD-sensitive and oligomycin-insensitive components of respiration. It is concluded that the effect of substrates on tubules respiration is the resultant of several phenomena occuring simultaneously at the membrane and mitochondrial levels, in a manner which is specific for each substrate: activation of the Na^+K^+ ATPase, activation or inhibition of the H^+ATPase, and changes of the non-phosphorylating respiration.

DECREASED CATABOLISM OF CELLULAR PROTEINS CONTRIBUTES TO THE EARLY HYPERTROPHIC RESPONSE OF CULTURED KIDNEY CELLS TO AMMONIUM CHLORIDE AND INSULIN. R. Rabkin, D. Dahl* and T. Tsao*. Stanford Univ. and VA Medical Center, Palo Alto, CA.

NH4Cl loading is a renal hypertrophic stimulus in intact animals and in cell culture. This is associated with an increase in cell protein content. Under certain circumstances insulin may also induce hypertrophy in cultured cells. Since both NH4Cl and insulin can inhibit proteolysis, altered protein catabolism may play a role in these hypertrophic responses. We tested this hypothesis in cultured opossum kidney cells. After 3 days of serum deprivation to render the cells quiescent, NH4Cl (20mM) or insulin (10^{-6} M) was added to the serum-free media. Two days later monolayers were analyzed for cell number, protein content, protein synthesis (^{14}C-valine incorporation), protein degradation (^{14}C-valine release), and DNA synthesis (^{3}H-thymidine incorporation). Results were as follows:

	Control	NH4Cl	Insulin
Cell number (x10^6)	2.21±.09	2.20±.05	2.42±.13*
Protein (pg/cell)	409±18	484±20*	452±20*
Prot syn (%/control)	100	105±3	141±3*†
DNA syn (%/control)	100	108±7	146±5*
Prot deg (%/hr)	2.8±.6	2.5±.5*	2.2±.4*

*p <.05 vs control, †p <.05 vs NH4Cl (paired t-test)

Thus NH4Cl produced an 18% increase in cell protein content and an 11% decrease in protein catabolic rate, but no significant change in cell number or protein synthesis. In contrast insulin, which was mitogenic, increased cell protein content 11%, inhibited proteolysis 21% and stimulated protein synthesis 41%. We conclude that inhibition of proteolysis plays an important role in the early hypertrophic response of kidney cells to NH4Cl and insulin.

COLLECTING TUBULE ATPase ACTIVITIES. S. Sabatini and N.A. Kurtzman, Depts of Internal Medicine and Physiology, Texas Tech Univ HSC, Lubbock, TX.

From a number of recent studies it is clear that "proton" ATPases are present in many tissues. They have been found in plasma membrane, endosomes, mitochondria, and subcellular organelles such as chromaffin granules and lysosomes. In microdissected rat cortical (CCT), medullary (MCT), and papillary collecting duct segments we previously demonstrated the presence of an NEM-sensitive "proton" ATPase, a Na-K ATPase, and a Mg-dependent ATPase; these activities vary widely according to the anatomic site dissected. In a series of experiments we examined the effects of several inhibitors on these three enzymes isolated from rat collecting tubule. Vanadate, CCCP, diethylstillbesterol, and valinomycin had no effect on NEM-sensitive or Mg-dependent ATPase activities in any of the collecting duct segments. Deoxycholate markedly decreased NEM-sensitive and Mg-dependent ATPase activity; the CCT was far more sensitive than the other 2 segments studied. In rat CCT and MCT, vanadate, CCCP, diethystillbesterol, and deoxycholate inhibited Na-K ATPase. ATP and GTP stimulated NEM-sensitive ATPase in CCT; only ATP stimulated the enzyme in MCT. These results demonstrate that differences in collecting tubule NEM-sensitive ATPase activity exist. The CCT is more sensitive to these inhibitors than is medullary or papillary collecting duct. Our results suggest that the NEM-sensitive ATPase is heterogeneous in the mammalian collecting tubule. We postulate that "proton" ATPase isoenzymes are present in collecting tubule as have been documented in a number of other biologic systems.

EFFECT OF HYPERTROPHY ON CRITICAL PO_2 OF MEDULLARY THICK ASCENDING LIMBS. P. Silva, R. Fuhro* and F.H. Epstein. Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA.

The critical pO_2 refers to that partial pressure of O_2 at which a declining O_2 supply first limits the rate of O_2 consumption and therefore reduces cellular metabolism. The critical pO_2 of intact cells is higher than that of isolated mitochondria, largely because of the slow rate of diffusion through cytoplasm and the effect of mitochondrial clumping within the cells. When O_2 supply is limited, a high critical pO_2 should render the cells more susceptible to ischemic dysfunction. We studied the effect of cellular hypertrophy produced by a high protein diet on the critical pO_2 of fresh isolated medullary thick ascending limb (mTAL) tubules from the outer medulla of rat kidneys. Continuous recording of ambient pO_2 and O_2 consumption was carried out with an O_2 electrode in a closed chamber with constant stirring of dispersed tubules in a buffered medium. Tubules from rats fed a diet containing 40% protein for 2 weeks were compared with those obtained from the kidneys of rats fed 5% protein for the same period. Mean diameter of the tubules was increased by 38% in rats fed high protein. Critical pO_2 of mTAL tubules from high protein rats was 24.6±5.0 mm, significantly higher ($p<0.01$) than that of low protein rats (9.2±1.3).

An increase in critical pO_2 produced by cellular hypertrophy thus poses a special risk of hypoxic injury to mTAL cells, located as they are in a region with low pO_2, and possessed of a high metabolic rate.

ROLE OF HEME OXYGENASE IN HUMAN KIDNEY ENDOGENOUS DRUG METABOLISM. Karim Solangi, Alvin I. Goodman, Richard D. Levere*, Pavel Martasek*, Michal L. Schwartzman* and Nader G. Abraham*. NY Med. College, Depts. of Med. and Pharm., Valhalla, NY.

We have recently shown that a reduction in cytochrome P450-dependent arachidonate metabolism via induction of heme oxygenase leads to a decrease in blood pressure of spontaneously hypertensive rats. It therefore seemed appropriate to investigate the characterization, activities and distribution of heme oxygenase within human kidney. Heme oxygenase, the rate-limiting enzyme in heme degradation, regulates the levels of a variety of drug metabolizing enzymes and other hemoproteins. Heme oxygenase activities were assessed in seven human kidneys; the range of heme oxygenase levels were from 0.295 to 0.781 nmole bilirubin formed/mg/hr. The activity in the cortex was higher than that in the medulla and about 70% of that present in human liver. Antibodies raised against human liver heme oxygenase cross react to human kidney enzyme protein. Both liver and kidney heme oxygenase have a molecular weight of 32,000 daltons. Several compounds have been studied for their ability to inhibit kidney heme oxygenase. It was found that a synthetic porphyrin, Zinc deuteroporphyrin IX, 2,4 bis glycol, at a conc. of 1μM inhibited 85% of renal heme oxygenase activity. This drug has been shown to be an excellent inhibitor of renal heme oxygenase in experimental animals. The observed suppressive effect of this inhibitor on human renal heme oxygenase supports the concept that these compounds might have a beneficial effect on certain renal dysfunctions such as that seen in hypertension and chronic renal failure.

EFFECT OF 5-AMINO-4-IMIDAZOLECARBOXAMIDE RIBONUCLEOSIDE (Z-RIBOSIDE) ON PURINE NUCLEOTIDE (PN) AND GROWTH OF NORMAL RAT KIDNEY (NRK) CELLS IN CULTURE. Beatrice States*, Marc Yudkoff*, Ilana Nissim* and Itzhak Nissim. Univ. of Penna, School of Med., Dept of Peds.. Philadelphia, PA

The de novo purine synthetic pathway supplies growing cells with the purines needed for the formation of ribonucleotides and ATP. We have examined the effect of Z-Riboside on the synthesis of PN and the growth of NRK-cells in culture. Experiment in the presence and absence of various concentrations of Z-Riboside were conducted with Dulbecco's Modified Eagle's medium supplemented with either 1mM [^{15}N]aspartate or [^{14}N]aspartate. GC-MS was utilized to monitor the flux of ^{15}N from aspartate to the 6-amino group of adenine nucleotides. The observations showed that following a 48h incubation with 50μM Z-Riboside there was a remarkable increase in PN and DNA concentration and in cell growth. However, with concentrations above 100μM there was a significant inhibition of cell growth and a depletion of the intracellular pools of PN and DNA compared with control incubations without Z-Riboside. The adenylate energy charge and the mass action ratio for the myokinase reaction remained relatively constant with varying Z-Riboside concentration in the incubation medium, indicating that the reaction was never far removed from equilibrium. Experiments with [^{15}N]aspartate indicated that the initial rate (0-24h) of 6-$^{15}NH_2$ production was 38.8 ± 9.6, 67.9 ± 12.5 and 20.1 ± 3.8 pmol $h^{-1}/10^6$ cells in the presence of 0, 50μM and 500μM Z-Riboside, respectively.

The current studies suggest that (a) Z-Riboside has a biphasic effect on the growth of renal cells in culture and on their PN and DNA pools, (b) Z-Riboside could be used as a factor mediating renal cell mitosis and (c) Z-Riboside has a possible therapeutic effect in case of renal failure associated with impaired renal energetics and reduced ATP levels.

MECHANISM OF LACTATE (LAC) TRANSPORT ACROSS THE BASOLATERAL MEMBRANE VESICLES (BLMV) OF DOG KIDNEY THICK ASCENDING LIMBS (TAL).

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LAC uptake by suspensions of dog TAL was shown to be depressed by furosemide, SITS or absence of Cl^-, suggesting a direct or indirect coupling between LAC uptake and the transepithelial flux of Cl^- (Kidney Int. 33: 395,1988). In order to test this hypothesis directly, BLMV were prepared from TAL and purified on a Percoll gradient. Based on Na^+K^+ATPase activity and its activation by 0.1% deoxycholate, BLMV showed 4-6 fold purification and 67% right-side-out configuration. LAC transport was studied by a rapid filtration technique. In this preparation, LAC transport was stimulated in the presence of a pH gradient (pH 8.0 in; pH 6.0 out) and showed a transient overshoot of intravesicular [LAC] which is characteristic of secondary active transport. This process was insensitive to SITS but inhibited by furosemide, OH-cyanocinnamate, CCCP, and inversion of the pH gradient. Neither inwardly nor outwardly oriented Na^+-, K^+, and Cl^-- gradients alone did stimulate LAC uptake. These results suggest the presence of a LAC/H^+ symport (or of a LAC/OH^- antiport) in BLM of dog TAL that is directly affected by furosemide. However a furosemide and SITS-sensitive transport of LAC was observed in Cl^--loaded BLMV incubated in the presence of 25 mM HCO_3^- (inside and outside). We thus propose that the activity of this transporter coupled to a SITS-sensitive Cl^-/HCO_3^- exchanger may explain the partial coupling previously observed between LAC and Cl^- fluxes in TAL tubules.

AMINO ACIDS MODULATE PROTEIN DEGRADATION IN CULTURED KIDNEY CELLS. T. Tsao*, G. E. Mortimore* and R. Rabkin. Stanford Univ. and VA Medical Center, Palo Alto, CA, and Penn. State Univ., Hershey, PA.

Cell protein content is determined by the balance between protein synthesis and degradation. It has been shown that in non-renal tissues certain amino acids (AA) regulate protein degradation while in the kidney nutritional deprivation is associated with enhanced protein degradation. In order to determine whether AA modulate renal protein degradation, studies were performed with isolated perfused rat kidneys and with a cultured proximal-like kidney cell line derived from the opossum kidney (OK). Cellular proteins were labelled with ^{14}C-valine for 20 hours prior to study. Protein degradation was determined by the rate of release of acid soluble radioactivity from the prelabelled proteins. In kidneys perfused with an albumin electrolyte solution containing glucose and AA, protein degradation averaged 1.3±0.06%/hr (equals 31%/day). In the absence of AA degradation increased by 26% to 1.7±0.06%/hr ($P< 0.01$). In OK cells cultured in medium containing AA and 10% serum, protein degradation averaged 1.8±0.12%/hr and increased to 2.8±0.24%/hr when AA were removed ($P < 0.01$). Deletion of both serum and AA accelerated protein degradation further to 3.8±0.24 %/hr; a situation analogous to nutritional deprivation. Replacement of the complete AA mixture reduced protein degradation to 2.3±0.14 %/hr. Each AA in the mixture was then added alone; but only LEU, LYS and PHE inhibited degradation ($P< 0.05$). LEU was most effective and accounted for 36% of the total AA effect. LEU is also known to regulate protein degradation in liver and muscle. Thus we conclude that 1) AA are involved in the regulation of protein degradation both in the intact kidney and in cultured kidney epithelial cells, and 2) only certain AA have regulatory properties. Accordingly, further study should lead to a more rational approach to the use of AA in renal nutritional therapy.

INFLUENCE OF LEUCINE AVAILABILITY ON ITS TRANS-AMINATION AND OXIDATION RATE (OR) IN THE RAT MEDULLARY THICK ASCENDING LIMB OF HENLE'S LOOP (MTAL). Marie-Marcelle Trinh*, Olivier Levillain*, Lise Bankir. INSERM U 90, Hôpital Necker, Paris, France.

We have shown that leucine is a potential energetic fuel for the MTAL (Pflügers Arch 1988;411: 676). As leucine plasma level (P-Leu) may vary widely according to protein intake, we investigated whether MTAL can adapt its capacity to utilize this branched chain amino acid to *in vivo* or *in vitro* alterations in its concentration. MTAL suspensions were prepared from rats fed a low (LP, 10% casein) or a high (HP, 32% casein) protein diet for 4-5 wk. Leucine transaminase activity was measured in both directions at maximal velocity on MTAL homogenates. OR rate in intact MTAL was determined from the amount of radioactive CO_2 produced from ^{14}C-labeled leucine (Leu) or glucose (Glu). Glu concentration was 5 mM and Leu was 0.2 or 0.5 mM to mimic plasma levels found in fasting or fed animals. HP diet significantly increased Leu transaminase activity toward deamination by 18% in MTAL without change in renal cortex or muscle. OR were (mean ±SE, pmol $CO_2/\mu g$ prot. hr):

Diet/[Leu]	LP/0.2mM	LP/0.5mM	HP/0.2mM	HP/0.5mM
Leu OR	46± 4	95± 8*	40± 5	70± 8*
Glu OR	148+17	162+22	154+19	145+21

* = $p<0.05$ for Leu 0.5 mM vs Leu 0.2 mM.

Glu OR. was not affected by previous protein intake or actual Leu concentration. Leu OR was slightly lower in HP than in LP for each *in vitro* leu concentration. However, *in vivo* (P-Leu = 0.5 mM in HP, 0.2 mM in LP), the contribution of leu to MTAL energetic metabolism is likely to be enhanced on HP diet or after acute protein intake.

NUCLEOSIDE TRANSPORT IN THE POSTISCHEMIC SYNTHESIS OF ATP FROM EXOGENOUS NUCLEOTIDES IN VIVO. **Aren van Waarde*, M.J. Avison, G. Thulin*, K.M. Gaudio, N.J. Siegel and R.G. Shulman*. Yale Univ. Sch. of Med., Depts. of Mol. Biophys. and Biochem. and Pediat, New Haven, CT**

To evaluate the metabolic effects of exogenous purines, we infused, i.v. during the first 30 min of reflow, radiolabeled ATP-$MgCl_2$ or adenosine (Ad)-$MgCl_2$ (50 µmol, 0.5 Ci/mol) in rats either alone or in combination with the nucleoside transport inhibitor NBTI (250 nmol). Two hrs after 45 min ischemia, a blood sample was drawn and the kidney was freeze-clamped and extracted with methanol:HCL:HCLO4. The specific radioactivities of plasma and kidney metabolites were measured, using reverse-phase HPLC.

ATP-$MgCl_2$ infusion caused a striking acceleration of the recovery of the renal ATP-pool, as expected. NBTI significantly depressed recovery of renal ATP (24%) and labeling of intracellular pool (44%). Plasma contained labeled Ad, inosine and bases.

Ad-$MgCl_2$ infusion did not accelerate the recovery of renal ATP, the incorporation of label into the cellular nucleotide pool was 60% of that observed after ATP-$MgCl_2$ infusion . NBTI did not depress the labeling of intracellular nucleotides while plasma had only labeled bases.

These data suggest: 1) The purine moiety of exogenous nucleotides is taken up by both NBTI-sensitive and NBTI-insensitive transport systems since inhibition was partial. 2) Ad transport by a NBTI-sensitive carrier protein contributes to beneficial effect of ATP-$MgCl_2$ infusions following ischemia.

EFFECT OF AGE ON GLOMERULAR GROWTH IN RATS. Gilberto Vera*, Francis Dumler, Pedro Cortes, and Ruth Osterby*. Depts. of Med. and Pathol., Henry Ford Hospital, Detroit, Michigan and Aarhus University, Aarhus, Denmark.

We have studied the effects of unilateral nephrectomy (Nx) and diabetes (Dm) on glomerular growth in 6 (young) and 18 (mature) weeks old male Fischer rats. Ninety days after Nx and/or streptozotocin injection, glomerular volume (GV), serum creatinine (SCr) and blood glucose (BG) concentrations were (mean±SD):

Young	Control(n=6)	Nx(n=6)	Dm(n=7)	Nx+DM(n=4)
GV(mm3)	47±5	76±2	42±6	52±8
SCr(mg/L)	2.3±0.9	3.1±0.7	3.5±0.2	4.6±1.3
BS(mg/dl)	157±15	130±16	372±101	402±103
Mature	**Control(n=7)**	**Nx(n=7)**	**Dm(n=6)**	**Nx+Dm(n=4)**
GV(mm3)	46±5	64±9	51±7	57±3
SCr(mg/L)	2.1±0.3	3.0±0.5	10.2±0.7	14.3±6.6
BS(mg/dl)	152±17	140±18	418±72	390±71

Nx increased GV in young ($P< 0.001$) and mature rats ($P< 0.01$), with changes being greater in the young ($P< 0.05$). Dm and Nx+Dm did not cause significant increases in GV in either group. SCr increased significantly ($P< 0.01$) with Dm, with renal function being worse in the mature group ($P< 0.001$). No additive effect between Nx and Dm was noted. In summary, glomerular hypertrophy following unilateral nephrectomy is greater in young than in mature rats, and long-term streptozotocin induced diabetes is not associated with increased glomerular volumes in this rat strain. The observed worsening of renal function with diabetes may be related in part to the absence of glomerular hypertrophy.

HYPERFILTRATION INCREASES APICAL MEMBRANE (Ap) Na/H ANTIPORTER AND BASOLATERAL MEMBRANE (Bl) $Na/3HCO_3$ SYMPORTER ACTIVITIES IN THE RAT PROXIMAL CONVOLUTED TUBULE (PCT). R.J. Alpern and P.A. Preisig. UT Southwestern Med Ctr, Dallas, TX.

To examine the mechanism by which chronic hyperfiltration (HF) stimulates solute absorption in the PCT, we compared Na/H antiporter and $Na/3HCO_3$ symporter activity in chronic uninephrectomized rats (12-18 days) ingesting a 40% protein diet with sham-operated rats ingesting a 24% protein diet (CON). Transporter activity was measured microfluorimetrically in the doubly perfused PCT using the pH-sensitive dye, BCECF.

Cell pH (pHi) under control conditions was higher in HF rats (7.30±0.03 vs 7.15±0.03, $p< 0.002$). Lowering luminal [Na] from 152 to 0 mM caused a ↓pHi which was faster in HF than CON (5.21±0.45 vs 3.44±0.52 pH units/min, $p<0.03$) and was larger (though NS) in magnitude (ΔpHi = 0.48±0.04 vs 0.36±0.04). Lowering peritubular [HCO_3] from 25 to 5 mM caused a ↓pHi which was faster in HF (8.02±0.38 vs 6.38±0.57 pH units/min, $p<0.03$) and was larger in magnitude (ΔpHi = 0.48±0.02 vs 0.39±0.02, $p<0.002$). Lowering capillary [Na] in the absence of Cl from 147 to 25 mM also caused a ↓pHi which was faster in HF (11.00 ±1.10 vs 7.29±0.50 pH units/min, $p<0.007$) and larger in magnitude (ΔpHi=0.46±0.01 vs 0.38± 0.03, $p<0.03$). There were no differences in cell buffer capacity, BCECF calibration or blood pH.

In conclusion, HF causes adaptive increases in the activities of the Ap Na/H antiporter and the Bl $Na/3HCO_3$ symporter. These adaptations are similar to those which occur in metabolic and respiratory acidosis, but in HF are associated with no change in blood pH and ↑ cell pH.

GLUCOSE TRANSPORT AND METABOLISM BY DOG PAPILLARY COLLECTING DUCTS IN SUSPENSION. Patrick Vinay, Alberto Tejedor, Josette Noel, Alfred Berteloot, Yvan Boulanger and André Gougoux. Nephrology service, Notre-Dame Hospital and Groupe de recherches en transport membranaire, Université de Montréal, Montréal, Canada.

Suspension of papillary collecting ducts (PCD) were prepared from the dog kidney papilla. PCD tubules presented the specific morphology and enzymatic content expected from papillary tissue. PCD adenylates were stable (>2 hours) and identical in presence or absence of oxygen. Glycolysis proceeded at similar and linear rate in both conditions, but the lactate accumulation/glucose utilization ratio was 1.6 in aerobiosis and 2.2 in anaerobiosis. A net utilization of endogenous glycogen was always observed in anoxia or in absence of glucose. Inhibition of the Na^+K^+-ATPase (by 1 mM ouabain) or H^+ ATPase (by 0.1 mM DCCD) reduced the PCD ATP turnover. The activity of Na^+K^+ and H^+ ATPases together explained 60% of the respiration in intact tubules. Activation of these ATPases respectively by nystatin and CCCP decreased the cell ATP in aerobic and anaerobic conditions, indicating that this segment presents limited glycolytic and phoshorylative capacities. When the ATP was severely reduced (nystatin), the glycolytic flux was also impaired. PCD respiration was modest and reduced when glucose was provided as substrate as compared to lactate. Phloretin inhibited markedly glucose metabolism. The study of glucose transport(rapid filtration technique) by PCD tubules and BLM vesicules prepared from these tubules confirmed that glucose entry in PCD is largely mediated by a phloretin and cytochalasin B sensitive, Na^+-independant, carrier-mediated diffusion (Km 2.4 mM). This preparation allows to study the functional and metabolic characteristics of the distal collecting duct in the dog and other species.

AXIAL HETEROGENEITY OF RABBIT PROXIMAL TUBULE ACIDIFICATION. Michel Baum, Univ. of Texas SW Med. Sch., Dept. of Ped., Dallas, Texas.

The present in vitro microperfusion study examined if the rates of the apical membrane Na^+/H^+ antiporter and basolateral membrane $Na(HCO_3)_3$ symporter vary along the length of the proximal tubule. Initial proximal convoluted tubules (PCT obtained within 0.5 mm from the glomerulus), mid PCT and proximal straight tubules (PST) were examined. Transporter activity was estimated from the initial rate of change of intracellular pH (pH_i) in response to a change in luminal or bathing solution composition. pH_i was measured fluorometrically using the pH sensitive dye BCECF. Na^+/H^+ antiporter activity was examined by changing the luminal sodium concentration and basolateral $Na(HCO_3)_3$ symporter activity was examined by changing either bath sodium or bicarbonate concentration.

	dpH_i/dt Initial PCT	Mid PCT	PST
luminal Na 142-->0	2.57±0.38	2.87±0.44	0.77±0.18*
Bath HCO_3^- 25-->5	4.01±0.29	4.57±0.31	2.90±0.26*
Bath Na^+ 147-->0	3.47±0.31	3.50±0.38	2.22±0.29+
	*$p<0.01$	+$p<0.05$	

There was no significant difference in buffer capacity in these segments.

There is a homogeneous rate of apical Na^+/H^+ antiporter and basolateral $Na(HCO_3)_3$ activity along the rabbit PCT. The rates of both transporters are decreased in the PST. The magnitudes of Na^+/H^+ antiporter and $Na(HCO_3)_3$ symporter activities may explain the previously observed axial changes in bicarbonate transport.

DEVELOPMENT OF NA/H ANTIPORTER ACTIVITY IN RABBIT RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV). J.C. Beck, M.S. Lipkowitz, and R.G. Abramson. Mt. Sinai School of Medicine, NY, NY.

We have reported that renal brush border Na glucose cotransport activity significantly increases postpartum. To determine if maturational changes occur in other transporters, the Na/H antiporter was examined in fetal (F) and adult (A) rabbit BBMV. When pHin < pHout, the 30" uptake of 1 mM Na^+ uptake was not significantly different in F and A BBMV and uptakes were unchanged when the P.D. was clamped with a protonophore. However, the amiloride-sensitive component of Na uptake was 50% lower in F BBMV [p<0.05]. These findings indicate that an electroneutral Na/H antiporter is present, but significantly diminished in activity in F BBMV while conductive Na^+ flux is significantly greater in F membranes. To assess the mechanism of reduced antiporter activity, its kinetics were examined using the fluorescent, pH sensitive probe, acridine orange. Conductive H^+ flux was estimated from the rate of dissipation of the pH gradient at varying $[K^+]$. Both Vmax (nmoles H^+/s/mg protein) and Km for Na (mM) were significantly lower in F BBMV [Vmax = 16.1 ± 1.2 vs. 40.5 ± 4.5, p<0.001; Km = 6.5 ± 1.2 vs. 12.3 ± 0.8, p<0.005]. Of note, the conductive H^+ flux (nmoles H^+/s/mg protein), like Na^+, was significantly greater in F BBMV [7.4 ± 1.5 vs. 0.97 ± 0.08 nmoles H^+/s/mg protein]. These studies suggest that there is a maturational increase in BB Na/H antiporter activity which derives from an increase in turnover number or increased recruitment of membrane carriers. These studies further suggest that the maturational reduction in membrane conductance to Na^+ and H^+ may increase the efficiency of the antiporter by limiting dissipation of both the Na^+ and H^+ gradients.

FUNCTIONAL EVIDENCE FOR PARALLEL BASOLATERAL Na^+/H^+ and $Cl^-/HCO3^-$ EXCHANGERS IN THE INNER STRIPE, OUTER MEDULLARY COLLECTING DUCT (OMCDi) OF RABBIT. M.D. Breyer, and H.R. Jacobson. Division of Nephrology, Vanderbilt Univ. and V.A.M.C., Nashville, TN.

The OMCDi is thought to acidify the urine via an apical H^+ATPase, and transfer the resultant intracellular $HCO3^-$ into the blood via $Cl^-/HCO3^-$ exchange. There is also electrophysiologic evidence for basolateral Cl^- conductance. We have recently demonstrated that the OMCDi cell pH (pHi) recovery from an acid load is reduced in the absence of Na+. Using BCECF to measure pHi, we now directly charactarized this dependence as basolateral Na^+/H^+exchange and provided further proof for basolateral $Cl^-/HCO3^-$ exchange in OMCDi.

In vitro microperfused rabbit OMCDi's were loaded from the bath with BCECF-AM. pHi was determined by the 495/440 ratio of BCECF fluoresence. In HCO3- free solutions Na^+/H^+ exchange was demonstrated by: 1) reversible cell acidification after replacement of bath Na with tetramethylammonium (pHi = 7.14 ± 0.09 ,125mM Na, vs.6.85 ±0.01, 0Na, p<0.005); 2) complete blockade by 0.2 mM amiloride of sodium dependent pHi recovery from an acid load. 3) dependence pHi recovery rate (from an NH4Cl-pulse acid load) on peritubular [Na]: apparent Km=9.1mM. Basolateral $Cl^-/HCO3^-$ exhange was demonstrated in HCO3- containing solutions by: 1. cell alkalinization by 0.1mM bath DIDS (7.14±0.09 to 7.34±.09, p<.005); 2)Alkanization by peritubular Cl- removal and 3) DIDS blockade of the alkalinization resulting from Cl- removal: ΔpHi (-DIDS) =0.363±.06 vs ΔpHi (+DIDS) =0.02±0.04, p<.005.

Preliminary studies examining the Cl- dependent quenching of 6-methoxy-N (3 sulfopropyl) quinolonium (SPQ) fluoresence in OMCDi's were also performed. OMCDi's were loaded with 30 mM SPQ for 60 minutes. Using 350 nm excitation, changes in $[Cl^-]i$ were determined as a function of fluorescent intensity of SPQ. In $HCO3^-$ free solutions, Cl^- removal significantly and reversibly increased fluoresence by 21.6±4.9% (p<.005). These results directly confirm the presence of basolateral Na^+/H^+ exhange, $Cl^-/HCO3^-$ exchange, and suggest the presence of a basolateral $HCO3^-$ independent Cl^- exit step.

Basolateral Na/HCO_3 Cotransport in the S3 Segment of the Rabbit Proximal Tubule. W.F. Boron & N.L. Nakhoul*. Yale Univ. Sch. of Med., New Haven, CT.

We studied the effect of basolateral HCO_3^- transport on intracellular pH (pH_i) in isolated S3 proximal tubules, calculating pH_i from absorbance spectra of dimethylcarboxyfluorscein. For 30 tubules in 5% CO_2/25 mM HCO_3^- (pH=7.4, 37°), the mean pH_i was 7.13±0.03. Lowering bath (B) pH from 7.4 to 6.7 by lowering $[HCO_3^-]_B$ from 25 to 5mM at constant pCO_2 caused pH_i to fall rapidly by 0.42±0.1 (4), whereas lowering luminal pH to 6.7 caused pH_i to fall by 0.17±0.02 (4). In both cases pH_i recovered upon restoring pH_o to 7.4. In the bilateral absence of Cl^-, lowering pH_B still caused pH_i to fall by 0.36±0.03 (5) and the initial rate of decline ($-dpH_i/dt$) was unchanged (106±22% of control). Bath Na^+ removal caused pH_i to fall reversibly by 0.26±0.03 (11) in the presence of HCO_3^-, but by only 0.08±0.02 (4) in the absence of HCO_3^-. The $-dpH_i/dt$ upon Na^+ removal was reduced by 72±8% (4) in the absence of HCO_3^-, and $+dpH_i/dt$ upon Na^+ readdition, by 87±2% (4). In the presence of 0.1 mM DIDS, the $-dpH_i/dt$ elicited by (B) Na^+ removal fell by 56±7% (5), and the $+dpH_i/dt$ upon Na^+ readdition fell by 70±8% (5). In the bilateral absence of Cl^-, (B) Na^+ removal still caused pH_i to fall by 0.23±0.08 (4). Both the rate of acidification (~38% > control) and recovery (~30% < control) were not significantly affected by Cl^- removal. Thus, the basolateral membrane of the S3 proximal tubule appears to possess a Na^+-dependent HCO_3^- cotransporter that is DIDS-sensitive and independent of Cl^-, presumably a Na/HCO_3 cotransporter.

DEVELOPMENT OF H^+ PUMP IN CULTURED INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. L.P. Brion, J.H. Schwartz, G.J. Schwartz, Dept. of Peds., Albert Einstein Coll. Med., Bronx, NY and Dept. of Med., Boston U. Sch. Med., Boston, MA.

Cultured IMCD cells secrete H^+. We tested whether location in the growing nest (center vs. periphery), time in culture medium, removal of fetal calf serum (FCS) for 1-2 days, and mitotic index (MI) were associated with development of the H^+ pump in monolayers plated on plastic at low density. H^+ pump activity was assessed from the slope of recovery of pH_i after acid loading in Na^+, CO_2 and HCO_3^- free buffers, while Na^+/H^+ exchange was assessed from the Na^+-dependent recovery of pH_i. Cells that showed H^+ pumping activity did not have Na^+/H^+ exchange (r=-0.68, n=245). Cells at the periphery of the growing nests always had MI> 1/1000 and only 3% showed H^+ pumps with slopes ≥0.10 pH U/min. After ≥7 days of culture, non-dividing center cells (MI< 1/1000) had greater H^+ pumping activity than dividing cells (MI>1/1000) (0.08±0.01 pH U/min, n=73, vs. -0.02±0.01, n=63, p<0.001), and 65% of them showed Na^+-independent recoveries of ≥0.10 pH U/min. Removal of FCS increased H^+ pump activity in non-dividing center cells from 0.06±0.01 pH U/min, n=43 cells in 10% FCS to 0.11±0.01, n=30 in 0.1% FCS (p<0.01), but it did not affect H^+ pumping activity in dividing cells (0.01±0.01, n=103 cells in 10% FCS vs. 0.01±0.01, n=203 in 0.1% FCS, p=0.85). We conclude that Na^+/H^+ exchange is prevalent in dividing IMCD cells. The cessation of cell division is associated with the differentiation of H^+ pumping activity.

ATP-DRIVEN H^+ TRANSPORT IN INTACT AND RECONSTITUTED RAT RENAL BRUSH-BORDER MEMBRANE VESICLES
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To prove the existence of an ATP-driven H^+ pump in the brush-border membrane (BBM) purified rat renal BBM vesicles were loaded by freeze/thawing with ATP, ATP-regenerating system, and 6-carboxyfluorescein as pH indicator. ATP-loaded vesicles alkalinize their interior, i.e. they eject protons. Alkalinization is stimulated by K^+/valinomycin and by permeant anions revealing the electrogenicity of the H^+ pump. In the presence of Na^+, D-glucose and L-phenylalanine stimulate ATP-driven H^+ efflux: Cotransport of these substrates with Na^+ provides charge compensation for ATP-driven H^+ efflux. This result proves the colocalization of H^+-ATPase and Na^+/substrate-cotransport systems in the same vesicles. Also with Na^+ present, inhibition of Na^+/H^+ exchange stimulates ATP-driven H^+ efflux indicating the coexistence of H^+ pump and antiporter in the same vesicles: protons pumped out by the ATPase recycle via the (uninhibited) Na^+/H^+ exchanger decreasing net intravesicular alkalinization. ATP-driven H^+ efflux is inhibited by DCCD, NEM and NBD-Cl, but not by oligomycin and vanadate. Thus, the BBM H^+ pump belongs to the "vacuolar type" ATPases. Solubilizing BBMV with cholate and overnight dialysis reorients the H^+ pump, its catalytic site now facing the outside: external ATP addition drives electrogenic, DCCD- and NEM-sensitive H^+ uptake. Our data provide direct evidence for an electrogenic ATP-driven H^+ pump in the BBM which, together with the Na^+/H^+ exchanger, accounts for proximal tubular H^+ secretion.

NORMAL DIET NACL VARIATIONS INFLUENCE RENAL SET-POINT FOR PLASMA pH-HCO_3^- MAINTENANCE DURING ALKALI INGESTION.
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Exogenously administered bicarbonate normally induces little change in plasma HCO_3^- concentration ($[HCO_3^-]_p$) or blood pH (pH_b) in subjects who ingest abundant NaCl. The possible modulating effect of a low NaCl diet has not been investigated, however. In five normal men ingesting a constant acid-producing diet of low intrinsic NaCl content, we measured plasma and urine acid-base composition and glomerular filtration rate (GFR) during four 7-day periods in which the diet was supplemented as follows: no supplements -> $NaHCO_3$ only -> $NaHCO_3$ plus NaCl -> NaCl only. (Each Na-salt: 2.0 mmol/kg body wt/day). With no supplements, $[HCO_3^-]_p$ was 25.0±0.4 meq/L and pH_b was 7.43±0.005. When $NaHCO_3$ only was supplemented, $[HCO_3^-]_p$ increased 4 meq/L (to 28.9±0.6 meq/L, $p<0.001$) and pH_b increased 0.02 (to 7.45±0.004, $p<0.01$). Since GFR changed little (102±6 vs. 98±6 ml/min, NS), that rise in $[HCO_3^-]_p$ was sustained predominantly by renal reabsorption of an increased filtered HCO_3^- load. When NaCl was supplemented, $[HCO_3^-]_p$ returned to the control (no supplements) level, whether $NaHCO_3$ supplements were continued (24.9±0.6 meq/L) or later stopped (24.1±0.5 meq/L). Thus, in humans, under conditions of moderate diet net base input (from $NaHCO_3$) but not under conditions of diet net acid input, diet NaCl level is a determinant of the set-point at which the kidney maintains $[HCO_3^-]_p$ and pH_b.

CHARACTERIZATION OF BICARBONATE TRANSPORT IN THE LOOP OF HENLE.
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To determine if the loop of Henle contributes to the regulation of acid excretion, rat superficial loops were pump microperfused in vivo from late proximal to early distal tubule. Perfusate was an end-proximal Ringer solution containing 15 mM $NaHCO_3$ and ^{14}C-inulin. Bicarbonate concentration in collected fluid was measured microcalorimetrically. In control, $JHCO_3$ was 150±6.0 pmol/min (n=30; mean±SEM). Perfusate containing the lipid soluble carbonic anhydrase inhibitor methazolamide (2.10^{-4} M) reduced $JHCO_3$ by 60% (46±8.1 pmol/min; n=13; $P<0.001$). Ethyl-isopropyl-amiloride (2.10^{-4} M), a specific blocker of Na^+-H^+ exchange, decreased $JHCO_3$ by 30% (97.1±10.5 pmol/min; n=24; $P<0.001$). Luminal bumetanide (10^{-6} M), while decreasing Jv by 20%, significantly increased $JHCO_3$ (198.8±11.0 pmol/min; n=14; $P<0.001$). Furosemide (10^{-4} M) also decreased Jv (30%), but had no effect on $JHCO_3$. Increasing bicarbonate load, either by raising bicarbonate concentration (15 to 30 mM) or perfusion rate (20 to 40 nl/min), stimulated $JHCO_3$ (208.3±14.0 and 194.2±15.6 pmol/min; n=13,18, respectively). Chronic potassium depletion did not change $JHCO_3$ (148±8.6 pmol/min; n=19). These results show that superficial loops of Henle contribute to renal acidification. Moreover, they indicate that loop bicarbonate transport is: (a) sensitive to carbonic anhydrase and Na^+-H^+ exchange inhibitors; (b) stimulated by bumetanide, but not by furosemide; (c) concentration- and flow-dependent; (d) unaffected by chronic potassium depletion.

EFFECTS OF CHRONIC HYPERKALEMIA (HK) ON RENAL AMMONIUM (Am) TRANSPORT.
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Although HK is associated with a reduction in urinary ammonium excretion ($U_{Am}V$), presumably by inhibition of renal ammoniagenesis, the effects of chronic HK on proximal tubule Am transport have not been examined. Free-flow micropuncture studies were performed in pair-fed Munich-Wistar rats receiving either a control (C) (0.6 gm KCl/100 gm) or high K+ (15.0 gm KCl/100 gm) diet for 10 days. Chronic K^+ loading was associated with HK (6.6 vs 4.1 mEq/L), a four-fold increase in K^+ excretion, systemic metabolic acidosis (pH=7.27 vs 7.33; HCO_3^-=18.6 vs 22.3 mM), an increase in urine flow (V), and a decrease in U/P inulin. Body weight, urine pH, and GFR did not differ. $U_{Am}V$ was reduced markedly in HK (0.42 µmol/min/kidney) vs C (0.74) ($p<0.005$). However, absolute Am delivery to both early (EP) and late (LP) proximal convoluted tubule did not differ in the two groups (EP:C=13.4±0.8 vs HK=11.8±0.8; LP:C=10.4±0.6 vs HK=9.9±0.6 pmol/min). Net Am secretion along the EP also did not differ (C= -11.5±0.7 vs HK= -10.1±0.7 pmol/min). In contrast, in situ pH was reduced significantly in HK rats at both EP (6.98±0.03 vs 7.06±0.02) and LP (6.58±0.02 vs 6.68±0.03) ($p<0.01$). $[NH_3]$ and total ammonia concentrations were similiar in both groups at each site. Therefore, in a model of chronic HK due to dietary K^+ loading: 1) $U_{Am}V$ was reduced despite systemic metabolic acidosis and an increase in V (factors that augment $U_{Am}V$ in the absence of HK); 2) the reduction in $U_{Am}V$ was not due to impaired Am secretion along the proximal convoluted tubule. The decrease in $U_{Am}V$ in HK may be due to a reduction in medullary trapping and impaired Am transfer from loop of Henle to medullary collecting duct.

CHANGES IN URINARY EPITHELIAL CELLS DURING ADAPTATION TO ACID-BASE IMBALANCE. John H. Durham,* (Intr. by M. Lipkowitz) Dept. Physiol. & Biophys., Mt. Sinai Sch. Med. N.Y.,N.Y. 10029

Bladders from NH_4Cl-loaded turtles do not secrete alkali and bladders from $NaHCO_3$-loaded turtles do not secrete acid while those from euhydric animals retain both functions. These adaptive changes could result from changes in the carbonic anhydrase (CA)-cell population (the probable source of acid and alkali secretion). The apical surfaces of bladders were stained with $AgNO_3$ and processed for carbonic anhydrase staining with Co using the Hanson technique and examined on a scanning electron microscope. X-ray microanalysis demonstrated that Ag selectively highlights the surface of CA-cells as defined by those cells which had acetazolamide dependent Co-accumulation (Rosen, J.His.Cy.,1972). In alkalosis, euhydria and acidosis respectively, the: %CA cells per total cells was 20 ±2%, 18 ±3%, and 18 ±2%; average CA cell area (sq. microns) was 45 ±5.3, 65 ±3.6 and 116 ±4; the %CA area/total area was 2 ±.3%, 7 ±2% and 9 ±1%. In all bladder types, the majority of CA-cells have the topography of the alpha-CA type (Stetson and Steinmetz, AJP 1985). It is evident that (i) no change in the number of CA cells occurs during adaptation to acid-base imbalance and (ii) the CA-cell area increases with in-vivo states of increasing acid excretion. It can be concluded that a decrease in alpha-CA cell area occurs concomitantly with a loss of acid secretion and it appears that the adaptation resulting in selective acid or alkali secretion during acid-base imbalance does not involve a change in the types of CA-cells present.

ACTIVATION OF A Na^+-INDEPENDENT Cl^-/HCO_3^- EXCHANGER IN PROXIMAL TUBULE CELLS UNDER ALKALINE LOAD CONDITIONS: DEPENDENCE ON pH_i. I. Fineman* and E.P. Nord. Division of Nephrology, HSC, SUNY at Stony Brook, NY.

The presence of a Cl^-/HCO_3^- exchange pathway, and the pH dependence of its activation under base-load conditions was examined in proximal tubule cells derived from monkey kidney (JTC). Changes in intracellular pH, (pH_i), were monitored in superfused monolayers using the pH-sensitive fluorescent probe, BCECF. An alkaline load was imposed by removing HCO_3^-/CO_2 from the bathing medium, and recovery from elevated pH_i followed in solutions with identical pH and in the absence or presence of Cl^-. In a solution buffered to pH 7.40 (pH_o) with 25mM HCO_3^-/5% CO_2, pH_i was 7.40 ± 0.04 (n=4) and increased to 7.86 ± 0.04 on removal of HCO_3^-/CO_2. Recovery towards basal pH_i was dependent upon Cl^-, blunted by 50μM DIDS, but independent of Na^+ or amiloride in the bathing medium. pH_o was subsequently changed to 7.0 or 6.7 by appropriate changes in HCO_3^- concentration in the presence of 5% CO_2. Acute removal of HCO_3^-/CO_2 resulted in an increment in pH_i from 7.03 to 7.40 (pH_o 7.0) and from pH_i 6.78 to 7.02 (pH_o 6.7) which failed to recover towards baseline in the presence of Cl^- in the bathing medium. Addition of 10^{-6}M TCS, a protonophore, collapsed the pH gradient. Increasing the buffer capacity by appropriate increments in HCO_3^-/CO_2 at a given pH failed to enhance the pH_i recovery process. Conclusions: 1. JTC cells possess a Na^+-independent Cl^-/HCO_3^- exchanger which allows for recovery from an alkaline load. 2. pH_i values above 7.4 are required for activation of the exchanger, even under base load conditions.

AXIAL HETEROGENEITY OF CORTICAL COLLECTING DUCT (CCD) CELL TYPES. C. Emmons, K. Matsuzaki*, J. Stokes, and V. Schuster, Dept. of Int. Med., Univ. of Iowa Coll. of Med., Iowa City, IA.

Rabbit CCD contains 3 cell types. Principal (P) cells transport Na and K (or Rb). Beta (β) intercalated cells absorb Cl. Alpha (α) intercalated cells secrete H. We used two approaches to assess axial distribution of each cell type. In a functional approach, we perfused rabbit CCDs in vitro and measured P cell function with the ^{86}Rb lumen-to-bath rate coefficient (K_{Rb}) and β cell function with ^{36}Cl (K_{Cl}). Then we halved tubule length by aspirating either the outer or inner half into the holding pipette. K_{Cl} and K_{Rb} were remeasured for the shortened segment, and then calculated for the aspirated tubule half. K_{Rb} for outer and inner CCD halves was not different (168±26 vs 149±13 nm/s). In contrast, K_{Cl} was twice as high in outer vs inner CCD (432±91 vs 195±41, p<0.05). In a histological approach, we fixed whole kidneys and cut slices from the surface and at depths of 1 mm (outer cut) and 2 mm (inner cut). The latter 2 depths correspond to the two halves of the perfused tubules. We stained the sections with both peanut lectin (L) and Hansson's carbonic anhydrase stain (H). Cells were identified as P cells (H-), α cells (H+,L-), or β cells (H+,L+). We found no difference in the fraction of total cells that were either P cells (0.63) or summed intercalated cells (0.37) at any depth. However, the β cell subtype significantly increased in the outer cortex (surface .13, outer .18, inner .12, p<0.005). Conclusions: 1) β cell number and function increase in outer CCD, at the expense of α cell number; 2) P cell number and function show no axial heterogeneity.

ROLE OF JUXTAMEDULLARY NEPHRONS IN THE CORRECTION OF CHLORIDE-DEPLETION METABOLIC ALKALOSIS IN THE RAT. IH Galla, DN Bonduris*, RG Luke. *Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, Alabama.*

In our model of acute chloride-depletion metabolic alkalosis (CDA) produced by peritoneal dialysis vs 0.15M $NaHCO_3$, delivery of Cl (180 ± 30 vs 167 ± 18 pmol/min) and tCO_2 (146 ± 23 vs 146 ± 20 pmol/min) out of the superficial cortical late distal tubule did not differ in maintained vs correcting CDA whereas urinary tCO_2 excretion increased in correcting animals (61 vs 209 neq/min/100g BW;P<0.01). To determine whether the juxtamedullary nephrons (JMN) contributed to this increment in urinary tCO_2 excretion, CDA was generated as before in young Munich-Wistar rats and, after 2 hrs of maintenance, either further maintained with 5% dextrose and 6% albumin (group DM; n=7) or corrected with 80 mM Cl added to that infusate (group CC; n=7). After an additional 30 min of infusion, JMN were sampled at the tip of Henle's loop during the succeeding 60 min. The results were as follows:

	P HCO3 (meq/L)	UtCO2V (neq/m)	SNGFR (nl/m/100g)	tCO2 del (pmol/m)	Cl del (pmol/m)
DM	40.1±1.1	356±137	30.4±2.9	202±56	1086±154
CC	28.2±1.0#	636±86	46.7±4.8#	145±18	889±248

Volume expansion was similar in both groups (final hematocrits 37.8±1.7% DM, 37.6±0.3CC); thus, the increment in SNGFR was probably due to diminished tubuloglomerular feedback, consistent with our previous findings.

These data suggest that delivery of tCO_2 out of JMN proximal to the tip of Henle's loop does not contribute to the bicarbonaturia that occurs during the correction of CDA by Cl. Thus, the primary site of the corrective response appears to reside in the collecting duct.

#P<0.05

Na$^+$-DEPENDENT, BASOLATERAL ACID-BASE TRANSPORT IN SUPERFICIAL AND JUXTAMEDULLARY RABBIT PROXIMAL S1, S2 AND S3 SEGMENTS. J. Geibel*, G. Giebisch & W.F. Boron. Yale Univ., Dept. Cell. & Mol. Physiol., New Haven, CT.

To compare Na$^+$-linked acid-base transport in proximal segments, we monitored intracellular pH (pH_i) of isolated perfused tubules loaded with BCECF. Cells at 37° were illuminated alternately with a 10-micron spot of 440-nm and 490-nm light. The emmission was monitored at 530nm. All cells were calibrated using the fluorescence excitation ratio method of Nigericin/high K$^+$. Bilateral Na$^+$ removal caused pH_i to fall from 7.24 to 6.75(n=15) in the superficial (SF) S1, 7.14 to 6.67(15) in SF/S2, and 7.09 to 6.69(10) in SF/S3. In juxtamedullary (JM) tubules, pH_i fell from 7.25 to 6.76(11) JM/S1, 7.16 to 6.71(11) in JM/S2, and 7.1 to 6.75(10) in JM/S3. In each case, pH_i recovered upon returning Na$^+$ to the bath. 50 µM DIDS blocked this Na$^+$-dependent pH_i recovery in SF/S1, SF/S2, SF/S3, and JM/S3, but had no effect in JM/S1 or JM/S2. Conversely, 50 µM EIPA blocked the Na$^+$-dependent pH_i recovery in JM/S1 and JM/S2, but had no effect on the other segments. In the DIDS-sensitive group, a 45-min preincubation in Cl$^-$-free solutions had no effect on the pH_i recovery induced by Na$^+$ readdition. We suggest that there are two Na$^+$-dependent acid-base transporters at the proximal basolateral membrane: (1) a Na/HCO_3 cotransporter in all SF segments and JM/S3, and (2) a Na-H exchanger in the JM/S1 and JM/S2.

EFFECTS OF CHRONIC METABOLIC ACIDOSIS (CMA) AND MINERALOCORTICOID ON H^+/HCO_3^- TRANSPORT IN RAT MEDULLARY THICK ASCENDING LIMB (MAL). D.W. Good and I. Kurtz. U. Texas Med. Br., Galveston, TX and UCLA, Los Angeles, CA.

Three series of experiments were performed to investigate regulation of H^+/HCO_3^- transport in the rat MAL. In series 1, MAL from control rats and rats drinking 0.28M NH_4Cl for 5-8 days (CMA) were perfused under standard conditions in vitro with 25 mM HCO_3^- in perfusate (P) and bath (B). Acidosis increased HCO_3^- absorption ($JHCO_3$, pmol/min/mm) from a control value of 11.3±1.2 to 17.1±1.1 (p<0.005). In series 2, MAL were perfused and bathed with HEPES-buffered, Na-free solutions (pH 7.4) and intracellular pH (pH_i) was measured using BCECF. When 20 mM NH_4Cl was removed from the P and B, pH_i fell by ≈1.0 unit and recovered at a rate of 0.07±0.01 pH unit/min. The pH_i recovery rate was reduced 60% by DCCD (1mM in P) and 71% by adding KCN (1 mM) and removing organic substrates from the P and B. Thus, under the conditions of these experiments, pH_i recovery required ATP, was Na-independent, and was inhibited by DCCD. CMA increased the pH_i recovery rate to 0.19±0.06 pH unit/min (p<0.05). In series 3, MAL from adrenalectomized (ADX) and sham-operated rats were perfused as described for series 1. ADX reduced $JHCO_3$ to 10.6±0.9 compared with 14.6±1.4 in sham-operated controls (p<0.05). In MAL from ADX rats, adding aldosterone (10^{-6}M) to the bath increased $JHCO_3$ significantly within 1-2 hr from an initial control value of 10.3±0.9 to 14.8±1.0, a rate not different from that in sham-operated controls. Conclusions: 1) $JHCO_3$ is increased in MAL from rats with CMA; 2) the MAL possesses a plasma membrane H^+-ATPase that can contribute to pH_i regulation and is stimulated by CMA; 3) $JHCO_3$ in the MAL can be modulated by aldosterone.

ROLE OF BASOLATERAL CHLORIDE ON tCO_2 TRANSPORT IN THE RAT CORTICAL COLLECTING TUBULE. D. Gifford*, K. Sharkins*, J.H. Galla, R.G. Luke, J. Work. *Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, Alabama.*

We have previously reported that tCO_2 transport in the rat cortical collecting tubule (CCT) is altered from absorption in control rats (CON, dialyzed with Ringers-HCO_3) to secretion in chloride-depletion metabolic alkalosis rats (CDA, dialyzed with 150 mM $NaHCO_3$). Removal of luminal chloride had no effect on net tCO_2 absorption in CON rats but abolished secretion and net tCO_2 flux in CDA rats. In order to determine the effect of basolateral chloride on tCO_2 transport, basolateral chloride was replaced with gluconate in tubules from both CON and CDA animals while luminal chloride was held constant at 105 mM. tCO_2 was measured by microcalorimetry. Results are expressed as Jnet tCO_2 (pmol•mm^{-1}•min^{-1}):

Bath Cl	*105 mM*	*0 mM*	*105 mM*
CON (n=4)	15.5 ± 4.9‡	-13.6 ± 9.2†	6.4 ± 2.2
CDA (n=6)	-12.1 ± 2.9¥	-26.1 ± 3.6†	-10.3 ± 3.3¥

‡ means ± SEM † p<0.05 105mM to 0mM ¥ p<0.05 CON vs CDA

When basolateral chloride concentration was reduced by half in a separate series, Jnet tCO2 decreased linearly (R=0.98) from net absorption to net secretion in CON tubules:

Bath Cl	*105 mM*	*52 mM*	*0 mM*
CON (n=3)	9.3 ± 1.6‡	0.5 ± 0.8§	-12.3 ± 0.2¶

‡ means ± SEM; § p< 0.05, 105mM vs 52mM; ¶ p<0.05 52 mM vs 0 mM

The data suggest that in the rat CCT: 1) decreasing basolateral chloride promotes net tCO_2 secretion, and 2) basolateral chloride may be necessary for net tCO_2 absorption. The resultant transepithelial chloride gradient may stimulate the apical anion exchanger while possibly inhibiting the basolateral anion exchanger.

CHLORIDE (Cl^-) MODIFIES THE EFFECTS OF cAMP ON ENDOSOMAL PROTON TRANSPORT. R. W. Gurich and T.D. DuBose, Jr. UTMB, Galveston, Texas.

cAMP has been demonstrated to either stimulate or inhibit endosomal H^+ transport depending on the concentration employed or fraction studied (Clin. Res. 519A, 1988). This disparate response could represent an effect of cAMP directly on H^+ ATPase, or alternatively, on Cl^- transport which could secondarily modulate H^+ transport. Thus, the effects of cAMP on H^+ transport under high (106 mM) [Cl^-] conditions was compared to the effects of cAMP on H^+ transport under conditions in which the Cl^- contribution to H^+ transport was minimized i.e. low (6 mM) [Cl^-] plus K^+/valinomycin voltage clamp to provide charge compensation. Endosomes were prepared from rabbit renal cortex by differential and sucrose gradient centrifugation. H^+ transport rates were measured using acridine orange. Experiments were performed in the presence or absence of 5 or 250 µM 8-Br-cAMP. Results are expressed as percent stimulation (+) or inhibition (-) by 8-Br-cAMP. * p<0.005 high vs low Cl^-.

Fx#	High Cl^- 5µM	Low Cl^- 5µM	High Cl^- 250µM	Low Cl^- 250µM
1	+13.1±1.3	-68.1±5.6*	-20.5±3.1	-97.5±8.6*
2	+14.3±2.1	-59.7±8.3*	-23.4±4.2	-94.8±9.7*
3	-1.5±3.0	-43.5±6.2*	-39.6±6.7	-83.5±6.6*
4	-11.3±1.5	-47.7±6.7*	-41.2±7.0	-93.7±9.3*
5	-10.7±1.7	-44.4±7.3*	-45.6±4.3	-88.3±7.2*
6	-12.8±1.3	-59.8±5.2*	-51.3±3.8	-85.8±5.3*

Inhibition of H^+ transport by cAMP is seen under conditions in which Cl^- transport is minimized, suggesting cAMP directly inhibits H^+ ATPase. cAMP inhibition of H^+ transport is augmented dramatically when [Cl^-] is reduced suggesting this response is Cl^- dependent. cAMP may enhance Cl^- entry and reduce the endosomal electrical gradient opposing H^+ entry.

Na-INDEPENDENT Cl/HCO_3 EXCHANGE IS THE MAJOR BASOLATERAL MEMBRANE HCO_3 TRANSPORT MECHANISM IN THE OUTER MEDULLARY COLLECTING TUBULE (OMCT). S.R. Hays and R.J. Alpern. UT-Southwestern Medical Center, Dallas, TX.

To examine the mechanism of basolateral membrane HCO_3 transport in the OMCT, cell pH (pHi) was measured microfluorimetrically in the rabbit OMCT (inner stripe) perfused in vitro using the pH-sensitive dye, BCECF. Under control conditions, pHi was 7.13±0.04. Acidifying the bath by 0.7 pH units caused pHi to decrease 0.24±0.04 pH units ($p<0.005$), while acidifying the lumen by 0.7 pH units caused pHi to decrease only 0.03±0.01 pH units ($p<0.05$). Isohydric bath Cl removal caused pHi to increase from 7.13±0.04 to 7.28±0.07 ($p<0.01$), an effect which was reversible, and completely inhibited by 100 µM DIDS. The effect of bath Cl removal on pHi was present in the complete absence of luminal and bath Na. To rule out the possibility that the apparent Cl/HCO_3 exchange was mediated by parallel Cl and HCO_3 conductances, two studies were performed. In cells voltage clamped by addition of 5 µM valinomycin and 125 mM extracellular K, bath Cl removal alkalinized cells by 0.48±0.06 pH units ($p<0.001$), an effect not consistent with parallel conductances. In addition, Cl removal inhibited basolateral membrane HCO_3 permeability by 99%.

In summary, the basolateral membrane of the OMCT possesses a Na-independent Cl/HCO_3 exchanger which forms the major pathway for basolateral membrane HCO_3 transport. As in the proximal tubule, basolateral membrane transporters are more potent determinants of pHi than are apical membrane transporters.

KCL DEPLETION PROMOTES METABOLIC ALKALOSIS CONSEQUENT TO PHOSPHATE ADMINISTRATION. Allen M. Kaufman, Daniel Kaw,* and Thomas Kahn. Bronx Vet. Adm. Med. Ctr. and Mt. Sinai Sch. Med., New York, NY.

Phosphate administration in normal animals increases net acid excretion (NAE) but not $PHCO_3$. These studies evaluate if KCl depletion (KCLD), commonly associated with metabolic alkalosis, permits phosphate administration to produce alkalosis.

KCLD was produced in rat by 5d on a KCl deficient diet. Then a)Na-neutral phosphate 4000 ueq /24h was added to the diet for 3d. In other groups small phos-induced K and/or Cl losses were replaced by adding b)KCl c)K_2SO_4 (K without Cl) or d)NaCl (Cl without K). $NAE=NH_4+TA-HCO_3$. Baseline in KCLD was (in ueq/3d): NAE=2640, K=132 and Cl=189. $PHCO_3$ after 3d was 26.7 meq/l.

TABLE: Phosphate Administration in KCl Depletion. ΔExcretion=ueq/3d, $\Delta PHCO_3$=meq/l.

Replacement	ΔNAE	ΔK	ΔCl	$\Delta PHCO_3$
a) none	+615*	+201*	+108*	+6.1*
b) KCl	+12	+201*(R)	+312*(R)	-0.9
c) K	+981*	+198*(R)	+153*	+7.9*
d) Cl	+21	+168*	+18(R)	+5.0*

(R)=phos-induced losses replaced; *=significantΔ.

In normals phosphate (data not shown) increased NAE +483 ueq/3d but not $PHCO_3$. a)In KCLD a phos-induced increase in NAE was associated with an increase in $PHCO_3$. b)Replacement of phos-induced K and Cl losses prevented the increase in NAE and $PHCO_3$. c)Replacement of K losses did not prevent the increase in NAE or $PHCO_3$. d)Without Cl loss NAE did not increase; nevertheless $PHCO_3$ rose, suggesting extra-renal factors increased $PHCO_3$.

Thus: 1)KCl depletion enhances the alkalosis-promoting effect of neutral phosphate. 2)Phos-induced increases in $PHCO_3$ may relate to increases in NAE and to extrarenal factors.

STUDIES OF THE CRITICAL LYSINE GROUP AT THE AMILORIDE BINDING SITE OF THE RENAL Na/H ANTIPORTER. Z-Q Huang* and D Warnock. NRTC and Nephrology Section, University of Alabama at Birmingham, Birmingham, AL.

Isothiocyanato (ITC) reagents attack a critical lysine group on the Na/H antiporter in brush border membrane vesicles (BBMV; Kid Int 33:401a, 1988). The present studies used an ITC derivative of 5'-(3-amino) amiloride (A35-NCS), described by Cassel et al (JBC 262: 4587, 1987), to characterize the amiloride binding site of the antiporter. When reacted with BBMV at pH 8.5, A35-NCS irreversibly inhibited the antiporter in a concentration dependent manner. The Vmax of the antiporter was reduced by 20% after 30 min treatment with 1.0 mM A35-NCS, but there was no significant effect on the Km for sodium. Amiloride protected the Na/H antiporter against attack by A35-NCS as well as other ITC reagents. Polyclonal antibodies were raised to 5'-(3-amino) amiloride coupled to hemocyanin, and then affinity purified. These antibodies were used in Western blots to label A35-NCS coupled to renal BBMV; labelling of a protein at 77 Kd indicated the putative amiloride-binding domain of the Na/H antiporter.

Conclusions: 1). ITC reagents attack a critical lysine group of the renal Na/H antiporter. 2). This group interacts with 5'-amino substituted forms of amiloride, and regulates the Vmax of the antiporter. 3). The amiloride-binding domain of the antiporter appears to be a 77 Kd protein.

Na-H ANTIPORTER IN LUMINAL MEMBRANES OF RENAL CORTEX, PARS RECTA AND MEDULLA: ROLE IN HYPERCAPNIA. F.T. Kear*, O.S. Ruiz*, Z. Talor and J.A.L. Arruda, Dept of Medicine, WSVAMC and Univ of IL, Chicago, IL.

The Na-H antiporter of renal brush border membranes has been well characterized and plays a role in adaptation to acidosis. The Na-H antiporter of other renal structures has not been well defined and its role in the adaptation to acidosis is unclear. We measured the Na-H antiporter in luminal membranes of the pars recta and medulla and in plasma membranes from the papilla and compared it to the Na-H antiporter of the cortex in control and in rabbits exposed to 10% CO_2 for 48 h. Chronic hypercapnic rabbits had significantly higher pCO_2 and plasma HCO_3 and lower urine pH than controls. Luminal membranes from the cortex and pars recta were enriched 10 fold in alkaline phosphatase while the luminal medullary membranes were enriched 20 fold in H^+-ATPase. In control animals, the magnitude of the Vmax of the Na-H antiporter (in fluorescence units/300 µg protein/ min) was 540 ± 44 in cortex, 196 ± 16 in pars recta, 117 ± 17 in medulla, and 141 ± 17 in the papilla. These values were significantly different from each other except between medulla and papilla. The Km for Na, however, was not different suggesting that the renal Na-H antiporter is basically the same in different nephron segments but displays different activity. Hypercapnia increased significantly the Vmax by 64% in the cortex but failed to alter the Vmax in pars recta, medulla and papilla. These results demonstrate that the activity of the Na-H antiporter varies in the different nephron segments but only the cortical Na-H antiporter adapts to hypercapnia.

ROLE OF Na/H EXCHANGE AND $H^{\pm}$ ATPase IN INTRACELLULAR pH (pHi) REGULATION IN INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. D. Kikeri* and M.L. Zeidel. Harvard Medical School, Boston, MA.

pHi and membrane potential (PD) were monitored in fresh rabbit IMCD cell suspensions using BCECF and DiS-C3-(5) respectively. Acid-loaded cells exhibited faster rates of pHi recovery in 130mM NaCl (.33±.02 pHU/min) than in 130mM KCl (.23±.01 pHU/min); 0.1mM amiloride inhibited Na-dependent pHi recovery (.20±.02 pHU/min). In addition, acidification of IMCD cells tripled ^{22}Na uptake rates. Varying PD (by setting K gradients) did not alter Na-dependent pHi recovery rates. Thus, fresh IMCD cells have an electroneutral Na/H exchanger. To examine the role of $H^{\pm}$ ATPase, studies were performed in the absence of extracellular Na. The rate of pHi recovery (.24±.02 pHU/min) was reduced by cellular ATP depletion (.13±.02) and by 1mM NEM (.16±.01). Graded hyperpolarization of PD reduced and then abolished pHi recovery. Cellular proton extrusion (measured using a pH stat) was inhibited by ATP depletion. Several lines of evidence indicate that the $H^{\pm}$ ATPase, but not the Na/H exchanger, participates in acid extrusion at the resting pHi (7.21±0.03) of IMCD cells: a) In nonacidified cells, membrane hyperpolarization but not removal of extracellular Na led to intracellular acidification. b) pHi increased equally rapidly in the presence or absence of Na when cells were diluted into medium of pH 8.3. We conclude that both Na/H exchange and a plasma membrane $H^{\pm}$ ATPase mediate pHi recovery in acidified fresh IMCD cells, and that the $H^{\pm}$ ATPase but not the Na/H exchanger extrudes acid at the resting pHi.

METABOLIC ALKALOSIS INCREASES THE NUMBER OF ß-CARBONIC ANHYDRASE (CA) CELLS. **D. Kniaz, R. Mola and J.A.L. Arruda, Dept. of Medicine, U of IL and WSVAMC, Chicago, IL.**

We studied the mechanism whereby the turtle bladder adapts to metabolic alkalosis. Turtles were made alkalotic by oral gavage with 30 mmol/day of $NaHCO_3$ for 3-4 days (blood pH 7.57 ± 0.03 vs 7.44 ± 0.02 as compared to controls). Urine pH increased from 6.36 ± 0.17 to 7.17 ± 0.18, $p < 0.0005$. HCO_3 secretion was greater in bladders from alkalotic turtles as compared to controls (1.23 ± 0.15 vs 0.73 ± 0.05 µmoles/hr, $p < 0.005$). H^+ secretion, however, was not different. The total number of CA rich cells was determined by fluorescence microscopy after mucosal staining with 6-carboxyfluorescein (6CF). The number of HCO_3 secreting cells (ß-CA) was quantified by mucosal staining with NBD-taurine and the number of H^+ secreting (α-CA) cells was calculated from the difference. The total cell counts per field were determined by ethidium bromide and results are expressed as % of total (*=$p < 0.01$ vs control).

	Total CA	ß Cells	α Cells
Control	4.6 ± 0.3	3.0 ± 0.3	1.6 ± 0.3
Alkalosis	6.5 ± 0.7*	4.9 ± 0.5*	1.8 ± 0.7

Metabolic alkalosis significantly increased 6CF positive cells and NBD-taurine positive cells. The increase in 6CF positive cells could be totally accounted for by the increase in NBD-taurine positive cells without an increase in the α cells. Thus, metabolic alkalosis selectively increases the number of ß-subtype of CA cells. The data show that in vitro turtle bladders "remember" the alkali environment and suggest that the adaptation is mediated through an increase in the number of HCO_3 secreting cells.

Cl^-/HCO_3^- EXCHANGE OF INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS IS PREDOMINANTLY A Na^+-INDEPENDENT PROCESS. C. Kim*, E.P. Nord and J.A. Kraut. Divisions of Nephrology, Wadsworth V.A. Medical Center, UCLA School of Medicine, Los Angeles, CA, and HSC, SUNY at Stony Brook, NY.

Previous studies from this laboratory have demonstrated the presence of a Cl^-/HCO_3^- exchanger in cultured IMCD cells. To determine the Na^+ dependence of this transporter, the effect on intracellular pH (pH_i) of Cl^- removal from a solution buffered to pH 7.4 with 25 mM HCO_3^-/5% CO_2 was assessed, in the presence and absence of Na^+. Changes in pH_i were measured using the pH-sensitive fluorescent probe, BCECF. In the presence of 140 mM Na^+, Cl^- removal led to a rise in pH_i which averaged 0.23 ± 0.03 pH unit (n=5). In the absence of Na^+, the identical experimental maneuver resulted in a rise in pH_i of 0.13 ± 0.03 pH unit, and in one of seven studies no increment in pH_i was observed. These data could suggest a requirement for Na^+ by the exchanger. Alternatively, the lesser rise in pH_i observed in the absence of Na^+ could be due, in part, to offsetting acidification of the cytosol by reversal of Na^+/H^+ antiport. Accordingly, cells were bathed in a Na^+-replete medium and switched to a Na^+-free medium. An abrupt fall in pH_i was observed which was attenuated by exposure of cells to 10^{-4}M amiloride. When Cl^- was subsequently removed from the Na^+-free, HCO_3^-/CO_2-buffered medium containing amiloride, pH_i rose 0.46 ± 0.02 pH unit (n=5). Conclusions: 1) Cl^-/HCO_3^- exchange in the IMCD cell is predominantly a Na^+-independent process. 2) In the absence of Na^+, reversal of Na^+/H^+ exchange or possibly lower pH_i could account for the blunted Cl^-/HCO_3^- exchange response.

BICARBONATE SECRETION: EFFECTS OF SODIUM. John D. Koethe, Robert L. Stephenson*, George M. Feldman. VA Med. Cntr. and Univ. of PA., Dept. of Med., Phila., PA.

Bicarbonate secretion ($JsHCO_3$) occurs in the cortical collecting duct as well as other tissues, including distal colon. To examine the role of Na in $JsHCO_3$, net HCO_3 flux was measured in segments of rat distal colon under short-circuited conditions; mucosal and serosal surfaces were bathed with symmetrical solutions of pH 7.4, containing 24 mM HCO_3, 147 mM Na and PCO_2 = 40 mmHg. Under these conditions $JsHCO_3$ was 1.0±0.2 µeq/h/cm^2. Ouabain (1 mM) reduced $JsHCO_3$ to 0.1±0.1 µeq/h/cm^2, demonstrating that the Na pump is required for $JsHCO_3$. While removal of Na from the bathing medium symmetrically (replacement with choline or N-methylglucamine) stopped $JsHCO_3$, replacing lumenal Na had no effect on basal $JsHCO_3$ (0.9±0.1 µeq/h/cm^2). On the other hand, removing basolateral Na reduced $JsHCO_3$ to 0.5±0.1 µeq/h/cm^2 ($p<0.01$), suggesting that basolateral Na is required for HCO_3 entry across the basolateral membrane. Na removal also increased total tissue resistance and the effect was greater when Na was removed from the basolateral side, consistent with a conductive step on the basolateral side (control, 149±19 Ω·cm^2; lumenal, 210±32; basolateral, 323±46). SITS (1 mM) on the basolateral surface reduced $JsHCO_3$ 60% ($p<0.01$), while SITS on the lumenal surface was without effect, suggesting that basolateral HCO_3 entry may be linked to Na. To examine the effect of membrane potential, bathing medium K concentration was increased from 5 mM to 20 mM and $JsHCO_3$ decreased from 1.1±0.2 to 0.7±0.2, suggesting that $JsHCO_3$ requires cell polarization. In conclusion, $JsHCO_3$ in rat distal colon depends on Na. These data are consistent with a linked Na-HCO_3 entry step across the basolateral membrane which may be conductive.

THE APICAL CL-HCO_3 EXCHANGER OF BICARBONATE SECRETING CELLS IN TURTLE BLADDER. Orly F. Kohn*, Peter P. Mitchell* and Philip R. Steinmetz, University of Connecticut Health Center, Division of Nephrology, Farmington, CT.

The apical anion exchanger of the β carbonic anhydrase (CA) cells differs from the basolateral exchanger of α CA cells in that it is less sensitive to disulfonic stilbenes and not immunoreactive with band 3 protein antibodies. To characterize the exchanger, we examined the effects on electroneutral HCO^-_3 secretion (J^nHCO_3) of Cl-replacement by Br^-, $SO^=_4$, NO^-_3 and gluconate and inhibition by 1) acetazolamide (ACZ) with and without NaN_3 2) furosemide (F) and 3) α-cyano-4-hydroxycinnamate (CHC). The Cl-dependent J^nHCO_3 was 0.72 ± 10 μmoles/hr, similar to the ACZ-inhibitable rate of 0.67 ± .09 μmoles/hr, with apparent Km near 5mM and maximal rate at 20mM luminal[Cl]. Maximal J^nHCO_3 was comparable at luminal pH 6.8 and 4.5. J^nHCO_3 was reduced to 31 ± 8% by Br^-, to 25 ± 9% by $SO^=_4$ and to 11 ± 3% by NO^-_3 substitution. ACZ inhibition occurred even after pretreatment with NaN_3 and presumably was not mediated by endocytosis. J^nHCO_3 was only slightly (14%) inhibited by F and not inhibited by CHC. In conclusion, the characteristics of the apical exchanger differ from the basolateral exchanger as well as the red cell and neutrophil exchangers. It is relatively inhibitor-resistant and selective for Cl^-. Its dependence on luminal Cl^- is such that its transport rate is closely regulated by luminal Cl^- at concentrations below 20mM.

MECHANISMS OF ADAPTATION TO CHRONIC RESPIRATORY ACIDOSIS IN THE PROXIMAL TUBULE (PT). Reto Krapf, UCSF, San Francisco. (Intro. by F.C. Rector*).

The hyperbicarbonatemia of chronic respiratory acidosis is maintained by enhanced bicarbonate reabsorption in the PT. To investigate the cellular mechanisms involved in this adaptation, cell and luminal pH were measured microfluorometrically using BCECF in isolated, microperfused S_2 proximal convoluted tubules from control and acidotic rabbits. Chronic respiratory acidosis was induced by exposure to 10% CO_2 for 52 to 56 hours. Arterial P_{CO_2} and HCO_3 were significantly higher in acidotic rabbits: 69 vs. 40.4 torr and 32.6 vs. 26.1 mM, respectively. All perfusion experiments were performed in the bilateral absence of chloride. Tubules from acidotic rabbits (perfused at 10 nl/min with 25 mM HCO_3 at P_{CO_2} of 40 torr) had a significantly lower luminal pH after 1 mm perfused length (7.03 ± 0.09 vs. 7.26 ± 0.06 in controls). Chronic respiratory acidosis increased the initial rate of cell acidification (dpH_i/dt) in response to luminal sodium removal by 63% and in response to lowering luminal pH (7.4 to 6.8) by 87%. Chronic respiratory acidosis also increased dpH_i/dt in response to peritubular sodium removal by 63% and in response to lowering peritubular pH by 93%. Baseline cell pH values and cellular buffer capacities were the same in tubules from normal and acidotic rabbits.

In conclusion, chronic respiratory acidosis induces a parallel increase in the rates of the luminal Na/H antiporter and the basolateral $Na/(HCO_3)_3$ cotransporter. The enhanced PT reabsorption of bicarbonate in chronic respiratory acidosis is mediated, at least in part, by a parallel adaptation of these transporters.

REGULATION OF INTRACELLULAR pH ($[pH]_i$) IN THE UPPER PART OF DESCENDING LIMB OF LONG-LOOPED NEPHRON (LDLu): A FLUOROMICROMETRIC STUDY. Chizuko Koseki, Yohkazu Matsushima, Koji Yoshitomi, and Masashi Imai (Intr. by G.Kimura). *Depts. of Pharmacol. and CV. Dynam., Natr. Cardiovasc. Ctr. Res. Inst. Osaka, Japan.*

The LDLu is rich in mitochondria and has high carbonic anhydrase in cytoplasm and Na^+-K^+ ATPase in basolateral membrane. These features suggest that the LDLu has active mechanisms in the regulation of transepithelial or transmembrane H^+ transport. To examine mechanisms of H^+ transport in the LDLu, we measured $[pH]_i$ as well as intraluminal pH ($[pH]_{TF}$) in the *in vitro* perfused segments from hamsters by microscopic fluorometry using BCECF as a fluorescent pH probe. In HCO_3^--free modified Ringer solution (pH7.4), $[pH]_i$ was 7.23±0.05 (n=18). Administration of 10^{-3}M amiloride (Amil) to the lumen decreased $[pH]_i$ from 7.15 ±0.10 to 6.95±0.10 (n=6, p<0.01). Addition of N-methylmaleimide (NEM) also decreased $[pH]_i$ from 7.23±0.12 to 7.01±0.09 (n=5, p<0.05). After H^+ was loaded into the cell by adding 20mM NH_4Cl, $[pH]_i$ was recovered, with an initial H^+ transport velosity (R_H:pH units/min) being 1.23±0.26(n=10). R_H was inhibited by 10^{-3}M Amil in the lumen from 1.80±0.36 to 1.02±0.18 (n=6), and by 10^{-3}M NEM (lumen, bath) from 1.02±0.24 to 0.42±0.06 (n=7). Under the stopflow condition, $[pH]_{TF}$ was rapidly equilibrated with bath pH, indicating that this segment is highly permeable to H^+. We conclude that both Amil- and NEM-sensitive mechanisms are operating in the LDLu to regulate $[pH]_i$ rather than transmural H^+ transport.

BASOLATERAL MEMBRANE Na^+- AND Cl^--DEPENDENT H^+/BASE TRANSPORT IN THE RABBIT S_3 PROXIMAL TUBULE. I. Kurtz. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

The basolateral membrane Na^+- and Cl^--dependent H^+/base transport processes were studied in the rabbit S_3 proximal tubule. pH_i was measured with BCECF. Tubules were perfused (L) and bathed (B) in 25mM HCO_3^-, pH 7.4 in the absence of organic anions and SO_4^{2-}. Reduction in HCO_3^- (B) to 5mM caused pH_i to decrease 0.81 pH/min, n=6. In the absence of Cl^- (L,B), the decrease in pH_i was 0.57 pH/min, n=7, p<0.01. In Na^+-free solutions (L,B), the rate was 0.33 pH/min, n=8, p<0.001. In Na^+- and Cl^--free solutions (L,B), the rate was 0.16 pH/min, n=5, p<0.001. Removal of Na^+ (B) caused pH_i to decrease 0.24 pH/min, n=4. Amiloride (1mM, B) decreased the rate to 0.11 pH/min, n=5, p<0.01, and SITS (1mM, B) decreased the rate to 0.13 pH/min, n=5, p<0.05. Cl^- removal (L,B) had no effect on the decrease in pH_i induced by Na^+ removal (B). In HEPES-buffered solutions (L,B), only an amiloride-sensitive component could be detected. Cl^- removal (B) in the absence of Na^+ (L,B) caused pH_i to increase 0.40 pH/min (n=5). DIDS (50 μM, B) completely inhibited the increase in pH_i induced by Cl^- removal (B). In HEPES-buffered solutions (L,B), the rate of increase in pH_i induced by Cl^- removal (B) was 0.45 pH/min, n=3. Conclusions: 1) the rabbit S_3 proximal tubule possesses a basolateral membrane Na^+/H^+ antiporter, Na^+/base cotransporter, and a Na^+-independent Cl^-/base exchanger; 2) 50% of base efflux is coupled to Na^+, 30% is Cl^--dependent, and 20% is Na^+ and Cl^--independent; 3) the Na^+-independent Cl^-/base exchanger, unlike the Na^+/base cotransporter, functions in the nominal absence of HCO_3^-.

AMILORIDE-SENSITIVE Na /H ANTIPORT IN CELLS OF MEDULLARY THICK ASCENDING LIMB (mTAL) OF RAT KIDNEY. F.Leviel*, E.Marty*, R.Costalat*,M.Paillard, and M.Bichara*. Lab. Physiologie Rénale, Hôp. L. Mourier, 92 Colombes, and INSERM, 75 Paris, France.

To characterize mTAL cell proton transport, we have monitored intracellular pH (pHi) in fresh suspensions of fragments of mTAL isolated from inner stripe of rat outer medulla using the fluorescent probe, BCECF. When mTAL cells were incubated in HCO_3^--free and Na-free tetramethylammonium medium (TMA-medium, pH=7.40), pHi was 7.18± 0.01; cells were acidified by the nigericin technique to pHi=6.87±0.04. Following hypertonic addition of TMA-Cl or when acidified cells were resuspended in isotonic TMA-medium, there was no pHi recovery. Hypertonic addition of NaCl to TMA- medium or resuspension of acidified cells in isotonic Na-containing medium caused pHi to recover within 1 or 2 min. Since cell-buffering power (βi) was measured under these kinetic conditions by the NH_4^+ technique, we were able to determine the initial (first 12s) proton efflux (JHi= dpHi /dt xβi, in mmol H^+ /liter/s). JHi exhibited saturability to external Na (Km for Na= 20.5-24 mM and JHi max = 0.35-0.45 in hypertonic and isotonic Na-media). Na-dependent pHi recovery was inhibited by amiloride in a dose-dependent manner by simple competitive inhibition (Ki=2.7-2.9 x10^{-5} M). Using valinomycin and various external K^+ concentrations, Na-dependent pHi recovery was not membrane potential-sensitive. We conclude that an electroneutral amiloride-sensitive Na^+/H^+ antiport mediates mTAL cell pHi regulation and may contribute to mTAL proton secretion in the rat in vivo.

IDENTIFICATION OF INTERCALATED CELLS (IC) IN RABBIT MEDULLARY COLLECTING DUCT. Kirsten M. Madsen, Jill W. Verlander*, Paul J. Linser*, and C. Craig Tisher. Div. of Nephrology, and C.V. Whitney Lab., Univ. of Florida, Gainesville, Florida

There is evidence that IC are involved in proton secretion in the collecting duct (CD) of both rat and rabbit. In the rabbit it is generally accepted that IC are present in the CD of the cortex (CCD) and the outer stripe of the outer medulla ($OMCD_o$). However, there is disagreement whether IC are present in the CD in the inner stripe of the outer medulla ($OMCD_i$). In a recent study IC were not observed in the $OMCD_i$, but the majority of cells in the $OMCD_i$ were positive for carbonic anhydrase (CA) by histochemical methods. Thus, controversy exists regarding the cell type responsible for proton secretion in the $OMCD_i$. We have used a mouse monoclonal antibody (Ab) against chick erythrocyte CA II in combination with an immunocytochemical peroxidase technique to identify CA-positive cells along the rabbit CD. In addition, the $OMCD_i$ was examined by scanning (SEM) and transmission electron microscopy (TEM). Rabbit kidneys were fixed by in vivo perfusion with paraformaldehyde-lysine-periodate fixative for immunocytochemistry and with glutaraldehyde for SEM and TEM. Light microscopy of paraffin sections incubated with Ab against CA II demonstrated that all cells with the appearance of IC were labeled. CA-positive cells accounted for 30-35% of the total cell population in the connecting tubule, CCD, and $OMCD_o$ and approximately 10% in the outer half of the $OMCD_i$. Principal cells and cells in the inner medullary CD were negative. By SEM most cells in the $OMCD_i$ had small microvilli on the surface, but a few cells in the outer $OMCD_i$ were covered with a mixture of microplicae and microvilli characteristic of IC. TEM confirmed the presence of two cell populations in the outer portion of the $OMCD_i$, whereas only one cell type was found in the inner portion of the $OMCD_i$. These results demonstrate that in the rabbit, CA-positive IC constitute approximately 10% of the cells in the outer $OMCD_i$, and only IC are positive for CA when using a monoclonal Ab against chick CA II.

ANGIOTENSIN II (AII) STIMULATES ACIDIFICATION IN EARLY (S_1) PROXIMAL CONVOLUTED TUBULE (PCT) BY DECREASING cAMP. F.-Y. Liu and M.G. Cogan. CVRI and Dept. Med., Univ. Calif., San Francisco.

We recently reported that AII potently stimulated bicarbonate absorption ($J_{HCO_3^-}$) in S_1 PCT cells, but the second messenger responsible is unknown. To explore the hypothesis that AII increases $J_{HCO_3^-}$ by diminishing intracellular cAMP, we performed in vivo microperfusion (30 nl/min) in S_1 PCT in 41 Munich-Wistar rats. We measured $J_{HCO_3^-}$ (microcalorimetry, peq/mm·min) and tubular fluid cAMP delivery ($TF_{cAMP}V$, RIA, fmol/mm·min), a reflection of cell cAMP level.

AII (20 ng/kg·min, i.v.) increased J_{HCO3} (348±11 to 588±8, $p<0.001$) and decreased $TF_{cAMP}V$ (18±2 to 12±2, $p<0.05$), while PTH (0.5 ug/kg·min, i.v.) had the expected opposite effects (351±5 to 194±12 and 18±1 to 24±1). Co-administration of AII and PTH cancelled each other's actions, returning J_{HCO3} (400±15) and $TF_{cAMP}V$ (16±1) to control values. Saralasin and PTH were additive on J_{HCO3} reduction (101±1) and TF_{cAMP} elevation (29±2); parathyroidectomy and AII also were additive on $J_{HCO_3^-}$ stimulation (609±11) and $TF_{cAMP}V$ lowering (9±1). Combining all results, there was an excellent inverse relationship of $J_{HCO_3^-}$ with $TF_{cAMP}V$ ($Y=-21.3X+740$, $r=0.86$). Clamping intracellular[cAMP] with luminal dibutyryl cAMP (10^{-5}M) abolished the AII-induced increase in $J_{HCO_3^-}$. Pertussis toxin (20 ug/kg, i.v., 3 days prior) significantly attenuated (by 20-30%) the AII-induced stimulation of $J_{HCO_3^-}$ and decrease in TF_{cAMP}, implying G_i-protein intermediation. In conclusion, these in vivo results suggest AII stimulates $J_{HCO_3^-}$ by a G_i-mediated depression in cAMP.

MECHANISM OF H-ION SECRETION IN CORTICAL DISTAL TUBULES IN THE RAT. Gerhard Malnic* and Miguel J. Lopes*(intr. by G. Giebisch). Depto. Fisiologia, Inst. Ciências Biomédicas, Univ. São Paulo, São Paulo, Brazil.

The nature of cortical distal tubule acidification in mammalian kidney is not well defined. We measured luminal and capillary pH in early (ED) and late distal (LD) segments of rat kidney using double barrelled H-resin/reference microelectrodes. Control transepithelial (TE) pH gradients were 0.70 ± 0.12 (n=16) in ED and 1.16 ± 0.016 (8) in LD. These gradients were abolished but not inverted in alkalotic, K-loaded rats. When luminal Na was lowered by capillary perfusion with low-Na solution, lumen pH rose by 0.22 ± 0.02 (9). During luminal perfusion with low Na plus 10^{-3} M amiloride lumen pH rose by 0.67 ± 0.01 (4) in ED, while TE PD was 14.5 ± 2.5 mV more positive. The observed TE PD changes were about 1/3 of the values expected from the pH changes. Luminal perfusion with 10^{-3} M n-ethylmaleimide increased luminal pH by 0.32 ± 0.08 (8) in LD against 0.20 ± 0.10 (7) in proximal tubules. These results suggest that cortical distal H-ion secretion has sodium-dependent and H-ATPase mediated components.

K-DEPENDENT H^+ SECRETION: AN ALDOSTERONE RESPONSIVE PATHWAY. David E. McBride# and Ronald D. Perrone. Renal Division, New England Medical Center, Boston, MA.

Recent studies from this laboratory have demonstrated that in vitro K^+ absorption in rat distal colon is markedly enhanced by CO_2 and aldosterone (aldo) when studied in Na-free Ringer. K^+ absorption appeared to be electroneutral and was inhibited by vanadate, mucosal but not serosal ouabain and the H^+-K^+-ATPase inhibitor SCH28080. These findings suggested the existence of a luminal H^+-K^+-ATPase that was stimulated by aldosterone. To determine whether aldo stimulated an H^+-K^+-ATPase, pH stat studies of luminal H^+ secretion (J_H) were performed in voltage-clamped segments of control and aldo-stimulated rat distal colon using HCO_3-free Ringer containing 140 mM Na^+ bubbled with 100% O_2. Secondary hyperaldosteronism was induced by feeding a Na-deficient diet. ATPase inhibitors and ion substitution were utilized to determine the mechanism of J_H. (J_H in ueq.h^{-1}. 1.13 cm^{-2})

	K^+ Ringer	K-free mucosal	K-free bilateral
Control	0.69±0.05	0.41±0.05+	0.18±0.04+
Aldo	0.99±0.07#	0.77±0.06#+	0.46±0.05#+

Means ± SE. N=3-8. # P<0.05 vs control, + P<0.05 vs K-Ringer.

The lesser inhibition of J_H with mucosal compared to bilateral K-free Ringer likely reflects the higher luminal $[K^+]$ (~0.5 meq/l) when 5 mM K^+ is present in the serosal bath. The ATPase inhibitors vanadate (100 uM to both baths) and mucosal ouabain (5 mM) significantly inhibited J_H in both control and aldo-stimulated colon. Luminal acidification in rat distal colon is K-dependent and inhibited by vanadate and mucosal ouabain suggesting the likely participation of an H^+-K^+-ATPase. The activity of this putative H^+-K^+-ATPase is increased by aldo.

DETERMINANTS OF AMMONIA (Am) ENTRY ALONG THE RAT PROXIMAL CONVOLUTED TUBULE (PCT) IN VIVO DURING CHRONIC METABOLIC ACIDOSIS (CMA). C. Merli*, E.E. Simon, J. Herndon*, E.J. Cragoe, Jr.*, L. L. Hamm. Jewish Hospital and Washington University, St. Louis, MO. and MSD Res. Lab., West Point, PA.

Studies in vitro have demonstrated significant PCT Am entry via the Na/H exchanger. The effects of flow rate, luminal pH, and the Na/H exchanger on Am entry along the rat PCT during CMA were examined using in vivo microperfusion. With a 5 mM HCO_3-containing perfusate, collected fluid [Am] remained constant with increasing flow rate (15, 30, and 45 nl/min); thus Am entry was highly flow-rate dependent. With a 25 mM HCO_3 perfusate, entry plateaued with increasing flow rate associated with increasing collected fluid $[HCO_3]$; this suggested inhibition of diffusion-trapping of Am. However, in all cases, Am entry was higher than in control rats. The effects of Na/H exchange inhibition on Am entry were examined using 5-(N-ethyl-N-isopropyl) amiloride. With a 25 mM HCO_3 perfusate at 30 nl/min, there was a significant (33%, $p<0.02$) decrease in $[HCO_3]$ reabsorption but a non-significant decrease in Am entry associated with a significant rise in collected fluid bicarbonate concentration. When the potential effects of decreased diffusion-trapping of Am were eliminated with a 5 mM HCO_3 perfusate, there was no effect of the amiloride analog on Am entry (23.2 ± 2.6 pmol/min/mm vs 20.1 ± 2.8 without analog). Thus, ammonia entry in CMA is highly flow-rate dependent, but there is a modest effect of tubule fluid pH. In vivo, there were no effects of Na/H exchange inhibition above those expected from inhibition of diffusion-trapping of Am.

EFFECTS OF GLUCAGON (GLU) ON BICARBONATE TRANSPORT IN THE DISTAL TUBULE. O.Mercier*, M. Bichara*, A. Prigent*, M.Delahousse*, F.Leviel*, and M. Paillard. Lab. Physiologie Rénale, Hop. L. Mourier, 92 Colombes, and INSERM, Paris, France.

We have shown that GLU increases urinary bicarbonate excretion (AJP 254:F762,1988). Paired experiments were performed in Wistar rats subjected to hypotonic volume expansion (EXP) (JCI 80: 621, 1987) to determine the effects on total CO_2 (tCO_2) transport in the distal tubule accessible to micropuncture of infusions of GLU and somatostatin (SOM) that inhibits GLU secretion. Systemic acid-base status was normal and stable throughout all experiments. Whole-kidney and single nephron GFR were not affected by GLU or SOM. GLU inhibited whereas SOM stimulated final urine acidification. Results (means±SE) obtained along the distal tubule are shown in the Table.

	FR H_2O	$[tCO_2]$, mM Early distal	Late distal	Net tCO_2 flux pmol/min
EXP	0.17±0.04	3.7±0.7	4.6±0.9	- 2± 7
SOM	0.20±0.10	5.1±0.6	4.3±0.6	25±13
	NS	P<0.01	NS	P<0.05
EXP+SOM	0.15±0.04	5.2±0.7	4.5±0.5	18± 8
GLU	0.11±0.03	4.4±0.5	6.3±0.7	-14± 4
	NS	NS	P<0.05	P<0.01

(FR, fractional absorption. Negative values denote secretion).

Chloride absorption along the distal tubule was not affected by GLU or SOM. We conclude that GLU stimulates bicarbonate secretion (and/or inhibits proton secretion) in the distal tubule and cortical collecting tubule, probably by acting directly on intercalated cells.

THE MECHANISM OF ACTIVATION OF THE Na^+/H^+ EXCHANGER BY CELL SHRINKAGE AND AGONISTS. Shmuel Muallem*, Jacob Green*, (Int. Charles R. Kleeman). Lab of Membrane Biology, Division of Nephrology, Cedars Sinai Med. Ctr., UCLA School of Medicine, L.A., CA.

The Na^+/H^+ exchanger in many cell types can be stimulated by various hormones, second messengers and hypertonic stress. We studied the mechanism of activation of the exchanger by various stimuli in the human epidermoid carcinoma cell line A431 and in peripheral blood mononuclear cells (PBM) by following pHi with BCECF. In A431 cells, epidermal growth factor (EGF) and bradykinin (BK) evoked initial acidification followed by amiloride-sensitive alkalinization. Phorbol ester (PMA) and osmotic shrinkage caused amiloride-sensitive alkalinization without prior acidification. In PBM, an activation of the Na^+/H^+ exchanger was evoked by Con A, PHA, PMA and osmotic shrinkage. Protein kinase C inhibitor, inhibited only PMA-mediated Na^+/H^+ exchange activation. Furthermore, stimulation by any agonist augmented exchanger activation by osmotic shrinkage. Hence, various stimuli appear to activate the Na^+/H^+ exchanger by different mechanisms.

Kinetic analysis of exchanger activation by agonists and cell shrinkage in A431 cells reveals that stimulation of the exchanger by various stimuli is followed by reciprocal changes in the apparent affinities for H^+_i and Na^+_i. While there is an increased apparent affinity for H^+_i, the apparent affinity for Na^+_i decreases. These findings suggest that there is a common mechanism of exchanger activation by stimuli acting to activate the exchanger by different biochemical pathways.

EFFECT OF ANGIOTENSIN II ON AMMONIA PRODUCTION BY ISOLATED PERFUSED MOUSE PROXIMAL TUBULES. Glenn T. Nagami, Med and Res Svcs, VAMC West Los Angeles and Dept of Med, UCLA School of Medicine, Los Angeles, CA.

Angiotensin II (AII) is a potent regulator of acidification in the proximal tubule. To determine the effects of AII on ammonia production (AP) by the proximal tubule, isolated perfused mouse proximal tubules were incubated in the presence of various concentrations of AII. Proximal tubule segments consisting of the late convoluted and early straight portions were perfused at luminal flow rates of 20 nl/min with Krebs-Ringer bicarbonate buffer (KRB) and were incubated at 37°C in KRB containing 0.5 mM L-glutamine and 1.0 mM acetate, equilibrated with 95% O_2: 5% CO_2, pH 7.4. AP rates were higher in proximal tubule segments incubated with 10^{-10} M AII (31.0±0.9 pmol/min per mm) and in tubules incubated with 10^{-9} M AII (31.0±1.2) than in tubules incubated with no AII (21.8±0.6 pmol/min/mm, $p<0.05$). Incubation of tubules with 10^{-12} M AII had no significant effect on AP. Incubation with 10^{-8} M AII inhibited AP by 20%. Incubating tubules with 10^{-7} M saralasin (SAR) alone had no effect on AP but addition of 10^{-7} M SAR reversed the stimulatory effect of 10^{-10} M AII on AP. Conclusions: 1) AP by isolated perfused mouse proximal tubules is affected by AII in a dose-dependent fashion such that AII at concentrations of 10^{-10} and 10^{-9} M stimulates AP while AII at a higher concentration of 10^{-8} M inhibits AP; and 2) SAR reverses the stimulatory effect of AII on AP.

REGULATION OF RENAL Na/H ANTIPORTER: INTERACTIONS OF METABOLIC ACIDOSIS (MA) AND K DEPLETION (KD). T. Northrup*, E. Perticucci*, JJ. Cohen, S. Garella Michael Reese Hosp, U of Chicago, Chicago, IL

Previous work has shown that enhanced Na/H antiporter activity (Na/H AA) in MA cannot be attributed to reduced blood pH. Because MA also causes KD, we examined whether KD mediates the MA-induced increase in Na/H AA.

Na/H AA and kidney K content were measured in 5 groups of rats: 1-Control (Normal diet, N=10); 2-KD (K-free diet, N=5); 3-MA with KD (Normal diet plus NH_4Cl, N=6); 4-MA without KD (Adrenalectomy [ADX], fixed dose dexamethasone and aldosterone plus NH_4Cl, N=6); 5-ADX Control (ADX, fixed dose dexamethasone and aldosterone as in group 4, N=6). Group 4 was designed to obviate the kaliuretic effect of NH_4Cl by combining ADX with low dose aldo replacement.

As shown in the table, groups 1, 2 and 5 maintained normal $[HCO_3]_p$ (and pH); groups 3 and 4 had reduced $[HCO_3]_p$ (and pH). Kidney K content was reduced only in groups 2 and 3, whereas Na/H AA was increased in groups 2, 3 and 4.

Group	$[HCO_3]_p$	Kidney K[a]	V_{max}[b]
1 Control	26.4±1.3	101±1	31.3±3.0
2 KD	25.9±0.7	94±2*	40.1±4.0*
3 MA with KD	19.6±0.8*	93±1*	45.8±6.2*
4 MA without KD	13.4±1.1*	111±6*	42.9±4.0*
5 ADX Control	27.7±0.8	114±6*	27.2±2.0

* $p < 0.05$ vs Group 1
([a] μmol/gm wet weight) ([b] units/sec/mg protein)

Conclusions: KD by itself increases Na/H AA; the enhanced Na/H AA seen in MA is, however, not mediated by KD.

Basolateral Na-independent Cl-HCO_3 Exchange in the S3 Segment of the Rabbit Proximal Tubule. N.L. Nakhoul*, L. Chen*, and W.F. Boron. Dept. Cell. Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT.

We examined basolateral HCO_3^- transport and the role of Cl^- in intracellular pH (pH_i) regulation of the S3 segment of the rabbit proximal tubule. pH_i was calculated from the absorbance spectra of the pH-sensitive dye dimethylcarboxyfluorescein. Tubules (S3 segments) were isolated and perfused at 37° with HCO_3^--containing solutions equilibrated with 5% CO_2. When bath (B) pH was lowered from 7.4 to 6.7 by reducing $[HCO_3^-]_B$ from 25 to 5mM at constant pCO_2, pH_i decreased rapidly and reversibly by 0.48±0.02 (n=13). When pH_B was again decreased, but in the bilateral absence of Na^+, pH_i still fell rapidly, indicating the presence of a Na-independent acid-base transporter. Removal of Cl^- from (B) caused pH_i to rapidly and reversibly increase by 0.23±0.03 (20); the inital rate was 100±8 x 10^{-4} pH/sec. When Cl^- was removed from the lumen (L), pH_i rose by only 0.07±0.01 (4), and the initial dpH_i/dt was just 18±3.9 x 10^{-4} pH/sec (4). In the nominal absence of HCO_3^-, removing Cl^- from (B) caused pH_i to increase by 0.15±0.06 (5), with an initial dpH_i/dt of only 22±6 x 10^{-4} pH/sec. In the bilateral absence of Na^+, but the same pH_i, (B) Cl^- removal still caused pH_i to rise (0.13±0.03, 4) with a dpH_i/dt of 120±22 x 10^{-4} pH/sec. In the presence of 0.1 mM DIDS, removal of (B) Cl^- elicited no change in pH_i. These data indicate that there is a Na-independent Cl-HCO_3 (base) exchanger at the basolateral membrane, but do not rule out the possibility of Na-dependent Cl-HCO_3 exchange.

HCO_3 TRANSPORT IN CELLS OF MEDULLARY THICK ASCENDING LIMB (mTAL) OF RAT KIDNEY. M.Paillard, F.Leviel*, E.Marty*, R.Costalat*, and M. Bichara*. Lab. Physiologie Rénale, Hôp. L.Mourier, 92 Colombes, and INSERM, Paris, France.

To characterize mTAL cell HCO_3 transport, we have monitored intracellular pH (pHi) in fresh suspensions of fragments of mTAL isolated from inner stripe of rat outer medulla using the fluorescent probe, BCECF. When mTAL cells were preloaded with HCO_3 in a Na-containing medium and then diluted into HCO_3-free Na-medium (pH=7.40), pHi rose rapidly to 7.89±0.08 (SE) (initial alkalinization) and then declined to 7.47±0.05 within 1 min (secondary acidification). When mTAL cells were alkalinized to pHi=7.90±0.05 by preincubation in HCO_3-free Na-medium at pH=8.2 and then diluted in HCO_3-free Na-medium at pH=7.40, there was no cell acidification. Thus initial alkalinization was attributed to rapid CO_2 efflux from the cells, and secondary acidification to specific HCO_3 efflux. The initial rate of secondary acidification ($dpHi/dt$= 0.018±0.003 pH/s) was not altered by 2x10^{-3}M amiloride or by replacing external chloride with gluconate, which excludes Na^+/H^+ and Cl^-/HCO_3 antiports as the mechanisms responsible for secondary acidification. When mTAL cells were preloaded with HCO_3 but Na-depleted in a Na-free tetramethylammonium (TMA) medium and then diluted into HCO_3-free TMA-medium (pH=7.40), pHi rose rapidly due to CO_2 efflux but subsequent HCO_3 efflux and thus secondary acidification did not occur. We conclude that a HCO_3 transport, Cl-independent but probably Na-dependent, mediates mTAL cell pHi regulation and may contribute to mTAL HCO_3 absorption in the rat in vivo.

FUNCTIONAL HETEROGENEITY OF ACID-BASE TRANSPORT IN THE AMPHIBIAN LATE DISTAL TUBULE (LDT). Marc Paulais*, Takis Anagnostopoulos and Gabrielle Planelles*. INSERM U.192, Hôpital Necker-Enfants Malades, Paris, France.

We studied the mechanism(s) of luminal acidification in the LDT of Necturus in vivo, by measuring luminal (pH_l) and capillary pH (pH_b) with double-barreled H^+-selective microelectrodes. Peritubular perfusion with a nominally CO_2-free TES-buffered solution (TES) at pH 7.5 produced two distinct patterns. Among 14 randomly selected LDT loops, 5 displayed an average transepithelial potential (V_T) of -14.8±5.5 mV (mean±SE) and pH_b-pH_l=0.64±0.08 ; the TES solution increased pH_l by 0.27±0.09. In the 9 other convolutions, in which V_T was -36.2±8.1 mV and pH_b-pH_l 0.87±0.07, the same TES solution lowered pH_l by 0.30±0.05. The former pattern suggests presence of luminal carbonic anhydrase (LCA), the latter absence of LCA. This was confirmed by studying 5 other tubules, each displaying several superficial convolutions ; TES perfusate was applied before and under luminal benzolamide (BZL) infusion, 50µM. In each tubule, convolutions with the largest pH_b-pH_l and most negative V_T responded to TES with acidification both before and during BZL. In contrast, loops with low V_T and small pH_b-pH_l yielded increase of pH_l upon exposure to TES before BZL, decrease of pH_l upon peritubular application of TES when the lumen was infused with BZL. Subsequent injection of a small oil droplet at the BZL perfusion site confirmed increasing length of LDT with lower steady state pH_l and larger V_T in each tubule. In conclusion : 1) Initial portions of the LDT, but not the late loops, contain LCA. 2) Lack of LCA in late loops leads to a steady state acid disequilibrium pH_l. 3) TES- induced acidification in these loops apparently dissipates a large cell-to-lumen HCO_3^- gradient (J. Physiol.393:73, 1987), thereby decreasing a HCO_3^- leak.

MECHANISM OF THE ACIDIFYING DEFECT AFTER RELEASE OF UNILATERAL URETERAL OBSTRUCTION (UUO). H.E. Purcell*, K.P.G.Harris*, I.Lim*, S.Klahr, S.Gluck. Renal Division, Washington Univ. St. Louis MO.

Following release of UUO there is a profound distal acidification defect. Since intercalated cell (IC) H^+ATPase is largely responsible for distal nephron acid secretion we studied the morphologic effect of UUO on this transporter using antisera against H^+ATPase. Measurements were made in sham operated rats and following 3hr, 5days and 10days of release of 24hr UUO (n=4 per group). Urinary pH was greater in the post-obstructed kidneys at 3 hours (5.72±.32 vs 7.83±.17 $p<0.001$) but not at 10days. Three IC staining patterns were identified: apical (A), diffuse (D) and basolateral (B).

	CORTEX			
	IC	A	D	B
	%tubular cells	%IC	%IC	%IC
SHAM	44±2.4	57±2.8	29±2.2	14±2.4
3 HR	42±2.6	60±5.2	34±4.7	6±6.5
5 Days	42±2.5	55±3.1	31±3.6	15±1.6
10 Days	44±.65	59±5.6	32±5.7	9±1.1
		MEDULLA		
SHAM	41±0.6	91±2.3	9±2.2	.2±0.2
3 HR	40±2.0	87±4.7	11±3.2	1±1.1
5 Days	46±2.0	92±3.7	7±3.8	1±0.7
10 Days	46±1.7	87±4.0	13±4.0	0

There were no significant differences between any of the time points post UUO and sham. Qualitative comparison of contralateral and experimental kidneys show no marked differences in fluorescent intensity. The above data imply that despite the presence of a distal acidification defect in this model, there is no significant change in IC number, H^+ATPase distribution or, on a qualitative basis, in H^+ATPase amount. This does not exclude inhibition of H^+ATPase or H^+ backleak.

CONTRIBUTIONS OF CELLULAR LEAK PATHWAYS TO NET $NaHCO_3$ AND NaCl ABSORPTION IN RAT PROXIMAL CONVOLUTED TUBULE (PCT). P.A. Preisig and R.J. Alpern. UT Southwestern Med. Ctr., Dallas, TX.

Apical (Ap) and basolateral (Bl) membrane proton (H) and formic acid (H_2CO_2) permeabilities were measured in the in vivo doubly microperfused rat PCT using the pH-sensitive dye, BCECF. Permeabilities were calculated from the rate of change in intracellular pH (dpHi/dt) in response to a rapid change in luminal or peritubular [H] or [H_2CO_2], using a measured buffer capacity (64±12 mM/pH units). All studies were performed in the absence of Na and Cl to eliminate contributions from Na/Cl-coupled transporters.

When perfusate pH was changed from 7.32→5.50, the calculated Ap and Bl H permeabilities (P_H) were 0.52±0.07 and 0.67±0.18 cm/sec, respectively. Using these permeabilities the calculated late PCT rate of H leak from lumen→cell is 17 pmol/mm·min (150% of measured net H secretion rate) and from peritubular fluid→cell is 4 pmol/mm·min. Ap and Bl H_2CO_2 permeabilities (P_{H2CO2}) measured at extracellular pH 6.62 were 6.1±0.6 x 10^{-2} cm/s and 9.1±2.0 x 10^{-2} cm/s, respectively. Control studies demonstrated that P_{H2CO2} was not underestimated by either the simultaneous movement of basic formate into the cell or the efflux of H_2CO_2 across the contralateral membrane. Using the measured Ap P_{H2CO2}, H_2CO_2 recycling is too slow for parallel Na^+/H^+-Cl/formate exchangers to mediate the majority of apical membrane NaCl absorption in the rat. If Ap P_{H2CO2} is used as an estimate of carbonic acid (H_2CO_3) permeability, carbonic acid recycling can account for all of carbonic anhydrase-independent HCO_3 absorption.

CHANGES IN APICAL MEMBRANE AREA ASSOCIATED WITH BICARBONATE SECRETION IN TURTLE BLADDER. A. Rich*, T.E. Dixon, and C. Clausen*. Dept. of Physiology and Biophysics, SUNY, Stony Brook, NY, and VAMC, Northport, NY.

The urinary bladder of the turtle possesses an electrogenic HCO_3^- secretory process, which is known to be stimulated by cAMP. Recent reports show that cAMP is associated with an increase in apical-membrane surface area specifically in carbonic-anhydrase-rich cells thought to be responsible for HCO_3^- transport. We investigated the association between HCO_3^- transport and the morphological changes. Transepithelial impedance was analyzed in order to measure changes in apical-membrane capacitance (Ca, proportional to membrane area) and conductance (Ga, proportional to ionic permeability). Bathing solutions were Cl^--free (to inhibit proton secretion) and contained 0.1 mM amiloride, and 20 mM HCO_3^-; they were bubbled with 5% CO_2. HCO_3^- secretion was stimulated with 1 mM 8-Br-cAMP (n=4) or DiButyryl-cAMP (n=2), in the presence of 0.5 mM IBMX, and resulted in an increase in Isc of 12±5 µA/cm². Concomitant with the increased rate of transport, we measured a 42±9% increase in Ca, and a 168±34% increase in area-normalized conductance Δ(Ga/Ca). Phorbol myristate acetate (PMA) has been reported to stimulate HCO_3^- secretion. Mucosal addition of 1 µM PMA resulted in an increase in Isc of 3.5±2.1 µA/cm² (n=3), and this was accompanied by a 26±9% increase in Ca and a 49±23% increase in Δ(Ga/Ca). We conclude that HCO_3^- secretion is accompanied by an increase in apical membrane area and conductance, suggesting that regulation of the process involves vesicle fusion.

CONTROL OF INTRACELLULAR pH (ipH) IN RAT LYMPHOCYTES (L). G. Rombola* and D.C. Batlle, Northwestern Univ. and Lakeside VA, Chicago, IL.

Since ipH regulation in L has been examined in the absence of external HCO_3 the possible participation of HCO_3-dependent acid transporting systems in the regulation of ipH has not been defined. To study this issue, acid extrusion was measured by monitoring ipH recovery after acid loading of L to a pH_i of about 6.5 using NH_4Cl. Thymus-derived rat L were loaded with BCECF-AM and fluorescence measured at two excitation wavelengths of 500 and 440 nm to calculate ipH. Steady-state pH_i was higher in HCO_3/CO_2 buffered medium than in HEPES buffered medium (7.24±0.05 and 7.06±0.04 p<0.01). The initial phase of ipH recovery from an acid load (first 1 min.) was fast and proceeded at a comparable rate in the presence and in the absence of external HCO_3 (0.19 and 0.26 pH/min. respectively). This rapid phase of pH_i recovery was completely obliterated by EIPA, a specific Na+/H+ exchange inhibitor. By 4 min., ipH increased by 0.46±0.08 and 0.37±0.03 pH units in the presence of HCO_3 and in its absence, respectively. ipH recovery was completely obliterated in the absence of external Na whereas EIPA obliterated it completely in the absence of HCO_3 but only partially in its presence (about 80% of control). As ipH approached normal steady-state pH_i (about 15 min.), recovery became less sensitive to EIPA but remained Na-dependent. We conclude that, in the presence of external HCO_3, regulation of ipH is completely Na+/H+ exchange-dependent at very low ipH. As pH_i approaches the physiological range, a Na-dependent EIPA-insensitive HCO_3 transporter(s) assumes an increasing role in the control of ipH in L.

PROXIMAL TUBULAR DYSFUNCTION IN CYSTINOSIS IS SECONDARY TO INHIBITION OF ACTIVE TRANSPORT. Richard F. Salmon* and Michel Baum, Univ. of Texas SW Med. Ctr., Dept. of Ped., Dallas, TX.

To examine the mechanism of proximal tubular dysfunction in cystinosis, the present in vitro microperfusion study examined the effect of intracellular cystine loading, using cystine dimethyl ester (CDME) on volume absorption (J_V), bicarbonate (J_{TCO2}), and glucose transport (J_{Glu}) in proximal convoluted tubules (PCT). PCT were perfused with an ultrafiltrate-like solution and bathed in a serum-like albumin solution. 0.5mM CDME (n=6) added to the bath inhibited J_V (0.67±0.07 to 0.15 ±0.09 nl·mm-1·min-1; p<0.001), J_{TCO2} (47.2±4.9 to 11.1±2.8 pmol·mm-1·min-1; p<0.001), J_{Glu} (34.1±1.5 to 19.6±1.5 pmol·mm-1·min-1; p<0.001), and transepithelial potential difference (-3.2±0.4 to -0.4±0.2 mV; p<0.001). As controls, the methyl esters of leucine (n=6), tryptophan (n=5), and cysteine (n=5) did not inhibit J_V, J_{Glu}, or J_{TCO2}. In tubules bathed in a solution containing 25mM HCO_3 with 1mM acetazolamide (ATZ) and perfused with a zero J_V solution containing 5mM HCO_3, 1mM ATZ and ^{14}C mannitol, addition of CDME had no effect on HCO_3 permeability, or mannitol permeability.

In conclusion, an in vitro model of cystinosis, achieved by intracellular cystine loading, inhibits volume absorption, as well as bicarbonate and glucose transport. This inhibition is not seen with the methyl esters of leucine, tryptophan, or cysteine. The decrease in proximal tubular transport in this model is not due to altered proximal tubular permeability, but rather to an inhibition in active transport.

H^+-ATPase IN DIFFERENT NEPHRON SEGMENTS. **O.S. Ruiz*, Z. Talor and J.A.L. Arruda, Dept of Medicine, WSVAMC and Univ of IL, Chicago, IL.**

An oligomycin resistant NEM sensitive H^+-ATPase has been described in luminal medullary membranes and has been postulated to play a role in urinary acidification. NEM-sensitive H^+-ATPase in other nephron segments has not been characterized in detail. Thus, we measured H^+-ATPase activity in presence of oligomycin and ouabain and in the absence of calcium and H^+-transport activity (by quenching of acridine orange in presence of ATP) in luminal membranes from cortex, pars recta and medulla of the rabbit kidney. Luminal membranes from pars recta were enriched 12 and 7 fold in alkaline phosphatase and H^+-ATPase, respectively. Medullary membranes were enriched 20 fold in H^+-ATPase activity. In the cortex, there was minimal H^+-ATPase activity and there was no H^+-transport in presence of ATP suggesting absence of functional H^+-ATPase in this nephron segment. Luminal membranes from the pars recta and medulla showed DCCD sensitive H^+-ATPase which was also sensitive to NEM and filipin. In presence of ATP, these membranes were capable of transporting H^+ as assessed by quenching of acridine orange. H^+-ATPase activity both by enzymatic as well as by transport assays was 3 fold greater in the medulla than in pars recta. H^+-ATPase activity in both pars recta and medulla was chloride dependent. Furthermore, the Km for chloride (by both enzymatic and transport assays) was very low (2-3 mM) indicating a H^+-ATPase with high affinity for chloride. In conclusion, a DCCD-sensitive H^+-ATPase with high affinity for chloride is present in luminal membranes from pars recta and medulla but not in the cortex.

FATE OF HCO_3 SECRETING INTERCALATED CELLS (IC) IN RESPONSE TO IN VITRO METABOLIC ACIDOSIS (MA). Lisa M. Satlin and George J. Schwartz. Albert Einstein Coll. of Med., Dept. of Peds., Bx, N.Y.

HCO_3 secretion by the cortical collecting duct (CCD) is accomplished by IC that bind rhodamine peanut agglutinin (PNA) to apical surface receptors. Cl removal from the lumen resulted in an increase in pH_i (BCECF) of 0.40±0.07 (SE) in PNA+ IC. Based on these experiments significant Cl/HCO_3 exchange was defined as an increase in cell pH of ≥0.2 pH units. After 20 hrs of in vivo MA there was a 28% reduction in the number of PNA+ IC per mm and reversal in direction of net HCO_3 transport in CCD. In an attempt to capture the cells during the remodeling process, we examined the changes in PNA+ IC in response to 3 hrs of in vitro MA in perfused CCD labeled with fluorescein-PNA (tCO_2=15 mM;pH 7.0) or control (tCO_2=26 mM;pH 7.4) conditions. The decrease in number of PNA+ IC per mm CCD after MA (41±5) exceeded control (9±5; p<0.05). After MA PNA cap size decreased by 35±4% vs. 16±4% in control (p<0.01). The pH of fluorescein-PNA caps (6.79±0.10) was lower than control (7.26±0.16; p<0.025), suggesting cellular removal of the PNA during MA. TEM confirmed that MA enhanced endocytosis of luminal electron-dense PNA. We then examined whether MA also affected apical Cl/HCO_3 exchangers. After MA 8/13 PNA+ IC failed to show Cl/HCO_3 exchange vs. 2/13 in control (p<0.025, X^2). We conclude that MA stimulates the removal of apical Cl/HCO_3 exchangers and membrane bound PNA ligand in HCO_3 secreting IC. We speculate that this cellular remodeling may mediate the reversal in CCD HCO_3 transport during MA.

CO-EXISTENCE OF H^+-ATPase AND BAND 3 Cl-HCO_3 EXCHANGER IN RABBIT COLLECTING DUCT (CD) INTERCALATED CELLS. V. Schuster, and S. Gluck. Univ. of Iowa Coll. of Med., Iowa City, IA, and Washington Univ. Sch. of Med., St. Louis, MO.

We and others have suggested that rabbit CD absorbs HCO_3 via "α" intercalated cells (proposed apical H^+-ATPase and basolateral Cl-HCO_3 exchanger) and secretes HCO_3 via "β" intercalated cells (opposite orientation). Although previous studies have found evidence for each of these separate transporters in CD intercalated cells, the co-existence of H^+-ATPase and Cl-HCO_3 exchanger at opposite membranes of the same α or β cell has not been shown. We perfusion-fixed rabbit kidneys with periodate-lysine-paraformaldehyde, cut 1 micron frozen sections, and double-labelled the same section with both 1) rabbit polyclonal antibodies to the 30 k, 56 k, or 70 k subunits of the bovine kidney medullary vacuolar H^+-ATPase; and 2) a mouse monoclonal antibody to human red cell "band 3" Cl-HCO_3 exchanger. In inner stripe, outer medulla about half the total cells labelled for band 3 at the basolateral membrane. Of these, 98% (n=47) also had apical H^+-ATPase. Antibodies to the three different subunits gave similar staining. In outer stripe, outer medulla each cell was also concordant for H^+-ATPase and band 3. However, 20/44 band 3-positive cells had apical H^+-ATPase, and 24/44 had diffuse cytoplasmic H^+-ATPase. Cortical CD was similar to outer stripe. We saw no preferential basolateral H^+-ATPase. No cells showed apical band 3. Conclusion: 1) The "α" cell model has been directly confirmed, i.e. apical H^+-ATPase and basolateral Cl-HCO_3 exchanger. 2) The "β" cell transporters may differ from those of α cells.

LUMINAL GLUTAMINE DEAMIDATION IN THE RAT PROXIMAL CONVOLUTED TUBULE (PCT) IN VIVO: ASSESSMENT BY A NEW MICROANALYTICAL GLUTAMATE ASSAY. E.E. Simon and L.L. Hamm. The Jewish Hospital of St. Louis and Washington University, St. Louis, MO.

The contribution of luminal ammoniagenesis in the late PCT via γ-glutamyl transferase (γ-GT) remains controversial, but if important, must rely on glutamine (gln) secretion since filtered glutamine is reabsorbed in the early PCT. Luminal gln loss is partially inhibited by phenylalanine (phe) and γ-GT is stimulated by hippurate (hip). Under these optimal conditions, luminal gln deamidation to glutamate (glu) would be favored. The luminal loss of glu may be abolished by aspartate (asp); thus the accumulation of glu would be an integrated index of luminal gln deamidation. This hypothesis was tested using in vivo microperfusion in conjunction with a new microfluorometric assay for glu (NADH generation via glutamate dehydrogenase). With a perfusion solution containing 10 mM hip, 10 mM phe, 10 mM asp and 0.5 mM glu, collected fluid [glu] averaged 0.58 ± 0.02 mM. Perfusion with a similar solution, but with 2 mM gln and 0 glu, resulted in a collected fluid [glu] of 0.77 ± 0.10 mM. However, when glu- and gln-free, collected fluid [glu] was only 0.12 ± 0.02 mM; glu delivery averaged 1.7 ± 0.4 pmol/min/mm. This represents a maximal value for the contribution of luminal gln deamidation in vivo since the glu accumulation may be partly a consequence of glu entry and the conditions favor gln deamidation. Am entry averages about 10 pmoles/min/mm under these same conditons. Thus, the luminal conversion of gln to glu via γ-GT must be a small component of total ammoniagenesis in this segment.

HEMODYNAMIC DIFFERENCES BETWEEN BICARBONATE (B) AND CARBICARB (C) TREATMENT IN AMMONIUM CHLORIDE (AC) ACIDOSIS. J.I. Shapiro, M. Whalen*, G. Filley*, N. Kindig*, and L. Chan. Dept. of Med. Univ. of Colorado Med. Sch. Denver, CO.

The deleterious effects of B during clinical therapy of acidosis have created considerable controversy regarding its use. We have previously shown that B and C have different effects on intracellular brain pH and arterial pCO2 in AC acidosis despite similar effects on blood pressure (Shapiro et al, Clin Research 1988). To more completely assess the hemodynamic effects of these agents, the following studies were performed on ventilated rats made acidotic with AC (10 mmol/kg). Cardiac outputs (CO) were measured using the indocyanine green method. In the first study, the rate of intravenous B administration was varied from rapid bolus, 2 minute and 10 minute infusions in a cross-over manner in 6 rats. The effects of different B infusion rates on CO were similar with the most negative effects on CO seen 10 and 15 minutes following infusion in each group (-50±15%, -35±10%, and -35±14%, respectively, all $p < .05$). In the second study, the effects of a 2 minute infusion of B or C on CO were compared using a cross-over design. B but not C therapy had a deleterious effect on CO 10-15 minutes following infusion as shown in the table below.

CO(ml/min)	Baseline	5 min	10 min	15 min
B(N=8)	94±10	89±8	74±7**	67±7**
C(N=7)	77±10	78±10	75±7	70±8

Results expressed as mean ± sem. ** $p<.01$ vs Baseline. These data suggest that B therapy is associated with decreases in CO regardless of the rapidity of administration where C does not appear to have any deleterious hemodynamic effects.

CHANGES IN TRANSMEMBRANE POTENTIAL(ψ) MEDIATE THE RISE IN INTRACELLULAR pH(pH_i) INDUCED BY IONOMYCIN IN INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. I.N. Slotki*, J.H. Schwartz and E.A. Alexander. Thorndike Mem. Lab., Renal Section, Boston City Hosp., Depts. of Medicine and Physiology, Boston Univ. School of Medicine, Boston, MA.

We have recently shown that the rise in intracellular calcium (Ca^{2+}_i) produced by ionomycin is associated with a rise in pH_i in cultured IMCD cells of the rat. This increase in pH_i is independent of Na^+/H^+ exchange and is not affected by inhibitors of a putative proton pump. The purpose of the present study was to explore further the mechanism of this ionomycin induced cell alkalinization. Confluent monolayers were made quiescent by incubation for 24 h in 0.1% serum prior to study. Changes in pH_i, Ca^{2+}_i and $\Delta\psi$ were measured in separate experiments with the fluorescent probes BCECF, fura-2 and bisoxonol respectively. In nominally bicarbonate free medium containing 110mM Na^+ and 1mM Ca^{2+}, addition of ionomycin 10^{-6}M produced an increase in pH_i from 7.21±0.04 to a steady state value of 7.44±0.09 (n=7) and completely depolarized the cells within 7 min. Depolarization induced by substitution of 110mM K^+ for Na^+ caused a rise in pH_i similar to that produced by ionomycin but did not alter Ca^{2+}_i. Valinomycin 1μM caused no further change in pH_i. The subsequent addition of ionomycin did not produce cytosolic alkalinization. We conclude that ionomycin causes intracellular alkalinization by collapse of $\Delta\psi$. This depolarization appears to be mediated by Ca^{2+} induced changes in membrane ionic conductance.

Na^+/H^+ EXCHANGE IS STIMULATED BY PROTEIN KINASE C ACTIVATION IN INNER MEDULLARY COLLECTING DUCT CELLS.

I.N. Slotki*, J.H.Schwartz and E.A. Alexander, Dept. of Medicine Boston City Hosp. and Boston Univ. School of Medicine, Boston, MA.

Intracellular pH (pH_i) and H^+ transport in cultured inner medullary collecting duct (IMCD) cells of the rat are regulated by a primary active H^+ pump and a secondary active Na^+/H^+ exchanger. In studies of non-renal epithelia, Na^+/H^+ exchange is activated by protein kinase C (PKC). The currrent study was undertaken to investigate the role of PKC in the activation of the Na^+/H^+ exchanger in IMCD cells. Confluent monolayers were made quiescent by incubation for 24 h in 0.1% serum. H^+ secretion was estimated by changes in pH_i measured with the fluorescent probe, BCECF. Phorbol-12 myristate-13 acetate (PMA), a synthetic analogue of diacyl glycerol was used to stimulate PKC. In nominally bicarbonate free Hepes buffer containing 110 mM Na^+ and 1mM Ca^{2+}, addition of PMA increased IMCD pH_i from 7.29±0.08 to a steady state value of 7.54±0.07 within 20 min (n=8). The increment in pH_i was completely inhibited by 1 mM amiloride (n=6) or in the absence of Na^+ (choline, n=3). Down regulation of PKC by overnight incubation of monolayers with PMA also prevented the rise in pH_i on subsequent challenge with PMA. An inactive analogue of phorbol ester did not stimulate Na^+/H^+ exchange. We conclude that in the IMCD cell, activation of PKC stimulates H^+ secretion via the Na^+/H^+ exchanger and may be important in mediating the hormonal control of pHi in this cell line.

ILEAL Na-H ANTIPORTER ACTIVITY IN CHRONIC HYPERCAPNIA.

Z. Talor, E. Sack*, O.S. Ruiz* and J.A.L. Arruda, Dept of Medicine, WSVAMC and Univ of IL, Chicago, IL.

The ileum, like the kidney, enhances HCO_3 absorption in response to acidosis. We reasoned that chronic hypercapnia might enhance the Na-H antiporter of ileal brush border membranes as it does in the kidney. Chronic hypercapnic rabbits (48 h inhalation of 10% CO_2) had significantly higher pCO_2 (83.6 ± 6.1 vs 44.7 ± 0.7 mmHg) and lower urine pH (5.44 ± 0.27 vs 7.87 ± 0.27) than controls. In both control and hypercapnic animals, renal and ileal brush border marker enzymes were enriched as compared with the appropriate homogenate. Alkaline phosphatase was enriched 10 fold and sucrase was enriched 8 fold in renal and ileal brush border membranes, respectively. The kinetic activity of the Na-H antiporter was measured by acridine orange fluorescence, by addition of different Na concentrations, using the pH jump technique. The Vmax of the Na-H antiporter in ileal brush border membranes was significantly higher in hypercapnia as compared with control (147 ± 9.7 vs 113.4 ± 11.7 fluorescence units/min/300 µg protein, $p < 0.05$). The K_m, however, was not different (13.8 ± 2.5 vs 11.5 ± 2.3 mM). The Vmax of the renal Na-H antiporter was also significantly enhanced in hypercapnia as compared with control (885.4 ± 33.7 vs 503.5 ± 31.7 fluorescence units/min/300 µg protein, $p < 0.001$). Thus, chronic hypercapnia induces enhancement of the Vmax of the Na-H antiporter in both renal and ileal brush border membranes, suggesting that in addition to the renal Na-H antiporter, the ileal Na-H antiporter also plays a role in the adaptation to chronic hypercapnia.

IDENTIFICATION AND PURIFICATION OF A STILBENE-BINDING PROTEIN WITH PROPERTIES OF THE RENAL $Na/CO_3/HCO_3$ COTRANSPORTER.

Manoocher Soleimani*, Gary V. Desir*, and Peter S. Aronson. Depts of Med., and Cell. & Mol. Physiol., Yale U., New Haven, Ct; and Dept of Med., Indiana U., Indianapolis, In.

The aim of this study was to identify and purify the $Na/CO_3/HCO_3$ cotransporter from rabbit renal basolateral membranes (BLM) by use of affinity chromatography. We found that the disulfonic stilbene DNDS reversibly inhibits the cotransporter by competitive interaction with CO_3. We then prepared a stilbene-affinity matrix by immobilizing 7 mM DNDS in polyacrylamide beads according to the method of Uchida and Filburn (JBC, 1984). BLM were solubilized with 0.6% Triton X-100, the solubilized extract was equilibrated with the affinity matrix for one hour, the matrix was washed extensively with buffer, and proteins were eluted with buffer alone or with buffer containing 5 mM DNDS. Eluates were concentrated and subjected to SDS-PAGE. The silver-stained gel revealed severa proteins in the molecular weight range 30-200 kD that were specifically eluted by DNDS. The presence of 4 mM free DNDS during equilibration of the solubilized BLM extract with the affinity matrix completely blocked adsorption of these proteins, confirming their identities as stilbene-binding proteins that likely correspond to the multiple stilbene-sensitive transporters present in BLM. The adsorption of the majority of these proteins, including a 71 kD protein, was blocked by the presence of 100 mM HCO_3/1 mM CO_3. However, only the adsorption of the 71 kD protein was blocked by 45 mM sulfite, which interacts at the CO_3 site of the $Na/CO_3/HCO_3$ cotransporter. The adsorption of the 71 kD protein was not blocked by 45 mM sulfate, which does not interact with the $Na/CO_3/HCO_3$ cotransporter. Kinetic studies revealed that harmaline and DNDS are partially exclusive inhibitors of the $Na/CO_3/HCO_3$ cotransporter. The presence of 2 mM harmaline selectively blocked the adsorption of the 71 kD protein to the DNDS-affinity matrix without effect on the adsorption of other stilbene-binding proteins. The 71 kD protein was not detected in brush border membranes by use of DNDS-affinity chromatography. These findings suggest that the 71 kD stilbene-binding protein in rabbit renal BLM is a structural component of the $Na/CO_3/HCO_3$ cotransporter.

MORPHOLOGICAL FINDINGS IN RAT COLLECTING DUCT DURING CHLORIDE-DEPLETION METABOLIC ALKALOSIS (CDA).

CC Tisher, JW Verlander*, KM Madsen, JH Galla, RG Luke, and D Bonduris*. Div. of Nephrology, Univ. of Florida, Gainesville, Florida and Univ. of Alabama, Birmingham, Alabama.

Bicarbonate secretion has been demonstrated in the isolated perfused rat cortical collecting duct (CCD). We have described 2 types of intercalated cells (IC) in the rat CCD, type A and type B. Type A IC appear to secrete protons, while it has been suggested that type B IC secrete HCO_3^-; however, there is no direct evidence to support the latter hypothesis. In this study we examined the ultrastructure of type A and type B IC during CDA which is known to be associated with augmented HCO_3^- secretion. Rats were subjected to peritoneal dialysis against 0.15 M $NaHCO_3$ to produce CDA, or Ringers HCO_3 to serve as controls (CON). Both groups then received an i.v. infusion of an 80 mM Cl^- solution for 90 min. Following collection of physiologic data, kidneys were fixed by perfusion with glutaraldehyde and processed for scanning and transmission electron microscopy. The means and SE of the physiologic parameters were: plasma total CO_2: CDA, 38.0 mEq/L±1.1 vs CON 27.8±0.6,$p<0.001$; mean urinary total CO_2 excretion rates: CDA, 141 nEq/m/100gBW±89 vs CON, 20±3; mean urinary Cl^- excretion rates: CDA, 20 nEq/m/100gBW±10 vs CON 486±144,$p<0.001$). With CDA, type A IC of the CCD and most IC of the outer medullary CD (OMCD) exhibited a reduced luminal surface with fewer and less prominent microprojections compared to CON rats. In contrast, type B IC in the CCD exhibited an enlarged luminal surface that was covered with more prominent microprojections and more extensive basolateral membranes when compared to CON rats. In summary, in rats stimulated to secrete HCO_3^-, the IC of the OMCD and type A IC of the CCD appear to decrease in both volume and luminal surface area, while these parameters and the basolateral membranes appear to increase in the type B IC. These morphologic changes suggest that proton secretion by type A IC is suppressed and that type B IC may participate in HCO_3^- secretion under these experimental conditions.

HCO_3^--SECRETING INTERCALATED CELLS IN CULTURE

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Intercalated cells (IC) of rabbit cortical collecting tubule secrete H^+ or HCO_3- depending on the hormonal and acid-base status of the animal. Using a purification scheme based on peanut lectin (PNA) affinity and Percoll gradient centrifugation, we developed primary cultures of HCO_3^--secreting IC. These cells grow as an epithelium on collagen-coated semipermeable supports in serum-free medium. The average transepithelial resistance of these monolayers is 595±75 ohm cm^2 (SE, n=7). Like HCO_3^--secreting IC *in vivo*, these monolayers have the following characteristics:

(1)apical 210 kDa PNA-binding protein

(2)DIDS-inhibitable Cl/HCO_3 exchange

(3)transepithelial Cl^--dependent HCO_3^- secretion. J_{HCO_3} was 4.0±0.4 nmol min^{-1} cm^{-2} in the presence of apical Cl^- and 0.6±0.3 nmol min^{-1} cm^{-2} in the absence of apical Cl^- (SE, n=5).

The basolateral Cl/HCO_3 exchanger of H^+-secreting IC is related to band 3, the Cl/HCO_3 exchanger of erythrocytes. Messenger RNA from cultured HCO_3^--secreting IC contains a 5 kb transcript which hybridizes with an erythrocyte band 3 probe. Further, using an antibody raised against the transmembrane region of band 3, we have immunoprecipitated an apical 70 kDa membrane protein from apically radioiodinated HCO_3^--secreting IC monolayers. This protein is a likely candidate for the apical Cl/HCO_3 exchanger responsible for HCO_3^- secretion.

NET ACID SECRETION IN ISOLATED PERFUSED RAT INNER MEDULLARY COLLECTING DUCTS.

S.M. Wall,* J.M. Sands, and M.A. Knepper. NHLBI, NIH, Bethesda, MD.

Micropuncture and microcatheterization studies have demonstrated significant net acid secretion in the inner medullary collecting duct (IMCD). Morphological studies have demonstrated that the terminal portion of the IMCD does not contain intercalated cells, the cell type believed responsible for proton secretion in other collecting duct segments. To determine whether net acid secretion occurs in the portion of the IMCD that lacks intercalated cells, tubules from the middle third of the rat IMCD were perfused in vitro. We confirmed the lack of intercalated cells in this portion of the rat IMCD by staining tubules with acridine orange. Perfusate and bath were identical physiological saline solutions containing 25 mM HCO_3^- and 6 mM NH_4^+ (pH 7.41). Bicarbonate absorption (pmol/mm/min) was significantly increased in response to in vivo NH_4Cl loading (control, 0.9 ± 0.2 [n=9] vs. NH_4Cl loaded, 2.6 ± 0.3 [n=7], $P<0.05$) or administration of deoxycorticosterone (DOC) (control, 1.6 ± 0.5 [n=6] vs. DOC treated, 4.2 ± 0.6 [n=5], $P<0.05$). In vitro addition of 10^{-8} M vasopressin to the bath caused bicarbonate absorption to increase from 1.0 ± 0.3 to 2.6 ± 0.4 (n=5, $P<0.05$), a response not seen in time controls. In other experiments, both in the presence and absence of bicarbonate, significant total ammonia secretion was found. We conclude that net acid secretion occurs in the portion of the IMCD that lacks intercalated cells. The rate of acidification can be increased by in vivo NH_4Cl loading, DOC administration, or addition of vasopressin in vitro.

IMMUNOCYTOCHEMICAL LOCALIZATION OF CARBONIC ANHYDRASE II (CA II) IN RAT COLLECTING DUCT (CD).

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Urine acidification occurs along the entire CD. We and others have provided evidence that intercalated cells (IC) participate in proton secretion. In the cortical collecting duct (CCD) we have described two forms of IC, type A and type B. Only type A IC appear to secrete protons. In the terminal inner medullary CD (IMCD), IC are rare, yet acidification has been reported. Histochemical and immunofluorescence studies in the rat CD have shown that IC are rich in CA, but these studies have not distinguished between A and B IC. The purpose of the present study was to localize CA along the rat CD using light (LM) and electron microscopic (EM) immunocytochemisty. Kidneys were fixed by *in vivo* perfusion with 1% glutaraldehyde or paraformaldehyde-lysine-periodate. Sections of tissue embedded in paraffin or Lowicryl K4M were labeled for CA II using the streptavidin-biotin-peroxidase technique for LM and the immunogold technique for EM. Both a monoclonal (mAb) and a polyclonal (pAb) primary antibody against chick RBC CA II were used. By LM CA-positive cells accounted for approximately 30% of the total cell population in the CNT and CCT, 40% in the CD in the outer stripe of the outer medulla (OM) and 35% in the CD in the inner stripe of the OM. By EM the pAb labeled the cytoplasm of IC in both the CNT and CD; however labeling was more prominent in A than B cells in the CCD. In summary, in the rat CD most IC from the CNT through IMCD, were positive for both immunolabels against CA II by LM immunocytochemistry. Less intense labeling was present in type B IC of the CCD by EM immunocytochemistry. We conclude that CA II is present in the cytoplasm of IC throughout the rat CD. No evidence was found for the presence of CA II in non-IC in the IMCD using these two antibody probes.

USE OF PHOTOAFFINITY ANALOGUES OF AMILORIDE TO CHARACTERIZE THE RENAL Na/H ANTIPORTER.

DG Warnock, EJ Cragoe Jr*, B. Rossier* and TR Kleyman. Univ. Alabama at Birmingham, AL, Univ. Of Lausanne, and Univ. Pennsylvania, Philadelphia, PA.

Nitromethoxy-benzene is a photoreagent which was coupled to the 5'amino group of amiloride (NENMBA), or to the guanidino group (NMBA) to produce inhibitors of the Na/H antiporter and Na channel. These agents reversibly inhibited the Na/H antiporter in rabbit renal brush border vesicles (BBMV) in the absence of UV light, with a potency of methyl isobutyl amiloride (MIBA) ≥ NENMBA > amiloride ≥ NMBA ≥ benzamil. With UV exposure, NENMBA caused irreversible inhibition of the Na/H antiporter (FASEB J. 2:2654a, 1988). The extent of inhibition depended on the concentration of NENMBA, and the duration of UV exposure. Protection against irreversible inhibition with NENMBA was seen with MIBA ≥ benzamil. Affinity purified polyclonal antibodies against 5'-(3-aminophenyl) amiloride detected NENMBA which was coupled to BBMV. Western blots revealed a 77 Kd protein labelled with NENMBA which showed a pattern of protection (MIBA >> benzamil) which was similar to that seen in the transport studies.

Conclusions: NENMBA is a photoaffinity probe for the renal Na/H antiporter. The Western blots and competition studies indicate that the amiloride-binding domain of the renal Na/H antiporter is 77 Kd.

INTRACELLULAR pH (pHi) REGULATION IN THE RABBIT CORTICAL COLLECTING TUBULE (CCT). ID Weiner,* LL Hamm. Washington Univ. School of Medicine, St. Louis, MO.

The fluorescent, pH sensitive dye 2',7'-bis-carboxyethyl-5(6)-carboxyfluorescein (BCECF) was used to study the mechanisms of pHi regulation in the different cell types of the isolated perfused CCT. Luminal loading of BCECF resulted in dye accumulation in intercalated cells (IC), while basolateral (bl) loading resulted in significant dye accumulation in all cells and was used for studying principal cells (PC). Intracellular acid loading was achieved via transient exposure to 10mM NH_4Cl. Baseline pHi of PC in CO_2 free solutions was 7.77 ± .08 and 7.50 ± .04 in CO_2 containing solutions. In PC bl Na^+ removal from CO_2 free solutions (without acid loading) resulted in intracellular acidification at a rate of .06 ± .02 pH units/minute (pHu/min). bl Na^+ removal resulted in a decrease in pHi recovery after an acid load from .23 ± .03 to .03 ± .04 pHu/min. IC were studied in CO2 containing solutions since baseline pHi in CO2 free solutions was 8.31 ± .02 and 7.55 ± .03 in CO2 containing solutions. HCO_3^- secreting IC (β-IC) were identified by intracellular acidification after bl Cl^- removal. Luminal Cl^- removal resulted in intracellular alkalinization and the inhibition of acidification after bl Cl^- removal. bl Na^+ removal from β-IC resulted in a decrease in pHi recovery after an acid load from .22 ± .08 to .01 ± .01 pHu/min. One millimolar bl amiloride similarly inhibited pHi recovery after an acid load.

We conclude: 1) Both PC and β-IC exhibit bl Na^+/H^+ exchange; 2) Luminal Cl^-/HCO_3^- exchange in series with bl Cl^- transport also participates in pHi regulation in β-IC.

INCREASED TUBULAR TOTAL CO_2 (TCO_2) REABSORPTION MAINTAINS CHLORIDE-DEPLETE CHRONIC METABOLIC ALKALOSIS (CMA) IN RATS. D.E. Wesson and H. Babino*. VA Med. Ctr. and Baylor Coll. of Med., Houston, TX.

Whether CMA is maintained by increased renal tubular TCO_2 reabsorption or by normal reabsorption associated with a depressed GFR which limits TCO_2 filtered load remains unclear. To explore this issue, free-flow micropuncture was done using tubular fluid collections which do not stimulate the tubuloglomerular feedback (TGF) response to more accurately determine in situ surface proximal tubule (SPT) TCO_2 reabsorption. Twelve Munich-Wistar rats were given intraperitoneal furosemide for two days and fed a low-electrolyte diet; six control (CON) and six CMA animals were supplemented with NaCl + KCl and $NaHCO_3$ + $KHCO_3$ respectively and studied 1 week into the protocol. Plasma TCO_2 was greater in CMA animals (41.5±2.1 vs 24.1±1.7 mEq/L, $p<0.001$) but early distal (ED) single nephron GFR (SNGFR) was less (22.2±1.7 vs 27.6±1.2 nl/min, $p<0.03$). Nephron TCO_2 filtered load calculated using ED SNGFR was greater for CMA vs CON (1004±105 vs 721±69 pEq/min, $p<0.05$). CMA late proximal (LP) TCO_2 concentration determined from fluid collected with minimal interruption of LP flow to avoid stimulation of TGF was less than that from fluid collected with complete interruption using an oil block (14±1.2 vs 19±1.5 mEq/L, $p<0.03$); these respective values were not significantly different in CON animals. SPT TCO_2 reabsorption calculated using the minimal interruption LP TCO_2 values and LP flow rate calculated from ED SNGFR was greater in CMA vs CON (903±106 vs 607±57 pEq/min, $p<0.04$). The data indicate that despite depressed GFR compared to CON, greater in situ SPT TCO_2 reabsorption contributes to the maintenance of CMA in rats.

DIACYLGLYCEROL REDUCES ANTILUMINAL GLUTAMINE UPTAKE IN ACIDOSIS. T.C. Welbourne and S. Joshi. LSUMC, Dept. of Physiol. & Biophys., Shreveport, LA 71130

Chronic metabolic acidosis opens a huge renal sink for interorgan glutamine, gln, subserving base generation. Quantitatively the bulk of the gln removed from the blood enters the proximal tubule cells across the basolateral membrane. Little is know concerning the in situ regulation of this flux during acidosis. There is increasing evidence that protein kinase C activation may play a role in transport regulation in the proximal tubule. Therefore the effect of diactanoylglycerol (DiC8) on renal glutamine uptake, at luminal and antiluminal sites, was investigated. Experiments were performed on kidneys isolated from chronically acidotic rats and perfused with 1 mM L-glutamine. After two consecutive 15 minute clearance periods, the perfusate was switched to one containing 9 μM DiC8 in addition to gln. Luminal gln uptake was determined as the difference between filtered and excreted gln; antiluminal gln uptake was estimated as the difference between gln extraction and luminal uptake. Comparisons are made between the second 15 minute periods of each 0.5h perfusion. Chronically acidotic rat kidneys removed 1619 ± 123 nmol gln/min while filtering 359 ± 109 of which 329 ± 114 nmol gln/min were reabsorbed; thus antiluminal uptake far exceeds maximal luminal uptake, 1290 vs 329 nmol/min. In the presence of DiC8 gln removal plunged to 788 ± 87 nmol/min in spite of maintained luminal uptake, 279 ± 79 nmol/min; accumulation of glutamate within the perfusion media suggests residual antiluminal removal reflects extracellular hydrolysis. These results are consonant with a major role of ionic gradients in regulating basolateral gln transport and hence ammoniagenesis in metabolic acidosis.

OMEPRAZOLE INHIBITS ACIDIFICATION IN RABBIT OUTER MEDULLARY COLLECTING DUCT: FUNCTIONAL ROLE FOR H-K-ATPase. Charles S. Wingo and Scott G. Straub,* Div.of Nephrology, University of Florida and VA Medical Center, Gainesville, Florida.

Previous studies (AJP 253:F418, 1987; JCI 81:1204, 1988) have demonstrated that: 1) the collecting duct possesses ouabain-insensitive, omeprazole-sensitive K-ATPase activity; 2) a low K diet stimulates K-ATPase activity in the cortical and the outer medullary collecting duct (OMCD). We have demonstrated (AJP 253:F1136, 1987) that the K absorptive flux of the inner stripe of the OMCD ($OMCD_i$) is enhanced when rabbits are fed a low K diet. In the present study we examined the effect of the gastric H-K-ATPase inhibitor omeprazole on acidification in the $OMCD_i$. Rabbits were fed a low K diet (0.55% K) for 7-10 days. Proton secretion was measured as net CO_2 flux (J_{CO2}) by microcalorimetry under symmetrical conditions. After control collections either vehicle or omeprazole (0.1 mM) was added to the perfusate. Listed below are the mean ± SEM transepithelial voltage (V_T, in mV) and J_{CO2} (in pmol·mm^{-1}·min^{-1}):

†p<0.05	Basal	Vehicle	Basal	Omeprazole
	N = 5		N = 6	
V_T	5.7±2.6	8.1±3.3	4.9±1.6	5.1±1.9
J_{CO2}	15.9± 4.3	20.6±5.5	14.5±5.5	-0.1±3.1†

Omeprazole consistently and significantly inhibited J_{CO2} without affecting V_T. In additional studies we observed significant net K absorption of 5.4 ± 1.1 pmol·mm^{-1}·min^{-1} under the same in vivo and in vitro conditions (N = 7).

The present observations demonstrate that the $OMCD_i$ possesses an omeprazole-sensitive acidification mechanism and absorbs K actively. These findings are consistent with the presence of H-K-ATPase activity in this nephron segment.

INHIBITION OF THE CLATHRIN COATED VESICLE PROTON PUMP (CCVPP) BY OMEPRAZOLE. F.R. Zuo*, X.S. Xie* and D.K. Stone, UT Southwestern Med. Ctr., Dallas, TX.

The CCVPP is composed of a cytosolic domain responsible for ATP hydrolysis and a trans-membranous proton channel. Investigations of the role of plasma membrane proton pumps in renal acidification have been hampered by the lack of an inhibitor which reacts externally with the proton channel, without producing intracellular effects. Omeprazole is an inhibitor of the H-K ATPase which requires acid-dependent activation to liberate a sulfhydryl-reactive group. The effects of omeprazole on the purified CCVPP have been tested with the following results: First, non-activated omeprazole (240 μM) had no effect on ATP hydrolysis catalyzed by the soluble CCVPP. Second, preactivated omeprazole (240μ) completely inhibited both ATP hydrolysis catalyzed by the soluble CCVPP and proton pumping catalyzed by the reconstituted CCVPP. Third, non-activated omeprazole (240μM) induced a time (and pH) dependent inhibition of proton pumping catalyzed by the reconstituted CCVPP. Extra-proteoliposome reducing agents did not protect against the latter effect, indicating that under these conditions, omeprazole did not react with a critical SH group within the 70 kDa subunit of the catalytic domain. These results are consistent with inactivation of the proton channel by omeprazole and support the utility of omeprazole as an extracellular inhibitor of luminal proton pumps in the kidney.

A COMPUTER SIMULATION OF GLOMERULAR DYNAMICS. R.Bernard* and E.J.Weinman. The University of Texas Health Science Center at Houston, Texas.

A computer model has been developed which graphically demonstrates the effects of mean arterial pressure (MAP), serum protein concentration (C(0)), hematocrit (hct), afferent resistance (AR), efferent resistance (ER) Bowman's pressure (BP), peritubular capillary pressure (PCP) and hydraulic permeability (K(f)) on the single nephron GFR (SNGFR), the glomerular blood flow (GBF) and the glomerular capillary pressure (GP).

All parameters can be varied independently. Format one plots the instantaneous net hydrostatic pressure, oncotic pressure and ultrafiltration rate against a normalized length of glomerular capillary. The second format generates a three dimensional curve of (SNGFR or GBF or GP) as a function of a continuum of any two variables (MAP, C(0), hct, AR, ER, BP, PCP, K(f)), and can be interrogated from most orientations.

The computer analysis was derived from an electrical equivalent model for SNGFR: MAP, BP and PCP are potential sources and AR, ER, R(glomerular capillary) and R(ultrafiltration) are variable resistors. Data points are calculated using recursive approximations.

This model was developed for teaching purposes. It should facilitate and understanding of how readily available clinical parameters (MAP, C(0), hct), parameters under pharmacologic control (MAP, AR, ER) and hydrodynamic parameters of pathologic significance (BP, PCP, K(f)) affect the GFR.

EFFECT OF ACUTE NEPHRECTOMY(NX) ON TUBULOGLOMERULAR FEEDBACK ACTIVITY(TGF) IN THE RAT. R.C.Blantz and O.W.Peterson*, Dept.of Med.,UCSD and VAMC, San Diego,CA.

NX results in increased single nephron filtration rate (SNGFR) by 24 hours,which must require modification or resetting of TGF. To examine the status of TGF early in this process before SNGFR was increased,we have evaluated SNGFR during microperfusion from late proximal tubule(LPT) at 0,10,20,30 and 40 nl/min in control two kidney rats(C),rats 2-3 hours after NX and in two kidney rats submitted to splenectomy (S),which served as controls for changes in systemic vascular resistance (SVR). *=p<0.05vs.0nl/min.

SNGFR(nl/min) at: LPT (Perfusion rates(nl/min)

	0	10	20	30	40
C	40±4	40±3	39±3	28±2*	29±3
S	37±3	32±3*	28±2*	28±3*	25±2*
NX	36±3	37±3	30±3*	28±3*	28±3*

The LPT:SNGFR relation was shifted leftward by both S and NX. We examined distal tubular flow rates (V_D) at 10-30 nl/min LPT perfusion to determine if loop reabsorption was altered by NX (*=p<0.05 vs. C).

V_D(nl/min) at: LPT (Perfusion rates(nl/min)

	10	20	30
C	5±1	14±1	25±2
NX	7±2	10±1*	18±2

The relation of V_D and LPT flowrate to SNGFR is markedly shifted to the left by NX compared to C. Conclusions:1)TGF sensitivity is increased by acute NX and this leftward shift is compatible with adaptations directed to renal volume conservation. 2)Studies in S suggest that increased TGF sensitivity is not specific to NX but an adaptation to acute changes in SVR.

DISSOCIATION OF EXTRACELLULAR FLUID VOLUME (ECF) AND RENAL FUNCTION IN EARLY UNTREATED (DM) AND TREATED (DM+I) AWAKE DIABETIC RATS.
R.C. Collins*,R.C. Blantz, and B.J. Tucker*. Univ of Calif, San Diego and VAMC, San Diego, Calif.

Glomerular hyperfiltration observed in early diabetes mellitus is due to increased renal plasma flow (RPF) which has presumed to be the result of ECF expansion. Male Wistar rats were chronically cannulated in the bladder, femoral artery and vein. Control measurements (C) were performed on 2 separate days prior to infusion of streptozotocin (S, 65 mg/kg BW, i.v.). Values for ECF, glomerular filtration rate (GFR), RPF, and sodium excretion were obtained in DM (n=6) and in rats moderately controlled with insulin (Ultralente)(DM+I, n=5) at 1,4,7,11,15 and 19 days after S. Blood sugar increased from 139±9 to 405±21 mg% in DM and 143±8 to 242±19 mg% in DM+I. (*P<0.05 to C, §P<0.05 to DM, values /100 gms BW)

	C	DAY 1	DAY 7	DAY 15
GFR(DM)	1.0±.1	1.3±.1*	1.5±.1*	1.7±.1ml/min*
GFR(DM+I)	1.0±.1	1.0±.1§	1.0±.1§	1.1±.1ml/min§
RPF(DM)	3.8±.1	4.6±.2*	4.4±.2*	5.6±.1ml/min*
RPF(DM+I)	3.4±.1	3.6±.2§	3.7±.1§	3.7±.3ml/min§
ECF(DM)	30±2	17±1*	18±2*	18±2%*
ECF(DM+I)	32±2	22±2*§	20±2*	21±4%*
$U_{Na}V$(DM)	0.4±.1	1.4±.2*	1.7±.2*	2.4±.1μeq/mn*
$U_{Na}V$(DM+I)	0.2±.1§	0.5±.1*§	0.6±.1*§	0.8±.1μeq/mn*

GFR and RPF increased progressively in DM but not DM+I rats during the first 15 days. ECF decreased within 24 hrs of S diabetes in both groups and remained lower than C. Therefore, mechanisms other than expanded ECF must be postulated to explain the hyperfiltration of DM. Also, renal function alterations are dissociated by time from ECF changes.

ALTERATIONS IN GLOMERULAR FUNCTION ACCOMPANYING ACUTE REDUCTION IN NEPHRON NUMBER. Robin G. Coombs* and Juan C. Pelayo. Univ. of Colorado, Sch. of Med., Dept. of Pediatrics, Denver, CO.

Compensatory renal hypertrophy of both structure and function occurs following nephron loss (Nx), as an essential adaptation for the long-term maintenance of body fluid homeostasis. The acute effects of Nx on glomerular function, however, are not well understood. To determine if acute Nx is associated with glomerular hyperfiltration prior to the development of structural hypertrophy, we studied glomerular dynamics at 24 hrs following 75% Nx in Munich-Wistar rats (Nx, n=8) and compared these data to sham-operated rats (Sh, n=8). Nx rats had marked elevation in single nephron GFR (SNGFR) when compared to Sh rats (49 vs 37 nl/min, $p<0.001$). Increases in both single nephron plasma flow (225 vs 144 nl/min, $p<0.01$) and glomerular capillary hydrostatic pressure difference (48 vs 40 mmHg, $p<0.0025$) were responsible for the glomerular hyperfiltration in remnant nephrons. Furthermore, these hemodynamic adjustments were principally due to a 45% reduction in afferent arteriole resistance ($p<0.0025$) because both mean arterial pressure and the glomerular ultrafiltration coefficient were the same in Nx and Sh rats. We conclude: 1) remnant nephrons exhibit a striking capacity to compensate rapidly for the loss of renal mass and excretory function by increasing SNGFR, 2) compensatory structural hypertrophy itself may not be a sine qua non for glomerular hyperfiltration and 3) this very early functional adaptation may be the consequence of alterations in the hormonal and/or biochemical environment of the nephrons at risk.

DOES TUBULOGLOMERULAR FEEDBACK (TGF) HAVE SUFFICIENT POWER TO DRIVE AUTOREGULATION? W.A. Cupples and D.J. Marsh. Dept. Physiol. and Biophys., Univ. So. Calif., Los Angeles, CA.

It has been suggested that TGF does not have the power required to account for renal autoregulation. A model describing pressure and flow in glomerulus and tubule was used to test the prediction (AJP 254: F601) that reduced proximal reabsorption caused by a step increase of arterial pressure (PA) could provide the needed power. TGF, specified by a logistic equation, was coupled only to afferent arteriolar resistance. The model was exercised with parameters for superficial (SF) and juxtamedullary (JM) nephrons over PA from 100 to 135 mm Hg. When run with SF parameters and without TGF it predicts autoregulatory indices (ARI) of 1.06, 0.94, and 2.45 for glomerular capillary pressure, blood flow, and filtration rate respectively. TGF reduced the ARI to 0.55, 0.50, and 1.51. Inclusion of PA-sensitive proximal reabsorption further reduced the ARI to 0.22, 0.23, and 0.43. When JM parameters were used, without TGF, the predicted ARI were 0.91, 1.11, and 2.01 for glomerular capillary pressure, blood flow, and filtration rate. TGF reduced the ARI to was 0.36, 0.45, and 0.87 respectively. Including PA-sensitive proximal reabsorption further reduced the ARI to 0.11, 0.15, and 0.15. PA-sensitivity of proximal reabsorption plays an important role in autoregulation.

EFFECTS OF A LIPID LOAD ON RENAL HEMODYNAMICS IN NORMAL MAN. Daniel J. Cordonnier*, Khaled Sirajedine* Claude Souvignet*, Monique Faure*, Marie-Claude Denis*, Paule Groslambert* (introduced by Gary Striker) Centre Hospitalier Régional Universitaire de Grenoble, France.

It has been reported that, in animals, a lipid enriched diet is deleterious for the glomerulus and that inhibition of cholesterol synthesis can delay renal failure. But the role of lipids on renal function in man is not known. Five healthy 18 to 21 years old people adhered volontarily to the protocol. The fasting water-loaded subjects infused with inuline and PAH completed 2 successive 30 min. clearance (C) periods before eating 1.5 g/kg. body weigth of butter (baseline). Four additional successive 30 min. periods were completed (1.30-2, 2-2.30, 2.30-3, 3-3.30 after lipid load). Blood cholesterol and triglycerides level were measured throughout the study. Mean values are shown for baseline periods, 2 and 3 hours after load.* $p<0,05$=vs baseline. Cin, CPAH, renal blood flow (RBF)=ml/mn/1.73m2; F.F=% TRR=mmHg/ml/mn ; triglycerides = g/l

	(baseline)	2 hrs	3hrs
Cin	109 ± 23	124 ± 19	120 ± 14
CPAH	521 ± 158	587 ± 168*	617 ± 146*
FF	23 ± 6	23 ± 7	20 ± 6
RBF	1025 ± 288	1157 ± 309*	1124 ± 255*
TRR	.093 ± .021	.0804 ± .015*	.0756 ± 0.013*
Trigl.	0.78 ± 0.040	2.03 ± 0.74*	2.06 ± 1.32

Butter absorption does not modify blood cholesterol but increases triglyceride levels up to a peak at 3 hours. It results in a fall in TRR with an increased RBF. FF tends to decrease but does not reach significance level. We conclude that lipid load in normal man increases triglyceride level which in turn increases RBF without modifying GFR.

SODIUM AND FLUID DELIVERY FROM THE PROXIMAL TUBULE TO THE LOOP OF HENLE INCREASES IN HEALTHY ADULT SUBJECTS FOLLOWING A MEAT MEAL. N.G.DeSanto, G.Capasso, P.Anastasio, S.Coppola, G.DeSimone, T.Coscarella, D.R.Giordano, P.Strazzullo, R.Iacone, G.Capodicasa, Università Federico II Napoli (Intr.by S.G.Massry)

To explain the renal hemodynamic response in healthy subjects to a protein meal, changes in the proximal fluid reabsorption have been hypothesized. The goal of this work is to validate this a hypothesis by investigating the function of proximal tubule in healthy adults by measuring Lithium Clearance (CLi), and by calculating 1. The fractional excretion from the proximal tubule (CLi/GFR), 2. the absolute reabsorption in the proximal tubule (GFR-CLi). A group of 8 healthy adult subjects of boths sexes were studied before (two 30 min clearance periods) and after a meat meal (5 additional clearance periods respectively 30, 60, 90, 120 and 180 minutes after the ingestion of 2g/Kg of protein in the form of cooked red meat). GFR and RPF were measured respectively as the inulin and PAH clearances, data are ± SD. Following the meat meal the well known usual increase of GFR ($p<0.0005$) and of RPF ($p<0.025$) was seen without changes in the Filtration Fraction. CLi increased from a baseline value of 24.2 ± 7.30 ml/min to a peak of 44.2 ± 24.2 ml/min ($p<0.01$). CLi/GFR increased from 0.20 ± 0.08 to 0.38 ± 0.21 ($p<0.025$) There was a transient increase lasting 60 minutes of GFR- CLi from 90 ± 19 to 117.2 ± 30 ml/min ($p<0.025$). The data point out that after a meat meal there is an increased delivery of Na and fluid to the loop of Henle associated with an increased absolute reabsorption in the proximal tubule.

NEW CONCEPTS OF GLOMERULAR PHYSIOLOGY AND PATHOLOGY DERIVED FROM COMPLETE 3-D MODELLING OF ALL GLOMERULAR COMPONENTS.

I. W. Dobbie. University Department of Medicine Glasgow Royal Infirmary, Scotland.

This study represents the first 3-D reconstruction at high magnification of all structural components (epithelial, endothelial and mesangial cells, mesangial matrix and basement membrane) in true spatial relationship to each other and to capillary lumena and urinary spaces in normal human and rodent glomeruli. Modelling began with serial 1 μm sections of resin-embedded whole glomeruli. By projection techniques a series of 6 scale 3-D models (mag x 10,000) were constructed for each glomerulus to display each structural component in contrasting spatial relationship to each and all other components. The original 1 μm sections were then re-embedded and serially cut for electron microscopy. These in turn provided serial templates for construction of models at higher magnification (x 100,000) of any region of the first series of models. Analysis of models reveals that current "flat earth" concepts of glomerular perfusion, and effect of arteriolar and mesangial contraction, is incompatible with the 3-D geometry of the glomerulus. The revealed spiral format of mesangial core, shared basement membrane by groups of parallel arched capillaries demand re-appraisal of our simplistic notions of glomerular function and of the effects of pathological change in each component.

RENAL AUTOREGULATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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Autoregulation of renal blood flow (RBF) was studied in male Wistar rats: 8 control rats; 8 rats with severe hyperglycemia induced by streptozotocin (diabetic group); and 7 moderately hyperglycemic rats made diabetic the same way but given insulin daily (insulin-treated group). 4-8 weeks after injection of streptozotocin, RBF was measured during stepwise reduction of renal perfusion pressure using an electromagnetic flow technique. Plasma glucose concentration avereged 122 ± 24,555 ± 26, and 254 ± 18 mg/dl in the control, diabetic, and insulin-treated group respectively. Whole kidney GFR in the insulin-treated group was significantly higher than in the other two groups. In the control group, RBF was effectively autoregulated at mean renal arterial pressures above 80 mmHg. However, in the diabetic group RBF readily fluctuated as blood pressure changed and autoregulation of RBF was impaired. In the insulin-treated group, autoregulatory capability was less attenuated than in the diabetic group. The autoregulatory index (ARI) was greater, on average,in the diabetic group than in the controls (0.59 ± 0.09 vs. 0.22 ± 0.03;P<0.05). There were no significant differences in ARI between the control and the insulin-treated groups. These results suggest that in uncontrolled diabetes, there may be diminished pretection against hyper- or hypoperfusion induced by alterration in blood pressure. Additionally, these findings may explain the well recognized acceleration of diabetic nephropathy during hypertension.

RENAL HEMODYNAMIC EFFECT OF GLUCAGON DURING "GLUCOSE CLAMPING" IN HUMANS.

H L Greene*, T Yoshioka, R Geer*, J Hill* and I Ichikawa. Vanderbilt University, Nashville, TN.

Although glucagon is well known to be an important physiological regulator of renal hemodynamics, results from animal studies conducted thus far have failed to reach a conclusion as to the mechanism of its vasodilative action, i.e., whether it is through glucagon's gluconeogenic action. In this regard, patients with glycogen storage disease (GSD), who lack enzymes involved in gluconeogenesis, provide a unique opportunity to address this issue. We therefore studied the effect of glucagon administration (20 ng/kg/min, i.v.) on renal plasma flow rate and glomerular filtration rate in 5 patients with Type 1 (age: 9-32 yr), 2 with Type 3 (8 and 13 yr) and 1 with Type 6 (8 yr) GSD, who were clinically free of renal abnormalities, i.e., hypertension, proteinuria or azotemia. {Mean values; statistical significance [* P <0.05 vs baseline (B)] was evaluated only for GSD Type 1}

	MGUR	P-lactate		P-glucose		C-PAH		C-inulin	
	mg/m2/min	mEq/l		mg/dl		----ml/min/1.73 m2----			
	B	B	G	B	G	B	G	B	G
GSD Type 1	4.0	2.3	7.5*	88	88	625	704*	116	139*
GSD Type 3	1.4	1.3	1.7	88	93	485	507	116	123
GSD Type 6	2.5	-	-	84	80	552	588	142	146

In contrast to Types 3 and 6, Type 1 (patients with G-6-Pase deficiency) had the characteristically high baseline minimum glucose utilization rate (MGUR), and elevated plasma lactate levels (P-lactate) which were further elevated during glucagon infusion (G). Plasma glucose level (P-glucose) remained essentially constant throughout the study, indicating that our method of supplemental i.v. glucose infusion and frequent monitoring of P-glucose was successful in achieving "glucose clamping". PAH clearance (C-PAH) and inulin clearance (C-inulin) increased significantly in response to G in Type 1. Likewise, C-inulin increased in the Type 3 patients although C-PAH decreased slightly in one. Both C-PAH and C-inulin increased in the Type 6 patient. Systemic blood pressure was unaffected throughout the study in all patients. When data were pooled, increases in both C-PAH and C-inulin during glucagon infusion remained significant (P<0.05 and P<0.025). We, therefore, conclude that glucagon has renal vasodilative actions which are independent of its gluconeogenic and hyperglycemic actions.

OSCILLATIONS OF PROXIMAL TUBULAR PRESSURE AND FLOW AND DISTAL CHLORIDE CONCENTRATION IN RATS.

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Previous experiments have shown prominent oscillations in proximal tubular pressure in Halothane anesthetized rats. Such oscillations should be due to local oscillations in flow rate and should cause periodic variations in early distal chloride concentration. We sought to test these predictions and to measure the amplitude and phase relations among the oscillating variables. In Halothane anesthetized Sprague-Dawley rats, distal tubular Cl concentration was measured with Cl-sensitive electrodes, and late proximal flow rate was measured by pulse injection of boluses of solutions containing rhodamine-dextran. Detection of bolus velocity was made by videomicroscopy; time resolution was 2 sec. All three variables oscillated with the same frequency, 36 ± 1.4 mHz. Amplitude of the flow and chloride variations was 50 % and 10 %, respectively, of the mean values. There were significant phase differences; fluid flow led tubular pressure by 1.5 ± .3 sec, indicating that forward propagation of the flow oscillation causes the pressure oscillation. The distal tubular chloride concentration lagged proximal flow by 10.4 ± .4 sec. The measured delay between proximal and early distal tubular events creates a lag in signal transmission in tubuloglomerular feedback, and this delay in the feedback loop is probably responsible for the observed oscillations.

2-CHLOROADENOSINE-INDUCED PROSTAGLANDIN PRODUCTION BY ISOLATED RENAL AFFERENT ARTERIOLES.

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The acute intrarenal infusion of adenosine causes a brief vasoconstriction followed by a return of renal blood flow to or above control values. We have shown in isolated canine afferent arterioles (AA) statically perfused in vitro that 2-chloroadenosine (2CA) produces transient vasoconstriction of 3-10 min duration followed by a return of lumen diameter to control values despite continued infusion of 2CA. After incubation of the AA in meclofenamate to block prostaglandin (PG) synthesis, 2CA (10^{-6}M) produces sustained vasoconstriction for the duration of 2CA infusion. This suggests that vasodilatory PGs act to attenuate the vasoconstrictor response to 2CA. In this study we directly measured PGE_2 and PGI_2 as 6-keto-$PGF_{1\alpha}$ (6K) production by isolated AA in the basal state and after administration of 2CA(10^{-6}M). Individual AA were obtained by microdissection. Pooled collections were preincubated at 37°C for 30 min. Two 30 min incubation periods followed: A-control and B-experimental. 2CA was added during period B. Total PG production was measured by RIA. Separate time-control experiments showed stable PG production during periods A and B. In table, *p< .025.

	Period A (control)	Period B (2CA)
6K	8.76 ± 0.22	10.36 ± 1.00
PGE_2	0.15 ± 0.02	0.53 ± 0.18*

PG production expressed as pg PG/30 min/incubation vial ± SEM. 2CA produces significant stimulation of PGE_2 production in isolated canine renal AA. Thus, we postulate that PGE_2 produced by the AA in response to 2CA acts to attenuate the vasoconstriction produced by 2CA.

RENAL, METABOLIC AND HORMONAL RESPONSES TO INGESTION OF PROTEIN OF DIFFERENT SOURCES IN NORMAL HUMANS

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Introduced by N. Zerefos. Unit for Metabolic Medicine, UMDS, Guy's Campus, London, England.

The renal effects of a vegetable (VPD) or animal protein diet (APD)were studied chronically (group A, 10 men)and acutely (group B, 7 men).1. The diets used in group A were a. APD(protein intake 76±20g/day) b. VPD (protein intake 71±15g/day)and c. APD supplemented with fibre (APD+F:protein intake 70±22g/day).The three diets were isocaloric. Each diet was taken by each individual for 3 weeks. GFR(inulin ilearance),RPF (PAH clearance),albumin excretion rate (AER), plasma glucagon (IRG), growth hormone (GH) and aminoacids (AA) were performed at the end of each diet. GFR and RPF were lower in VPD (110±14vs 121±11ml/min/1.73m^2;p 0.001 and 558±83vs 643±90ml/min/1.73m^2;p 0.01 respectively).AER was also lower in VPD (7.4±2.9 vs 13.0±6.3mg/24h;0.01). IRG nad GH did not differ significantly. Plasma levels of lysine and valine were higher in APD. In APD+F the parameters were similar to APD. 2. Subjects of group B were given an equivalent load of 80 g of protein;meat (MM) or soya (SM) and renal function was assess ed one hour before and 3 hours after the meal. After the MM GFR,RPF and AER rose significantly while SM did not change any of these parameters. In conclusion the source of dietary protein affects renal function in healthy humans. Differences in plasma AA concentration may contribute to this. Fibre supplementation on APD has no effect on renal haemodynanics .The type of protein acutely affects the renal vascular response as well.

COLLOID OSMOTIC PRESSURE AND RENAL FUNCTION IN THE YOUNG ANALBUMINEMIC RAT.

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Colloid osmotic pressure (COP) was measured in plasma and interstitial (sc. wick) fluid in young Nagase Analbuminemic rats (NAR) and Sprague Dawley rats. GFR and ERPF were measured using ^{51}Cr-EDTA and ^{125}I-hippuric acid. At a body weight of 300 g plasma COP was 15.8 mm Hg in the male NAR as compared to 19.8 mm Hg in the controls (P<0.01) and interstitial COP was 5.3 mm Hg in the NAR and 9.6 mm Hg in the SDR (P<0.01). In the female NAR plasma and interstitial COP were 2-3 mm Hg lower than in the controls. Thus the transcapillary COP gradient was very similar in both strains. Plasma and extracellular fluid volumes measured by isotope dilution and tail cuff pressure were also similar.

	males		females	
strain(N)	SDR(6)	NAR(6)	SDR(6)	NAR(6)
GFR (n=24,	0.98	0.85	0.99	0.85*
ml/min/100 g)	(0.06)	(0.04)	(0.04)	(0.04)
ERPF (n=12,	1.72	1.56	2.05	1.64*
ml/min/100 g)	(0.17)	(0.11)	(0.14)	(0.08)
GFR/ERPF	0.59	0.54	0.54	0.53
(n=12)	(0.02)	(0.01)	(0.02)	(0.01)
kidney weight	0.42	0.30*	0.40	0.32*
(n=6, g)	(0.01)	(0.01)	(0.02)	(0.01)

* P < 0.05 as compared to SDR

The renal function (related to body weight) was slightly reduced in NAR as compared to SDR, but the filtration fraction was similar. In relation to kidney weight, however, renal perfusion and filtration are high in NAR. The low plasma COP in NAR theoretically enhances capillary and glomerular filtration. This is probably averted by respectively a decrease in interstitial COP and increase in preglomerular vasoconstriction.

CIRCADIAN RHYTHM OF THE GLOMERULAR FILTRATION RATE IN NORMAL INDIVIDUALS.

M.G. Koopman, G.C.M. Koomen, R.T. Krediet and L.Arisz, intr. by L.W. Statius van Eps. Academic Medical Centre, Amsterdam, The Netherlands.

Circadian variations in glomerular filtration rate (GFR) were studied in 11 normal individuals (6 male, 5 female; age 19–37 years) during a regimen of bedrest and identical meals every 3 hrs. GFR and effective renal plasma flow (ERPF) were measured as inulin and para-aminohippurate clearance. After equilibration blood and urine samples were taken every 3 hrs during one day. In all individuals GFR had a circadian rhythm. Maximum was 127 ± 7 ml/min (mean ± SEM) (orthophase 13.50 ± 1.00 hr) and minimum 95 ± 4 ml/min (bathyphase 2.45 hr ± 40'). The relative amplitude (max–min as % of mean i.e. A/M) of the rhythm was 28 ± 3%. All subjects had a rhythm for ERPF as well with an A/M of similar magnitude (24 ± 3%). However, both orthophase (18.40 hr) and bathyphase (5.50 hr) were later (p<0.01) when compared with the GFR rhythm. Filtration fraction had a rhythm in 9 persons, often with a biphasic orthophase. Maximum was 0.22 ± 0.02, minimum 0.15 ± 0.02 and A/M 40 ± 4%. A circadian rhythm for urinary albumin excretion was present in 8 persons, in 3 it was undetectable by a steep fall in excretion during bedrest. The phase of albumin and GFR rhythm was the same, but the A/M was higher (75 ± 9%, p<0.01). Urinary beta$_2$–microglobulin excretion was not "posture dependent" and had a rhythm in all individuals with the same phase as the GFR and albumin rhythms; the A/M was 68 ± 8%.

In conclusion, GFR has a definite circadian rhythm in normal individuals with a maximum in daytime and a similar phase as the rhythms for albumin and beta$_2$–microglobulin excretion. The ERPF also has a rhythm, but its orthophase and bathyphase are later than the GFR rhythm. The changes as percentage of the mean are less pronounced for GFR and ERPF than for albumin and beta$_2$–microglobulin excretion.

ATRIAL NATRIURETIC FACTOR ATTENUATES HYPERTONIC SALINE AND ADENOSINE MEDIATED REDUCTIONS IN RENAL HEMODYNAMICS. J. P. Loftus*, M. Redfield*, W. Miller*, and J. C. Burnett Jr., Mayo Clinic, Rochester, Minnesota.

Tubuloglomerular feedback (TGF) is activated by intrarenal infusion of hypertonic saline (HS). The intrarenal production of adenosine (AD), by constricting the afferent arteriole, has been implicated as a mediator of TGF. Atrial natriuretic factor (ANF) has been shown to attenuate reductions in single nephron glomerular filtration rate (GFR) during increased fluid delivery to the macula densa. In the intact dog, we hypothesized that ANF attenuates reductions in renal blood flow (RBF) and GFR in response to HS (n=12) and to AD (n=12). HS (16% NaCl, ir) or AD (0.1 μmol/min, ir) was infused in the presence or absence of intrarenal ANF (0.1 μg/kg/min)

Compared to HS, HS+ANF attenuated reductions in GFR (HS, -39.6±9.8 ml/min vs HS+ANF, -14.3±4.6 ml/min, $p<.05$) and in RBF (HS, -143±35 ml/min vs HS+ANF, -5±22 ml/min, $p<.05$). GFR was reduced by AD (-9.2±3.0 ml/min, $p<.05$), but maintained by AD+ANF (-0.4±2.0 ml/min, NS). A transient vasoconstriction associated with AD was markedly attenuated by AD+ANF (AD, -54.5±3.6 ml/min vs AD+ANF, -3.7±3.1 ml/min, $p<.005$).

We conclude that ANF importantly attenuates both hypertonic saline-induced and adenosine-mediated reductions in renal hemodynamics. This study suggests that ANF acts as a modulator of whole kidney TGF and that ANF antagonizes the effector limb of the TGF mechanism.

INTRARENAL HEMODYNAMICS IN PRIMARY ALDOSTERONISM T Nagai*, G Kimura, * M Imanishi*, Y Kawano*, S Kojima*, K Yoshida*, H Abe*, T Ashida*, H Yoshimi*, M Kawamura*, M Kuramochi*, and T Omae*. Dept. of Medicine, Natl. Cardiovascular Ctr., Osaka, Japan

Intrarenal hemodynamics were estimated in primary aldosteronism(PA) using Gomez's equations and by analyzing the renal function curve.

Two-week studies were performed in 6 patients with PA who were given a regular sodium diet in the 1st week and a sodium restricted diet in the 2nd week. Afferent(R_A) and efferent(R_E) arteriole resistances, and glomerular pressure (P_G) were calculated using Gomez's equations. $U_{Na}V$ was plotted on the ordinate as a function of MAP on the x-axis. Assuming that the difference between MAP and the x-intercept of this renal function curve represents the effective filtration pressure, R_A, P_G and gross filtration coefficient(k_F) were also calculated.

GFR and RBF were 102±6, 780±46 ml/min. Estimated R_A(6600±700 dyns.sec.cm^{-5}) was markedly elevated, while R_E(2500±100 dyns.sec.cm^{-5}) and P_G (57±2 mmHg) and k_F(0.195±0.041 [ml/sec]/mmHg) remained normal. Estimations by analyzing the renal function curve were consistent with those by Gomez's equations.

In DOCA-salt hypertension, which is an animal model analogous to PA in humans, increases in GFR, RBF and P_G are reported, suggesting that renal damage in PA may be due to the elevation in P_G. However, the increase in R_A was as marked as seen in essential hypertension, and further the increases in GFR, RBF and P_G were not significant in this study. Effect of mineralocorticoid on intrarenal hemodynamics seems different between acute and chronic phases.

VASOCONSTRICTIVE FAILURE OF AFFERENT ARTERIOLE IN HYPERTENSIVE PATIENTS WITH DIABETES MELLITUS(DM) H Matsuoka*, G Kimura, T Nagai*, T Sanai*, M Imanishi*, Y Kawano*, S Kojima*, M Kuramochi*, T Omae*. Dept. of Medicine, Natl. Cardiovascular Ctr., Osaka, Japan

Characteristics on intrarenal hemodynamics in DM were studied by comparing essential hypertensive patients(EHT) with DM to those without DM.

Intrarenal hemodynamic parameters such as afferent(R_A) and efferent(R_E) arteriole resistances and glomerular pressure(P_G) were estimated clinically by Gomez's equations. EHT were divided into two groups, non-DM and DM(subclinical, non-insulin dependent).

Group	MAP	PRA	GFR	RPF	R_A	R_E	P_G
non-DM	126	1.0	82	311	9900	3000	57
(n=25)	±3	±0.3	±9	±15	±500	±200	±1
p	NS	<.05	NS	<.005	<.05	<.01	NS
DM	125	0.4	88	397	8300	2300	57
(n=10)	±3	±0.1	±9	±30	±1000	±200	±2

Although MAP, GFR and P_G were comparable in both groups, RPF was significantly higher in DM, while R_A, R_E, and PRA were lower in DM.

It is suggested R_A can not rise high enough to keep P_G normal when DM coexists with systemic hypertension. Relative reduction in R_E in DM compared with non-DM may act to lower P_G, which would otherwise be raised by this vasoconstrictive failure of afferent arteriole against systemic hypertension. The fact P_G was not elevated in this study, different from reports in animal DM models, may suggest that vasodilation of efferent arteriole can completely compensate the vasoconstrictive failure of afferent arteriole and keep P_G normal in such an early stage of DM as non-insulin dependent DM.

NEUROGENIC MEDIATION OF ANTINATRIURESIS FOLLOWING REDUCTION OF SODIUM INTAKE IN CONSCIOUS DOGS. Jeffrey L. Osborn. Med. Coll. Wis., Dept. of Physiol., Milwaukee, WI.

The influence of renal nerve activity (RSNA) on the control of sodium (Na) balance has not been determined in conscious dogs. The present study evaluated the relationship between RSNA and sodium excretion (UNaV) when Na intake was decreased from 5.0 mEq/hr (HNa) to 0.09 mEq/hr (LNA). Dogs (N=6) were surgically prepared for chronic study and placed on HNa by i.v. infusion (5 days). A renal nerve bundle then was prepared for recording RSNA. Water was allowed ad lib. Renal hemodynamics and urinary excretions were measured hourly during HNa at which time Na intake was reduced to LNa and measurements were continued for 30 hours. Renal DNX then was performed and the study was repeated. After switching to LNa, dogs with INN kidneys significantly decreased UNAV in 6 hours. This antinatriuresis was associated with increased RSNA by hour 4 to 240±26% of control. RSNA was maximally elevated by 649±42% by hour 11. Cumulative Na loss of DNX dogs was significantly greater than that of INN dogs beginning at hour 8 and continuing through hour 10 ($P<0.05$). Renal blood flow, plasma renin activity, potassium excretion and arterial pressure were not altered by LNa in 24 hrs. These results demonstrate that following reduction of Na intake from HNa to LNa, increased RSNA is associated with decreased UNaV. During Na depletion, cumulative Na loss in dogs with renal DNX exceeds that in INN dogs for at least 24 hours. Thus in dogs, activation of RSNA accelerates the rate of achieving Na balance when Na intake is reduced.

DISSOCIATION BETWEEN PLASMA VOLUME EXPANSION (PVE) AND INCREASES IN GFR DURING PREGNANCY IN THE RAT. Jane Reckelhoff*, Lennie Samsell*, Chris Baylis. Physiol Dept, West Virginia Univ., Morgantown, WV 26505.

Normal pregnancy is characterized by progressive PVE and also by increases in GFR and renal plasma flow (RPF). By day 9 of pregnancy (9P;term = 22 days) in the Munich Wistar (MW) rat, the PVE is only 20% (=+1ml) of that seen at term whereas the maximum (+30%) increment in GFR has already occurred. These studies were performed to assess whether a 30% rise in GFR could result from this +1ml PVE. Using virgin female MW rats, micropuncture measurements were made of single nephron (SN)GFR; glomerular capillary and proximal tubule hydrostatic pressures, P_{GC} and P_T; also GFR, RPF and hct during a euvolemic control period (C) and after +1 ml PVE. Data: n=9; mean±SE; ¶p=<0.05,paired t-test.

	hct (vol%)	RPF (ml/min)	GFR (ml/min)	SNGFR (nl/min)	P_{GC} mm Hg	P_T
C	48+1	3.0+0.2	0.88+0.04	32+2	48+1	13+1
PVE	44+1¶	3.5+0.2¶	0.87+0.03	33+2	51+1¶	15+1¶

With PVE a fall in hct occurred similar to that seen at 9P. RPF rose slightly but GFR and SNGFR were unchanged. In the pregnant rat, 30% rises in GFR and SNGFR occur solely as a result of increases in plasma flow due to parallel falls in tone in pre-and post glomerular resistances; thus P_{GC} is maintained constant. Here, acute PVE led to rises in P_{GC} but since P_T was also elevated, glomerular pressure difference (ΔP) was not altered. The glomerular hemodynamic changes seen here with acute PVE suggest that PVE does not cause the gestational rise in GFR.

EFFECTS OF ANGIOTENSIN II (AII) AND NOREPINEPHRINE (NE) ON ISOLATED RAT AFFERENT (AA) AND EFFERENT ARTERIOLES (EA). J.B. Robinette*, B.H. Yuan*, J.D. Conger**. UCHSC and VAMC, Denver, CO.

Disagreement persists regarding the sensitivity of AA and EA to AII and NE in rat kidneys based on in vivo measurements of glomerular hemodynamics. Differences in results may be due to the known effects of experimental variations in renal perfusion pressure and the intrarenal neuro-hormonal environment. In order to determine vessel-specific sensitivities to AII and NE, AA and EA were isolated from similarly hydrated, litter mate rat kidneys and perfused in vitro at 80 and 30 mmHg, respectively. Dose response curves to AII and NE (10^{-12} to 10^{-5} M) for each vessel type were measured. The molar doses required for half-maximal contraction (EC_{50}) were as follows:

Vsl	n	AII	Vsl	n	NE
AA	(15)	$1.0\pm1.9\times10^{-9}$	AA	(13)	$3.3\pm1.1\times10^{-7}$
EA	(14)	$5.3\pm2.2\times10^{-11}$▼	EA	(16)	$1.4\pm2.4\times10^{-8}$▼

▼Different from AA at $p< 0.0001$.

Isolated EA were 20-fold more sensitive to AII, but only 4-fold more sensitive to NE than AA. The magnitude of sensitivity differences between AA and EA for the two hormones was significant at $p< 0.01$. It is concluded that (1) rat EA are more sensitive than AA to both AII and NE; (2) the AA to EA sensitivity difference is greater for AII and (3) the differences in AA and EA sensitivity are independent of perfusion pressure and the perivascular neuro-hormonal environment.

EFFECT OF LOW-PROTEIN DIET ON SIZE AND CHARGE PERMEABILITY IN EXPERIMENTAL NEPHROSIS.

A. Remuzzi*, C.Battaglia* and G. Remuzzi*. Mario Negri Institute for pharmacological Research, Bergamo, Italy. (intr. by R.J. Glassock).

Conflicting results have been obtained on the causes of altered glomerular permeability in ADR nephrosis. We addressed 1) whether proteinuria in ADR treated animals (5 mg/Kg i.v.) is due to size or charge defect and 2) whether the protective effect of low-protein diet (LPd) on proteinuria restores one or both determinants of gomerular functional barrier. Three weeks after injection, ADR animals (n=12) fed a standard diet (Sd) showed abnormal proteinuria (460 ± 200 mg/day, m ± SD); low-protein feeding was associated with a significant reduction of proteinuria (109 ± 88 mg/day). GFR was significantly reduced ($p< 0.01$) in ADR animals as compared to controls, while RPF did not changed significantly. LPd did not significantly influenced GFR. Fractional clearance of neutral and charged dextrans were as follows:

	Neutral Dextrans			
r(Å)	36	44	52	60
Controls	.123± .048	.024± .008	.0047± .0013	.0007± .0001
ADR + Sd	.142±.035	.042±.018*	.0133±.0081*	.0035± .0027*
ADR + LPd	.104± .039	.020±.007	.0035±.0012	.0004± .0003

* P< 0.05 ADR + Sd Vs. Controls

	Dextran Sulfate			
r(Å)	28	32	36	40
Controls	.056± .021	.0056± .0019	.0012± .0005	.0005± .0002
ADR + Sd	.085± .056	.0184±.0072*	.0056±.0015**	.0033± .0009**
ADR + LPd	.165± .100	.0229±.0141°	.0028±.0014°	.0008± .0005

* P< 0.05 ADR + Sd Vs. Controls, ** P< 0.01 ADR + Sd Vs. Controls
* P< 0.05 ADR + LPd Vs. Controls

Thus both size and charge dependent permeability are significantly altered in ADR nephrosis. The antiproteinuric effect of low-protein diet is associated with a complete recovery of glomerular size selectivity. Charge dependent permeability normalized only for the largest dextran molecules.

N^6-CYCLOHEXYLADENOSINE (CHA)-INDUCED RENAL VASOCONSTRICTION IN STREPTOZOTOCIN-TREATED DIABETIC RATS (STZ). N. Rossi,* J. Dunbar, V. Ellis, and P. Churchill. Wayne State U. School of Medicine, Detroit, Michigan, USA

Renal hemodynamics are altered in diabetes mellitus, however the vasoactive substance(s) responsible for these changes in renovascular resistance are not known. We examined the effects of CHA, an adenosine analogue selective for A_1 adenosine receptors, in rats treated with streptozotocin 50 mg/kg body wt i.v. and in age-matched, sham-treated control rats (C). Kidneys were perfused at constant flow in a non-recirculating system using a modified Krebs-Henseleit buffer containing 3.5% Ficoll and 1% albumin. Basal perfusion pressure in C was 103±3 mm Hg and perfusate flow rate 8.7±0.7 ml/min/g kidney wt; and did not differ significantly from the STZ groups on days 1, 3, 5, or 42. Plasma glucose was 117±8 in C and 436±38 mg/dL in STZ by day 1 ($p < 0.0005$), and remained stable.

CHA (uM)			Perfusion Pressures (mm Hg) 0.1	1.0	10
	day	N			
C		17	140±6	137±8	89±3
STZ	1	6	125±6*	119±6*	88±3
STZ	3	7	148±6	153±7*	111±8*
STZ	5	7	156±10*	166±7*	103±6
STZ	42	5	163±18*	158±11*	92±8

* $p < 0.05$ versus C; values are mean ± SEM.

Diminished vasoconstriction to A_1 adenosine receptor activation was followed by an increased response. A_2 adenosine receptor-induced vasodilation was briefly decreased, but returned to control levels. We conclude that renal hemodynamic alterations occur due to changes in renal adenosine receptor characteristics in diabetes mellitus.

GLOMERULAR HEMODYNAMICS AND FLUID COMPARTMENTS IN THE ANALBUMINEMIC RAT (ANALB) - NFT Sanfelice*, CK Fujihara*, M Marcondes*, RM Padilha*, MC Pires* and R Zatz - University of São Paulo Medical School, São Paulo, Brazil.

The ANALB rat, a mutant of the Sprague-Dawley (SD) strain described by Nagase et al (Science 205:590,79) exhibits no circulating albumin due to a genetic defect. Based on this characteristic, we investigated: 1) Whether ANALB rats have increased extracellular fluid volume (ECFV) as a consequence of low plasma oncotic pressure. 2) Whether glomerular ultrafiltration coefficient (K_f) is increased as a consequence of the complete absence of albumin in the capillary wall. Fluid volumes and plasma renin activity (PRA) or glomerular hemodynamics were studied in 28 male ANALB and 29 control (SD) rats at the age of 3 months. Results (Mean±SE, *=p<0.05, PO=plasma oncotic pressure, SNGFR=single nephron glomerular filtration rate, $\overline{\Delta P}$=glomerular hydraulic transcapillary gradient):

	PO	ECFV	SNGFR	$\overline{\Delta P}$	K_f
	mmHg	ml/kg	nl/min	mmHg	nl/s/mmHg
SD	19±1	301±14	42±2	39±1	0.07±0.01
ANALB	15±1*	312±17	52±3	33±1*	≥0.11±0.01*

In addition, blood volume and APR in ANALB rats were no different from values observed in SD rats. Since ECFV was also identical to control, these results indicate that persistent hypooncolicity alone is insufficient to promote appreciable hemodynamic derangement or edema formation. Since filtration pressure equilibrium was the rule in ANALB rats, only minimum values for K_f could be determined. Even these minimum values were higher than unique values obtained in SD rats, indicating that albumin is important in restricting the passage of water through the glomerular wall.

RESTORATION OF TUBULOGLOMERULAR FEEDBACK RESPONSES IN SALINE EXPANDED RATS BY ANGIOTENSIN INFUSION. Jurgen Schnermann, Jo Ann Davis*, and Josephine P. Briggs. University of Michigan, Depts. of Physiology and Int. Med., Ann Arbor, Michigan.

The reason for the blunting of tubuloglomerular feedback (TGF) responses in acute isotonic saline expansion is unknown. We examined whether infusion of angiotensin II (AII) restores TGF reactivity in saline expanded rats. The effect of raising loop of Henle flow rate from 0 to 45 nl/min on SNGFR and stop flow pressure (Psf) was assessed in rats continuously infused with isotonic saline at a rate of 3% BW/hr. AII was infused at 50 or 100 ng/kg min while renal arterial pressure (AP) was held constant with a suprarenal aortic clamp. When loop flow was increased from 0 to 45 nl/min, SNGFR fell 4.4 ± 2.2 nl/min (from 37.5 to 31.6 nl/min). During AII infusion the response increased to 14.8 ± 2.8 nl/min (from 37.8 to 23.0 nl/min). The effect of AII on PSF responses was as follows:

	Psf-0	Psf-45	d Psf	AP
Control	46.7±1.7	42.4±1.9	- 4.3±0.3	94±2
AII-50	50.1±3.4	40.3±4.1	- 9.9±1.1	98±2.7
AII-100	60.1±1.1	45.3±3.5	-14.8±2.6	92±4

(all values mm Hg ± SEM)

The change in TGF responses was observed within minutes after starting or terminating angiotensin infusion. Our results demonstrate that AII rapidly restores TGF responsiveness despite continued volume expansion and without alterations in arterial pressure. We conclude that variations of plasma AII concentration alter the responsiveness of the TGF system. Low plasma angiotensin II levels may contribute to resetting of TGF sensitivity during volume expansion.

DIET-INDUCED HYPERCHOLESTEROLEMIA ELEVATES GLOMERULAR CAPILLARY PRESSURE. P.G. Schmitz*, M.P. O'Donnell, B.L. Kasiske, W.F. Keane, Hennepin Co Med Ctr, Univ. of Minn., Mpls, MN.

Hypercholesterolemia and glomerular hypertension frequently accompany renal disease and both may contribute to progressive glomerular injury. We have previously shown that rats fed a high cholesterol (C) diet develop early mesangial expansion, albuminuria and accelerated focal glomerulosclerosis (FGS). The present study was designed to assess the influence of increased serum C on glomerular hemodynamics in rats with either normal or reduced renal mass. Ten-week old, male rats fed a 4% C diet underwent unilateral nephrectomy (UNx) (C-1K, n=5) or sham surgery (C-2K, n=8). Rats fed standard (S) diet also underwent UNx (S-1K, n=5) or sham surgery (S-2K, n=6). Micropuncture studies were performed 4-6 weeks after surgery, prior to development of FGS. Results (mean ± SE):

Group	Serum C (mg/dL)	SNGFR (nL/min)	SNPF (nL/min)	P_{GC} (mmHg)
C-1K	266±44	75.1±4.5	278±28	60.3±0.7
C-2K	233±41	51.3±4.3	155±18	58.5±1.2
S-1K	52±4	69.5±4.0	262±31	50.5±0.7
S-2K	52±5	42.2±2.8	137±8	53.0±0.7

High C diet caused increased glomerular capillary pressure (P_{GC}; p<0.001 by two-way ANOVA), which was not further exaggerated by reduced renal mass. High C did not affect glomerular function (SNGFR, SNPF) in 2-K rats, and did not alter compensatory increases in SNGFR and SNPF after Nx. Increased P_{GC} precedes C-induced glomerulosclerosis and may contribute to C-induced glomerular injury.

DEVELOPMENTAL CHANGES IN RENAL BLOOD FLOW AND ITS INTRACORTICAL DISTRIBUTION IN CONSCIOUS DOGS. Mouin G. Seikaly and Billy S. Arant, Jr., Dept. Peds., UT-Southwestern Medical Ctr. Dallas, TX.

Previous studies have shown a preferential distribution of renal blood flow (RBF) to juxtamedullary nephrons. Most studies have been performed in animals stressed by anesthesia or surgery which alter RBF. In the present experiments, RBF was measured by the radiolabeled microsphere technique in conscious, unstressed mongrel puppies (2-30 days old) either before (n=8) or after (n=10) 14 days of age (when the adult complement of glomeruli/kidney had been attained) and in 6 adults. Total RBF (2 kidneys) increased from 18.8±6.0 to 41.3±15.5 (p<.001) in puppies and was 358±150 ml/min in adults. RBF factored for kidney weight did not change in puppies (1.7±0.4 v. 2.0±0.6; p=NS) but was increased to 4.2±1.0 ml/min/g in the adult (p<.001). Renal vascular resistance decreased from 3.8±1.3 to 1.8±0.8 (p=.001) and further to 0.4±0.1 mmHg/ml·min in the adult (p<.001). In the adult kidney, the outer third of the cortex received 41±7% of RBF and the inner third was 21.5±4.7%. In contrast, the intracortical distribution of RBF in puppies did not change when nephrogenesis was completed: the outer third or least mature nephrons received 63±8 v.59±19% (p<.05 compared to adult) while the inner third or most mature nephrons received 8.3 v.9.1% (p<.001 compared to adult). We conclude that unlike stressed animals, awake puppies have a preferential distribution of renal blood flow to immature superficial nephrons.

MEASUREMENT OF RENAL SYMPATHETIC NERVE ACTIVITY IN NEWBORN AND YOUNG ADULT SHEEP. Francine G. Smith* and Jean E. Robillard. Dept. of Pediatrics, University of Iowa, Iowa City, Iowa.

The role of renal sympathetic nerves in the control of renal function and hemodynamics in adult animals is well described. However, little is known of the role of the renal nerves during development. This study describes the direct recording of renal sympathetic nerve activity (RSNA) from six chronically instrumented newborn lambs (aged 3-10 days; 3.2-7.4 kg) and one young adult sheep (10 kg). RSNA was inhibited with incremental doses of the pressor agent norepinephrine (Levophed, 0.25-10 µg/kg/min). Each dose was repeated 2-3 times and the mean rise in systemic arterial pressure (AP) and mean fall in RSNA were calculated. The percent inhibition of RSNA was proportional to the rise in AP in both groups of animals:

% ↓ AP	0-20	21-40	41-60	61-80	
% ↓ RSNA newborn lamb	13.6 (7.6)	19.4 (2.9)	27.1 (4.2)	26.5 (1.3)	$\bar{x}$ (sem)
% ↓ RSNA young adult	18.0	26.3	29.2	23.0	

Thus, baroreflex control of RSNA appears to be similar in newborn lambs and young adult sheep. These measurements of RSNA in conscious newborn and young adult animals provide a new avenue for determining the role of the renal nerves during development.

ENHANCED GFR RESPONSE TO ORAL VS INTRAVENOUS (IV) ARGININE ADMINISTRATION. William E. Smoyer, and Ben H. Brouhard. Univ. of Texas Med. Br., Dept. of Pedi., Galveston, Texas.

Oral protein and intravenous mixed aminoacids as well as arginine alone can increase GFR and plasma glucagon. To determine if the response to the oral ingestion (30 gm) and the IV infusion of arginine (30 gm) produce similar renal and hormonal responses, we measured the GFR (inulin clearance) and glucagon concentrations for five 30 minute clearance periods after the oral ingestion or IV infusion of arginine and compared these data with the infusion of glucagon for three clearance periods (10 ng/kg/min) in 6 normotensive subjects. Administration of arginine and glucagon increased GFR and plasma glucagon at various time periods. Maximal increases, regardless of time after administration, are noted below:

	Peak GFR		Peak Glucagon
	Absolute (ml/min/m^2)	Percent	pg/ml
IV arginine	9±2*	13±5	369±92
Oral arginine	23±6**	42±15	130±40
Glucagon	28±4**	53±12	510±176

*x±SEM ** p<0.05 vs IV

We conclude that the GFR response to oral arginine differs significantly from that of IV arginine with higher peak GFR's despite lower peak glucagon concentrations. Thus although mediated in part by glucagon, these data suggest the influence of other factors in the GFR response which appear to be stimulated by oral ingestion.

ANGIOTENSIN-ADENOSINE INTERACTIONS IN THE RENAL VASCULATURE: MECHANISMS OF CALCIUM ACTIVATION. Cathy D. Smith*, H. Leland Mizelle*, Drew A. Hildebrandt*, and John E. Hall. Dept. Physiol. & Biophys., Univ.Miss.Med.Ctr., Jackson, MS

In most physiological conditions, angiotensin II (ANGII) preferentially constricts efferent arterioles. However, increased intrarenal adenosine levels markedly sensitize preglomerular vessels to ANGII. The aim of this study was to determine the role of extracellular Ca^{++} in mediating ANGII-adenosine constriction of pre and postglomerular vessels. In all experiments, endogenous ANGII formation was blocked with captopril (14 µg/kg/min iv) and intrarenal adenosine was increased by renal arterial adenosine infusion (1.0 µM/min) throughout the experiment. In 6 dogs, ANGII infusion (20 ng/kg/min iv) during adenosine infusion decreased renal blood flow (RBF) by 46±3% and GFR by 28±1% while increasing pre and postglomerular resistances by 37±9% and 35±5%, respectively. Intrarenal infusion of the calcium channel blocker verapamil (100 µg/min) completely abolished the ANGII-adenosine mediated decrease in GFR and the increase in preglomerular resistance, while blunting the decrease in RBF (14±3%). In order to prevent possible tubuloglomerular feedback mediated changes in the renal vasculature during ANGII-adenosine infusions, the kidneys of 6 different dogs were made non-filtering by occluding the ureter during mannitol diuresis. ANGII infusion (20 ng/kg/min) during intrarenal adenosine in nonfiltering kidneys decreased RBF (39±6%) and ureteral stop-flow pressure (6.3±1.0 mmHg), while increasing pre and postglomerular resistances (105±6% and 39±10%, respectively). After verapamil infusion, the ANGII-adenosine mediated increase in preglomerular resistance was completely abolished, although postglomerular resistance still increased markedly (39±4%). Verapamil infusion also blocked the reduction in ureteral stop-flow pressure caused by ANGII-adenosine infusion. Thus, Ca^{++} entry blockade prevents the preglomerular constrictor response to ANGII-adenosine but does not alter postglomerular vasoconstriction, suggesting that preglomerular vessels depend on extracellular Ca^{++} whereas postglomerular vessels mobilize Ca^{++} through a different mechanism.

VERAPAMIL DECREASES RENAL VASCULAR RESISTANCE AND FILTRATION FRACTION IN NORMOTENSIVES WITH NORMAL RENAL FUNCTION. D. Sullivan,* M.R. Weir, D.K. Klassen, and S.Y. Shen. Univ. of Maryland Hospital, Dept. of Medicine, Renal Division, Baltimore, MD.

Verapamil(V) has been reported in hypertensives to lower blood pressure(BP), reduce renal vascular resistance(RVR), and increase or not change renal blood flow(RBF) and glomerular filtration rate(GFR). We studied the effects of 240mg sustained-release V daily for 10 days on renal hemodynamics in healthy humans. V reduced BP(mmHg) from 119.2±5.2/73.2±7.5 to 107.4±4.0/67.0±2.3. Renin(ng/ml/hr), aldosterone (ng/ml), and angiotensin II(pg/ml) levels did not change significantly: pre 1.1±0.3/post 1.4±0.3, pre 8.6±2.4/post 8.5±1.7, pre 38.6±8.5/post 44.3±8.8, respectively(R). Urine sodium and potassium in meq/24 hours changed slightly: pre 93.0±10.8/ post 125.0±21.1, pre 43.7±5.2/ post 45.7±6.7, R. V lowered GFR ($p.<05$) from 108.8±2.5 to 99.5±3.7 ml/min, yet improved RBF (ml/min), pre 479.6±24.9 vs post 499.0± 27.5, and reduced RVR (dyne sec/cm^5/1.73m^2) from 14810±1155 to 13069±819. Filtration fraction (FF) decreased from .23 to .20. These changes were not associated with any changes in serum creatinine (0.9±0.1mg/dl), thromboxane B_2, 6-keto-prostaglandin $F_{1\alpha}$, or prostaglandin E immunoreactivity in the urine.

The decreases in RVR and FF (an index of post glomerular vascular resistance) may be important in hyperfiltering patients in whom the pathogenesis of progressive renal dysfunction may be related to elevated intraglomerular pressure.

ROLE OF REGULATORS OF SODIUM EXCRETION IN PRESSURE-NATRIURESIS. Y.Takamitsu*, S.Yuasa*, T.Sumikura*, S.Miki*, T.Yura*, and H.Matsuo*. (intr. by F.Marumo). Kagawa Medical School,Dept. of Medicine, Kagawa, Japan.

Pressure-natriuresis has been well recognized, but the mechanisms remain pooly understood. The purpose of this study was to evaluate the role of several regulators of sodium reabsorption (dopamine, angiotensin II (AII), prostaglandins (PGs) and calcium) in pressure-natriuresis in uninephrectomized and adrenalectomized rats. Neural and hormonal influences on the kidney were minimized by renal denervation and infusion of norepinephrine, ADH, cortisol and aldosterone. The addition of SCH23390(D1 receptor antagonist) failed to inhibit the pressure-natriuresis response. AII infusion(40ng/kg/min) decreased the sodium excretory response to altered renal perfusion pressure. In hydrated rats, indomethacin did not inhibit the pressrure-natriuresis response, but indomethacin with a small amount of AII(5ng/kg/min) showed an inhibitory effect on sodium excretion. In unhydrated rats indomethacin alone inhibited the pressure-natriuresis response. However, the administration of captopril with indomethacin allowed sodium excretion rates to rise to near-normal levels. These data suggest that some amount of AII is needed for the inhibitory effect of indomethacin in which the action of AII is unmasked. We found the pressure-natriuresis response was reduced by dietary hypocalcemia. Calcium reduction has been reported as stimulating AII. These results indicate that AII and PGs play the role of modulators in the pressure-natriuresis mechanism, and that the D1 receptor is uninvolved.

EFFECTS OF ACUTE INSULIN (I) INFUSION ON GLOMERULAR HEMODYNAMICS IN NORMAL (C) AND DIABETIC (D) RATS. B.J. Tucker*, R.C. Blantz, and R.C. Collins* Univ. of Calif, San Diego and VAMC, San Diego, CA.

Treatment of diabetes mellitus with I utilizing current therapies requires greater amounts of I than that produced endogenously. Insulin may induce changes that affect glomerular function. Micropuncture studies were performed in 6 C and 6 non-I treated D rats (streptozotocin, 65 mg/kg i.v.,7-10 days prior). Measurements of single nephron filtration rate (SNGFR), single nephron plasma flow (SNPF), glomerular hydrostatic pressure gradient (ΔP), and afferent (AR) and efferent (ER) arteriolar resistance were obtained before and after infusion of I (5-8U, Humulin-R, i.v.). Glucose was infused in both groups after I to prevent hypoglycemia. Glomerular filtration rate was 0.97±.05 in C compared to 1.19±.06 mls/min in D (P<0.05). (*P<0.05 to respective non-I period, §P<0.05 to C+I)

	Blood Sugar [mg%]	SNGFR [nl/min]	SNPF [nl/min]	ΔP [mmHg]	AR [Gdynes·sec·cm^{-5}]	ER [Gdynes·sec·cm^{-5}]
C	99±6	32±2	93±7	34±1	30±4	19±3
C+I	82±7*	38±2*	125±7*	40±1*	19±2*	15±2*
D	342±15	31±2	99±8	33±1	28±4	15±2
D+I	75±5*	25±1*§	71±5*§	28±1*§	38±4*§	19±3

Mean arterial pressure was not different between C and D and was unchanged after I. The contrasting effects of I on glomerular hemodynamics in C and D are mediated by AR but may derive from two different mechanisms. Stimulation of growth hormone by I in C may contribute to the increase in SNGFR, whereas in D, the dominant factor reducing SNGFR via decreases in SNPF and ΔP could be an I induced extra- to intracellular fluid shift, acutely decreasing plasma volume.

INFLUENCE OF MENTAL STRESS AND EPINEPHRINE ON RENAL HEMODYNAMICS AND RENIN RELEASE IN HUMANS Bo Tidgren and Paul Hjemdahl. (Int.C.Kjellstrand) Clinical Physiology and Clinical Pharmacology, Karolinska Hospital, Stockholm, Sweden

Sympatho-adrenal control of renal blood flow and renin release during mental stress was evaluated by comparing the effects of a color word test (CWT) and epinephrine (Epi; 0.03, 0.1 & 0.3 nmol/kg min) on renal blood flow (thermodilution measurements), net renal venous overflows of norepinephrine (NE) and dopamine (DA) and renin release in 12 healthy male subjects.

CWT caused rapid increases of heart rate (22 bpm), mean arterial pressure (20 mm Hg),renal vascular resistance (RVR; 48 %), arterial NE (from 0.78 to 1.34 nM), renal venous NE (from 1.09 to 2.93 nM) and arterial Epi (from 0.26 to 0.68 nM). Renal venous overflows of NE and DA (corrected for the renal extraction of arterial NE and DA by the extraction of Epi) increased from 252±29 to 708±79 pmol/min and from 24±2 to 32±4 pmol/min. Renin release increased by 65 %. The increase in RVR correlated to the increase in NE overflow. Renin release responses correlated to increases in mean arterial pressure, and to renal DA overflow more closely than to NE overflow. Neither responses correlated to changes in Epi. Epi infusions increased arterial Epi to 0.89, 2.49 and 6.43 nM. Epi did not change mean arterial pressure, RVR or renal venous NE overflow, but increased arterial PRA and renin release dose-dependently.

We conclude that the kidney is a target organ for mental stress, since renal vasoconstriction is linked to markedly increased renal nerve activity (NE release) and since renin release is enhanced.

EFFECT OF INTRAVENOUS BRANCHED CHAIN-ENRICHED AMINO ACIDS(BCAA)ON RENAL FUNCTION IN PATIENTS WITH CHRONIC LIVER DISEASE (CLD)
Andrés Valdivieso, *Helia Morales, *Alberto Maiz, *Salvador Vial. Catholic Univ.Chile,Med. School. Depts.Nephrourology and Nutrition, Santiago, CHILE.

The purpose of this study was to compare the effect of a standard(Aminoplasmal 10%-Braun)(AA), and BCAA (Hepamino-F 4,5% Braun)amino acid infusion on glomerular filtration rate(GFR), renal plasma flow(RPF) and fractional sodium excretion (FeNa%)in Child-B, CLD and normal volunteers(N). Each subject was randomly assigned and studied on two occasions, separated by 48 hrs, while on a 60 mEq/day sodium diet. Four CLD and 6 N received 40gr of AA and BCAA for 6 hrs in an isovolemic infusion. GFR and RPF were assessed by inulin and PAH clearances, respectively (ml/m/1.73 m^2). Data taken before(B)and between the 2nd-4th hr of infusion(I)are presented and expressed as mean ± se.

		CL		N	
		AA	BCAA	AA	BCAA
GFR	B	94± 16	86± 12	92± 11	106± 8
	I	89± 21	94± 19	*109± 7	90± 7
RPF	B	477±152	438±135	415± 79	296± 84
	I	344±122	360±140	**366± 84	253±150
FeNa	B	0.6±0.3	0.8±0.3	0.9±0.2	0.7±0.04
	I	**0.9±0.3	0.9±0.4	0.9±0.1	0.8±0.1

* = p < 0.05 vs B ** = p < 0.025 vs B

These data show that:
a)AA increased GFR by 18% in N.
b)Unlike N, patients with CLD did not increase their GFR after a standard AA load; however in these patients, AA produced a small natriuretic effect.
c)Given at this dose, BCAA did not change GFR, RPF or FeNa in N or in CLD.

THE ACUTE EFFECT OF CYCLOSPORINE ON THE INNER MEDULLARY MICROCIRCULATION IN THE NORMAL RAT KIDNEY. Yoram Yagil, Tylbert Schabel,* and Rex L. Jamison. Univ of Rochester, Rochester, NY.

Acute cyclosporine-A (CysA) nephrotoxicity has been attributed to renal vasoconstriction; it is not known if the vasoconstriction is uniform or regional. To study its effect on the inner medulla, CysA was infused for 20 min in anesthetized rats. Vehicle was infused in controls. Blood flow in ascending (Qavr) and descending vasa recta (Qdvr) was determined in the exposed renal papilla by fluorescence videomicroscopy prior to (Period I) and 45 min after CysA (Period II). In parallel studies, inulin (Cl_{In}) and PAH clearances (Cl_{PAH}) were measured. Results (mean ±SE; N=5-6 per group).

Period	Cl_{In} I	Cl_{In} II	Cl_{PAH} I	Cl_{PAH} II	Qavr I	Qavr II	Qdvr I	Qdvr II
	(ul/min)		(ul/min)		(nl/min)		(nl/min)	
Vehicle .9%NaCl	1018 ±68	993 ±93	3582 ±277	3492 ±174	4.8 ±.7	5.6 ±.8	10.4 ±2.7	11.7 ±3.0
CysA 4mg/kg	1094 ±153	1295 ±161	3556 ±375	3985 ±421	6.1 ±.7	6.8 ±1.1	12.6 ±3.5	14.6 ±4.2
CysA 20mg/kg	1129 ±97	1164 ±83	3796 ±368	3232* ±315	4.7 ±.8	4.2 ±.7	7.0 ±1.8	7.2 ±1.4

* p<0.01 Period I vs Period II, paired analysis

Low dose CysA (4 mg/kg) had no effect. At 20 mg/kg CysA, there was a rise in MAP, no change in Cl_{In}, a reduction in Cl_{PAH}, and an increased filtration fraction. In contrast, Qavr and Qdvr did not change. We conclude that the medullary circulation escapes the CysA-induced net post-glomerular vasoconstriction.

CHLORIDE TRANSPORT MECHANISMS IN AMBYSTOMA KIDNEY PROXIMAL TUBULE. Solange Abdulnour-Nakhoul* and Emile L. Boulpaep, Yale Univ. Sch. Med., Dept. of Cellular and Molecular Physiology, New Haven, CT.

Modes of chloride transport were examined in isolated perfused proximal tubules, using determinations of basolateral V_1, luminal V_2 and transepithelial V_3 as well as of intracellular chloride activity (aCl_i). In control substrate Ringer aCl_i is 11.8±0.6 mM (n=39). Diphenylamine-2-carboxylate (DPC) added to the lumen increases aCl_i by 2.4±0.6 mM (n=22). Addition of DPC to the bath increases aCl_i by 2.9±1.0 mM (n=6). This finding is consistent with the presence of Cl^- channels at the apical and basolateral cell membranes. Addition of furosemide, bumetanide or hydrochlorothiazide to the lumen had no significant effect on aCl_i, suggesting that the dependence of aCl_i on luminal Na reported earlier is not caused by an apical Na-Cl or Na-K-Cl symport. The removal of HCO_3^- from the lumen decreases the aCl_i by 1.1±0.3 mM (n=12). This finding is incompatible with any Cl^--HCO_3^- antiport on the luminal membrane. Additional removal of HCO_3^- from the bath increases aCl_i by 1.5±0.5 mM (n=11) an effect due to Na/HCO_3^--Cl/H antiport. In HCO_3-free Ringer, the addition of DIDS to the lumen increases aCl_i by 1.7 ± 0.6 mM (n=10), which is incompatible with luminal Cl^--$Base^-$ antiport. In HCO_3-free Ringer, DIDS in the bath decreases aCl_i by 2.4 ±0.9 mM (n=9). After addition of DIDS to lumen and bath, V_2 is 46 ± 5.5 mV not significantly different from the equilibrium potential for Cl of 43 ± 3.8 mV (n=10). We conclude: 1) Cl^- channels are present on both apical and basolateral cell membranes. 2) basolateral Cl transport occurs both through Cl^--HCO_3^- and other Cl^--$Base^-$ exchange.

SELECTIVE ARTERIOLAR CONSTRICTION TO NERVE STIMULATION IN RAT HYDRONEPHROTIC KIDNEYS. C. Zhang, J.P. Porter and J.T. Fleming: (intr. by J. Passmore) University of Louisville, School of Medicine, Louisville, KY.

This study was conducted to quantitate the constrictor response of pre- and postglomerular arterioles in the rat hydronephrotic kidney during nerve stimulation. Six to 8 weeks after surgical induction of unilateral hydronephrosis, six female Wistar rats (240-270g) were anesthetized with Inactin. The hydronephrotic kidney, with intact blood supply, was exteriorized in a 37°C and pH controlled bath for direct visualization of the microvessels using TV microscopy. From each kidney the baseline diameters of a single interlobular artery (INT), afferent (AFF) and efferent (EFF) arterioles (at sites near the glomeruli) were measured. Then the severed splanchnic nerve was stimulated for 15 seconds at frequencies of 2 to 8 Hz (duration 1mS, 0.3mA). Control diameters and diameter changes during nerve stimulation (expressed as percent of control ± SEM) are given below. * p<0.05)

	Control µm	2Hz %	4Hz %	6Hz %	7Hz %	8Hz %
INT	15±2.1	86+3*	74+4*	61+4*	54+3*	47+2*
AFF	9±0.5	85+3*	81+3*	73+4*	62+4*	59+6*
EFF	9±0.4	99+1	92+3	94+3	92+5	87+6

The INT and AFF arterioles constricted to the nerve stimulation in a frequency-dependent fashion. At the higher stimulation frequencies (4-8 Hz), the INT were more responsive than the AFF. In contrast, the EFF did not significantly constrict. These data from the rat hydronephrotic kidney indicate that a brief increase in efferent renal nerve activity selectively constricts the preglomerular vessels.

ß-ADRENERGIC AGONISTS INCREASE Na, Cl, Ca AND Mg TRANSPORT IN ISOLATED MOUSE THICK ASCENDING LIMB. C. Bailly*, M. Imbert-Teboul*, N. Roinel*, C. Amiel*. INSERM U. 251 and Univ. Paris 7, France. (Intr. by Christian Le Grimellec).

Because ß-adrenergic agonists stimulate adenylate cyclase (AC) in several segments of the mammalian nephron, we tested the effect of isoproterenol (Iso) on AC of the Swiss mouse nephron : Stimulation was x 9 in the cortical (cTAL) and x 5 in the medullary (mTAL) part of the thick ascending limb (TAL). We investigated the effects of Iso (10^{-7} M) on electrolyte transport in isolated cTAL and mTAL perfused and bathed with isoosmotic, Hepes-buffered solutions. Concentration difference between perfused and collected fluid was as follows for cTAL :

	Control	Iso	Recovery
ΔNa,mM	56 ± 8	68 ± 7*	40 ± 5§
ΔCl,mM	57 ± 8	67 ± 8*	39 ± 6§
ΔCa,mM	0.23±0.07	0.39±0.06*	0.17±0.04§
ΔMg,mM	0.11±0.04	0.25±0.05*	0.08±0.02§
ΔK ,mM	0.17±0.38	-1.4 ±0.60*	-0.65±0.26

* p < 0.01 Iso/Control ; § p < 0.01 Iso/Recovery

The transepithelial voltage (Vt) was transiently increased from 11.6 ± 2.4 in the last control period to 14.4 ± 2.6 mV during Iso.

In mTAL, a similar Iso effect was observed on Vt, ΔNa (54 ± 8* vs 40 ± 6) and ΔCl (55 ± 7* vs 42 ± 6). However, because of widely scattered data, no net basal or stimulated transport of K, Ca, and Mg could be evidenced.

It is concluded that Iso enhances Ca and Mg reabsorption in cTAL and Na and Cl reabsorption in the whole TAL. Thus, besides polypeptidic hormones, ß-adrenergic agents contribute to the multihormonal modulation of the TAL function.

DA$_2$ DOPAMINE RECEPTOR MEDIATED RENAL EFFECTS OF LY 141865 IN RATS SUBJECTED TO ACUTE UNILATERAL RENAL DENERVATION. Alan S. Bass. Committee on Clinical Pharmacology, University of Chicago, Chicago, Illinois.

We reported previously that LY 141865 increased urinary sodium excretion (UNaV) in the innervated kidney and decreased UNaV in the denervated kidney of anesthetized rats subjected to acute unilateral renal denervation and extra-cellular fluid volume expansion (Pharmacologist 27:168, 1985). In addition, LY 141865 increased urinary potassium excretion (UKV) in the innervated kidney without significantly affecting UKV in the denervated kidney. These effects were associated with a decrement in mean arterial pressure (MAP) without a change in glomerular filtration rate (GFR). In the present experiments I extended this study further by examining the effect of the DA$_2$ dopamine (DA$_2$) receptor antagonist, S-sulpiride (S), on the renal responses to LY 141865. S (25 mg/kg iv) blocked the increment in UNaV and UKV produced by LY 141865 (750 µg/kg iv) in the innervated kidney and the decrement in UNaV produced by LY 141865 in the denervated kidney. S, alone, had no effect on UNaV and UKV in the innervated kidney, but decreased UNaV in the denervated kidney. The decrement in MAP produced by LY 141865 was attenuated by S. S, alone, produced a smaller decrease in MAP than LY 141865. Neither LY 141865 plus S nor S, alone, affected GFR. In conclusion, the natriuresis and kaliuresis produced by LY 141865 in the innervated kidney was due to activation of neuronal DA$_2$ receptors. Elucidation of a mechanism for the antinatriuresis produced by LY 141865 in the denervated kidney requires further study.

RAPID AND REVERSIBLE DOWN-REGULATION OF THIAZIDE DIURETIC RECEPTORS BY ACUTE RENAL ISCHEMIA. Kevin Beaumont,* Andrzej Maciejewski,* Duke A. Vaughn,* and Darrell D. Fanestil. Univ. of Calif., San Diego, Div. of Nephrology/Hypertension, La Jolla, CA.

This laboratory recently identified receptors for thiazide diuretic drugs in rat renal cortex through binding of ^{3}H-metolazone, a potent diuretic with a thiazide-like mechanism of action (PNAS 85: 2311, 1988). We now describe the rapid and reversible alterations that occur in thiazide receptors following acute renal ischemia in the rat. The apparent density of thiazide receptors in kidney membranes, as measured by saturation analysis of equilibrium binding of ^{3}H-metolazone, was reduced by 90% (from 0.555±0.052 to 0.058±0.01 pmol/mg protein; n=5) following 10 minutes of renal ischemia produced by clamping the renal pedicle. With release of the clamp and subsequent reperfusion for 10 minutes, thiazide receptor density returned to within 40% of control levels (0.347±0.086; n=3). Ischemia did not alter the apparent affinity of receptors for ^{3}H-metolazone. Tissue sections prepared from renal cortex and incubated in oxygenated media in vitro displayed similar rapid changes in thiazide receptors: hypoxia of 10-30 minutes duration produced by incubating sections in vitro in nitrogen-saturated media caused a significant decrease in binding of ^{3}H-metolazone that was reversible with return to oxygenated media. Similar decreases were obtained in oxygenated sections that were incubated with mitochondrial inhibitors (dinitrophenol or rotenone), but not in sections incubated with ouabain. These results indicate that renal thiazide diuretic receptors are rapidly regulated and that the supply of metabolic energy is crucial in this process.

IN VITRO GLUCOCORTICOIDS (G) RAPIDLY INDUCE THE NA-H ANTIPORT. C. P. Bastl, Temple Univ. Health Sci. Ctr. Philadelphia, PA.

In vivo studies (J.C.I. 80,348,1987) suggest G rapidly induce Na absorption in colon of adrenalectomized (ADX) rats. It is not known if this rapid induction is a systemic or direct tissue effect or which pathway is induced. Distal colon of ADX rats was studied in Ussing chamber under short circuit current (ISC) in the following groups: 1) ADX alone 2) ADX & Dexamethasone (Dex 2.5x10^{-8}M) 3) ADX & Dex & N-ethyl-N isopropyl amiloride (NENIA), the Na-H antiport inhibitor and 4) ADX & Dex & amiloride. Mucosal to serosal (m→s) and net Na absorption significantly increased fifteen minutes after addition of Dex while ISC was unchanged. NENIA completely blocked this increase.

uEq/h/cm^2	ADX	ADX & DEX	ADX & DEX & NENIA 10^{-5}M
Na m→s	5.7±0.7	9.8±1.1*	4.3±0.6
Na net	0.9±0.6	5.1±0.9*	-1.8±1.3
Cl m→s	10±0.7	14±1 *	8.5±1
Cl net	-0.02±0.7	4.2±1 *	-2.6±1.6
Isc	2.0±0.1	1.9±0.1	1.7±0.1

* p<0.01 compared to other groups

Serosal to mucosal flux, residual ion flux and tissue conductance were similar in all three groups. PD was 2mV higher with ADX alone (p<0.05). Amiloride 10^{-5}M did not decrease Na m→s, (Δ +1.7±1.4) or Isc (Δ -0.2±0.1 uEq/h/cm^2. Ion flux values were similar to those obtained with in vivo administration of low dose DEX for 24 hours. Thus, receptor specific doses of G increase electroneutral NaCl absorption within minutes by a direct tissue effect. The pathway induced appears to be the Na-H antiport.

BASOLATERAL MEMBRANE CONDUCTANCES AND INTRACELLULAR pH (pH$_i$) OF THE PRINCIPAL CELL (PC) IN MONOLAYERS OF CORTICAL COLLECTING DUCT (CCM). Elsa Bello-Reuss. University of Texas Medical Branch, Dept. of Med., Galveston, Texas.

Conventional and double-barrel microelectrodes were used to determine the basolateral membrane voltage (V_b) and pH$_i$ in PC cells of CCM. Previously, we have shown that the morphology, hormonal responses and apical membrane conductances allow for the recognition of PC and intercalated cells. PC cells, identified by Hoffman modulation contrast, constituted 70% of the monolayer. Studies were performed in a modified Ussing chamber; apical and basal sides were superfused with a HCO_3^--salt solution, pH 7.40 at 37°C. Reducing bath Cl^- concentration from 110 to 11 mM (cyclamate replacement) resulted in a transient depolarization of V_b from -80±2 to -63±3 mV (n=5), (p<0.001) followed by hyperpolarization. This pattern has been observed in other cells in which K^+ and Cl^- conductive pathways coexist in the same membrane. There was a concomitant increase in transepithelial resistance (R_t) from 820±200 to 1400±200 ohm·cm^2 (p<0.01). Increasing the K^+ concentration in the bath from 5 to 50 mM resulted in depolarization of V_b from -79±3 to -60±2 mV (n=6) (p<0.001) and in a decrease in R_t from 1200±200 to 1100±200 ohm·cm^2 (p<0.01). In 3 experiments, pH$_i$ was 7.20±0.02 under control conditions. The cells alkalinized to 7.39±0.02 and 7.45±0.03 when the basolateral side was exposed to low [Cl^-] or high [K^+], respectively. It is concluded that the basolateral membrane of the PC has both Cl^- and K^+ conductances. In addition, the changes in pH$_i$ upon ionic substitutions suggest the presence of a voltage-dependent mechanism of acid extrusion at the basolateral membrane.

ACTIVATION OF PROTEIN KINASE C(PKC) INHIBITS Na,K-ATPase ACTIVITY IN RAT KIDNEY PROXIMAL TUBULES (PT). Alejandro Bertorello* and Anita Aperia. Dept of Pediatrics, Karolinska Institute, Stockholm, Sweden.

Activators of PKC inhibit Na transport in PT (AJP 254:F9, 88). This study evaluates the effect of PKC activators on the mediator of active Na transport, Na,K-ATPase. Enzyme activity was determined in microdissected, permeabilized PT as ouabain sensitive ^{32}P-ATP hydrolysis under Vmax for Na,K and ATP. In vehicle incubated tubules Na,K-ATPase (pmol Pi/mm tubule/h) was 1403±128, n=5. The endogenous PKC activator diacylglycerol 10^{-4}M significantly inhibited Na,K-ATPase to 673±51, n=5. Phorbol 12,13 dybutyrate (PDBu) induced a time and dose-dependent inhibition of Na,K-ATPase. Inhibition was significant at 15 and maximal at 20 min. In PCT incubated 20 min with PDBu Na,K-ATPase was: 796±171 (10^{-8}M); 570±198 (10^{-7}M); 484±130 (10^{-6}M). PBDu inhibition of NaKATPase is not due to a non-ionic detergent effect. The inactive 4α-phorbol didecanoate did not inhibit Na,K-ATPase. Sphingosine (SP), a PKC inhibitor, abolished the inhibitory effect of PBDu on Na,K-ATPase in a dose-dependent manner (1102±78, PDBu+SP10/uM; 1356±114, PDBu+SP50/uM). PDBu 10^{-7}M had no effect on Na,K-ATPase purified from rat renal cortex. DA is a physiological inhibitor of Na,K-ATPase in PT (AJP 252:F39, 87). The effect involves a pertussis toxin sensitive G-protein. DA (10^{-5}M) inhibition of Na,K-ATPase was abolished by SP in a dose-dependent manner: 816±26 (DA+SP1/uM); 1237±75 (DA+SP10/uM); 1327±16 (DA+SP50/uM). Conclusion: Activation of PKC inhibits Na,K-ATPase activity in renal PT cells.

PURIFICATION OF A DIPHENYLAMINE-2-CARBOXYLATE (DPC)-BINDING PROTEIN WITH PROPERTIES OF A CHLORIDE CHANNEL. Gary V. Desir*, Thecla C. Massad* and Peter S. Aronson. Depts of Medicine, and Cellular & Molecular Physiology, Yale School of Medicine, New Haven, CT.

DPC-sensitive Cl channels have been described in the thick ascending limb and collecting duct of the mammalian kidney. The aim of this study was to identify and purify DPC-sensitive Cl channels from rabbit renal outer medullary membrane vesicles (OMMV).

DPC-sensitive, electrogenic ^{36}Cl flux was demonstrated in OMMV. The rank order for inhibition of this flux by Cl transport blockers was: 5-Nitro-2-(4-phenyl-butylamino)-benzoic acid [#145] > 5-Nitro-2-(3-phenyl-butylamino)-benzoic acid [#144] > 4,4'-dinitrostilbene-2,2' disulfonic acid [DNDS] ≈ DPC. These agents inhibited electroneutral Cl-Cl exchange in OMMV with a different rank order: #144 > #145 > DPC > DNDS.

We then synthesized a DPC-affinity column by immobilizing 2 mM DPC in polyacrylamide according to the method of Uchida and Filburn (JBC 259:12311, 1984). OMMV were solubilized with Triton (0.6% w/v) and the solubilized extract was equilibrated with the DPC-affinity matrix by a batch method. Following extensive washing of the matrix with buffer, proteins were eluted with buffer alone or with buffer containing 2 mM DPC. Eluates were concentrated and subjected to SDS-PAGE. Proteins were visualized by silver-staining. A 67 kD protein was specifically eluted from the affinity matrix by 2 mM DPC. Adsorption of this protein to the DPC-affinity matrix was completely blocked by #145 (250 μM). When different Cl transport inhibitors were compared, the rank order for inhibition of adsorption of the 67 kD protein to the DPC-affinity matrix was #145 > #144 > DNDS ≈ DPC, which was identical to that displayed for inhibition of conductive ^{36}Cl flux in intact OMMV. Amino acid analysis of the purified 67 kD protein indicated a yield of 5 pmoles/mg OMMV and a content of 60% hydrophobic amino acids, consistent with an intrinsic membrane protein.

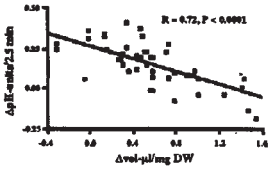
These findings strongly suggest that the 67 kD protein is a structural component of a DPC-sensitive Cl channel present in the outer medulla of the rabbit kidney.

CELL VOLUME REGULATES Na^+-H^+ EXCHANGE IN PROXIMAL TUBULE. S. Blumenthal and J. Kleinman. VAMC & Med Coll of Wisc Milwaukee, WI.

The purpose of this study was to examine the relationship between alterations in cell volume and Na^+-H^+ activity in renal proximal tubule. Suspensions of rabbit cortical tubules were solute depleted by incubation with rotenone in a Na^+-free medium. Cell volume was estimated as cell water/dry weight (μL/mg) and Na^+-H^+ exchange by the rise in cell pH (DMO) upon exposure to 135 mM Na^+ (ΔpH/2.5 min). In choline Cl (Group I), cell volume fell from 2.7 to 2.2 μl/mg; in choline SO_4 (Group II), cell volume decreased to 2.0 μl/mg ($P<0.001$ for both). To change cell volume, the predominant anions accompanying Na^+ were varied. ΔpH and increases in cell vol upon exposure to Na-anion pairs are shown:

Gr	Δ	SCN^-	NO_3^-	Cl^-	$Isethio^-$	$SO_4^=$
I	pH	0.07	0.17		0.31	0.37
	vol	0.71	0.39		0.35	0.07
II	pH		0.03	0.06	0.09	
	vol		1.16	0.95	0.66	

When individual data points were plotted, a significant reciprocal relationship between the ΔpH and the change in cell volume from its nadir was revealed:



In summary, cell volume and activity of the proximal tubule Na^+-H^+ exchanger are inversely related. Cell volume may quantitatively regulate Na^+-H^+ exchange in proximal tubule.

WITHDRAWN

CELL VOLUME REGULATION IN PROXIMAL TUBULE: SEQUENTIAL ACTIVATION OF K AND Cl CHANNELS. L. Dube*, L. Parent and R. Sauve* (intr. by J-Y. Lapointe), Membrane Transport Research Group, Universite de Montreal, Montreal, P.Q.

The role of ionic channels in cell volume regulation was studied using whole-cell and cell-attached configurations of the patch-clamp technique. Cell monolayers from proximal tubule in primary culture were perfused alternatively with normal and dilute (1:2) Earle solution. Membrane potential was monitored in whole-cell experiments during the hypoosomotic shock. Mean resting potential was -42 mV (n=10) when measured in the control medium. Cells exposed to test medium transiently hyperpolarized to -70 mV after which potential plateau at -20 mV. When the external medium is changed back to control, cells depolarized to -8 mV and then repolarized to a mean potential of -20 mV (n=8). Cell-attached experiments clearly showed the sequential activation of K, Cl and cationic channels. The channel active during the transient hyperpolarization phase was shown to be a classic K(Ca) with a slope conductance evaluated to 185 pS (n=15). It is activated by membrane potential and internal Ca (0.1 to 10 µM) in a bell shaped relationship. Cl and cationic channels that become active during the subsequent phases were found to be of intermediate conductance (70 and 100 pS). All the channels involved seem to operate within the same calcium range although the calcium sensitivity of the latter channels remains to be precisely characterized.

CULTURED RABBIT DISTAL CONVOLUTED TUBULE CELLS TRANSPORT SODIUM AND POTASSIUM. David H. Ellison & Heino Velázquez. Yale University & VA Med Center, West Haven, CT.

Cellular heterogeneity has made it difficult to investigate functional properties of individual renal distal tubule cells. We explanted discrete tubule segments to obtain primary cultures of distal convoluted tubule (DCT) cells. DCTs, identified by contiguity with thick ascending limb, were dissected from rabbit kidneys and cut proximal to the connecting tubule. Cells were grown on collagen coated glass or permeable supports (Costar® Transwells™) in medium containing 140 mM Na, 5 mM K, and 3% fetal calf serum. For comparison, cortical collecting tubules (CCT) were dissected from medullary rays and grown in a similar manner.

Cell outgrowth was observed in 35% of plated DCT (N=295) and 81% of CCT (N=127, P<0.001). Outgrowth occurred at 4 ± 0.2 (SE) days after plating DCT and 3 ± 0.2 days after plating CCT (P<0.001). Transmission electron microscopy showed uniform cell morphology; cells were 4-6 µm high with tight junctions, basolateral membrane amplification and apical microvilli. When grown on Transwells™, DCT cells reached confluence after 7-14 days. In 3 plates (confluent), the ability to transport was assessed by replacing apical and basolateral solutions and measuring [Na], [K], and transepithelial (TE) gradient (in mM) 24 hours later:

	Apical	Basolateral	TE Gradient
[Na]	124 ± 5	142 ± 2	-18 ± 5
[K]	10 ± 3	5 ± 1	5 ± 4

In apical solution, [Na] fell by 16 mM and [K] rose by 5 mM; because Δ[Na] does not equal Δ[K], transport of other ions (e.g. Cl^-, HCO_3^- or H^+) must have occurred. In summary, 1) primary cultures of rabbit DCT cells can be established from well defined microdissected tubule segments, 2) cell morphology reflects a single population of cells, and 3) cultured DCT cells transport Na and K actively.

LITHIUM (Li) CLEARANCE AND ATRIAL PEPTIDE (ANF) IN PREGNANCY (P), EFFECT OF VOLUME EXPANSION. J. Durr*, N. Miller*, L. Ganousis*, A. Alfrey, M. Lindheimer, J. Davison*. Dpt. Med Univ. Colorado, Univ. Chicago and Univ. Newcastle upon Tyne (U.K.).

Plasma volume rises in P while P_{Na} and BP decrease. Thus "effective blood volume" was described as both "over-" and "underfilled". Tubular and humoral indices of volume homeostasis were studied in six subjects during P and the non-pregnant (NP) state. Creatinine clearance (GFR) increased by 58 and 49 % in early (E) and late (L) P (p<.009). The rise in GFR was paralled by a 58 % decrease of endogenous serum Li (116 ± 23 in NP to 50 ± 3 nM/l in P, p<.05), consistent with unaltered (16.4 ± 3, NP; 16.2 ± 2, EP; and 16.4 ± 1 % in LP) fractional excretion of this trace element (FE_{Li}), a marker of proximal tubular fluid reabsorption. Plasma ANF, 19 ± 7 pg/ml (NP), increased to 40 ± 6 (p=.002) in EP and decreased again to 28 ± 5 during LP. ANF and tubular responses to central volume expansion were studied during water immersion. After 1 hour, ANF increased from 17.4 ± 6 to 26 ± 3 pg/ml (NS) in NP, from 41 ± 8 to 89 ± 19 (p<.02) in EP and from 22.2 ± 6 to 40.5 ± 11 (NS) in LP. An additional infusion of.06 ml/kg/min 5 % NaCl for 2h increased ANF to 60 ± 6 in NP (p=.002), to 118 ± 11 in EP (p=.000), and to 126 ± 24 pg/ml in LP (p =.004) and ANF rose more during P (ANOVA). Fractional excretion of Na (FE_{Na}), FE_{Li}, and ANF correlated significantly. Conclusion: ANF rises transiently in pregnancy while fractional proximal tubular handling of fluid (FE_{Li}) is unaltered. Strong evidence against a vascular "underfill" in P. Absolute tubular Na reabsorption is increased markedly and proportionally to GFR in both proximal and distal nephron sites during P.

THE EFFECT OF RINGERS EXPANSION ON PROXIMAL TUBULE DELIVERY IN THE AWAKE MICROPUNCTURED RAT. TA Fried, JR Lugon*, RW Osgood* and JH Stein. Univ of Texas Health Science Center, San Antonio, Tx

Both anesthesia and surgical stress are associated with a number of hormonal and hemodynamic changes which lead to salt retention. Because traditional micropuncture techniques are performed under these conditions, the mechanisms and tubular sites involved in excreting an acute volume load in the awake state remain incompletely understood. Indeed, the micropunctured animal excretes a very small amount of a volume load. We have recently developed a technique which allows micropuncture experiments to be carried out in awake rats 48 hours following surgical manipulation and anesthesia.

Three animals had chronic vascular catheters placed and their left kidneys "exteriorized". Forty-eight hours later they were put in restraining cages (to which they had previously become accustomed) and underwent baseline collections from their late proximal tubules (LPT). They then had a 1.3% per body weight Ringers expansion over 40 minutes followed by recollections from the same tubules. (Data expressed as mean ± SE)

	SNGFR nl/min	$(TF/P)_{IN}$	EPFR nl/min	Na inf uEq/min	UNaV uEq/min
Baseline	30±3	1.55±.1	20±1	2.5±.1	2.7±.3
2% VE	27±4	1.37±.1	20±2	10.4±.3	8.5±.8

(EPFR=end proximal flow rate, Na inf=sodium infusion rate, UNAV=sodium excretion rate).

These preliminary data suggest that awake rats quickly come into sodium balance following Ringers expansion. In addition, since the delivery from LPT doesn't change, the superficial proximal tubule does not appear to play an important role in the increase in salt excretion following mild acute volume expansion in the unanesthetized rat.

THE EFFECT OF DIET ON NA^+ CHANNEL DENSITY IN RAT CORTICAL COLLECTING TUBULE.

G. Frindt, L.G. Palmer and H. Sackin. Cornell Univ. Medical College, Dept. of Physiology and Biophysics, New York, N.Y.

Isolated rat cortical collecting tubules (CCT) were split to expose the apical membrane surface and bathed with 140mM NaGluconate medium at 23°C. Micro-injection of fluorescein into individual cells revealed no cell to cell coupling. Furthermore, the average cell capacitance of 36±7pF (n=8 tubules), measured under whole-cell clamp conditions with KGluconate in the pipette, is consistent with the capacitance expected for isolated rat CCT cells. The whole-cell Na conductance (G_{Na}) was defined as the amiloride (10μM) sensitive slope conductance near zero holding potential. Rats maintained on a *low Na diet* (n=12 tubules) exhibited a G_{Na} of 6.0±2 nS/cell compared with a G_{Na} of 0.06±0.1 nS/cell for rats maintained on a *normal diet* (n=14 tubules). The amiloride *insensitive* conductance (G_L) averaged 9.1±2 nS/cell, with no significant difference between *low Na* and *normal* groups. This relatively high value of G_L compared to G_{Na} is consistent with an important K conductance in rat CCT. The channel density (N) was determined from the relation: $G_{Na} = g_{Na} \cdot N \cdot P_o$, where P_o is the open probability of the channel (=0.5) and g_{Na} is the single channel conductance (=4pS) [J. Gen. Physiol. 92:121-138, 1988]. Consequently, the present study indicates that rats on a *low Na diet* have a Na channel density of N=3000 channels/cell compared to N=30 channels/cell for rats on a *normal diet*.

ADH ENHANCES NaCl ABSORPTION IN MOUSE MEDULLARY THICK ASCENDING LIMBS (MTAL) WITHOUT INCREASING O_2 CONSUMPTION (QO_2).

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In the mouse MTAL, ADH doubles the rate of furosemide(F)- and ouabain(O)-sensitive(sens) NaCl absorption. Thus we evaluated the effect of ADH on F-sens QO_2 in a purified suspension of mouse MTAL tubules. Either 1mM F or 5mM O reduced QO_2 by 55%. However, 10^{-7}M ADH (+1mM IBMX), which caused a 6-fold increase in cAMP accumulation, had no effect on O-sens QO_2 [45.6 ± 7.2 (-ADH) vs 54.7 ± 6.7 (+ADH) nmol $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$; p>0.2]. However, increasing cell Na entry with nystatin doubled QO_2 either with or without hormone. Thus, ADH stimulation of NaCl absorption in mouse MTAL occurs without increasing O_2 consumption. Since ADH appears to change the K^+ requirement for F-sens, apical Na,Cl cotransport in perfused mouse MTAL tubules [Sun & Hebert, ASN, 1988], we evaluated the effect of 10^{-7}M ADH [+10^{-6}M forskolin(FSK)] on F-sens Rb^+ uptake in mouse MTAL suspensions. F-sens Rb^+ uptake was linear over the first 60s. In the absence of ADH F-sens Rb^+ uptake at 60s was negligible [1 ± 15 $cpm \cdot \mu g\ protein^{-1}$] but increased to 79 ± 9 after a 10 min exposure to ADH(+FSK) [p< 0.01 vs no hormones]. Thus ADH may increase NaCl absorption in mouse MTAL with no increment in QO_2 by altering the K^+ affinity of the apical symporter: -ADH the F-sens symporter operates in a Na:Cl mode so that most Na^+ traverses the cellular pathway while +ADH the F-sens symporter operates as Na:K:2Cl symporter so that 50% of Na^+ traverses the cell utilizing Na:K-ATPase and apical K^+ recycling contributes to the lumen-positive transepithelial voltage driving the remaining 50% of Na^+ via the paracellular route

REGULATION OF IONIC CONDUCTANCES IN COLLECTING DUCT PRINCIPAL CELLS(CDP)CULTURED FROM NEWBORN RABBITS.

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The control of electrolyte transport across CDP has not been clarified.We therefore used a model epithelium of cultured CDP to evaluate putative stimuli of such transport by measurements of transepithelial voltage(V_{te})and resistance(R_{te}). - We observed:Co-incubation of CDP with aldosterone(10^{-6} M)for 10 days provided a stimulus to Na reabsorption(control:V_{te} -1.1+2.8 mV;R_{te} 0.4+.1 kΩ cm^2;n=7; aldosterone:V_{te} -43+5.5;R_{te} 0.9+.1;p<.05 both parameters).Co-incubation with aldosterone plus amiloride(10^{-6}M)for 10 days was associated with a further augmentation of Na transport in subsequent acute experiments without amiloride(aldosterone:V_{te} -26+3.9 mV;R_{te} 0.6+.05 kΩ cm^2;n=6;after aldosterone plus amiloride:V_{te} -49+3.8;R_{te} 0.45+.05;p<.05 both parameters).PGE_2(10^{-10}M)perfused at the basolateral cell membrane in acute experiments reduced sodium reabsorption(control:V_{te} -43.6+4.2 mV;R_{te} 0.8+.1 kΩ cm^2;n=8;PGE_2:V_{te} -39.7+3.8;R_{te} 0.86+.1;p<.05 both parameters).In contrast an increase of potassium and/or chloride conductivity was indicated by observations using basolateral h-α ANP(10^{-10}M)acutely(control:V_{te} -35.9+5.5 mV;R_{te} 1+.6 kΩ cm^2;n=8; ANP:Vte -32.7+5;Rte 0.98+.1;p<.05 both parameters), as well as a DA-1 agonist(10^{-10}M)(control:V_{te} -43.9 +6 mV;R_{te} 1.1+.2 kΩ cm^2;n=8;Fenoldopam:V_{te} -39+5.3; R_{te} 1+.2;p<.05 both parameters),AVP or angiotensin II(all 10^{-10}M). - It is concluded that aldosterone and PGE_2 primarily regulate sodium transport,whereas h-α ANP,AVP,A II and DA-1 agonist primarily control potassium- and/or chloride-conductivity in this collecting duct principal cell epithelium.

CHANGES IN INTRACELLULAR Na^+ (Na^+_i) IN THE PROXIMAL TUBULE MEASURED WITH AN EXTRACELLULAR Na^+ ELECTRODE.

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Regulation of Na^+_i in the mammalian proximal tubule is poorly understood largely because routine measurement of changes in Na^+_i have not been feasible. To address this problem, we used an extracellular Na^+ minielectrode coupled to a computerized data acquisition and analysis system to monitor continuously net Na^+ fluxes in suspensions of rabbit proximal tubules. The electrode displayed a Nernstian response to Na^+, exhibited a high selectivity for Na^+, and had a response time of < 2 s. This system could reliably resolve changes of 0.2 mM Na^+ in the presence of 145 mM extracellular Na^+ which represents a change of ≈ 5 mM Na^+_i using a 5% cytocrit. Ouabain inhibition of Na^+,K^+-ATPase activity caused a dose-dependent (K_i = 2.6 μM) net Na^+ influx with a Vmax of 232 nmol Na^+/min/mg protein. Using an extracellular K^+ electrode, ouabain promoted a net K^+ efflux with a similar K_i (2.5 μM) and a Vmax of 165 nmol K^+/min/mg. Based on these initial rates, the calculated $Na^+:K^+$ coupling ratio was 3:2, in agreement with previous measurements. Inhibition of K^+ permeability with 5 mM Ba^{2+} caused a Na^+,K^+-ATPase-mediated net K^+ uptake (103 ± 7 nmol/min/mg) and a simultaneous net Na^+ efflux (104 ± 6 nmol/min/mg). Therefore, extracellular electrode measurements of Na^+ and K^+ provide routine and sensitive measurements of ion transport and allow direct measurement of net ion transport coupling ratios in suspensions of intact cells.

DIMINISHED FUROSEMIDE EXCRETION CONTRIBUTES TO BLUNTED DIURETIC EFFECT IN HYPOKALEMIA. Max Hropot*, Wolfgang Schulz,* Erik Klaus, and Gerhard Giebisch. Hoechst AG, Frankfurt am Main, FRG; Yale Univ. Sch. of Med., New Haven, CT.

The salidiuretic effect of furosemide was studied in unanaesthetized potassium depleted rats. Hypokalemia was induced by feeding the rats a low-potassium diet for three weeks. Compared to controls (K: 4.35-4.65 mM, pH: 7.43, HCT: 0.46-0.47) the following parameters were measured in the blood of potassium depleted rats: K^+ 2.65-2.70 mmol/l, HCT 0.52-0.53 and pH 7.49. Rats were deprived of food 18 hours before the diuresis experiment, but had free access to tap water. On the day of the experiment rats were given furosemide (Lasix) as a single dose 32 mg/kg body weight. The salidiuretic effect and urinary excretion of furosemide were observed during a period of five hours and compared with results of a control group on a standard diet. In the hypokalemic group the diuretic and natriuretic effects of furosemide were sharply reduced, two- and five-fold, respectively. The excretion of furosemide, measured in urine by high pressure liquid chromatography, was decreased in the hypokalemic group about three-fold as compared to control rats.

In conclusion, decreased furosemide excretion in hypokalemic rats contributes to the diminished diuretic and saliuretic effect of this loop diuretic.

BASOLATERAL Cl EXIT IN RABBIT PROXIMAL CONVOLUTED TUBULES (PCT) PERFUSED IN VITRO. K. Ishibashi*, F. Rector, and C. Berry. UC, San Francisco.

To examine basolateral Cl exit mechanisms intracellular Cl and K activities (act.) were measured with double-barreled Cl or K selective microelectrodes. When PCT were perfused with high Cl, low HCO_3 and bathed with an ultrafiltrate -like solutions, Cl act. was 27mM and the basolateral membrane potential difference (Vbl) was -46mV. In the absence of ambient Cl, Cl act. was 7 mM. Subtracting this value from measured Cl act. still gave 1.2 times higher Cl act. than electrochemical equilibrium across basolateral membrane. Possible basolateral Cl exit mechanisms which we examined are: KCl symporter and $Na(HCO_3)_2/Cl$ countertransport. KCl symporter was suggested by: (1) basolateral Cl removal significantly decreased K act. from 52 to 49mM and (2) increasing bath K to 60mM increased Cl act. from 31 to 46mM. $Na(HCO_3)_2/Cl$ countertransport was suggested by: in the absence of luminal Cl, bath Na removal or HCO_3 reduction (25 to 5mM) increased Cl act. from 23 to 30 or from 27 to 31 mM, respectively. Predominance of SITS-sensitive Cl exit was suggested by: bath Cl removal reduced Cl act. from 28 to 12 with a rate constant of 18mM/min. 1mM bath SITS decreased Cl act. from 34 to 19mM and increased K act. from 50 to 74mM. Despite relatively constant driving force for KCl exit, bath SITS reduced the rate constant for Cl exit by 78% to 4mM/min.

We conclude that (1) Cl act. is above electrochemical equilibrium across basolateral membrane; (2) both KCl symporter and $Na(HCO_3)_2/Cl$ countertransport are present; (3) SITS-sensitive Cl exit mechanisms dominate.

GLUCOCORTICOIDS STIMULATE NA TRANSPORT BY RAT PAPILLARY COLLECTING DUCT CELLS (RtPC) IN CULTURE. R.F. Husted and J.B. Stokes. Univ. of Iowa Coll. of Med., Iowa City, IA.

RtPC play an important role in determining the final concentration of Na in the urine and steroids regulate Na transport in a number of epithelia. We investigated the steroid effects on Na transport by RtPC obtained from Wistar rats and cultured on filter-bottom cups. Cells were grown in a serum-free medium (Dulbecco's/Ham's F12 supplemented with insulin, transferrin, triiodothyronine, hydrocortisone [HC] and albumin) for 3 days and for 2 days in SF (serum-free medium less HC and albumin). The next 24 hr the cells were grown either in SF or in SF supplemented with various steroids (10^{-8}M). The short-circuit current (Isc, $\mu A/cm^2$) was stimulated by dexamethasone (DEX; 32.2±5.1) and HC (21.1±7.3) while progesterone, corticosterone, and aldosterone were similar to control (8.8±2.2). The glucocorticoid antagonist RU38486 blocked the stimulation of Isc by DEX and HC. Dose-response analysis of the DEX-stimulated Isc indicated half-maximal stimulation between 1-10 nM. Amiloride (100 μM) reduced the Isc in both control and DEX groups to ~1 $\mu A/cm^2$. The IC_{50} for amiloride on Isc was less than 1 μM. ^{22}Na uptake across the apical membrane was measured in cells exposed to DEX for 24 hr. Na uptake under conditions set to minimize tracer leak from the cells was equivalent to >90% of the Isc. This fact and the sensitivity of the stimulated Isc to amiloride indicate that the measured Isc represents primarily Na transport. The data demonstrate glucocorticoid stimulation of electrogenic Na transport by these cells.

INTERACTIONS BETWEEN Cl^- AND OTHER HALOGENS IN THE HAMSTER ASCENDING THIN LIMB OF HENLE (ATL). Taisuke Isozaki, Koji Yoshitomi, and Masashi Imai (Intr. by Genjiro Kimura). *Dept. of Pharmacol., National Cardiovascular Center, Osaka 565, Japan*

A highly conductive Cl^- transport system exists in the ATL. To examine the specificity and selectivity of the transporter, ion permselectivities and flux coefficients for ^{36}Cl and ^{125}I ($K_{Cl(LB)}$, $K_{I(LB)}$, $10^{-7}cm^2\ s^{-1}$) were measured in hamster ATL perfused *in vitro*. The relative permeabilities for halogen X to sodium ($P_{X/Na}$), as determined by biionic diffusion voltage (n=6), were 3.23±0.22, 3.23±0.26, 0.47±0.09, and 0.47 ±0.11 for $P_{Cl/Na}$, $P_{Br/Na}$, $P_{F/Na}$ and $P_{I/Na}$, respectively. The treatment of ATL with 0.1 M glutaraldehyde decreased both $P_{Cl/Na}$ and $P_{Br/Na}$ to 1.13±0.13 and 1.15±0.11, respectively. $K_{Cl(LB)}$ and $K_{I(LB)}$, simultaneously determined, were 88.0± 4.0 and 14.8±3.5, respectively (n=7). An addition of 100 mM NaCl or NaI to both the lumen and bath decreased $K_{Cl(LB)}$ from 124.8±14.3 to 87.7±13.0 or to 41.0±11.6 without affecting $K_{I(LB)}$, indicating that I^- is less permeable but inhibitory to the Cl^- pathway. An addition of 10^{-4}M 5-nitro-2-(3-phenyl propylamino)-benzoate (NPPB), a Cl^- channel blocker, to the bath decreased $K_{Cl(LB)}$ from 67.0±11.7 to 29.5±7.9 (n=5) without significant change in $K_{I(LB)}$ (18.4±3.0 vs 15.4± 5.5). We conclude : 1) ATL is permselective only to Cl^- and Br^-; 2) ATL is less permeable to I^- and F^-: 3) I^- inhibits Cl^- conductance: 4) Our previous conclusion on general halogen permselectivity in ATL was in error due to neglection of the inhibitory effect of other halides on the Cl^- conductance.

RENIN-ANGIOTENSIN-SYSTEM (RAS) AND WATER-ELECTROLYTE METABOLISM WITH CHRONIC HYPOXIA IN NEONATAL RATS. Shashi Jain*, Lee Wilke and Alan Tucker*, Colorado State Univ., Dept. of Physiol., Fort Collins, CO.

Neonatal rats were exposed to conditions simulating 10,000 ft altitude from 2 days of age for 30, 60 or 90 days. Plasma renin substrate (PRS) concentration increased with age but was reduced with altitude exposure Lung angiotensin-converting-enzyme (ACE) activity was lower at 30 and 60 days of age. Serum ACE and plasma renin activity were not affected by high altitude exposure. Water and electrolyte metabolisms were strikingly affected. Higher 24 hr water intake, urine output, and urinary sodium and potassium excretions were especially evident in early stages of altitude exposure. The results (means±SD) were:

		AGE		
		30	60	90
Water intake#	(C)	17.1±4.9	11.3±3.6	7.9±2.6
	(HA)	24.8±5.7*	20.4±4.9*	9.9±3.9
Urine output#	(C)	4.7±1.5	4.7±0.9	4.4±1.3
	(HA)	7.7±2.9*	10.7±4.1*	5.3±1.2
urinary Na^+ ##	(C)	0.9±0.4	0.7±0.1	0.5±0.1
	(HA)	1.9±1.5*	0.9±0.3	0.5±0.1
Urinary K^+ ##	(C)	1.4±0.5	1.3±0.2	0.9±0.2
	(HA)	2.0±0.8*	1.1±0.2	0.8±0.1

#ml/24 hr/100 gm BW; ##mEq/24 hr/100 gm BW; C control; HA high altitude; *p<0.01 vs control.

Right ventricular hypertrophy was evident at all ages with high altitude exposure, which is indicative of pulmonary hypertension. It was concluded that the effects of chronic hypoxia on lung ACE and water and electrolyte metabolism were transient. It is unclear if the development of pulmonary hypertension and the alterations in RAS and water-electrolyte metabolism are related.

EFFECTS OF CATECHOLAMINES ON SODIUM (Na) AND POTASSIUM (K) FLUX IN THE CORTICAL COLLECTING TUBULE (CCT). James S. Kaufman and Robert J. Hamburger. Boston VA Med. Ctr., Boston, MA.

We examined the role of catecholamines in modulating Na and K flux in the isolated perfused CCT of the rabbit. Tubules were perfused at 5-8 nl/min. Na and K concentrations were measured using electron probe microanalysis. After a control period the bath was exchanged for one containing norepinephrine (NE), epinephrine (E) or isoproterenol (I) in varying concentrations. In time controls there were no changes in Na or K flux. The results for the specific hormones are shown below (fluxes are in pmol mm^{-1} min^{-1} ± SEM):

Hormone	n	J_K	J_{Na}
Control	15	-16.00±9.40	46.18±11.37
NE 10^{-8} M	7	-15.15±2.49	52.97±19.33
NE 10^{-6} M	20	-12.17±1.08*	62.94±15.77
NE 10^{-4} M	10	-7.92±1.87*	36.95±12.99
E 10^{-5} M	7	-12.05±3.01*	16.51±4.67
I 10^{-6} M	5	-13.72±4.12*	23.10±14.84

*significantly different from control (p<.05)

These results indicate that the endogenous catecholamines, norepinephrine and epinephrine, inhibit K secretion without affecting Na reabsorption. Since a similar effect was noted with isoproterenol, the probable mechanism is stimulation of a beta adrenergic receptor.

EFFECT OF RENAL INTERSTITIAL INFUSION OF ARACHIDONIC ACID ON PROXIMAL TUBULE SODIUM REABSORPTION: MICROPUNCTURE STUDY. Y. Kinoshita*, J. C. Romero, and F. G. Knox, Dept. of Physiology and Biophysics, Mayo Clinic, Rochester, MN

The objective of the present study was to investigate whether proximal tubule sodium reabsorption in Sprague-Dawley rats is altered by the renal interstitial infusion of arachidonic acid and whether the effect of arachidonic acid is mediated by the production of prostaglandins. Continuous infusion of 10^{-4}M arachidonic acid directly into the kidney interstitium through an implanted matrix significantly increased the tubular flow rate and the fractional delivery of sodium at the superficial late proximal tubules (FD_{Na}) from 26.5±1.9 to 35.2±1.1 nl/min (n=6, p<0.01) and from 47.8±5.9 to 58.3±4.6% (n=6, p<0.01) respectively. In the presence of indomethacin, arachidonic acid failed to augment tubular flow rate and FD_{Na} (from 31.4±3.3 to 29.4±3.6 nl/min and from 46.5±5.0 to 47.7±5.9%). Arachidonic acid infusion increased 6-keto $PGF_{1\alpha}$ and PGE_2 excretion in urine (from 7803±1581 to 15223±3884 pg/30 min, p<0.05, and from 551±337 to 776±326 pg/30 min, p<0.01, respectively) and indomethacin blocked the stimulatory effect of arachidonic acid on prostaglandin excretion. Additional experiments indicated that the infusion of PGI_2 and PGE_2 also altered the sodium reabsorption by proximal tubules.

In summary, this study demonstrates that cyclooxygenase products affect proximal tubule sodium reabsorption.

REGULATION OF INTRACELLULAR K^+ (K^+_i) BY THREE K^+ LEAK PATHWAYS IN RABBIT PROXIMAL TUBULE (PT): EVIDENCE FOR AN INDUCIBLE, FUROSEMIDE (F)-SENSITIVE PATHWAY. B.C. Kone, H.R. Brady*, and S.R. Gullans, Harvard Medical School, Boston, MA.

The mechanisms by which the PT maintains K^+_i are largely unknown. Using an extracellular K^+ electrode, we characterized the K^+ leak pathways of PT suspensions and studied their contributions to K^+_i homeostasis. Under basal conditions, barium (Ba, K_i=0.4 mM) or quinine (Q, K_i=0.2 mM) caused a dose-dependent, net K^+ uptake mediated by the Na^+, K^+-ATPase. Based on the additive responses to Ba and Q, as well as differences in initial K^+ uptake rates and maximum K^+ accumulated (Ba=206±9 nmol K^+/mg prot; Q=113±6 nmol/mg, n=5), Ba and Q inhibited separate pathways. F (1 mM) alone had no effect on K^+ transport under basal conditions, but promoted a net K^+ influx (24±4 nmol/min/mg, n=5) in the presence of Ba (5 mM) and Q (1 mM), indicating the expression of a third K^+ leak pathway. In the presence of Ba (5 mM), the initial increase in K^+_i was followed by the gradual loss of cell K^+ which restored K^+_i to its basal level. This K^+_i recovery (nmol/min/mg, see table) was partially inhibited by Q (1 mM) or F (1 mM) and completely blocked by Q+F (n=5):

	Ba alone	Ba+Q	Ba+F	Ba+Q+F
K^+_i recovery	34±4	17±3	9±2	0

In conclusion, three separate K^+ leak pathways are present in the PT. Both the F-sensitive pathway, which appears to be induced, and the Q-sensitive pathway, can restore K^+_i in the presence of Ba. K^+_i appears to be exquisitely regulated by the coordinated actions of these three pathways.

THE MEMBRANE CROSS-TALK IN MAMMALIAN PROXIMAL TUBULE. J.Y. Lapointe and L. Garneau*. Membrane Transport Research Group, Univ. of Montreal, Montreal, P.Q.

The present study is aimed at establishing the presence of a membrane cross-talk when the transepithelial Na transport rate (J_{Na}) is reduced by the partial replacement of glucose, alanine and Na in the lumen of the rabbit proximal convoluted tubule perfused in vitro. When J_{Na} is reduced, the basolateral membrane potential, V_{BL}, (-42.5 ± 3.2 mV in control conditions, n=17) hyperpolarizes transiently by 12.6 ± 3.0 mV and the ratio of basolateral to apical membrane conductance (G_{BL}/G_A) doubles (initial value = 1.26 ± 0.15, n=7). The apparent transference number for K through the basolateral membrane (G_K/G_{cell}) decreases by 43 ± 12 % (n=6) in the first 3 min of J_{Na} reduction (initial value = 0.15 ± 0.02) and the cellular volume (CV) decreases by 13 ± 2 % (n=8). While the initial effects on V_{BL} and G_{BL}/G_A are well explained by the reduction in the apical rate of Na entry, the slower decrease in G_K/G_{cell} clearly represents a cellular response helping the cell to regulate the membrane potential and the ionic content of the cytosol. The time courses of the changes in G_A, CV and G_K ($t_{\frac{1}{2}}$ = 18, 41 and 72 s respectively) suggest that the reduction in CV could be the signal triggering the decrease in G_K as a part of a volume regulatory mechanism.

REGULATORY ROLE OF APICAL SODIUM (NA) ON SINGLE NA CHANNELS IN A6 EPITHELIA: ROLE OF PROTEIN KINASE C (PKC) . B. N. Ling,* D. C. Eaton,* (intr. by W. E. Mitch). Emory Univ., Dept. of Med. and Physiol., Atlanta, GA.

Na "self-inhibition" in tight epithelia describes the observation that increasing external Na_o concentration inhibits apical membrane Na permeability. We examined the effects of Na_o on single Na channel events in A6 amphibian renal cells by the patch clamp technique. After cell-attached patches [pipet solution 129mM NaCl] were obtained, the 129mM NaCl (high Na_o) apical bath outside the patch was replaced with 3mM NaCl (low Na_o). There was an increase in open channel probability (Po) by 25-75% and appearance of 2-5 "new" channels (#chs). A similar increase in Po and #chs occurred when apical Na entry was blocked by adding 10uM amiloride to the high Na_o apical bath. To investigate the mechanism for Na "self-inhibition", 0.1uM 4B-phorbol 12-myrisate 13-acetate was added to the basolateral bath after apical low Na_o or amiloride. PKC activation resulted in a gradual decrease in the stimulated Po and #chs over 15-20 mins. Conversely, 0.1uM 4a-phorbol, an inactive phorbol ester, did not decrease channel activity. Conclusions: 1) modulation of apical membrane Na permeability by Na_o does not require direct interaction with the Na channel protein but regulation involves an indirect intracellular pathway, 2) Na permeability modulation alters both the number and kinetics of single Na channels, 3) increases in Na permeability by low Na_o must be modulated via decreased apical Na entry and intracellular Na since amiloride yielded similar results, 4) the effects of decreasing Na_o are reversed by phorbol ester suggesting a possible role for PKC in Na "self-inhibition".

ATRIAL NATRIURETIC PEPTIDE (ANP) AND CYCLIC-GMP INHIBIT A RENAL CATION CHANNEL. D. Light,* E. Schwiebert,* K. Karlson,* and B. Stanton. Dept. of Physiol., Dartmouth Med. Sch., Hanover, NH.

In a previous study we characterized a 28 pS cation channel in the apical membrane of isolated inner medullary collecting ducts (IMCDs) and in IMCD cells grown in primary culture (Light, et al., J. Gen. Physiol. 90:28a, 1987). The channel is inhibited by amiloride and mediated electrogenic Na uptake across the apical membrane. We now report the effects of ANP and its second messenger, cGMP, on the cation channel in rat IMCD cells grown in primary culture. ANP (10^{-11} M) in the bath reduced the channel open probability (Po) from 0.64 to 0.44 in cell-attached patches (n=4; $P<0.05$). Maximum inhibition by ANP was observed at 10^{-7} M (Po=0.05). Dibutyryl-cGMP (10^{-4} M) in the bath also decreased the Po from 0.58 to 0.20 in cell-attached patches (n=4; $P<0.05$). In contrast, LY83583 (10^{-6} M), a compound which lowers intracellular cGMP, activated quiescent channels in cell-attached patches: Po changed from 0 to 0.26 (n=7; $P<0.05$). Finally, cGMP inhibited the channel in inside-out patches. Addition of cGMP (10^{-5} M) to the solution bathing the cytoplasmic surface of the membrane decreased the Po from 0.70 to 0.53 (n=6; $P<0.05$). Maximum inhibition by cGMP was observed at 10^{-4} M. 8-bromo-cGMP (10^{-4} M), a poorly hydrolyzable analog of cGMP, also decreased the Po from 0.58 to 0.36 (n=6, $P<0.05$). Summary and conclusion: ANP, via cGMP, inhibits a cation channel in the apical membrane of IMCD cells in culture. cGMP also inhibits the channel in inside-out patches. These results suggest that ANP increases urinary Na excretion in part by inhibiting electrogenic Na absorption by the IMCD.

INCREASED CORTICAL Na,K-ATPase DOES NOT FACILITATE K EXCRETION (KE) IN NEWBORN DOGS. John M. Lorenz*, Margery A. Manuli*, Lyle E. Browne*, Katherine Markarian*, Leonard I. Kleinman. Dept of Pediatr, SUNY at Stony Brook, NY and Dept of Biochemistry & Molec Biophysics, Coll of P&S, Columbia Univ, NY.

We have shown that KE during acute K loading is lower in newborn (Nb) than in adult dogs (AJP 251: F513, 1986. This is due to decreased K secretion in late distal and cortical collecting tubules (lDT/CCT). To determine if the less efficient response of the Nb to acute K loading could be improved by increasing Na,K-ATPase activity in lDT/CCT cells, cortical Na,K-ATPase activity and KE during acute K loading were determined in litter and age-paired non-K-adapted (NKA) and K-adapted (KA) Nb dogs (n = 10 pairs). K-adaptation consisted of the administration of 3-4 mmole K/kg BID for $\geq$ 7 days prior to acute K loading. NKA animals received no K prior to acute K loading. This protocol resulted in a 50% increase in oubain sens cortical Na,K-ATPase activity [mean ± SEM = 7556 ± 2273 (NKA) v 11578 ± 3604 (KA) nmole P_i/ mg protein/hr, $p<0.05$]. However, this increase was not associated with an increase in KE during a 3 hr infusion of KCl at 20 umole/kg body/min [7.7 ± 0.7 (NKA) v 7.1 ± 1.0 (KA) umole/Kg body/min].

Clearance data were incomplete in another 6 NKA and 6 KA animals who developed K toxicity (bradyarrythmia and anuria) during the 3hr KCl infusion. There was no significant difference in cortical Na,K-ATPase between 10 of these animals and 10 of the above animals, for whom this comparison was valid. These results are consistent with the hypothesis that lDT/CCT Na,K-ATPase activity is not the rate limiting factor in the less efficient response of the newborn to acute K loading.

KIDNEY MICROPUNCTURE IN THE AWAKE RAT. JR Lugon*, RW Osgood*, TA Fried and JH Stein. Univ of Texas Health Science Center, San Antonio, Tx

Micropuncture studies have considerably advanced our understanding of renal physiology. Unfortunately these studies have only been possible in the anesthetized state shortly following major surgery, a state associated with hormonal and hemodynamic changes which result in profound salt retention. We have developed a technique to micropuncture awake rats. Chronic vascular catheters are inserted and the left kidney is placed into a plastic chamber which is filled with agar and attached to the animals' skin. During micropuncture the animal is held in a restraining cage to which it was previously conditioned. Rats were infused with tritiated inulin in Ringers. Studies were performed 5 (N=4), 24 (N=6) and 48 (N=2) hours post operatively, and following one hour of the infusion. Results are expressed as mean ± SE. Data are from exteriorized kidney.

	BW	BP	GFR	Na inf	UNaV	TT	PT
	Kg	mmHg	ml/min	(uEq/min)		LP/ED	mmHg
5hr	.27±.2	117±6	0.9±.1	6.4±.4	0.2±.1	6±.3/26±3	16±1
24hr	.20±.1	124±4	0.6±.1	2.4±.2	0.2±.1	6±.3/27±1	17±1
48hr	.18±.1	122±4	1.2±.1	1.9±.5	1.0±.3	6±.3/24±1	16±1

(BW=body weight, Na inf=sodium infusion, UNaV=sodium excretion, TT=transit time (sec), PT=proximal tubule pressure, LP=late proximal and ED=early distal).

In other 6 rats studied at 48hr (whole kidney data from bladder catheter- 2 kidneys):

BW	GFR	Na inf	UNaV	(TF/P)in	SNGFR (nl/min)	
Kg	ml/min	(uEq/min)		LP	Prox	Distal
.18±.01	1.9±.1	2.5±.1	2.7±.3	1.49±.09	32±1	28±2

We conclude that in contrast to micropuncture in the anesthesized rat: 1. The TT is shorter and the PT higher; 2. The fractional reabsorption of water in superficial proximal tubule is lower; and 3. At 48hr the animals are in Na balance during the study with the exteriorized kidney excreting half of the Na infusion.

A NEW FLUORESCENT PROBE FOR MEASURING POTASSIUM IN AQUEOUS SOLUTIONS. D. Masilamani*, M.E. Lucas*, K. Golchini* and I. Kurtz. Biosciences Division, Allied-Signal, Morristown, NJ and Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

A new fluorescent probe, 6,7-(4-methyl) coumaro-[222] cryptand, has been developed for measuring K^+ in aqueous solutions. This compound was made by fusing [222] cryptand with 4-methyl-coumarin through 2 ethoxy bridges at the 6 and 7 position. The spectral properties of the probe were characterized in a HEPES (5 mM) buffered solution, pH 7.4. The probe has a fluorescent excitation peak at 340 nm and an emission peak at 417 nm. Increasing the K^+ concentration from 0 to 10 mM caused the fluorescence intensity to increase by 143%. The $\log_{10}$ equilibrium constant for complexation (Ka) of K^+ in 50% ethanol/water, 9.74, was greater than the Ka for Na^+, 7.20. Unlike K^+, Na^+ did not increase the fluorescence of the probe. However, 10 mM Na^+ by displacing K^+ from its binding site quenched the fluorescence by 6.5% in the presence of 4 mM K^+. Ca^{2+} (1 mM) and Mg^{2+} (1 mM) did not alter the fluorescent intensity of the dye in the presence or absence of K^+. NH_4^+ (10 mM) in the absence of K^+ increased the fluorescence intensity minimally (16%), and did not alter the fluorescence intensity in the presence of K^+. The fluorescent measurement of K^+ agreed favorably with the flame photometric technique. Conclusions: 1) A new fluorescent probe has been developed for measuring K^+ from 0-10 mM in aqueous solutions. 2) The selectivity of the dye for K^+ over Na^+ is 350:1. 3) This probe will be useful for measuring K^+ transport across epithelial membranes.

REDUCED MAXIMUM OUABAIN (OU) BINDING (BMAX) IN RED CELLS OF HIV INFECTED PATIENTS. J.K. Maesaka, J.M. Piccione* F.P. Siegal* A.W. Dreisbach* Dept. of Med. Long Island Jewish Med. Ctr., New Hyde Park, NY.

We investigated OU binding in red cells of randomly selected HIV infected patients which included AIDS, ARC, and those that were asymptomatic. Binding experiments were conducted after 2 hour incubation with 23 nM H..3..OU (specific activity: 19.9 Ci/mmole) and varying concentrations of cold OU ranging from .0025 uM to 100 uM. The binding parameters were determined by modified Scatchard analysis using the NIH Ligand program. The results showed a 35% reduction in mean Bmax (x±sem; control: 384±17 sites/cell, n=10, HIV: 294±19 sites/cell, n=21, $p<.00002$) without a significant change in dissociation constant (Kd) (control: 9.1±1.6 nM, n=11, HIV: 10.8±1.1 nM, n=21, NS). Of the 21 HIV infected patients studied, 6 showed no reduction in Bmax. Intracellular sodium (IC Na) was found to be significantly increased (control: 8.0±1.0 mmole/L cell H..2..O, n=9, HIV: 14.4 mmole/L cell H..2..O, n=11, $p<.009$). In the HIV infected patients the increase in IC Na was negatively correlated with Bmax ($r=-.73$, n=8, $p<.04$). There was no significant change in cell H..2..O (control: 63.5±.5% PRBC, n=10, HIV: 63.0±.1% PRBC) n=12, NS). This decrease in Bmax without a change in Kd is consistent with an actual reduction in receptor number or may represent an apparent reduction due to the presence of a circulating factor with OU binding activity. The negative correlation of Bmax with IC Na suggests a functional effect on cation transport in the red cells of patients with HIV infection.

STIMULATION OF Cl SELF EXCHANGE BY INTRACELLULAR HCO_3 $[HCO_3^-]_i$ IN RABBIT CORTICAL COLLECTING DUCT (CCD). Kenzo Matsuzaki*, John B. Stokes, and Victor L. Schuster, Dept. of Int. Med., U. of Iowa College of Medicine, Iowa City, IA.

Cl tracer flux (K_{Cl}, nm/s) occurs across ß intercalated cells via an apical anion exchanger and a basolateral Cl channel. The system exhibits self exchange and is stimulated by bath HCO_3/CO_2. Here we examine the individual components of the CO_2/HCO_3^- system that stimulate K_{Cl}. 0.5% CO_2 added to a HEPES bath (pH=7.24) stimulated K_{Cl} by 70±19 nm/sec, a ΔK_{Cl} comparable to that induced by 1% CO_2 (pH 7.12), 6% CO_2 (pH 6.6), or 6% CO_2/25 mM HCO_3^- (pH 7.4). Such extracellular CO_2 addition should lower intracellular pH (pH_i) and raise $[HCO_3^-]_i$. We examined the roles of pH_i and $[HCO_3^-]_i$ more directly by clamping pH_i using K/nigericin. Increasing pH_i from 6.9 to 7.6 without exogenous CO_2/HCO_3^- increased K_{Cl} by 71±17 nm/sec, suggesting that pH_i might regulate anion exchange. However, a pH_i shift would also increase $[HCO_3^-]_i$ derived from metabolic CO_2. To determine if increasing $[HCO_3^-]_i$ without pH_i could stimulate K_{Cl}, we replaced HEPES isohydrically with 6% CO_2/5 mM HCO_3^- (pH_i clamped at 6.9). With this increase in $[HCO_3^-]_i$ at constant pH_i, K_{Cl} increased by 51±10 nm/sec. These maneuvers had negligible effects on Cl diffusion and $Cl^- - HCO_3^-$ exchange. We conclude that increases in $[HCO_3^-]_i$ of <2 mM stimulate transepithelial anion exchange. Because our previous experiments showed that HCO_3^- markedly attenuated the inhibitory effect of Cl channel blockers on K_{Cl}, we postulate that HCO_3^- stimulates K_{Cl} at the basolateral Cl channel.

Cl^- FLUX IN BASOLATERAL MEMBRANE VESICLES (BMV) FROM RABBIT RENAL MEDULLA (RRM) IS MEDIATED VIA CONDUCTIVE AND K^+-DEPENDENT NONCONDUCTIVE MECHANISMS. PS Mehta* and DA Molony, University of Texas Medical School-Houston, Houston, Texas.

Cl^- efflux from medullary thick ascending limb (mTAL) cells is largely conductive; efflux via electroneutral KCl cotransport is controversial. To document both modes of Cl^- efflux, we measured Cl^- and K^+ fluxes in RRM-BMV prepared by differential centrifugation. Conductive Cl^- flux was measured as the valinomycin (VAL) stimulated component of $^{36}Cl^-$ uptake in the presence of a .15 M inwardly directed K^+ gradient. VAL (2 uM) stimulated $^{36}Cl^-$ uptake by 42% at 30 s, from 2112 ± 323 to 2992 ± 455 pM/mg protein (n=4); the VAL-dependent increase in Cl^- uptake was abolished by 0.1 mM diphenylamine-2-carboxylate (DPC).

Rb^+ uptake into RRM-BMV was determined in the presence of a .15 M outwardly directed K^+ gradient, 0.1 uM DPC, 1 mM ouabain, and 0.1 mM furosemide. 5 mM Ba^{++} inhibited $^{86}Rb^+$ uptake by 21.6 ± 5.1 % at 30 s in the absence of Cl^- but had no effect in the presence of 0.15 M Cl^- (n=4). $^{86}Rb^+$ uptake (pM/mg protein) with zero Cl^- was 1440 ± 130; increased significantly with 10 and 20 mEq/L Cl^- to 1712 ± 109 and 1871 ± 121, respectively, but did not increase further with 140 mEq/L $[Cl^-]$ (1913 ± 110) (n=5).

Thus, RRM-BMV exhibit conductive Cl^- and K^+ fluxes that are abolished by DPC and Ba^{++} respectively. Rb^+ uptake is also mediated by a DPC insensitive, Cl^- dependent pathway with an apparent K_m for Cl^- of 9 mM. These results are consistent with KCL cotransport and parallel Cl^- and K^+ conductances across the BM of the mTAL.

MEASUREMENT OF INTRACELLULAR Cl^- ($[Cl^-]_i$) IN ISOLATED PERFUSED MOUSE MEDULLARY THICK ASCENDING LIMBS (mTAL) WITH THE FLUORESCENT DYE 6-METHOXY-1-(3-SULFONATOPROPYL)QUINOLINIUM (SPQ). DA Molony and MD Breyer, Univ. of Texas Med. Sch., Houston, Tx and Vanderbilt Univ., Nashville, Tn.

$[Cl^-]_i$ will affect critically transcellular Cl^- reabsorption by the mTAL, a principal site of renal Cl^- regulation. To probe the modulation of $[Cl^-]_i$, we measured quenching of SPQ-fluorescence by $[Cl^-]_i$ in the mTAL perfused in vitro in HCO_3^- free solutions. Tubules were loaded from the bath with 30 mM SPQ for 1 hr.; excited at 350 nm; fluorescence emission >435 nm was measured by photon counting (Illsley & Verkman, Biochem. 26:1215). Fluorescence in 110 mM Cl^- bath was 4.1 ± 1.3 fold above background and decayed by 50 % over 16.1 ± 3.6 min (n=6). Bath Cl^- deletion produced a prompt (30 seconds), reversible 58.9 ± 15.4 % increase in fluorescence (n=9). Bath 60 mM K^+ or 5 mM Ba^{++} resulted in reversible declines in SPQ signal of 25.8 ± 5.6 % (n=4) and 21.8 ± 4.6 % (n=3). SPQ fluorescence was calibrated to $[Cl^-]_i$ using a double ionophore technique with 10 uM tributyltin (Cl^-:OH^- exchanger), 7 uM nigericin (K^+:H^+ exchanger) in 100 mM K^+ bath. This technique yielded a relation of change in fluorescence to $[Cl^-]_i$; slope of -19 % /10 mM/L $[Cl^-]$. After Ba^{++} washout apparent $[Cl^-]_i$ was 39.7 ± 9.2 mM/L (n=3).

Thus, these studies show that: 1) SPQ can be used successfully to measure $[Cl^-]_i$ in the mTAL, 2) $[Cl^-]_i$ is above electrochemical equilibrium and 3) the changes in SPQ fluorescence with bath Ba^{++} and 50 mM K^+ are compatible with either conductive or KCL cotransport mediated Cl^- exit from the mTAL.

ACTION OF ALDOSTERONE ON CITRATE SYNTHASE IN CULTURED RENAL COLLECTING DUCT CELLS.
W. Minuth*, M. Struck*, M. Zwanzig* and P. Gross*†. University of Heidelberg, Depts. of Anatomy and Medicine†, Heidelberg, FRG. (intr. by R.J. Anderson).

Stimulation of Na^+-transport by the steroid hormone aldosterone requires an increased amount of ATP supplied to the Na^+/K^+-ATPase pump. ATP, an energy rich molecule is preferably generated by the tricarboxylic acid cycle and by oxidative phosphorylation. These mechanisms can be accelerated by increased citrate synthase (CS) activity. We therefore used cultured renal collecting duct cells (Minuth, W.W. - Differentiation 36:12-22 (1987)) to investigate the question whether aldosterone controls CS during increase of Na^+-transport. The experiments demonstrated for the first time in cultured collecting duct cells that aldosterone indeed has an influence on CS enzyme activity. While controls showed basal values of 71±3 mU/mg protein (n, 28), hormone treated epithelia had increased citrate synthase activity of 79±6 mU/mg after 1 h (n, 5), 88±6 mU/mg after 2 h (n, 6), and 93±8 mU/mg protein after 3 h (n, 5) of administration ($p \leq 0.05$, paired t-test). After that period the activity during the 4th and 6th hour was found to be decreased to basal values. The significant 30 % increase in citrate synthase enzyme activity within 3 h after aldosterone administration could be blocked by spironolactone. Regarding the time course of enzyme stimulation, the results indicated that aldosterone activates citrate synthase during the physiological early response phase. Radioactive labelling experiments further showed that hormone stimulated increase of CS activity seems to be related to an activation of preformed molecules, and not to a new synthesis of CS.

ELECTROGENIC APICAL AND BASOLATERAL L-LACTATE (LAC) TRANSPORT IN ISOLATED PERFUSED AMBYSTOMA PROXIMAL TUBULES. Nikolas S. Morgunov* (intro. by D.J. Hirsch). Dept. Physiol. & Biophys., Dalhousie Univ., Halifax, N.S., Canada.

Apical and basolateral Lac transport was characterized in isolated perfused (HCO_3 Ringer solutions) salamander proximal tubules by monitoring basolateral cell membrane potential (V_{bl}) and intracellular sodium activity (a_{Na}). Organic substrate deletion from the luminal perfusate rapidly hyperpolarized V_{bl} by 16.4±1.1 mV ($p<0.01$:n=7) and decreased a_{Na} by 50% ($p<0.01$: n=4) from control values of -64 ± 2mV and 18.2± 1.8 mM respectively. In contrast, substrate deletion from the bath superfusate gradually depolarized V_{bl} by 11.5 ± 0.8 mV ($p<0.01$:n=12) but only produced a small, transient increase in a_{Na}. Lac (5 mM) addition to the lumen or bath solution, depolarized V_{bl} by 7.9±0.7 mV and 8.5± 0.8 mV respectively ($p<0.01$:n=7). The apical electrogenic Lac response was associated with a 2.3±0.2 mM increase in a_{Na}. However, the basolateral response decreased a_{Na} by 1.9±0.1 mM ($p<0.01$:n=3). Yet both responses exhibited a marked Na dependence: 80% reduction (n=7) in Na-free solutions. The data suggest the following: (1) Ambystoma proximal tubule cells possess an apical electrogenic Na-Lac cotransporter associated with net entry of Na into the cell. (2) Basolateral Na-Lac transporter exerts an inhibitory affect on apical Na-Lac cotransport via V_{bl} depolarization and increases in a_{Lac}.

GLUCOCORTICOIDS INCREASE Na^+ AND K^+ TRANSPORT IN PRIMARY CULTURES OF CORTICAL COLLECTING TUBULE CELLS (CCTC). A. Náray-Fejes-Tóth* and G. Fejes-Tóth. Hypert. Res. Div., H. Ford Hosp. Detroit, MI

In addition to aldosterone, glucocorticoids can also modulate Na^+ and K^+ excretion. To examine their possible direct effects on CCT, we used primary cultures of immunodissected rabbit CCTC. CCTC in culture retained their glucocorticoid receptors as indicated by the presence of specific high-affinity binding sites for dexamethasone (10,500/cell; K_d=5.1 nM). When grown on permeable supports CCTC actively reabsorbed Na^+, secreted K^+ and developed a lumen negative transepithelial potential difference (PD). 50 nM of dexamethasone or RU 28362 (a pure glucocorticoid which does not bind to mineralocorticoid receptors) caused marked increases in Na^+ reabsorption, K^+secretion, PD and short-circuit current (SCC) comparable to the effect of 5 nM aldosterone.

	Control	DEX	RU 28362	Aldo
Na^+ reabs.*	6.5±1.1	17.2±2.9	15.2±1.5	17.4±0.9
K^+ secr.*	1.3±0.2	3.3±0.3	3.4±0.4	3.2±0.2
PD (mV)	13.8±0.02	37.5±3.8	37.4±2.6	37.9±3.9
SCC ($\mu A/cm^2$)	9.6±1.1	21.0±1.8	20.9±1.9	25.1±2.1

(* $\mu mol/cm^2/24$ h)

These effects were detectable after 3 h and increased further up to 24 h. Addition of 1 μM RU 486 (glucocorticoid receptor antagonist) prevented the effect of RU 28362, while 1 μM of ZK 91587 (mineralocorticoid receptor antagonist) did not. We conclude that besides the well-known effect of aldosterone on Na^+/K^+ transport, glucocorticoids can also increase Na^+reabsorption and K^+secretion in CCTC. This effect seems to be mediated through specific glucocorticoid receptors.

LUMEN CALCIUM INHIBITS POTASSIUM SECRETION BY RAT RENAL DISTAL TUBULE.

M. D. Okusa*, H. Velázquez, D. H. Ellison, and F. S. Wright. Yale University School of Medicine and VA Medical Center, New Haven, CT.

Calcium is important in the regulation of ion transport in both epithelial and nonepithelial tissue. To investigate the role of Ca on K secretion by the renal distal tubule, in vivo microperfusion experiments were performed in male Sprague Dawley rats. The control perfusion solution resembled interstitial fluid to minimize transepithelial ion gradients and contained (in mM): 150 Na, 4 K, 119 Cl, 5 HEPES. Experimental perfusion solutions were prepared by adding 0.4, 0.8 or 1.5 mM Ca to the control solution. In paired comparisons, the effect of lumen Ca on net K flux (J_K, indicates secretion) is shown in the following table:

Lumen [Ca]	0.4	0.8	1.5
J_K (control)	-89	-97	-120
J_K (Ca)	-94	-65	-78
P (control vs Ca)	NS	<0.005	<0.005

Whereas 0.4 mM Ca did not affect net J_K, both 0.8 and 1.5 mM Ca decreased net J_K. Since net K secretion is the sum of two unidirectional (absorptive and secretory) fluxes, we sought to determine the effect of 1.5 mM Ca on these components. A tracer for K (^{86}Rb) was added to the control and 1.5 mM Ca solutions. 1.5 mM Ca did not affect lumen-to-blood K flux (26 ± 2.8 pmol/min vs 27 ± 2.7 pmol/min; NS) but decreased blood-to-lumen K flux (110 ± 14.5 vs 90 ± 12.9 pmol/min, P< 0.05). We conclude that : 1) lumen Ca in concentrations normally present in the distal tubule decreases net K secretion, and 2) the decrease in net K secretion is a result of inhibiting unidirectional secretory K flux. The mechanism by which this occurs may involve inhibition of K or Na channels in late distal tubule cells.

K-DEPENDENT Cl^- ABSORPTION: AN ALDOSTERONE RESPONSIVE PATHWAY. Ronald D. Perrone and David E. McBride.* Renal Division, New England Medical Center, Boston, MA.

Cl^- absorption in rat distal colon is mediated via neutral NaCl absorption (linked Na^+-H^+ and Cl^--HCO_3^- exchange). Aldosterone (aldo) suppresses neutral NaCl absorption and stimulates conductive Na^+ absorption. Recent studies from this laboratory have demonstrated that in vitro K^+ absorption in rat distal colon is markedly stimulated by CO_2 and aldo when studied in Na-free Ringer. K^+ absorption was electrically silent and was inhibited by vanadate, ouabain and the H^+-K^+-ATPase inhibitor SCH28080. The relationship between K^+ and Cl^- absorption under Na-free conditions, the effect of CO_2 and the effect of aldo have not been evaluated. To address these questions, unidirectional fluxes of ^{36}Cl were determined in voltage-clamped segments of rat distal colon from control and aldo-stimulated rats. Secondary hyperaldosteronism was induced by feeding a Na-deficient diet. Bathing solutions contained 25 mM HCO_3^- bubbled with CO_2; all Na^+ was replaced with choline. Data are J_{Cl}^{net} ($ueq.h^{-1}.cm^{-2}$). Means ± SE. N=7-8.

	1% CO_2	5% CO_2	10% CO_2
Control	-0.8±0.9	0.7±0.9	2.6±0.7
Aldo	0.2±0.5	2.7±1.3	5.3±1.0

PD and I_{sc} approximated zero in the absence of Na^+. Cl^- absorption varied significantly with pCO_2 in both control and aldo-stimulated colon, but aldo exceeded controls at all CO_2 levels. In 10% CO_2, J_{Cl}^{net} was markedly reduced when studied in the absence of both Na^+ and K^+: 0.3±0.6 in controls and 1.0±0.6 in aldo-stimulated colon. In the absence of Na^+ in vitro, Cl^- absorption varies directly with the pCO_2 and is stimulated by aldo in a manner similar to that previously demonstrated for K^+. A close coupling between Cl^- and K^+ absorption is revealed by the marked inhibition of Cl^- absorption under K-free conditions.

WITHDRAWN

VOLUME REGULATION IN S_2 SEGMENTS: ANALYSIS OF INTRACELLULAR OSMOLAL AND ANION GAPS. L. Rome*, J. Grantham, V. Savin, and C. Lechene. Univ. of Kansas Med. Ctr., Kansas City, Kansas; Harvard Medical School, Boston, Massachusetts.

We previously showed that, in butyrate-supplemented medium, isolated, non-perfused S_2 segments maintained constant volume when external osmolality was changed at 2 mOsm/min from 167 to 450 mOsm. Proximal cells lost KCl during volume maintenance in hypotonic media and gained NaCl during volume maintenance in hypertonic media. We used electron probe analysis (EPA) to quantitatively assess changes in intracellular K, Na and Cl content in S_2 segments during gradual 100-150 mosm change in external osmolality. Na_i and Cl_i were calculated from EPA results assuming control K_i equals 140 mEq/L. Intracellular osmolality (Osm_i) was assumed equal to bath osmolality. Osmolal gap (O.G.) = Osm_i - (K_i+Na_i+ Cl_i). Anion gap (A.G.) = (K_i+Na_i) - Cl_i.

	N	Osm	K_i	Na_i	Cl_i	O.G.	A.G.
		mosm/L	meq/L	meq/L	meq/L	mosm/L	meq/L
Hypo	12	295	140	8.3	37.4	108	112
Hypo	12	208	133	6.5	19.5	49	120
Hypo	11	158	122	6.5	16.3	13	112
Hyper	20	295	140	9.5	30.2	115	119
Hyper	17	400	143	30.2	63.6	153	110

Hypo-osmotic volume maintenance was accompanied by a loss of KCl and undetermined osmolytes but no change in A.G. Hyperosmotic volume maintenance was accompanied by a gain of NaCl and undetermined osmolytes but no change in A.G. The magnitude of change in O.G. and the stability of the A.G. suggest that other factors act in conjunction with net movement of Na, K and Cl to maintain constant cell volume in anisotonic fluids.

IONOMYCIN AND CYCLIC-AMP ACTIVATE Cl^- CHANNELS IN APICAL MEMBRANE OF SHARK RECTAL GLAND PRIMARY CULTURES (SRGPC). S. Sansom, M. Moran*, Q. La*, and J. Valentich*. Departments of Medicine and Physiology, University of Texas Medical School, Houston, TX and MDIBL, Salsbury Cove, ME.

Previous microelectrode experiments showed that forskolin activated a Cl^- conductance in the apical membrane of SRGPC grown to confluence. We used microelectrode and patch-clamp methods to examine the effects of ionomycin on the activation of apical Cl^- channels. In microelectrode experiments (absence of ionomycin), activation of the apical membrane voltage (V^a) was -74 mV, the apical membrane fractional resistance (fR^a) was 0.90, and a 10-fold reduction of apical Cl^- concentration had no effect on V^a. Apical ionomycin (1 uM) depolarized V^a by 13 mV and reduced fR^a to 0.20. In the presence of ionomycin, Cl^- removal depolarized V^a by 23 mV, revealing activation of an apical Cl^- conductance. In the patch-clamp experiments, SRGPC were grown to confluence on glass slides. With 280 mM KCl in the pipette and normal shark Ringers in the bath, the apical membrane was patched in both cell-attached and excised configurations. We found reversal potentials and current amplitudes consistent with both low and high conductance Cl channels (slope conductances were 6 and 40 pS, respectively). In cell-attached configuration, the open probability of the high but not the low conductance channel was increased by either 1 uM ionomycin or 0.1 mM dibutyrl cAMP. These studies indicate that increasing either intracellular Ca^{++} activity or cAMP concentration activates the apical Cl^- channel of SRGPC.

A STRETCH-ACTIVATED K^+ CHANNEL THAT RESPONDS TO CHANGES IN CELL VOLUME.

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Swelling is known to increase potassium (K) conductance in a variety of cell types. Subsequent exit of K down its electrochemical gradient, together with an accompanying anion, would restore swollen cells to their original volume, if these ion fluxes are followed by osmotic water flow. The role of K channels in cell osmoregulation was investigated using the patch-clamp technique. In cell-attached patches from amphibian proximal tubule the short open-time K channel at the basolateral membrane has a conductance of 42pS, and a K to Na selectivity of 15 to 1. The channel can be stretch-activated by pipette suction, where a negative pressure of 6cm H_2O is sufficient to increase the open probability of the channel by a factor of 4.0±0.8 (n=7 tubules). Exposing unperfused *Necturus* proximal tubules to a 100mOsm hypotonic bath increases cell volume by 66±10% and increases the K channel open probability by a factor of 5.8±1.4 (n=7), in the same set of cell-attached patches that were initially subjected to suction. Although single channel currents were recorded during large changes in osmolarity, calculations based on a simple model indicate that K channel activity could increase significantly with as little as a 1% increment in cell volume. Furthermore, the similar effect of both swelling and suction on the open probability and kinetics of the channel suggests that stretch-activated K channels might be responsible for the increase in macroscopic K conductance that occurs when proximal tubules are subjected to hypotonic media.

K TRANSPORT MECHANISMS IN RABBIT PROXIMAL STRAIGHT TUBULE CELLS. Sei Sasaki, and Fumiaki Marumo. Dept. of Internal Medicine, Tokyo Medical and Dental Univ. Tokyo, Japan

K transport mechanisms across luminal and peritubular membranes of rabbit proximal straight tubule cells(S2), and their relation to Cl reabsorption were examined by monitoring intracellular K activity (aK). Isolated tubules were perfused in vitro and aK was determined by double barreled ion selective electrode. Luminal perfusate was a high Cl (HCO3 5mM) solution and formate (0.5mM) was included in luminal and bath fluids. aK in a steady-state condition was 59.9±2.0mM(basolateral membrane potential; -45.5±1.5mV, n=32). A rapid addition of 0.1mM oubain (Ou) to the bath caused an immediate reduction of aK (estimate of NaK ATPase pump rate) at 22.4±1.3mM/min (n=24). An addition of 2mM barium (Ba) to the bath rapidly increased aK (estimate of K flux through basolateral K conductance) at 15.9±2.1mM/min (n=12). A simultanous application of Ou and Ba to the bath caused a aK reduction (estimate of K flux independent of K conductance, possibly through KCl cotransport) at 7.8±0.8mM/min (n=15). aK reduction rate in response to Ou was significantly slowed from 21.6±3.2 to 14.6±2.8mM/min by a total removal of luminal Cl (n=4). After treatment with bath Ba, an addition of Ba to the lumen or changing luminal K concentration (0 and 15mM) did not significantly alter aK.

These data suggest that 1)K transport across luminal membrane is unmeasurably small, 2)a 70% of K taken up by NaK pump exits through the basolateral K conductance, and 3)one-thirds of NaK pump rates is coupled to Cl reabsorption.

EFFECTS OF INTRA-RENAL NOREPINEPHRINE INFUSION ON FETAL RENAL FUNCTION. Tadashi T. Sato*, Jon Klinkefus*, Oliva J. McWeeny*, Francine G. Smith*, and Jean E. Robillard. Dept. of Pediatrics, University of Iowa, Iowa City, Iowa.

Renal nerve stimulation and increased levels of circulating catecholamines in adult animals give rise to sodium retention, due probably to a direct tubular action of norepinephrine (NE). We studied effects of circulating catecholamines on fetal renal function by measuring the response to intra-renal infusion of NE. Chronically instrumented fetal sheep aged 130-141 days were studied at least 3 days following surgery. During experiments, NE (levophed) was infused into the renal artery for 20 min at a rate of 0.0025-0.02 µg/kg/min. Each animal received 3 incremental doses. Measurements were made at the completion of each infusion for 40 min. Intra-renal infusion of NE did not alter fetal blood gas status or hematocrit. Mean arterial pressure following NE infusion also remained constant. Glomerular filtration rate in fetal sheep remained at control levels of between 2.23±0.73 SD (n=4; prior to lowest dose of NE) and 3.48±0.99 SD (n=6; prior to highest dose of NE). Control sodium excretion was 96.16±80.72 SD (n=4; prior to lowest dose of NE) and 42.27±18.02 SD (n=6; prior to highest dose of NE). Sodium excretion did not change after NE infusion. Similar results were achieved when experiments were repeated during α-blockade with 5 µg/kg/min phentolamine mesylate (Regitine). Thus, basal sodium reabsorption in fetal sheep does not appear to be affected by increased levels of circulating catecholamines.

RENAL INTERSTITIAL HYDROSTATIC PRESSURE AND SODIUM EXCRETION DURING VOLUME EXPANSION IN THE DEVELOPING PIGLET. M.J. Solhaug*, J.P. Granger, M.R. Wallace*, Dept. Pediatrics and Physiology Eastern Virginia Med. Sch., Norfolk, VA 23501.

The role of renal interstitial hydrostatic pressure (RIHP) in the regulation of sodium excretion (UNaV) in the developing kidney is not known. The purpose of this study was to determine RIHP and Na excretory responses to acute volume expansion (VE). The effect of 5% VE was determined in adult pigs (A), and piglets ages, 26-43 days, and 18-25 days. Fractional excretion of lithium (FeLi) was utilized as an indicator of proximal tubular sodium reabsorption.

	n	CRIHP (mmHg)	ΔRIHP (mmHg)	n	ΔUNaV (µEq/min/gkw)	ΔFeLi
A	4	7.89 ±1.70	4.86 ±0.20	6	3.87 ±1.07	21.7 ±5.6
18-25	5	5.96 ±1.41	6.84 ±2.05	5	2.94 ±0.78	35.7 ±6.68
26-43	7	6.09 ±1.21	2.59* ±0.89	7	1.50* ±0.45	21.4 ±6.37

Increases in UNaV were similar in A and 18-25 d piglets but diminished in 26-43 d piglets vs A. Although ΔFeLi was slightly higher in the 18-25 d piglets, there were no significant differences between all groups. Control RIHP (CRIHP) was not significantly different. The peak increase of RIHP during VE (ΔRIHP) was not different between A and 18-25 d group, but significantly less in the 26-43 d group vs A. Conclusions: 1) RIHP demonstrates maturational changes in a pattern similar to sodium excretion in the developing piglet during VE. 2) RIHP may participate in the sodium excretory response to VE in the developing piglet.

CHANGES IN CELL VOLUME INDUCED BY K AND CL IN THE ISOLATED AND PERFUSED RABBIT PROXIMAL TUBULE. Laurent Schild*, Peter S. Aronson, and Gerhard Giebisch. Dept of Cellular & Molecular Physiology, Yale Univ. School of Medicine, New Haven, Ct.

We used real-time recordings of cell volume to determine the pathways for K and Cl transport across the basolateral membrane of the proximal tubule cell. Raising bath [K] from 3 to 30 mM resulted after 20 sec in a 11.35 ± 1.02% increase in cell volume. The cell swelling induced by increased bath [K] was inhibited 71.6% by 1 mM Ba in the bath. K-induced cell swelling was inhibited 77.8% by replacement of lumen and bath Cl with cyclamate, but was unaffected by replacement of Cl in the lumen only. At normal bath [K], inhibiting K efflux from the cell with bath Ba caused only a 5.03 ± 1.14% increase in cell volume. These findings suggest that the cell swelling induced by increased bath K reflects KCl entry across the basolateral membrane. The Ba-sensitivity of this KCl entry suggests that it is mediated by parallel K and Cl conductances.

At normal bath [K], replacing bath Cl by cyclamate caused a 2.50 ± 1.17% decrease in cell volume. Importantly, in the presence of 30 mM K in the bath, replacing bath Cl by cyclamate caused a much larger decrease in cell volume (7.83 ± 1.52% decrease). Thus, increasing bath K stimulated the efflux of Cl. This is consistent with exit of Cl through a conductive pathway activated by cell swelling and/or membrane depolarization.

In conclusion, measurements of cell volume changes suggest that KCl transport across the basolateral membrane of the proximal tubule cell occurs predominantly by a Ba-sensitive K conductance in parallel with a volume- and/or potential-sensitive Cl conductance.

STIMULATORY AND INHIBITORY INTERACTIONS BETWEEN α-ADRENOCEPTORS AND THE RENAL Na/H ANTIPORTER. J. W. Strandhoy & F. A. Gesek. Wake Forest Univ. Med. Center, Winson-Salem, NC 27103.

The mechanisms by which α-adrenoceptors regulate Na reabsorption in the nephron were examined in rat proximal tubule segments. Influx of ^{22}Na through a Na/H antiporter was decreased by ethylisopropyl amiloride and increased by the phorbol ester, PMA. Na/H activity was increased similarly by the α_1 agonists tested (cirazoline, phenylephrine, NE), but variably increased by α_2 agonists. B-HT 933 maximally stimulated influx 98% while guanabenz (GBZ) only increased Na/H by 22%, with an order of stimulation: BHT > α-methylNE > NE > clonidine-UK 14304 > guanfacine > GBZ. Heterogeneity of α_2 dependence on pertussis toxin (PT)-sensitive G proteins was tested in other rats. In tubules prepared from rats treated 5 days earlier with PT, PMA and α_1 responses were unaffected, but stimulation by α_2 was blocked. When the antiporter was increased directly by PMA, GBZ but not BHT inhibited the response. Therefore, α_2 agonists *stimulate* Na/H through PT-sensitive pathways but some agonists also *inhibit* the antiporter through a PT-resistant mechanism. Antagonism of PMA by α_2 agonists was prevented by α_2 antagonists or brief trypsin pretreatment (50 µg/ml x 10 min). When tubules from normal (no PT) rats were pretreated with trypsin, GBZ could stimulate Na uptake as well as BHT. Thus, the net effect of α_2 agonists on Na influx related to separate interactions at stimulatory and inhibitory sites. These findings may help explain the differing propensities of α_2 agonists to cause Na retention and pseudotolerance.

OUABAIN-INDUCED CELL SWELLING IN RABBIT CORTICAL COLLECTING TUBULE (CCT). Kevin Strange, Wright State University School of Medicine, Department of Physiology & Biophysics, Dayton, Ohio.

Peritubular application of 10^{-4}M ouabain to DOCA-treated CCT had no effect on intercalated cell volume but caused rapid swelling in principal cells (PC) at a rate of 66.5 ± 6.7%/min. PC swelled 145.5 ± 16.0% and then downregulated their volume at a rate of -3.1 ± 0.5%/min to a new steady-state volume 13.3 ± 3.8% above control. Luminal Na^+ removal or 10^{-5}M amiloride addition caused PC to shrink -11.8 ± 2.3 and -7.7 ± 2.5%, respectively. In the absence of perfusate Na^+ ouabain swelling is completely blocked while amiloride inhibits the rate 95.0 ± 1.2%. Luminal or bilateral Cl^- removal caused PC to shrink -9.2 ± 1.1% and -12.0 ± 0.9% and inhibited the rate of ouabain swelling 70.2 ± 2.3% and 98.7 ± 0.8%, respectively. Perfusion of CCT with 0.1 mM acetazolamide or 0.1 mM diphenylamine-2-carboxylate caused PC shrinkages of -7.4 ± 2.4% and -6.5 ± 1.2% and inhibited ouabain swelling by 58.7 ± 3.8% and 67.4 ± 6.2%, respectively. Bilateral CO_2-HCO_3 removal inhibited the rate of swelling by 68.1 ± 4.7%. Ouabain swelling in CCT dissected from acidotic (NH_4Cl loading) DOCA-treated rabbits was inhibited 50.7 ± 5.4% compared to controls. Conclusions: 1) ouabain swelling is due to cytoplasmic NaCl accumulation, 2) Na^+ enters the cell primarily through apical Na^+ channels, 3) Cl^- enters the cell at least in part through the apical membrane, 4) apical Cl^- entry may be mediated by an anion exchanger.

POTASSIUM (K) IS SECRETED IN RABBIT PROXIMAL CONVOLUTED TUBULES (PCT) IN VITRO. Kaoru Tabei*, Hiroaki Furuya*, Shigeaki Muto*, Yasushi Asano*. Jichi Med. Sch., Unit of Nephrol., Dept. of Med., Tochigi, Japan (Intr. by Eiji Kusano)

Previous micropuncture studies in rats suggested that K is reabsorbed in PCT in vivo, although the transport mechanism has remained unclear. To examine the K transport mechanisms in PCT, rabbit PCT was perfused in vitro by using micropefusion technique. PCT was dissected from New Zealand white rabbits and perfused by standard micropefusion method. Tritiated Inulin was used as a volume marker and K concentration in collected fluid was measured by ultramicro-flamephotometer. In unidirectional K flux study, $^{86}RbCl$ was used instead of ^{42}K. Reflection coefficient to KCl was 0.86±0.03, and hydraulic water permeability was 2.31±0.06x10^{-5}cm/sec /atm/cm^2. Efflux coefficient (Ke) was increased from 2.87±0.60x10^{-5}cm/sec to 4.16±0.28 in the presence of 10^{-5}M ouabain in the bath, whereas Influx coefficient (Ki) did not change (4.45±1.03 vs 4.98±0.96). Measured net K flux (JK) was -15.01±4.51pEq/min/mm when Jv was 1.03±0.19nl/min/mm and perfusion rate was 12.96±0.96nl/min. 10^{-5}M ouabain decreased JK to -0.83±2.53. When 10^{-5}M amiloride or 2 mM Ba^{++} were added to the perfusate, net K flux did not changed significantly, however, 2 mM Ba in the bath increased net K flux from -9.59±5.36 to -24.51±9.07 pEq/min/mm. These results may suggest that potassium is secreted in rabbit proximal convoluted tubules in vitro. Barium enhances K secretion from the basolateral side indicating that basolateral membrane of PCT has a Ba sensitive conductive pathway.

ADH ALTERS THE K^+ REQUIREMENT FOR THE LUMINAL, FUROSEMIDE-SENSITIVE NaCl SYMPORTER IN MOUSE MEDULLARY THICK LIMBS (MTAL). Adam M. Sun* and Steven C. Hebert. Brigham & Women's Hosp. and Harvard Med. School, Boston, MA.

The K^+ requirement for luminal, furosemide-sensitive NaCl symport in the MTAL remains controversial. The present study assessed the K^+ requirement for Na,Cl cotransport activity with and without ADH in mouse MTAL tubules perfused in vitro at 37°C. Video imaging techniques were used to measure cell swelling resulting from both 1 mM ouabain (O) addition and K^+ removal ($-K^+_B$) from peritubular bath. [O,$-K^+_B$]-induced cell swelling was inhibited by 0.1 mM furosemide addition to, or Na^+ removal from, perfusate, indicating that cell swelling was due to Na entry via the luminal Na:?K:Cl symporter. Thus, the rate of [O,$-K^+_B$] -induced cell swelling ($nl \cdot min^{-1} \cdot cm^{-1}$) was used as an index of luminal Na:?K:Cl symporter activity. Studies were performed using symmetrical solutions and luminal 20 mM Ba^{2+} was used to reduce K^+ entry into the perfusate via apical K^+ channels. In the absence of ADH, luminal K^+ (K^+_L) had no significant effect on the rate of [O,$-K^+_B$]-induced cell swelling [0.318 ± 0.090 and 0.199 ± 0.046 with and without K^+_L, respectively (p=NS; n=3)]. Addition of 250 μU/ml ADH increased the transepithelial voltage (mV) from 4.4 ± 1.7 to 8.8 ± 0.6 (p<0.05;n=3), and luminal K^+ removal now inhibited [O,$-K^+_B$]-induced cell swelling [0.016 ± 0.004 ($-K^+_L$) vs 0.473 ± 0.060 ($+K^+_L$); p<0.05, n=3]. These studies indicate that in the absence of ADH the luminal symporter in the mouse MTAL operates in a Na:Cl mode and that ADH modifies the symporter to become K^+ dependent. This alteration in the luminal symporter has important implications for energy consumption related to NaCl absorption.

NA-K-ATPASE ACTIVITIES IN INNER MEDULLARY RENAL TUBULE SEGMENTS OF RATS. Y. Terada* and M.A. Knepper. National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland.

General agreement has not been reached regarding the mechanism by which an axial osmolality gradient is generated in the renal inner medulla. Various proposed models assume active NaCl transport either in the thin descending limb (TDL), the thin ascending limb (TAL), or the inner medullary collecting duct (IMCD). In this study, we have measured the activities of Na-K-ATPase (the primary Na pump) as a function of inner medullary depth for each inner medullary tubule segment. Measurements were made in microdissected segments from collagenase-treated kidneys of Sprague-Dawley rats using the technique of O'Neil and Dubinsky (A.J.P. 247:C314). Inner medullary position is designated as percent of the total inner medullary length where 0% is the inner-outer medullary junction and 100% is the papillary tip. Activities in thin limb segments (pmol/min/mm) were: TDL(0-25%), 2.2±0.6; TDL(50-100%), 3.2±1.0; TAL(0-25%), 3.6±1.3; TAL(50-100%), 4.2±0.9 (all P<0.05, vs. 0). The activity in outer medullary TDLs (long-looped nephrons) was significantly greater than inner medullary TDL values (5.8±1.0). Values in collecting ducts were: outer medullary CD, 21±5; IMCD(0-25%), 35±6; IMCD(25-50%), 20±3; IMCD (50-75%), 17±2; IMCD(75-100%), 16±1. Deoxycorticosterone administration to the rats increased Na-K-ATPase activities in all collecting duct segments. CONCLUSIONS: 1) Measurable Na-K-ATPase activities are present in all inner medullary tubule segments. 2) Axial differences in thin limb activity, postulated in some models of the inner medulla, were not observed. 3) The activity in the initial IMCD is greater than in the terminal IMCD.

THE ROLE OF Na^+ TRANSPORT IN THE CEREBRAL ADAPTATION TO CHRONIC HYPERNATREMIC DEHYDRATION (CHD). H.Trachtman, R.Del Pizzo & E.Cragoe,Jr. Dept. of Peds., SUNY-Stony Brook, Schneider Child. Hosp. of LIJMC, New Hyde Park, NY.

Increased cerebral cytosolic ionic content is an important adaptive mechanism to minimize cell shrinkage during CHD. Na^+ content may increase via the Na-H antiport exchange or Na^+ channels. We explored the relative importance of these transport pathways using amiloride (A) analogues.

Rats were pretreated for 6 days either with A, a more potent inhibitor of the Na^+ channel,10 mg/kg/day or with 5-(N,N-hexamethylene)-amiloride (HMA),a more potent inhibitor of the Na-H antiporter,1 mg/kg/day. Both drugs were dissolved in a 0.5% DMSO drinking water solution. Control rats received 0.5% DMSO solution as drinking water for 6 days. CHD was induced by total water deprivation for 24 hr followed by daily injections with 1M NaCl to raise serum Na^+ to 180-190 mmol/L over 72 hr. Brain tissue was removed at death for measurement of cerebral water compartment sizes(ml/100 gm dry weight). (Results: Mean ± SEM).

	A (N=9)	HMA (N=8)	CONTROL (N=5)
Mortality	9/9[a]	0/8	1/5
Na^+, Initial	142±2	144±1	145±5
Final	182±7	187±4	189±4
TTW*	344±2[a]	361±5	373±2
ECW*	131±3[a]	134±4	137±4
ICW*	213±1[a]	227±2	237±3

*Abbrev: TTW-total tissue water;ECW-extracellular water,ICW-intracellular water;[a]$p < 0.01$,A vs HMA&C.

These results indicate that in vivo, the Na^+ channel is a more important mechanism than the Na-H antiporter for increasing cerebral cell Na^+ content during CHD.

DIFFERENCES IN Na+/H+ ANTIPORT BETWEEN BRUSH BORDER MEMBRANE VESICLES (BBMV) ISOLATED FROM OUTER CORTEX (OC), INNER CORTEX (IC), AND OUTER MEDULLA (OM) OF DOG KIDNEY. Stephen T. Turner, Molly A. Van Norman*, Thomas P. Dousa, Jonathan M. Chen*. Mayo Foundation, Division of Hypertension, Rochester, Minnesota.

To assess possible cellular basis for heterogeneity of sodium (Na) reabsorption by proximal tubules located in different kidney zones, we studied Na/H antiport and other Na-gradient dependent transport systems in BBMV prepared from OC, IC, and OM of mongrel dogs. Initial ameloride sensitive Na/H antiport and Na-dependent uptake of proline and glucose were significantly greater in BBMV-IC than in BBMV-OC or BBMV-OM:

	Transport Rate (pmole/mg protein/5sec)			
	Na-H	Uptake of		
BBMV	antiport	L-Proline	D-glucose	phosphate
OC	199	148	47	303
(n=4-7)	±38	±17	±7	±32
IC	339‡†	267‡†	132‡†	362†
(n=4-7)	±42	±26	±25	±26
OM	111	36	28	77
(n=4-7)	±20	±2	±6	±20

‡Different from OC; †Different from OM, ($p<0.05$).

Kinetic studies revealed significantly greater Vmax for Na/H antiport in BBMV-IC compared to BBMV-OC or BBMV-OM. Transport differences were not due to differences in intravesicular volume or purity of BBMV. Higher capacity for Na/H antiport by luminal membranes may contribute to greater Na reabsorption by proximal tubules in IC.

EFFECT OF SODIUM, CHLORIDE AND BICARBONATE ON BINDING OF METOLAZONE TO THE THIAZIDE DIURETIC RECEPTOR. Johan Tran,* Kevin Beaumont,* and Darrell D. Fanestil. Univ. of Calif., San Diego, Div. of Nephrology/Hypertension, La Jolla, CA

This laboratory recently identified receptors for thiazide diuretic drugs via the binding of ^{3}H-metolazone (MTZ). We now report effects of selected ions on the binding of MTZ. Rat kidney cortex was homogenized in Tris-phosphate buffer and centrifuged at 120 x g, and 48,000 x g to yield membranes. Binding of MTZ was relatively insensitive to changes in pH over the range 6.0 to 9.0 The maximal binding capacity (B_{max}) was 0.8 pmol/mg protein and the equilibrium dissociation constant (K_d) was 3.3 nM. Addition of NaCl produced a biphasic effect: the binding of MTZ was increased by concentrations of NaCl <20 mM, but was inhibited at higher concentrations; whereas $NaHCO_3$ produced only the stimulatory effect. Other salts of Na^+ that stimulated MTZ binding included sulfate, methylsulfate, acetate and lactate. Saturation analysis of the binding of MTZ indicated that Na^+ (50 mM $NaHCO_3$) did not alter B_{max}, but lowered the K_d to 1.4 nM. The concentration of Na^+ producing this apparent increase in affinity for MTZ was half-maximal at about 10 mM. In contrast, Cl^-, Br^- and I^- inhibited the binding of MTZ. NaCl (50 mM) did not alter B_{max}, but increased the apparent K_d to 9.5 nM, a finding consistent with binding of chloride and MTZ being competitive (or mutually exclusive). The concentration of NaCl required to produce a two-fold increase in the K_d of binding of MTZ is 26 mM. Thus, the conformation of the thiazide diuretic receptor is sensitive to Na^+ and Cl^- over the concentration ranges expected for these ions in the lumen of the distal convoluted tubule.

THE EFFECT OF CHRONIC POTASSIUM DEPLETION ON POTASSIUM, SODIUM AND WATER TRANSPORT IN THE LOOP OF HENLE. Robert Unwin*, Giovambattista Capasso*, Kristina Lujic* & Gerhard Giebisch. Dept. Cell. & Mol. Physiol., Yale Univ. Med. Sch., New Haven, CT.

To determine the effect of chronic potassium depletion on loop electrolyte transport, rat superficial loops were pump microperfused in vivo from late proximal to early distal tubule. Two groups of rats were studied: (1) control rats on a standard diet (C); (2) rats on a low potassium diet for at least 3 weeks (LK). The perfusate in each group was an end-proximal Ringer solution containing either 2 or 4 mM potassium and ^{14}C-inulin. Potassium and sodium concentrations in collected fluid were measured by furnace atomic absorption spectrophotometry. The results are summarized below as mean±SEM:

Perfusate [K]	J_K (pmol/min)	J_{Na} (nmol/min)	J_V (nl/min)	n
C 2 mM	15.7±2.1	1.95±0.06	7.4±0.2	15
LK 2 mM	22.7±1.7*	1.74±0.10	8.3±0.4	23
C 4 mM	44.2±3.6	2.02±0.09	8.4±0.4	15
LK 4 mM	53.1±2.6*	1.58±0.09*	6.9±0.4*	21

(*$P<0.05$ vs. C; n = number of loops perfused)

The data show that in potassium-depleted animals, loop potassium reabsorption (J_K) is significantly increased. Moreover, they suggest that loop sodium and water reabsorption (J_{Na} and J_V) are decreased in LK when the perfusate potassium concentration is "normalized".

FLUORESCENCE MEASUREMENT OF INTRACELLULAR CHLORIDE IN MONOLAYER CULTURED CELLS. A.S. Verkman, Mary Sellers*, R. Ketchum*, J.H. Widdicombe* and A.C. Chao*. CVRI and Pharm. Chem., UCSF, CA.

Methodology has been developed for measurement of cell Cl in cultured cells using entrapped Cl-sensitive fluorophores. An established renal cell line (LLC-PK1) and a primary culture of dog tracheal epithelium were grown to confluency on thin glass cover slips. Cells were effectively loaded with the relatively impermeable fluorophore SPQ by a hypotonic loading procedure (5mM, 2min, 37°C, 150mOsm), or with more permeable compounds synthesized with cleavable esters (6-methoxy-N-[aceto-pivolic ester] quinolinium and 6-oxyaceto-pivolic ester-N-[3-sulfopropyl] quinolinium) by 10min incubation at 37°C. Cells were mounted in a chamber designed for high N.A. objectives and rapid (<1s) fluid changes. Cell fluorescence was quantitated by epifluorescence microscopy using UV optics and photomultiplier or SIT camera detection. Photobleaching was eliminated by excitation of fluorescence at 350 nm using a 100 watt stabilized tungsten source and O.D. 2 filter. SPQ leakage from cells was <10% in 30min at 23°C and ~20% at 37°C. Intracellular calibration was performed using high K buffers containing the ionophores nigericin and tributyltin. Fluorophore signals decreased by 50% at cell [Cl]=20-50mM. Transients in cell Cl ($T_{½}$ 5-30s) were measureable following changes in solution Cl. In tracheal cells having a cAMP-dependent Cl channel, 10 μM isoproterenol gave a 3-5 fold increase in Cl transport rates. These results establish methodology for direct measurement of cell Cl by fluorescence microscopy for use in defining Cl transport and regulatory mechanisms.

RAPID VIDEO MEASUREMENT OF KCl REFLECTION COEFFICIENT ACROSS BASOLATERAL CELL MEMBRANE OF S_2 PROXIMAL RENAL TUBULE. Larry W. Welling, Dan J. Welling, and Toni Ochs*. Laboratory and Research Services, VA Medical Center, Kansas City, MO, and Depts. of Pathology and Physiology, Univ. of Kansas Medical Center, Kansas City, KS.

We previously have reported the use of acute, osmotically induced tubule volume changes and rapid video techniques to measure the hydraulic conductivity (L_pA) and the reflection coefficient (σ) for NaCl across the basolateral cell membrane of lumen collapsed proximal tubule segments of rabbit. We now report similar studies in which S_2 segments were equilibrated in Ringers medium (285 mOsm/kg) before and after being challenged in sequence by the same medium made hypertonic (+60, +100, or +140 mOsm/kg) by the addition of either raffinose or KCl. For each tubule the test media were of the same osmolality and in half of the studies a KCl challenge preceded and followed a raffinose challenge. In the other half the sequence was reversed. Before and after results were not different in either case. Volume changes were measured at 1/60 s intervals and L_pA was calculated from data obtained in the first 3/60 to 5/60 s. Assuming $\sigma = 1.0$ for raffinose, the ratio of the L_pA measured using the same measured osmolalities of raffinose and KCl media in the same tubule is equal to σKCl across the basolateral membrane of that tubule. The ratios were 0.65 ± 0.04 at +60 (n=10), 0.72 ± 0.05 at +100 (n=12), and 0.73 ± 0.02 at +140 mOsm/kg (n=12). These values are not statistically different. Combining all 34 studies, σKCl is 0.70 ± 0.02 at the S_2 basolateral cell membrane. Our reported value for σNaCl at that membrane is 0.56 ± 0.07.

THE ROLE OF INTRACELLULAR pH IN ALDOSTERONE-INDUCED INCREASE OF K^+ CONDUCTANCE IN EARLY DISTAL RENAL TUBULE CELLS (EDC) OF FROGS. Wenhui Wang*, John Geibel*, and Gerhard Giebisch. Yale Univ., Med. School, Dept. Cell. & Mol. Physiol., New Haven, Connecticut.

Previous studies had shown that an increase of K^+ conductance(gK) of EDC by aldosterone results in part from the rise of cell pH, and that this increase is largely due to the increase of the K^+ channel numbers (Wang, W. et al., Kidney Int 33: 429, 1988). The present study was designed to further test whether cell pH could be a signal to regulate gK. Experiments were performed in isolated EDC of frogs using the whole-cell perfusion-recording technique. Cell pH was kept constant(7.30) during all measurements of Ba^{2+}-inhibitable gK. The intracellular pH(pH_i) was measured using the fluorescent dye BCECF-AM. Aldosterone(1 um, 20-28 hrs) increased pH_i from control value 7.43 (pH of incubation solution 7.60) to 7.55 and gK increased from 4.2±0.7(n=9) to 12.0±0.7 nS(n=17). When cells were incubated in pH 8.00 solution for 28 hrs, pH_i increased to 7.70 and gK was 8.2±0.7 nS(n=8). When cells were first incubated in pH 7.60 solution containing aldosterone for 20 hrs, then changed to pH 6.60 aldosterone-free solution for 8 hrs, pH_i dropped to 7.10, and gK decreased to 3.7±0.4 nS (n=8). In contrast, gK of cells still incubated in pH 7.60 aldosterone-free solution was 8.80±1.1(n=7). The results suggest that intracellular pH plays an important role in aldosterone-induced increase of gK. Aldosterone alone or cell-alkalosis stimulates cells to recruit K^+ channels into membranes. In contrast, intracellular acidosis removes K^+ channels induced by aldosterone and alkalosis.

ION CHANNELS IN CULTURED RABBIT RENAL COLLECTING DUCT CELLS. Stanley J. White*, Robert M. Henderson*, Emile L. Boulpaep and Gerhard H. Giebisch., Yale School of Medicine, Dept. of Cellular & Molecular Physiology, New Haven, Connecticut.

Recent observations (Bello-Reuss & Weber, AJP. 252: F899-909, 1987), suggest that cortical distal nephron fragments grown in culture may be a suitable model for the Cortical Collecting Duct (CCD) in vitro. Distal fragments were isolated by isotonic Percol density-gradient centrifugation and grown to confluence on rat-tail collagen membranes. By six days, Monolayers developed a pd of -3.6+0.24mV (n=11), apical side negative. The (apical/basal)[Na^+] was 0.92+0.02 (n=11) and (apical/basal)[K^+] was 1.34+0.02) (n=11).
Patch clamp experiments were carried out on the apical membrane of the majority cell type. In excised "inside-out" patches, three types of channel have so far been observed. The first, (n=6) has a single channel conductance of 160pS, is activated by depolarising potentials and is inhibited by either reducing Ca^{2+} below 1μM or by adding 100μM Ba^{2+} to the cytoplasmic side of the patch. This channel conducts outward currents of K^+ but not Rb^+ or Na^+ currents. The second channel has a conductance of 40pS (n=3) and conducts both Na^+ and K^+ but is not inhibited by Ba^{2+} at the cytoplasmic side. The third channel has a conductance of 10pS (n=4) and conducts outwards current of both K^+ and Rb^+ and is not inhibited by Ba^{2+} at the cytoplasmic side. We conclude that these cells in culture express some channels observed in CCD (Hunter, et al, PNAS 81:4237-39 1984) and therefore, may be a suitable model for studying CCD apical membrane channels.

ELECTROPHYSIOLOGICAL EVIDENCE THAT TRICHLORMETHIAZIDE (TCM) ACTS ON THE LUMINAL MEMBRANE OF THE CONNECTING TUBULE (CNT) CELL. Koji Yoshitomi, Toshikatsu Shimizu, and Masashi Imai (Intr. by Genjiro Kimura). *Dept. of Pharmacol., National Cardiovascular Center, Osaka 565, Japan*

We have previously reported that TCM acts on the connecting tubule (Kidney Int 33:426, 1988) which consists of electrophysiologically distinct two cell types, the CNT cell and intercalated (IC) cell (Kidney Int 33:430, 1988). To identify the exact cell type and mechanism of action of TCM, we examined effects of TCM on electrophysiologically defined 4 cell types from rabbit distal nephron segments: the distal convoluted tubule (DCT) cell, CNT cell, IC cell and collecting duct (CD) cell. When the tubules were perfused with a HCO_3^-/CO_2 buffered solution, 10^{-4}M TCM in the lumen hyperpolarized the basolateral membrane voltage (V_B) of the CNT cell from -71.0 ± 3.2 to -77.3 ± 3.0 mV (n=12, P<0.001) but not of the DCT cell ($\Delta V_B=-0.2\pm0.1$mV,n=18), IC cell ($\Delta V_B=0.1\pm0.1$mV,n=15), and CD cell ($\Delta V_B=-0.4\pm0.3$mV,n=9). TCM did not affect the transmural voltage in all segments. While the luminal addition of 10^{-3}M SITS hyperpolarized V_B of the CNT from -67.0 ± 3.6 to -70.9 ± 3.6 mV (n=10, P<0.005), the combined adminstration of 10^{-3}M SITS and 10^{-4}M TCM further hyperpolarized the V_B to -75.5 ± 3.9 mV (n=10, P<0.005). TCM also hyperpolarized the V_B of the CNT cell in the absence of HCO_3^-/CO_2 (-81.1 ± 1.6 vs -84.5 ± 1.3, n=15, P<0.01). We conclude: 1) TCM acts on the luminal membrane of the CNT cell, 2) TCM inhibits an electroneutral Na^+ transport system that is distinct from the parallel antiport of Na^+/H^+ and Cl^-/HCO_3^-.

OBSERVATION OF TRIMETHYLAMINE OSMOLYTES IN HUMAN KIDNEY IN VIVO BY ^{1}H NMR. Malcolm J. Avison*, D.L. Rothman*, R.G. Shulman* and N.J. Siegel, Yale Univ. Sch. of Med., Dept. of Peds. and Mol. Biophys. and Biochem., New Haven, CT

There has been renewed interest in the osmoregulatory role of the trimethylamines (-NMe_3) glycerophosphorylcholine (GPC) and betaine, in the protection of the cells of the renal medulla from the effects of the large variations in extracellular tonicity which accompany normal regulation of water balance. We report here the direct observation of these compounds in the normal human kidney by volume localized ^{1}H nuclear magnetic resonance. ^{1}H NMR images were obtained at 89.43 MHz using a 10 cm surface coil, and used to define and position the volume of interest (VOI) at the magnet isocenter. Spectra were obtained using the same coil and a pulse sequence consisting of 1) A composite θ pulse. 2) A low power pulse to presaturate the water resonance. 3) The volume selective ISIS sequence. 4) A surface nulling $\theta/3$ pulse followed by a dephasing magnetic field gradient. 5) A sinc pulse delivered in a magnetic field gradient, adjusted to suppress signal from tissue lying between the coil and the VOI. 6) A semiselective spin echo sequence to suppress the water resonance and provide maximal excitation of the -NMe_3 resonance at 3.25 ppm. This approach yielded excellent spectra of the -NMe_3 resonance in 5 mins.

THE KINETICS OF Na^+/H^+ EXCHANGE IN RAT RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV) DOES NOT CHANGE WITH AGE. Israel Zelikovic,* Peter Lohstroh,* Christine Iwahashi,* Russell W. Chesney. Depts. Pediatrics, Univ. CA., Davis, CA, and Univ. Tennessee, Memphis, TN.

We have recently demonstrated an increased Na^+/H^+ exchange activity in neonatal rat renal BBMV (Zelikovic, The Physiologist 30:148, 1987). We also observed that in contrast to increased amiloride-sensitive peak Na^+ accumulation by neonatal vesicles, the enhancement of initial rate uptake was accounted for by the amiloride insensitive component of uptake. In order to further explore the ontogeny of Na^+/H^+ exchange we determined the kinetics of this antiport system in rat renal cortical BBMV isolated from 7-day-, 21-day-old and adult rats. Initial rate (2 sec) accumulation of ^{22}Na, at a concentration range 1-20mM, into BBMV was determined using a "rapid reaction" millipore filtration technique. Experiments were performed under pH gradient conditions (pHi=5.5, pHo= 7.5) in the presence and absence of 3mM amiloride in the external medium. Amiloride insensitive ^{22}Na uptake was linear with Na concentration in all age groups suggesting complete inhibition of Na^+/H^+ exchange at the concentration range tested. Higher total and amiloride insensitive ^{22}Na uptakes were found in 7-day-old rats when compared to the two other groups. Lineweaver-Burk analysis of amiloride-sensitive ^{22}Na accumulation (obtained by subtracting uptake values in the presence of amiloride from values in its absence) revealed Km 19.27±5.49, 19.86±2.72,16.53± 4.92 mM (nonsignificant) and Vmax 7.45±1.65, 15.23±3.41, 14.47±3.22 nMol/mg protein/2 sec (nonsignificant) for 7- day-, 21-day-old and adult rats, respectively. We conclude from these data that unlike the enhanced Na^+/H^+ exchange activity which is responsible for augmented peak Na^+ uptake into neonatal rat renal BBMV, a "leaky", highly permeable membrane to Na^+ probably accounts for the increased initial rate Na^+ accumulation in neonatal vesicles. These two factors may operate in concert to rapidly dissipate the electrochemical Na^+ gradient across the luminal membrane during early life, thereby contributing to neonatal aminoaciduria.

SIMULTANEOUS MEASUREMENT OF THE DIFFUSIONAL WATER PERMEABILITY (P_{DW}) OF THE LUMINAL AND BASOLATERAL MEMBRANES OF THE ISOLATED PERFUSED PROXIMAL TUBULE. Delon W. Barfuss, Georgia State University, Biology Department, Atlanta, Georgia 30303

Isolated S1 and S2 segments of the rabbit proximal tubule were isolated and perfused with H^3-D-aspartic acid which is actively transported at the luminal membrane into the tubule cells. Cellular D-amino acid oxidase rapidly deaminates the aspartic acid which liberates the ^{3}H from the 2nd and 3rd carbons. This process generates three ^3H_2O molecules for every aspartic acid oxidized that are free to diffuse out of the cell across the luminal and basolateral membranes. By measuring the appearance rate of ^3H_2O in the flow through bathing solution and in the collected perfused fluid, and by measuring the cellular concentration of ^3H_2O, the diffusional water permeability of the luminal and basolateral membranes was measured simultaneously. The ^3H_2O was separated from other ^{3}H by allowing in to evaporate in a closed container and trapped by the hygroscopic effects of $MgCl_2$. The separated ^3H_2O was then quantitated in a scintillation counter. A mathematical model was developed to account for the effect of perfusion rate and back leak on the appearance of ^3H_2O in the collectate. The measured P_{DW}'s for the luminal membrane of the S1 and S2 segments were both $7.7\pm0.2*10^{-5}$ cm/sec while the basolateral P_{DW} for both was $2.7\pm0.5*10^{-6}$ cm/sec. Given that the reported transepithelial P_{DW} for the proximal tubule is reported to be $5*10^{-3}$ cm/sec(Berry,AJP,Nov,1985), it is concluded that greater than 98% of diffusional water crosses the epithelium via paracellular routes.

HIGH PROTEIN (HP)INTAKE ENHANCES RENAL CONCENTRATION CAPACITY IN THE RABBIT: POSSIBLE ROLE OF MEDULLARY PROSTAGLANDINS (PGE_2). J.E. Benabe, F. Aruz, H. Cordova and M. Martínez-Maldonado. Dept. of Med, VAMC and UPR Sch of Med, San Juan, P.R.

We have shown that NZW rabbits kept on a normal rabbit chow diet (16% protein) are unable to raise fractional free water reabsorption (TcH_2O/GFR) as the fractional osmotic load ($Cosm/GFR$) is increased. Acute administration of urea (400 mOsm/L) or indomethacin (2 mg/kg IV) during these clearance studies corrects this defect. Moreover, rabbits kept for 2 weeks on a 40% protein diet(HP) had marked improvement in their renal concentration capacity. Since renal PG s are known to modulate both medullary solute transport and ADH actions, we studied in vitro Angiotensin II (5 μg/ml) stimulation of PGE_2 production in medullary slices obtained from rabbits kept on a 16% protein diet (Group I), 16% protein diet and acute infusion of urea (vide infra) (Group II) and 40% protein diet (Group III). Results were as follows:

	PGE_2 Production (ng/ml/mg protein)		
Group	15′	30′	60
I	770±103	950±214	1733±110
II	336±82*	405±102*	813±76*
III	326±13*	412±9*	423±11*+

n=5-6 slices, 2 rabbit/group; * $p<0.01$ vs I; + $p<0.01$ vs II

These results show that HP intake blunts the A II stimulated medullary PGE_2 production. These suggest that a reduction in medullary PGE_2 production after HP or acute urea administration may facilitate the hydroosmotic effect of ADH in these rabbits and improve the renal concentration capacity.

ACUTE CHANGES IN INTRACELLULAR SODIUM (Na_i) DURING THE HYDROOSMOTIC RESPONSE TO VASOPRESSIN (VP). A.S. Brem, K.L. Matheson*, K.C. Inman*, and R.G. Lawler*. Brown University, Depts. of Pediatrics and Chemistry, Providence, Rhode Island.

Several lines of evidence support the presence of an electro-neutral Na-Ca exchange process along the basolateral membrane of uroepithelial cells including toad bladder epithelial cells (Am J Physiol 252:F1028, 1987). We hypothesized that VP stimulation might promote Na-Ca exchange leading to a small but measurable increase Na_i during peak hormone-induced water flow. Changes in Na_i were monitored over time in individual hemibladders using ^{23}Na NMR. Hemibladders were mounted as bags on glass pipets and filled with distilled water. During NMR studies, the serosal bath consisted of aerated 2.4 mM HCO3 amphibian Ringer (pH 8.1) made up with 15% D2O containing the shift reagent Dysprosium tripolyphosphate (1 mM). This reagent allows for visualization of Na_i by shifting the extracellular Na signal; it does not affect basal or VP stimulated water flow or high energy phosphate metabolism as seen by ^{31}P NMR. The relative change in Na_i was determined by integrating the area under the unshifted Na peak for each measurement; the areas were expressed in arbitrary units. The initial Na_i signal from unstimulated hemibladders averaged (mean ± SE) 3.22±0.48 (n=18) and the signal remained stable in these tissues over at least 60 min, 3.50±0.30 (n=8). Within 30 min of VP (20 mU/ml) exposure, the Na_i peak increased from 3.03±0.45 to 4.92±0.95 (n=6, $p<0.05$). Thus, Na_i rises during peak VP stimulated water flow. The Na mostly likely originates from the serosal bath since no Na is present in the mucosal bath. These findings could be consistent with a putative Na-Ca exchange process which is activated by VP.

INHIBITION KINETICS OF CATIONIC DRUGS (CD) ON N-METHYLNICOTINAMIDE (NMN) UPTAKE BY BRUSH BORDER MEMBRANE VESICLES (BBMV) FROM DOG KIDNEY CORTEX. R. Bendayan*, E.M. Sellers* and M. Silverman, Fac. of Pharm. and Depts. of Pharmacol. and Med., Univ. of Toronto, and Addiction Research Foundation, Toronto.

The purpose of the present study was to establish an in vitro methodology for drug screening to predict the potential for clinical pharmacologic interactions based on drug competition for common renal secretory transport systems. We chose as an experimental model system, the organic base transporter in renal BBMV prepared from dog kidney cortex. A rapid Millipore filtration technique (25°C) was used to assay the effect of various CD on the uptake of NMN. NMN uptake was found to be saturable, and exhibited osmotic and proton gradient dependence. All the CD showed competitive inhibition of NMN uptake indicating that they share the organic base transporter. Apparent inhibitory constants (ki) calculated from kinetic analysis of NMN uptake (10 sec uptake corrected for the passive diffusion component; proton gradient conditions) are: quinidine (0.7μM), trimethoprim (1.3μM), cimetidine (2.0μM), famotidine (3.0μM), quinine (4.0μM), amiloride (5.8μM) and procainamide (19.7μM). Correlation of the chemical structure of the CD with their Ki indicates the importance of both positive charge and adjacent hydrophobic moieties. Our results demonstrate the utility of BBMV as an in vitro empirical system for predicting drug interactions in vivo.

THE ROLE OS LIPID PEROXIDATION ON THE ACTION OF VASOPRESSIN. C.P. Carvounis, S. Bernstein,* and M. Oros.* VAMC and SUNY, Syracuse, NY.

Oxidative processes and their products lipid peroxides occur widely in most cells and epithelia. In the present study we evaluated the role of lipid peroxide production, as measured by malonyl-dialdehyde (MDA), in the action of vasopressin (V) 30 mU/ml in the toad urinary bladder. We found that addition of V in the absence of osmotic gradient for 30' resulted in a significant increase in MDA (8.7±0.9 vs 5.7±0.8 nM/mg protein, n=6, $p<0.05$). A similar increase was noted following stimulation with V as early as 10' (10.6±2.1 vs 5.0±1.0, n=6, $p<0.05$). Stimulation with 8 bromo-cyclic AMP and theophylline, also resulted in increased tissue MDA (7.9±0.9 vs 5.4±0.3, n=6, $p<0.05$ and 13.6±1.1 vs 7.8±1.0 nM/mg protein, n=6, $p<0.05$ respectively) suggesting that lipid peroxidation occurs at steps subsequent to cAMP generation. The effect of V on MDA production was reversible since removal of V for 30' after 30' of V stimulation significantly decreased the bladder's MDA concentration (6.2±1.1 vs 8.7±1.5 nM/mg protein, n=6, $p<0.05$). It has been previously shown that the reducing agent GSH decreases the hydro-osmotic action of V. We found that 2 mM GSH also greatly diminishes 8 Br cAMP stimulated water flow (13.9±0.9 vs 50.0±2.6 μL/min, n=4, $p<0.001$). We suggest that V stimulates lipid peroxidation reversibly by an action localized at steps that follow cAMP generation. The occurrence of the phenomenon before 10' suggest it may be responsible for some of the steps leading to increased permeability. This is further supported by the inhibitory action of agents that reduce oxidation. The exact importance of our findings cannot be identified at the present time.

VASOPRESSIN (AVP)-STIMULATED cAMP LEVEL IS INHIBITED BY DIFFERENT WAYS IN THE RAT MEDULLARY COLLECTING TUBULE (MCT) : ROLE OF PGE_2. D. Chabardès* and M. Montégut* (intr. by T. Anagnostopoulos). Collège de France, Paris, France.

The accumulation of cAMP was measured by radioimmunoassay in single isolated MCT incubated in the presence of indomethacin and of a phosphodiesterase (PDE) inhibitor : either IBMX (1mM) which blocks all types of cyclic nucleotide PDE, or Ro 20-1724 (Ro, 50 μM) known to block mainly the type III PDE. In the presence of Ro, 0.3 μM PGE_2 inhibited the response to 1 nM AVP in 8 experiments (N) : AVP = 50.9 ± 6.3 (fmmol/mm/4min ± SEM), AVP + PGE_2 = 24.1 ± 3.3, $p<0.005$. In the same experiments, the use of IBMX relieved the PGE_2 inhibition : AVP = 108.9 ± 9.4, AVP + PGE_2 = 95.4 ± 6.0, N=8. Adenosine A_1 agonist (PIA) decreased also the response to AVP ; however the effect of PGE_2 was not mediated by intracellular adenosine since 1) the addition of 0.1 μM PACPX (specific A_1 antagonist) blocked the effect of 0.1 μM PIA but did not affect the PGE_2 inhibition : AVP + PGE_2 = 62.9 ± 2.1% inhibition, AVP + PGE_2 + PACPX = 61.4 ± 9.9%, N=4 ; and 2) simultaneous addition of 0.3 μM PGE_2 and 0.1 μM PIA (both eliciting maximal effects) induced cumulative inhibition on the response to 1 nM AVP : AVP + PGE_2 = 60.9 ± 2.8% inhibition ; AVP + PIA = 50.0 ± 4.8% ; AVP + PGE_2 + PIA = 76.9 ± 3.8%, $P<0.01$ and <0.001 when compared to PGE_2 and PIA inhibition respectively, N=7. A similar cumulative inhibition was found with 0.3 μM PGE_2 and 1 μM clonidine (α_2 adrenergic agonist, N=5).These data suggest a specific PGE_2 inhibition in rat MCT ; this inhibition is not mediated at the level of cAMP formation but results from an activation of cAMP hydrolysis.

SUCCESSFUL, SPECIFIC TREATMENT OF NEPHROGENIC DIABETES INSIPIDUS (NDI) IN MICE. A.K. Coffey*, D.J. O'Sullivan*, S. Homma*, T.P. Dousa, and H. Valtin. Dept. Physiol., Dartmouth Med. Sch., Hanover, NH, and Nephrol. Res. Unit, Mayo Clinic, Rochester, MN.

Mice of the DI+/+ Severe strain (NDI mice) have vasopressin-resistant diabetes insipidus. The defect appears to lie in abnormally high activity of cAMP-phosphodiesterase isozyme type III (PDE-III), and hence in lack of intramembranous particle (IMP) clusters in apical membranes of inner medullary collecting ducts (IMCDs). When IMCDs are incubated in vitro with two inhibitors of PDE-III, Rolipram (R) and Cilostamide (C), IMP clusters reappear (Clin.Res. 36:593A, 1988). We now report that treatment of NDI mice with R and C in vivo ameliorates the diabetes insipidus.

Seven NDI mice were kept in separate metabolic cages. Following a Control period of 6 days, 5 of the mice were then given a solution of R and C for 4 days (10 mg/kg•day of each), while the remaining 2 animals simultaneously drank the vehicle (0.5% ethanol). Results are shown in the Table.

	Body Weight (%)	Water Intake (ml/24 hours)	Urine Flow	Urine Osmolality (mosm/kg H_2O)
Control (7)*	100	18.4	12.9	608
Vehicle (2)	101.1	21.8	12.9	705
R and C (5)	101.6	7.7†	4.3†	1,095†

*number of mice †<0.05 vs Control or Vehicle

Conclusions: 1. Oral administration of two inhibitors of PDE-III to NDI mice corrected their high fluid turnover. 2. Extrapolating to humans, in those patients with NDI who have increased activity of PDE, inhibitors of PDE may improve their condition.

INHIBITION OF UREA TRANSPORT IN RAT INNER MEDULLARY COLLECTING DUCTS BY UREA ANALOGS AND PHLORETIN. C.-L. Chou* and M.A. Knepper. National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland.

Vasopressin (AVP) increases the urea permeability of the rat inner medullary collecting duct (IMCD) to levels much greater than can be explained by lipid-phase permeation or paracellular diffusion, indicating the presence of an AVP-stimulated facilitated transport pathway. We tested whether inhibitors of carrier-mediated urea transport in erythrocytes also inhibit urea transport in the isolated perfused IMCD. Apparent urea permeability (Purea) was determined by measuring the flux due to an imposed 5 mM concentration gradient. Inhibitors were added from the "cis" side of the epithelium with respect to the flux. Urea analogs (200 mM) in the bath inhibited Purea (thiourea, 76% inhibition; methylurea, 65%; acetamide, 35%). Urea analogs in the lumen decreased Purea with the same order of potency. The inhibitory $K_{1/2}$ for thiourea in the lumen was 27±2 mM and did not change with 0.1 nM AVP (28±3), despite a 4-fold higher Purea with AVP. Phloretin (0.25 mM) added to the lumen decreased AVP-stimulated Purea (91±4 to 45±4 $x10^{-5}$ cm/s), but did not alter osmotic water permeability (505 to 532 μm/s). CONCLUSIONS: The urea transport pathways in the IMCD and erythrocytes are functionally similar with regard to inhibitor effects, suggesting that the IMCD transporter could be a carrier like that in the erythrocyte. Inhibition of Purea by phloretin without an effect on water permeability indicates that the urea pathway is unlikely to be the vasopressin-stimulated water channel. The lack of an effect of AVP on $K_{1/2}$ for thiourea indicates that urea probably penetrates via the same pathway in the presence and absence of AVP.

HUMORAL AND VOLUME EFFECTS ON OSMOREGULATION IN PREGNANCY (p). J.M. Davison* E.A. Shiells* M.D. Lindheimer. Human Reproduction Group, Newcastle upon Tyne U.K., U. of Chicago, Chicago, IL.

Osmoregulation changes in p; plasma osmolality (P_{osm}) and osmotic thresholds (T) for vasopressin (AVP) release and thirst decreasing in early p, while rates of rise for P_{AVP} per unit increase in P_{osm} ($\Delta P_{AVP}/\Delta P_{osm}$) decrease in the 3rd trimester [J Clin Invest 81:798,88]. This study, designed to characterize mechanisms for these changes, first tested the hypothesis; "effective" intravascular volume is decreased in p (despite absolute hypervolemia), resulting in non-osmotic AVP release, and/or a lower T_{AVP} and T_{thirst}. Hypertonic saline was infused into 6 women in the presence and absence of water immersion (WI); in early and late p, and when not pregnant. P_{osm} and both T were ~ 10 mOsm/kg lower throughout p ($p<0.01$). WI consistently decreased $\Delta P_{AVP}/\Delta P_{osm}$ ($P<0.01$) but had no effect on either T_{AVP} or T_{thirst}; evidence against the above hypothesis. Next, influence of the pregnancy hormone chorionic gonadotropin, (hCG), was tested in 5 nongravid women. Each subject received hypertonic saline during a luteal cycle on 2 occasions, (randomly), once after 15,000 IU, in divided doses, 5 and 3 d before the test. Hormone levels averaged 4.8± 2.3 u/ml after hCG, while P_{osm}, and T_{AVP} and T_{thirst} decreased 6, 5, and 6 mOsm/kg respectively ($p<0.01$). AVP abscissal P_{osm} intercepts, regression slopes, and r values averaged 280, 0.54, 0.93, hCG; vs 285, 0.57, 0.92 control. Conclusions: Central volume expansion by WI failed to alter basal P_{osm}, T_{AVP} or T_{thirst} in p, evidence against a role of "underfilling" in the osmoregulatory alterations. Chorionic gonadotropin, however, may play a role in these changes.

CELL SWELLING INDUCED BY AN ORGANIC ANION. George M. Feldman, Fuad N. Ziyadeh, George W. Booz*, Arnost Kleinzeller*. Mt. Desert Isl. Biol. Lab., ME., VAMC and Univ. of PA, Depts. of Med. and Physiol., Phila, PA

Many cells swell when bathed in media containing organic anions, including formate, acetate and propionate (PROP). To examine the mechanism of swelling, slices of shark rectal gland, a NaCl transporting tissue, were incubated in iso-osmotic solutions containing Na^+ salts of PROP or Cl^- at the same pH. After 3 hours in PROP medium, cell H_2O increased 60% and Na^+ and K^+ concentrations increased 38% and 46%, respectively. Cell volume and contents recovered after returning to Cl^- medium. Ouabain (1 mM) inhibited PROP-induced K^+ accumulation, but Na^+ accumulated and cells swelled to the same degree, demonstrating that Na^+ pump activity was not required for swelling. The mechanism of PROP-induced Na^+ entry into cells was examined. Na^+ substitution blocked PROP swelling, and amiloride (1 mM) decreased swelling 53%, suggesting a role for Na^+/H^+ exchange. Cell pH was measured in suspensions of rectal gland tubules loaded with BCECF. At medium pH 7.4, cell pH initially decreased from 7.1 to 6.7, and then, recovered to 7.0 over 10 minutes. Supporting a role for Na^+/H^+ exchange, amiloride blocked the recovery of cell pH. The mechanism of PROP-induced K^+ accumulation was examined. PROP did not alter $^{86}Rb^+$ uptake, but $^{86}Rb^+$ efflux decreased 59%, suggesting that reduced K^+ leak accounts for K^+ accumulation. This reduction in K^+ leak was pH independent, since increasing cell pH to 7.6 in the presence of PROP did not return $^{86}Rb^+$ efflux to control values. In conclusion, PROP, a model organic anion, induces cells to accumulate solute and swell. PROP enters as the undissociated acid and acidifies the cell, accelerating Na^+/H^+ exchange and Na^+ entry. PROP decreases K^+ leak by a pH independent mechanism and, thus, induces cells to accumulate K^+ when the Na^+ pump is active.

GLYCINE (G) TRANSPORT IN NECTURUS (N) PROXIMAL TUBULE (PT) Todd Gehr and Donald Oken, Dept. of Med. McGuire VAMC and Med. Col. of Va., Richmond, Va.

Few methods have directly assessed amino acid transport processes in vivo. Microperfusion utilizing a split-droplet technique with N Ringers solution containing physiologic quantities of 14C labelled G was performed in 3 N PT. Total G was measured simultaneously in tubular fluid and plasma water(glomerular ultrafiltrate) in nl samples utilizing 3H dansylation and microchromatography. Water absorption was measured separately in 10 PT and found to be <6% for the duration of the experiment. Perfusate dwelled for 4-5 min., aspirated, and then analyzed for 14C activity and total G. PT cellular G was .692±.058 mM. Results from microperfusions appear below:

[G]total mM		14C G cpm/nl		[G]ufr mM
pre	post	pre	post	
.024	.068	7.8	4.1	.023
.024	.078	7.8	5.6	.023
.023	.073	7.7	4.1	.035

Although G efflux from the PT reflected by the 41% reduction in 14C activity was substantial, G influx presumably via facilitated diffusion across the cell membrane surpasses efflux in the normal N PT during the short dwell time of these experiments.

PRE-STEADY-STATE ANALYSIS OF WATER TRANSPORT REGULATION IN KIDNEY COLLECTING TUBULE. K. Fushimi*, Michio Kuwahara* and A.S. Verkman. CVRI, UC San Francisco, CA 94143.

Water transport across the mammalian collecting tubule is regulated by vasopressin-dependent water channel insertion into and retrieval from the cell apical membrane. The time course of osmotic water permeability (P_f) following addition and removal of VP and 8-Br-cAMP was measured continuously by quantitative fluorescence microscopy using an impermeant fluorophore perfused in the lumen. Tubules were subject to a 120mOsm bath-to-lumen gradient at 37°C with 10nl/min lumen perfusion and 15ml/min bath exchange rate. With addition of VP (250μU/ml), there was a 23±3s (SEM, n=16) lag in which P_f did not change, followed by a rise in P_f (initial rate $1.4\pm0.2\times10^{-4}$cm/s/s) to a maximum of $265\pm10\times10^{-4}$cm/s. With addition of 8-Br-cAMP (0.01-10mM) there was a 11±2s lag. For [8-Br-cAMP] = 0.01, 0.1 and 1mM, the initial rate of P_f increase following the lag was (units 10^{-4}cm/s/s): 1.1±0.1, 1.2±0.1 and 1.7±0.3. Maximum P_f was (units 10^{-4}cm/s): 64±4, 199±9 and 285±11. With removal of VP, P_f decreased to baseline (12×10^{-4}cm/s) with a $T_{1/2}$ of 26min; removal of 0.1 and 1mM 8-Br-cAMP gave $T_{1/2}$ of 5 and 9min. These results demonstrate (1) a brief lag in the P_f response, representing the pre-steady-state kinetics of hydroosmosis, (2) similar initial dP_f/dt (water channel insertion) over a wide range of [8-Br-cAMP], and (3) more rapid P_f decrease (water channel endocytosis) with removal of 8-Br-cAMP than with VP. These pre-steady-state data provide evidence that water channel exocytosis is not the step by which VP regulates P_f and provide a novel approach to study the vasopressin hydroosmotic response.

A NEW FLUORESCENCE METHOD FOR THE QUANTITATION OF ORGANIC ANION TRANSPORT IN SINGLE, ISOLATED, PROXIMAL TUBULES. J.A. Grantham,* L.P. Sullivan, L. Rome,* and J.J. Grantham. Depts. of Medicine and Physiology, Univ. of Kansas, Kansas City, KS 66103.

Fluorescein and fluorescein congeners (BCECF) are organic anions at physiologic pH. We determined if fluoresceinate (FLS) was accumulated by lumen-collapsed rabbit proximal tubules in isotonic HCO_3, Ringer's medium containing citrate, alanine, lactate, and glucose at room temperature and pH 7.4. Tubules were attached to the coverslip of an incubation chamber with poly-L-lysine. Medium was exchanged continuously. Tubules were viewed in an inverted Nikon fluorescence microscope fitted with a digital photometer; excitation 500 nm and emission 520 nm; exposure time 2 sec.; recording window 25 μm. FLS (0.1-10μM) added to medium caused tubule fluorescence above background in $S_2>S_1 = S_3$ proximal tubules, but uptake was not seen in TAL or CCT. Cell FLS levels were determined in relation to glass microcapillaries of tubule diameter filled with a range of FLS concentrations (2-400μM). The rate of FLS uptake was concentration dependent and the affinity for transport was <50μM. Maximal FLS levels were 80-fold greater than medium. Photobleaching was minimal. Removal of medium FLS caused washout of tubule fluorescence. Probenecid (10^{-3}M) and ouabain (10^{-4}M) inhibited FLS accumulation. These studies indicate that FLS is transported with high affinity into proximal tubules by the classical organic anion system. FLS is uniquely suited for kinetic quantitation of organic anion transport in proximal tubules.

SORBITOL METABOLISM IN INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS OF DIABETIC RATS. R. Willi Grunewald* and Rolf K.H. Kinne. Max-Planck-Institut für Systemphysiologie, Dortmund, FRG

Changes in metabolism of the organic osmolyte sorbitol have been implied as cause for complications in diabetes. Since inner medullary necrosis is frequently observed in diabetic patients it was investigated whether inner medullary metabolism of sorbitol is altered in diabetic animals.
Male Wistar rats (150 g b.w.) were treated with streptozotocin (60 mg/kg b.w., one injection i.p.). 6-8 weeks later blood sugar was 332±102 mg/dl in diabetic (D) animals compared to 113±17 mg/dl in control (C) animals. Sorbitol content in inner medullary tissue derived from D rats was 1.8 times higher than from C rats (134±17 µmol/g prot. vs. 74±22 µmol/g prot.; $p < 0.0025$). Uptake kinetics of D-glucose, the major metabolic precursor of sorbitol, were not affected by stretozotocin treatment. However, the maximal velocity of aldose reductase, the main pathway for sorbitol synthesis, was 4.2 fold higher in D than in C animals (97±22 U/mg prot. vs. 23±7 U/mg prot.; $p < 0.001$) while the apparent affinity, as determined with DL-glyceraldehyde remained unchanged. Sorbitol release from IMCD cells, recently shown to be involved in osmoregulation of these cells (Grunewald, R.W. et al., Pflügers Arch. 441:S87, 1988), was significantly reduced in D animals. These studies demonstrate that in streptozotocin-induced diabetes the inner medullary sorbitol content is elevated by a combination of increased substrate availability, increased V_{max} of the aldose reductase, and an impaired capability to release sorbitol when exposed to osmotic stress. These alterations may contribute to the development of inner medullary necrosis in diabetes.

A HUMAN RENAL Na^+/GLUCOSE COTRANSPORTER CLONE. Matthias A. Hediger*, Eric Turk*, Hsin-Shung Lee*, and Ernest M. Wright. Department of Physiology, School of Medicine, University of California, Los Angeles, CA 90024-1751.

We have recently isolated a cDNA clone encoding a human renal Na^+/glucose cotransporter. A lambda gt 10 library was constructed from renal cortex and screened using the rabbit small intestine Na^+/glucose cotransporter clone (Hediger et al. 1987, Nature 330: 379). We isolated a 2.6 kb clone and subcloned it in Bluescript plasmid. Northern analysis of human kidney cortex RNA with this clone as a probe shows 3 bands at 2.4, 3.0, and 3.6 kb. Western analysis of rabbit renal brush border membranes was carried out using an antibody to a peptide synthesized based on the intestinal transporter sequence. The blot shows three bands between 70 to 75 kDa which were all blocked with the peptide. This size range is consistent with previous biochemical studies on the renal Na^+/ glucose cotransporter. Preliminary expression experiments with Xenopus oocytes show that RNA synthesized from the clone in vitro can induce ^{14}C-alpha-methyl-D-glucopyranoside uptake. We conclude that we have isolated a clone encoding a human kidney Na^+/glucose cotransporter. Further, these experiments indicate that there may exist more than one species of the transporter. This is consistent with results of transport studies in the kidney.

EFFECTS OF PGE2 ON CORTICAL COLLECTING TUBULE (CCT) INVOLVE MULTIPLE SIGNALING MECHANISMS. R.L. Hebert, H. R. Jacobson, and M.D. Breyer. Div. of Nephrol., Vanderbilt Univ. and V.A.M.C., Nashville, Tn.

PGE2 both inhibits Na absorption in the CCT, a cAMP independent process, and modulates vasopressin- stimulated water flow, a cAMP dependent process. While recent biochemical studies document PGE2's abiltity to activate both the stimulatory and inhibitory guanine nucleotide binding proteins, Gs and Gi, other signal transduction mechanisms, responsible for inhibition of Na transport remain uncharactarized. The present studies provide functional evidence for both Gs and Gi activation by PGE2 and demonstrate that an additional intracellular signaling mechanism, elevation of cell calcium, $[Ca^{++}]i$, is involved in PGE2's action.

Utilizing in vitro microperfusion of rabbit CCTs at 37°C , we measured the hydraulic conductivity response (Lp x 10^{-7} cm/atm/sec) to 10µU/ml bath arginine vasopressin (AVP) in 4 protocols: 1. AVP alone; 2. pretreatment with 10^{-7}M bath PGE2 followed by AVP; 3. AVP followed by 10^{-7}M bath PGE2; 4. pre-treatment with pertussis toxin (PT, 100ng/ml) followed by AVP and then 10^{-7}M bath PGE2. We found: 1) PGE2 alone significantly increases Lp from 11.4± 4.5 to 81±8; 2) PGE2 pretreatment inhibits AVP induced-Lp: 220±12.5 AVP alone vs.153.7±4.6 PGE2+AVP ($p<.01$unpaired t) 3) PGE2 post- treatment inhibits Lp: 153±9.9 AVP alone to 74±11.4 AVP+ PGE2 ($p<.005$, paired t); 4) PT significantly but incompletely reverses the inhibitiory effect of PGE2 post-treatment: 74±11.4 AVP + PGE2 vs. 118± 20 PT+AVP+PGE2. These findings confirm dual stimulatory and inhibitory effects of PGE2 on AVP stimulated Lp and that at 37°C there appears to be a pertussis toxin sensitive component to the inhibitory effects of PGE2.

To examine the potential role of $[Ca^{++}]i$ in mediating PGE2's effects, we measured $[Ca^{++}]i$ in FURA-2 loaded rabbit CCTs. 10^{-7}M bath PGE2 abruptly and transiently increased $[Ca^{++}]i$ to 350% of basal levels. This was followed by a sustained increase in $[Ca^{++}]i$ at 142% of basal levels. We conclude that PGE2 enlists multiple intracellular signaling mechanisms in its actions on CCT.

IDENTIFICATION OF HYPERNATREMIA-INDUCED OSMOLYTES IN RAT BRAIN. C.W. Heilig*, M.E. Stromski*, J.B. Blumenfeld*, J.P. Lee*, and S.R. Gullans. Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

Unidentified osmolytes, known as idiogenic osmoles, accumulate in the brain in response to hypernatremia. We used high resolution ^{1}H NMR spectroscopy and enzymatic assays of PCA extracts to identify and quantify the specific osmolytes which accumulate in brain in response to 5 days of salt loading (SL) or 3 days of water deprivation (-H_2O). Salt-loading involved a daily gavage (6 ml of 10% NaCl) plus 320 mM NaCl drinking water ad libitum. P_{Na} were as follows: controls, 141 ± 1 meq/l; -H_2O, 151 ± 4*; SL, 161 ± 3*. The table indicates the brain contents of those solutes which changed.

	Control	-H_2O	SL
Glutamate	80 ± 6	68 ± 4	101 ± 7*
Glutamine	36 ± 4	36 ± 8	58 ± 5*
PCr+Cr	19 ± 2	19 ± 1	26 ± 1*
GPC	8 ± 1	8 ± 1	13 ± 1*
Betaine	3 ± 1	1 ± 1	3 ± 1
Choline	2 ± 1	2 ± 1	5 ± 1*
myo-Inositol	62 ± 5	58 ± 2	90 ± 8*
Sorbitol	0.5±0.1	0.5±0.1	0.4±0.1

(Mean ± SEM in nmol/mg protein; * $p<0.05$)

The total amino acids, methylamines, and polyols were all higher with SL but not -H_2O. Of these glutamate, glutamine, and myo-inositol increased the most. Glycerophosphorylcholine (GPC) and myo-inositol, prominent osmolytes in the renal medulla, were elevated in the brain with SL suggesting similar osmoregulatory roles in the two organs. In contrast to renal medulla, brain betaine and sorbitol were not elevated by SL or -H_2O.

MERCURIALS INHIBIT FLOW THROUGH ADH INDUCED WATER CHANNELS IN TOAD BLADDER. B.S. Hoch, H.I. Lipner, P.C. Gorfien*, A. Eres*, and S.D. Levine. Dept. Med. Maimonides Medical Center, Brooklyn, NY and Albert Einstein Coll. Med., Bronx, NY.

Mercurial reagents inhibit the water permeability of erythrocytes (RBC) and proximal renal tubule. We examined the effect of these agents on water flow in bladders which had been fixed with N-ethylmaleimide (NEM) or glutaraldehyde after stimulation with ADH. PCMBS inhibited flow in tissues which had been fixed with NEM but not in glutaraldehyde fixed tissues. $CdCl_2$, $CuCl_2$, $LaCl_3$, $ZnCl_2$, and cisplatin did not inhibit flow in glutaraldehyde fixed tissues.

In contrast, $HgCl_2$ inhibited flow in tissues which had been fixed with glutaraldehyde after stimulation with ADH. Sucrose permeability, a measure of epithelial integrity, was not increased in these tissues. $HgCl_2$ did not diminish the frequency or area of luminal membrane aggregates observed by freeze-fracture electron microscopy. $HgCl_2$ also did not affect amphotericin induced water permeability in glutaraldehyde treated tissues, suggesting that it did not diminish the permeability of cellular barriers to flow.

These results parallel those reported by other investigators for water flow across RBC and proximal renal tubule and suggest that mercurial reagents can directly block the vasopressin induced water channel. The water channel at the apical membrane of the toad bladder may prove to share structural similarity with that constantly present in RBC and proximal renal tubule.

COMPARISON OF THE EFFECTS OF AVP AND dDAVP ON RABBIT CORTICAL COLLECTING TUBULE (CCT) AT PHYSIOLOGICAL TEMPERATURE. Maurilo Leite, Jr.,* and W.N. Suki, Baylor Coll. of Med., Houston, TX.

Whereas AVP in CCT increases both cAMP (V2 receptor) and intracellular Ca^{2+} (V1 receptor), the V2-selective analogue dDAVP only increases cAMP. Since a Ca^{2+}-activated pathway has been shown to inhibit the hydroosmotic effect of AVP, we compared the effects of these two agonists on rabbit CCT's perfused in vitro at 38°C. Both agonists increased osmotic water permeability (Pf,$\times 10^{-4}$ cm/sec) steadily reaching a peak which was maintained for 150 min. At a concentration of 10 uU/ml the peak Pf value, of 191±38.5 (n=6) for AVP and 193±16.3 (n=5) for dDAVP, was reached at 40 min. Using 100 uU/ml the peak Pf value, of 191±30.0 (n=5) for AVP and 180±12.6 (n=5) for dDAVP, was reached at 20 min. There was no significant difference between peak Pf values comparing either the 2 agonists or the 2 concentrations used for each agonist.

We conclude that: 1) 10 uU/ml is a maximal concentration for the hydroosmotic effect of both AVP and dDAVP, 2) at 38°C the hydroosmotic effect of AVP and dDAVP is stable, and 3) V1 receptor-activated pathways have no net effect on the hydroosmotic response of the CCT to AVP. The differences in time required to reach peak Pf may be a function of the different concentrations.

LUMINAL MEMBRANE STRUCTURAL FEATURES OF ADH-TREATED TOAD URINARY BLADDER REMAIN CONSTANT WITH TIME. W.A. Kachadorian, J. Muller,* and V.A. DiScala. GRC, NIA, Baltimore, MD, FDA, Bethesda, MD, and Winthrop-Univ. Hosp., Mineola, NY

ADH-induced water permeability across toad bladders prepared with steep transmural gradients (e.g., 175 mOsm) achieves maximum 10-15 min after hormone addition, and decreases by 1/3 - 1/2 to a steady level by 40 min. The mechanism for this decrease, which is not seen in bladders with less steep gradients (e.g., 100 mOsm), is unknown. We analyzed freeze fracture data from 137 bladders treated with ADH (20mU/ml) for 2.5, 5, 10, 20, 30, 60, and 240 min (n per group, 12-31). Initial gradient was 175 mOsm. Fused aggrephore and aggregate frequencies were maximal (111±7 and 12±1 per 100 μm^2) by 5 min, and remained unchanged thereafter even though by 240 min gradient decreased by a factor of 2, to 84±9 mOsm. The proportion of fused aggrephores with apparently emerging aggregates was maximal at approximately 70% at 2.5, 5, and 10 min, but declined to 44±3% by 20 min and to 16±4% by 240 min. Though renewal by turnover would not be excluded, these findings indicate that for bladders with initially steep transmural gradients, the number of aggrephores fused with and aggregates in the luminal membrane during the course of ADH action, after onset, remain constant. The reduction of water permeability early on in such tissues would be explained by a decrease in permeability of luminal membrane water conducting pathways or a more limiting resistance distal to them.

EFFECT OF SANGIVAMYCIN (SA), A SELECTIVE INHIBITOR OF PROTEIN KINASE C, ON WATER FLOW IN TOAD URINARY BLADDER. S.D. Levine, H. Goldin*, and M. Jacoby*. Albert Einstein College of Medicine, Bronx NY.

The kinase C inhibitor H-7 enhances water flow elicited by the submaximal stimulants 1mU/ml vasopressin (VP), 2uM forskolin (FSK) and 12mM cAMP, but not flow elicited by 20mU/ml VP. (Levine & Jacoby, Clin Res 36:595A) Complicating these data, H-7 inhibits both kinase C and cAMP-dependent kinase A to approximately the same extent.

SA inhibits kinase C as effectively as H-7, but is a far less potent inhibitor of kinase A (Loomis & Bell, J.Biol.Chem. 263:1682). Like H-7, SA did not alter basal water flow, and enhanced water flow stimulation by 1mU/ml, but not by 20mU/ml VP, in both the presence and the absence of naproxen. In contrast to H-7, however, SA did not enhance water flow elicited by either cAMP or FSK. The kinase A activity ratio, an estimate of in-vivo kinase A activity, was increased by SA in VP-stimulated bladders (.39±.07 vs .58±.06), but not in the absence of VP. The discrepancy between the VP- and FSK- elicited flows suggests that VP, but not FSK, activates phospholipase C in the intact tissue, leading in turn to diminished activation of kinase A. Inhibition of flow, how-ever, can be overcome by SA or high levels of VP.

Fluoride (F^-) stimulates both adenylate cyclase and phospholipase C. Despite a large increase in cell cAMP levels, F^- enhances flow to only a small degree, and VP-elicited flow is even inhibited. In contrast, SA greatly enhanced flow in F^--treated tissues, both in the presence and absence of VP. These data suggest F^--activation of phospholipase C (and thereby protein kinase C) inhibits flow at an additional site distal to cAMP generation.

CHARACTERIZATION OF BRAIN IDIOGENIC OSMOLES (IO) ACCUMULATED IN UREMIA (U) AND HYPERNATREMIA (H). Y.H. Lien,* J.I. Shapiro and L. Chan. Dept. of Med., Univ. of Colorado Med. Sch.,Denver, CO.

IO have been postulated to develop in the brain cells of experimental animals and patients subjected to chronic elevations in extracellular osmolality. Recently, increases in betaine (B), glycerophosphocholine (GPC), inositol (I) and sorbitol (S) concentrations have been observed in the renal medullas of antidiuretic animals (Bagnasco et al, J Biol Chem, 1987). We studied the perchloric acid extracts of brains obtained from Sprague-Dawley rats weighing approximately 350 gms subjected to U (induced by bilateral nephrectomy 3 days prior to study), H (gavaged with 2 ml/100 gm/day of 5% NaCl for 7 days) or no insult (C) with conventional chemical methods and H-1 magnetic resonance spectroscopy (MRS). U rat brains were not found to accumulate any IO compared with C. H rat brains were found to have marked increases in B (213 ± 17 vs 164 ± 10 μg/ g wet weight, $p<.05$) and GPC (283 ± 80 vs 183 ± 12 μg/ g wet weight, $p<.05$) determined by conventional chemical methods. These findings were confirmed by H-1 MRS which also detected significant increases in I in the H compared with C rat brains. No resonance attributable to S was observed in either C or H rat brains. These data suggest that conditions associated with acute increases in the concentration of freely permeable osmoles (e.g. urea) may not result in an increase in brain IO where IO are generated in conditions characterized by chronic elevations in extracellular sodium. Important differences in the IO developed by the brain from those observed in renal medulla are also noted.

IDENTIFICATION AND PURIFICATION OF A STILBENE-BINDING PROTEIN FROM RABBIT RENAL BRUSH BORDER MEMBRANES (BBM) THAT HAS AFFINITY FOR OXALATE. Kevin R. McConnell*, Gary V. Desir*, Shiu-Ming Kuo*, and Peter S. Aronson. Depts. of Medicine, and Cellular & Molecular Physiology, Yale School of Medicine, New Haven, Ct.

Stilbene-sensitive anion transporters, such as the Cl-formate exchanger, the Cl-oxalate exchanger, and the SO_4-HCO_3 exchanger, have been described in renal BBM. We have attempted to identify and purify stilbene-sensitive anion transport proteins from rabbit renal BBM by use of affinity chromatography. An affinity matrix was synthesized by immobilizing the disulfonic stilbene DNDS (10 mM) in polyacrylamide by the method of Uchida and Filburn (JBC 259:12311, 1984). BBM were solubilized in 0.6% Triton X-100, and then centrifuged at 100,000xg for one hour. The supernatant was equilibrated with the affinity matrix for 30 minutes, the matrix was washed extensively with buffer, and proteins were eluted with buffer or buffer containing 5 mM DNDS. The eluates were concentrated and subjected to SDS-PAGE. Silver-stained gels revealed several proteins in the 30-200 kD range that were specifically eluted with DNDS. Binding of these proteins to the matrix was completely inhibited by 5 mM DNDS, confirming their identities as stilbene-binding proteins. Substrates for various BBM anion exchangers were tested for their abilities to block adsorption of particular proteins to the DNDS-affinity matrix. The adsorption of a 37 kD protein was blocked by 45 mM oxalate but not by 45 mM acetate, chloride, formate, gluconate or sulfate. This 37 kD protein was not detected on silver-stained SDS-polyacrylamide gels of solubilized BBM, indicating that it was highly purified in the eluate from DNDS-affinity chromatography. Its migration was similar under reduced and nonreduced conditions.

In conclusion, we have identified and partially purified a stilbene-binding protein from rabbit renal BBM that may be a structural component of an oxalate transporter, such as the Cl-oxalate exchanger.

RENAL TUBULAR ABSORPTION OF FLUID IN THE ABSENCE OF PERITUBULAR ONCOTIC PRESSURE. M.L. MacDougall, L.W. Welling, and T.B. Wiegmann*, Univ. of Kansas, Kansas City, KS, and VA Medical Center, Kansas City, MO.

Absorption of tubular fluid into peritubular capillaries normally depends upon the balance of hydrostatic and oncotic forces within the tubule, interstitium, and peritubular capillaries. No satisfactory explanation has been given for the absorption of fluid in the absence of peritubular oncotic forces. Isolated rat kidneys were perfused with solutions containing protein (IPK-P) or without protein (IPK-NP). Bulk reabsorption in IPK-NP was equal to that in IPK-P (0.42 ml/min vs 0.47 ml/min) despite much lower fractional reabsorption (30.6% vs 90.8%). IPK-NP had a 21.6% increase in weight over IPK-P after 1 hour of perfusion. Morphometric point count evaluations of transverse kidney sections (x31) demonstrated a 26.1% increase in tubule lumen area in the cortex and a 12.1% increase in the tubule lumen area in the medulla in IPK-NP as compared to IPK-P. Tubule cell area was decreased by 18.2% in IPK-NP. There was no change in interstitial area. Since tubules account for about 80% of total cortical area, the increased luminal area of 26.1% can account for the increase in measured kidney weight. We propose that proximal tubule fluid absorption in the absence of peritubular oncotic pressure results from increased intraluminal hydrostatic pressure secondary to high tubular flow rate and limited distensibility of the tubule. This process may be facilitated by an increase in tubule hydraulic conductivity as previously observed to occur during conditions of increased luminal pressure and decreased peritubular protein concentration.

BRUSH BORDER MEMBRANE D-MANNOSE TRANSPORT IS INDEPENDENT OF THE D-GLUCOSE TRANSPORTERS. B.C. Mendelssohn* and M. Silverman, Membrane Biology Group, University of Toronto, Toronto, Ontario.

Dog renal brush border membrane vesicles (BBMV) from whole kidney cortex contain both low affinity, high capacity and high affinity, low capacity Na dependent D-glucose (D-glc) transporters (Biochim. Biophys. Acta 507:470, 1978). D-mannose (D-man) differs in structure from D-glc only at the C2 position of the pyranose ring. Uptakes of radioactive sugars into BBMV were performed by standard millipore filtration to determine whether D-man shares either, or both, of the D-glc carriers, or if it is transported by an independent system. Transport of D-man occurs into an osmotically active space, is saturable and Na^+ dependent with a 1:1 Na:D-man stoichiometry, Km=0.067μM, Vmax=3.8μM/mg/min, 25°C, pH 7.4. When an NaSCN electrochemical gradient was present, an "overshoot" was demonstrated indicating active cotransport. Up to 50 mM D-man had no affect on Na^+ dependent ^{3}H-D-glc and ^{3}H-α-methyl glucoside (αMG) uptake (0.01-20mM). Na^+ dependent D-man uptake was not inhibited by phloretin, cytochalasin B, L-man, galactose and 3-O-methyl-glucoside. Preliminary data indicate phlorizin, 2-deoxy-D-glc, 2-fluoro-2-deoxy-D-glc, fructose and mannoheptulose are specific competitive inhibitors, while D-glc and αMG compete only by dissipating electrochemical gradients. The results suggest that D-man and D-glc have different Na^+ cotransport systems. Ultimately their comparison should provide new insights into the molecular basis of sugar-transporter interaction.

EFFECT OF ARGININE VASOPRESSIN ON TRANSEPITHELIAL RESISTANCE IN INNER MEDULLARY COLLECTING DUCT CELLS. D. R. Mishler*, J. A. Kraut, and G. T. Nagami. Medical and Research Svcs, VA Med Center West Los Angeles and Dept of Med, UCLA Sch of Med, Los Angeles, CA.

The inner medullary collecting duct (IMCD) is an important site of action for arginine vasopressin (AVP). To examine the mode of action of AVP in this segment, we measured the change in transepithelial resistance of cultured rat IMCD cells grown to confluence on collagen-coated Millicell culture plate inserts in response to AVP. Resistance was measured using an EVOM voltage-ohm meter. AVP at 10^{-11} to 10^{-8} M caused a fall in resistance of 28±8 and 40±13 ohm/cm^2, respectively ($p<0.01$ vs. no AVP), which was reversed by removal of AVP or addition of 10^{-6} M amiloride. Addition of 10^{-8} M AVP or 10^{-6} M forskolin increased adenylate cyclase activity by 175% or 1079%, respectively. Stimulation of endogenous cAMP generation by forskolin or the addition of exogenous 8-bromo cAMP caused no change in resistance. To examine the relationship between intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) and AVP action, the response of $[Ca^{2+}]_i$ to AVP was measured using fura-2. AVP induced no change in $[Ca^{2+}]_i$. These data suggest that AVP reduces transepithelial resistance in IMCD cells by augmenting movement of sodium through conductive channels. This effect of AVP does not appear to be mediated by changes in $[Ca^{2+}]_i$ or cAMP.

EFFECTS OF INCREASING NaCl OR UREA CONCENTRATIONS ON ADH-STIMULATED WATER PERMEABILITY (P_f) IN RAT INNER MEDULLARY COLLECTING DUCT (IMCD). Steven P. Nadler, Dept. of Medicine, University of Ottawa and Ottawa General Hospital, Ottawa, Canada.

To assess the effects of hypertonicity on water reabsorption (J_V) in IMCD, ADH-stimulated J_V and P_f were determined in microperfused IMCD dissected from the mid-inner medullary region of rat kidney. IMCDs were exposed initially to a 150 mOsm/kg H_2O gradient in isotonic bath at 37°C. Osmolality (OSM) was then increased by symmetric addition of 75 mM NaCl or 150 mM urea to perfusate and bath, thus preserving the initial osmotic gradient. Each tubule served as its own control. In isotonic bath, 10^{-11}M ADH-stimulated J_V (nl·mm^{-1}·min^{-1}) averaged 5.48 ± 0.64(SE) and P_f (μm/s) 719 ± 93. When OSM was increased with NaCl, P_f increased by 56 ± 11 % (n=6, $p<.01$), and returned to initial values when initial conditions were restored. J_V did not change, but would have decreased by 16% ($p<.01$) had P_f not increased, due to the greater absolute axial increase in luminal tonicity with more hypertonic luminal solutions. When OSM was increased with urea, there was no significant change in P_f, which averaged 97% of control values (n=5). In IMCD exposed to 10^{-4}M 8-(CPT)-cAMP, increasing OSM with NaCl also resulted in a 76% increase in P_f. These results suggest that increased NaCl, but not urea, augments ADH-stimulated P_f in rat IMCD, at a site beyond cAMP generation. This enhancement may contribute to maintenance of medullary interstitial tonicity during antidiuresis by ensuring that most water reabsorption occurs more proximally within the IMCD.

HIGH EXTRACELLULAR NaCl STIMULATES SYNTHESIS OF ALDOSE REDUCTASE, AN OSMOREGULATORY PROTEIN, IN RENAL MEDULLARY CELLS. Toshiki Moriyama*, Arlyn Garcia-Perez*, and Maurice Burg. NHLBI. Bethesda, MD.

During antidiuresis renal inner medullary cells contain a high level of sorbitol which helps balance the high extracellular osmolality. GRB-PAP1 is a continuous line of epithelial cells derived from rabbit renal inner medulla. It was previously shown that when medium osmolality is increased by raising the NaCl concentration, these cells osmoregulate by accumulating sorbitol. The sorbitol is synthesized from glucose, catalyzed by aldose reductase. When the medium is made hyperosmotic, aldose reductase activity increases because of a large increase in the amount of enzyme protein. The purpose of the present studies was to test whether increased aldose reductase protein synthesis is involved in this process. GRB-PAP1 cells were grown in isosmotic (300 mosmol) or hyperosmotic (500 mosmol, high NaCl) medium, and the rate of synthesis of aldose reductase protein was measured, as follows. During the last hour of incubation, ^{35}S-methionine was added to the incubation. Aldose reductase protein was immunoprecipitated from cell extracts using a goat, anti-rabbit-aldose reductase antiserum. The proteins were separated by SDS-PAGE and quantified by laser scanning densitometry of the aldose reductase band. The average rate of synthesis of aldose reductase protein in cells grown in hyperosmotic medium was 7.0 ±1.4 (n = 3) times that of control cells grown in isosmotic medium. When control cells were switched to hyperosmotic medium the relative rate of synthesis increased 9.6-fold after 12 hours and 14.9-fold after 24 hours.

CONCLUSION: A large increase in the rate of synthesis of aldose reductase protein contributes to the high aldose reductase activity and consequent sorbitol accumulation that occur in these renal medullary cells when the extracellular fluid is hyperosmotic.

OSMOREGULATORY ACCUMULATION OF BETAINE BY MDCK CELLS IN HYPEROSMOTIC MEDIUM INVOLVES INCREASED BETAINE TRANSPORT. Takeshi Nakanishi*, R. James Turner and Maurice B. Burg. NHLBI, Bethesda, MD.

During antidiuresis renal inner medullary cells contain a high level of betaine which helps balance the high extracellular osmolality. We previously found that MDCK cells also contained a high level of betaine if grown in hyperosmotic medium. We have now studied the mechanism of betaine accumulation. With medium containing only that betaine added in the 10% serum (≈50 μM), cell betaine was < 10 mM in cells grown in isosmotic medium and > 60 mM in hyperosmotic medium. In defined medium, the cells accumulated betaine only when it was added to the medium and betaine was much higher in cells grown in hyperosmotic than in isosmotic medium. Thus, betaine apparently accumulated by transport from the medium. ^{14}C-betaine was used to study the transport. Betaine influx was >90% sodium dependent in hyperosmotic medium. The kinetics of Na-dependent uptake were measured using an assay medium with the same osmolality as the growth medium.

Osmolality mosm/kg	300	600	600
NaCl in assay mM	150	150	300
Mannitol in assay mM	0	300	0
Vmax pmoles/min/mg	370	3230	4230
Km mM	1.97	3.47	2.81

Vmax was much greater in cells grown in hyperosmotic medium, but Km was not significantly different. When NaCl was high in the assay medium, Vmax increased even further. The higher Vmax in cells grown in hyperosmotic medium is consistent with an increased number of functional transporters (or, less likely, with an increased transport rate by each). After switching to higher osmolality, betaine transport reached a maximum within one day.

CONCLUSIONS: 1) MDCK cells accommodate to elevated osmolality by accumulating high levels of betaine, as do cells in the renal inner medulla. 2) The betaine is accumulated because of increased Na-dependent uptake from the medium.

IMPORTANCE OF BUTYRATE IN HYPERTONIC VOLUME REGULATION OF CORTICAL COLLECTING TUBULE (CCT). E. Natke, Jr.*, R. Terranova*, and V.A. DiScala. Winthrop-Univ. Hospital, Mineola, N.Y.

Studies done with collapsed CCT's bathed in hypertonic media have shown that CCT's shrink but do not volume regulate. To test whether this was due to some factor missing from our bathing media we added a short-chain fatty acid, butyrate (1mM), to both the isotonic and the hypertonic media. Rabbit CCT's were studied in vitro at 37°C. Tubular diameter (D) was measured with an image splitting eyepiece. Tubular volume was calculated by $\pi D^2 L/4$. When media osmolality was increased by 150 mOsm by the addition of NaCl both the butyrate-treated (BT) and the non-butyrate control (C) CCT's shrank to 74±2% and 79±2% of their isotonic volumes. As evidence of volume regulation, BT CCT's (n=7) swelled while still bathed in hypertonic media, recovering in 30 min 78% of their volume lost due to osmotic shrinkage. In contrast C CCT's showed very little recovery. After 30 min. in hypertonic there was a significant difference in tubular volume between BT and C CCT's (94±3% vs. 79±3%, $p<0.01$ of the isotonic volume). Both groups of CCT's displayed an overshoot and a gradual return to control isotonic volume when rebathed in isotonic media. However, BT CCT's had a significantly larger overshoot than non-butyrate CCT's (125±10% vs 106±2% of isotonic volume $p<0.05$). These results suggest that the presence of butyrate permit CCT's to volume regulate in hypertonic media. Since the concentration of butyrate was 1mM, it must exert its effect by either being metabolized and/or accumulated in CCT's bathed by hypertonic media.

FUNCTIONAL PROFILE OF FLUID REABSORPTION IN REMNANT NEPHRONS: ROLE OF PERITUBULAR STARLING FORCES. Juan C. Pelayo. Univ. of Colorado, Sch. of Med., Dept. of Pediatrics., Denver, Colorado.

Acute nephron loss (Nx) is followed promptly by natriuresis and diuresis, thereby contributing to the control of extracellular fluid volume. The mechanisms for and sites of regulation remain controversial. Using micropuncture techniques in Munich-Wistar rats with 75% Nx (Nx, n=8) and sham controls (Sh, n=8), single nephron GFR (SNGFR), absolute (AR) and fractional (FR) tubular fluid reabsorption in proximal (p) and distal (d) nephron segments, and peritubular Starling forces (PSF) were examined at 24 hrs, thereby allowing the calculation of effective reabsorptive pressure (ERP) and peritubular capillary reabsorptive coefficient (LpAr). *=p<0.05 vs Nx.

	SNGFR [nl/min]	FRp	FRd	LpAr [nl/(min.mmHg)]	ERP [mmHg]
Sh	37.5	0.38	0.65	0.96	16.2
Nx	49.1*	0.37	0.56*	0.93	20.5*

ARp increased proportionally to the rise in the filtered load in Nx. This adjustment was associated with an augmentation in ERP, which was primarily due to a higher interstitial hydrostatic pressure (Nx vs Sh, $p<0.0001$). In Nx, the increased delivery of fluid into distal tubules accompanied by a decreased FRd produced no changes in ARd, but a significant elevation in distal tubular fluid flow rate as compared with Sh ($p<0.001$). These results suggest: 1)Glomerulotubular balance is intact in Nx via adaptations in PSF. 2)Increases in the filtered load coupled with reduction in tubular fluid reabsorption in nephron segments beyond the late proximal tubule are, in part, responsible for the adaptive diuresis in Nx.

EFFECT OF CORTICOMEDULLARY OSMOTIC GRADIENT ON TRANSCAPILLARY FLUID MOVEMENT IN DESCENDING VASA RECTA (DVR). Thomas L. Pallone, Yoram Yagil, and Rex L. Jamison. University of Rochester School of Medicine. Departments of Medicine and Nephrology, Rochester, NY.

To test the hypothesis that small solutes (ss) (e.g. NaCl) influence transcapillary water movement (Jv) in DVR of the renal medulla, DVR were punctured at the base (B) and tip (T) of the exposed papilla of rats during the presence (hydropenia, H) and furosemide induced absence (F) of a medullary axial osmotic gradient. DVR plasma protein concentration [Pr], g/dl, oncotic pressure (Po), mmHg, osmolality [Osm], mOsm/Kg H20, sodium concentration [Na], mEq/l, (N=10) and hydraulic pressure (Ph), mmHg (N=8) were determined. The results (means, * P<.05 B vs T):

		[Osm]	[Na]	[Pr]	Po	Ph
H	B	573	200	5.2	16.7	9.5
	T	1011*	322*	6.8*	25.1*	9.1
F	B	356	167	5.4	17.6	12.2
	T	377*	174	5.5	18.2	11.2

The rise of [Pr] during H indicates Jv occurred along DVR in the presence of an axial gradient for both [Osm] and [Na] from B to T. In fact DVR tip [Pr] was correlated with [Osm] (R=.72, P<.05). In contrast [Pr] did not increase from B to T when ss gradients were abolished (F). These results support the hypothesis that ss influence Jv across the DVR.

HYDRAULIC CONDUCTIVITY OF THE ISOLATED PERFUSED MACULA DENSA. Maria Ribadeniera* and Delon W. Barfuss, Georgia State University, Biology Department, Atlanta, Georgia 30303

The hydraulic conductivity of the macula densa and its corresponding thick ascending limb was determined using the isolated perfused tubule technique. An osmotic gradient of 230 mOsmol, lumen to bath, was imposed on the preparation. Diffusional water flow was quantitated by measuring the lumen-to-bath flux of ^3H-H_2O. Bulk water flow was determined by measuring the difference between perfusion rate and collection rate using ^{14}C-PEG as a volume marker. The H_2O flow across the macula densa is the difference between the water flow of the thick ascending limb with and without the macula densa segment.

Hydraulic conductivity (L_p), osmotic water permeability (P_f), and diffusional water permeability (P_{DW}) were calculated for the thick ascending limb (TAL) and macula densa plaque (MD). These values are: for the TAL, L_p=1.5 x 10^{-4} nl min^{-1} mm^{-1} $mOsmol^{-1}$, P_f=16.13 µm sec^{-1}, P_{DW}=12.66 µm sec^{-1}; for the MD, L_p=18.4 x 10^{-2} nl min^{-1} mm^{-1} $mOsmol^{-1}$; P_f=4581 µm sec^{-1}, P_{DW}=55.30 µm sec^{-1}. The values obtained for the Lp of the macula densa are remarkably higher than those reported for the proximal convoluted tubule (7.2 x 10^{-2} nl min^{-1} mm^{-1} $mOsmol^{-1}$). The magnitude of this transepithelial H_2O flow could offer an explanation for the structural changes previously observed during imposition of an osmotic gradient (Kirk et al, 1984). This great capacity to allow H_2O outflow further implicates this segment in the regulation and/or initiation of tubular glomerular feedback.

SUCCINYLACETONE (SA) INDUCED RENAL BRUSHBORDER FUNCTION ALTERATIONS. Karl S. Roth*, Marvin [redacted] and Steven M. Schwarz*, Departments of Pediatrics, Medical College of Virginia, Richmond, VA and New York Medical College, Valhalla, N.Y.

We have previously shown that SA, a compound excreted in excess in patients with hereditary tyrosinemia, reversibly inhibits amino acid and sugar uptake by rat renal brushborder membrane vesicles. DPH fluorescence anisotropy measurements suggested that transport dysfunction was associated with increases in membrane fluidity (decreased r). To further characterize the nature of SA influence on vesicle solute uptake, transport kinetics were determined. For L-glycine, both the high and low capacity transport systems were competitively inhibited by 4 mM SA (Vmax unchanged). Conversly, a-CH_3-D-glucoside (AMG) transport inhibition was non-competitive (Km unchanged).

	L-Glycine		AMG
	Km1(mM)	Km2(mM)	Vmax(nmol/mg/30s)
Control	0.18	6.78	8.25
4mM SA	0.33	28.57	6.25
p <	0.05	0.01	0.01

In contrast to our earlier membrane fluidity data, DPH fluorescence anisotropy measurments (5-40°C) of liposomes from extracted BBM lipid demonstrate no SA-related lipid fluidity changes (SA vs control, r@25°C= .213 vs .214, p=n.s.). CONCLUSIONS: 1) In vitro SA-induced inhibition of BBM vesicle solute transport is associated with variable effects on uptake kinetic parameters; 2) SA-related changes in fluidity are a consequence of altered hindrance of probe motion by membrane proteins. SPECULATION: SA-induced BB membrane transport and fluidity changes are related to alterations in bilayer protein-lipid interactions.

INTRACELLULAR OSMOLARITY GRADIENTS IN ISOLATED PERFUSED CORTICAL COLLECTING TUBULES DURING ADH-INDUCED WATER FLOW. Walter Schratt,* Roger Rick,* Jack T. Walker,* and Donald R. DiBona.* Dept. of Physiology and Biophysics, Univ. of Ala. at Birmingham, Birmingham, AL, and Dept. of Anatomy and Cell Biology, Med. Univ. of South Carolina, Charleston, SC (intr. by James A. Schafer).

Quantitative electron microprobe analysis was employed to record intracellular osmolarity gradients in cortical collecting ducts during ADH-induced osmotic water flow. Osmolarity was estimated from the sum of Na, K and Cl concentrations as measured from 0.3 μm thick, freeze-dried cryosections by energy-dispersive x-ray microanalysis. Isolated tubules from NZ white rabbits were perfused using a specially designed microperfusion system. The tubule was visualized by Nomarski optics using a long distance condenser and a 100x dry objective lens. After establishing transepithelial water flow, the bathing fluid was rapidly aspirated and the tubule was frozen by a vigorous jet-stream of pre-cooled propane (~188°C). For cryosectioning the tubule was mounted using a newly developed fluid-clamping technique. Ice crystal size was small enough to allow analysis of luminal and bathing fluid, in addition to cellular measurements. In the presence of a lumen-to-bath osmotic gradient (130 to 290 mM) and ADH (0.1 mU/ml) a marked reduction in the intracellular ion concentrations of the apical cytoplasm was observed, giving rise to a basal-to-apical intracellular ion concentration gradient. No gradients were detectable when the lumen was perfused with hypotonic fluid without ADH, or when ADH was applied without osmotic gradient. Intracellular vacuoles observed during ADH-induced water flow showed a typical extracellular composition of electrolytes with high Na and Cl concentrations. These results suggest the existence of a significant intracellular water activity gradient during osmotic water flow. Thus, in addition to the two limiting cell membranes, the cytoplasmic pathway provides resistance to transepithelial water flow.

ALDOSE REDUCTASE ACTIVITIES IN MICRODISSECTED RAT NEPHRON SEGMENTS. J.M. Sands, Y. Terada,* L.M. Bernard,* M.B. Burg, and M.A. Knepper. NHLBI, NIH, Bethesda, MD.

Osmoregulation in inner medullary cells depends in part on cellular accumulation of sorbitol, whose production is catalyzed by aldose reductase (AR). To identify nephron segments that contain AR, we developed an ultra-micro assay to measure AR activity in microdissected nephron segments from collagenase-treated kidneys of Sprague-Dawley rats. Tubules (total length 3-5mm) were dissected, permeabilized by hypotonic shock and freeze-thawing, and incubated with 10mM DL-glyceraldehyde and 0.25 mM NADPH. NADP production was measured by converting NADP to a fluorescent form and measuring the fluorescence. Tubules were dissected from the inner stripe of the outer medulla (OM), and two regions of the inner medulla (IM), the outer 25% of the IM (IM-base) and the inner 50% of the IM (IM-tip). The AR activities were (pmol/min/mm):

	Desc. Limb	Asc. Limb	Coll. Duct
OM	0.5±0.2	0.3±0.1	0.3±0.1
IM-base	0.9±0.3	1.7±0.2	1.8±0.7
IM-tip	3.3±1.0	2.4±0.7	15.1±2.2

$P < 0.05$ vs. 0 (N=6) for all values. Little or no AR activity was measured in glomeruli or any distal cortical segment. Activities in the three proximal tubule subsegments (S-1, S-2, S-3) were 6-10 pmol/min/mm, but were negligible when the substrate was changed to D-xylose, consistent with the presence of aldehyde reductase, not AR. Conclusions: (1) AR activity can be measured in individual nephron segments. (2) AR activity is present in substantial amounts in inner medullary collecting ducts, thin descending limbs, and thin ascending limbs. (3) AR activity increases with medullary depth in each medullary segment.

VASOPRESSIN REGULATED UREA PERMEABILITY OF CULTURED RAT INNER MEDULLARY COLLECTING DUCT (IMCD) CELL MONOLAYERS. J.H. Schwartz, H.H. Bengele, E.R. McNamara* & E.A. Alexander. Renal Section, Boston City Hosp., Depts of Med. & Physiol., Boston University Medical School, Boston, MA.

The distal IMCD is critical in the urinary concentrating process in part because it is the site of vasopressin (AVP) regulated permeability to urea. The purpose of these experiments was to develop a cell culture model of the IMCD on permeable structure and to characterize the responsiveness to AVP. Rat IMCD cells were grown to confluence on collagen coated millipore filters glued onto plastic rings. To assess the time required to achieve confluence, the transepithelial resistance was measured periodically and was found to be stable after 2 weeks at a maximal value of 595±22 Ω-cm^2. In separate monolayers the effect of AVP on inulin and urea permeability was determined. Inulin permeability was unchanged, 2.2 ±0.5 $x10^{-6}$ before and 1.6±0.3 $x10^{-6}$ $cm\text{-}sec^{-1}$ after AVP. Urea permeability increased after AVP, however, from 3.0±0.4 to a peak of 8.4±1.2 (10nM); 13.3±1.6 (1μM) and 15.9±2.9 (10μM) $x10^{-6}$ $cm\text{-}sec^{-1}$ (n=24). In 10 additional monolayers, the addition of 1mM 8-br-cAMP increased urea permeability from 2.6±0.3 to 8.1±1.6 $x10^{-6}cm\text{-}sec^{-1}$. We conclude that rat IMCD cells grown in culture exhibit the characteristics of a "tight" epithelium. Inulin and urea permeability are not different in the absence of AVP, consistent with high resistance junctional complexes. Furthermore IMCD cells retain the capacity for AVP regulated urea permeability, a characteristic feature of this nephron segment *in vivo*.

FUNCTIONAL COLOCALIZATION OF WATER CHANNELS AND PROTON PUMPS IN APICAL ENDOSOMES FROM KIDNEY PROXIMAL TUBULE. Lan-Bo Shi*, Rengao Ye*, Wayne I. Lencer*, and A.S. Verkman. CVRI, UC San Francisco, CA and Harvard Med Sch, Boston, MA.

The apical membrane of proximal tubule undergoes rapid cycling by exocytosis and endocytosis. Osmotic water and ATP- driven proton transport were measured in endocytic vesicles from rabbit renal cortex labelled in vivo with the fluid phase marker fluorescein-dextran (F-D). Morphological studies showed that endosomes were derived from the apical membrane of proximal tubule. Osmotic water permeability (P_f) was determined from the time course of F-D fluorescence following an inward osmotic gradient. P_f was 0.02 cm/s (23°C) and independent of osmotic gradient size. P_f was inhibited reversibly by $HgCl_2$ (K_I=0.2 mM) and had an activation energy of 5.3±0.3 kcal/mole (15-35°C). Endosomes contained an NEM-sensitive, oligomycin-insensitive H ATPase which acidified the endosome ~1.2 pH units. Na/H exchange and Na/glucose cotransport were absent in endosomes. To examine whether water channels and the H ATPase were present in the same endosome, F-D fluorescence was measured in response to an osmotic gradient in the presence and absence of ATP. ATP did not alter endosome P_f, however the amplitude of the fluorescence signal, indicative of the internal pH of endosomes containing water channels, decreased by 54±4%; the signal decrease was reversed by nigericin. Therefore >90% of endosomes containing a water channel contain a H ATPase. These findings show selective endocytic uptake of water channels and H pumps in proximal tubule with exclusion of apical Na/H and Na/glu transporters.

BOMBESIN INHIBITION OF STIMULATED CHLORIDE SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS. P Silva**, S Reichlin* and FH Epstein**, Dept of Med., Beth Israel Hosp, Harvard Med. School, Boston, MA, and Mount Desert Island Biological Laboratory, Salsbury Cove, ME.

Bombesin is a tetradecapeptide present in nerve fibers in the rectal gland of the shark together with vasoactive intestinal peptide (VIP). Bombesin suppresses the effect of VIP to stimulate chloride secretion. Bombesin also inhibits the stimulation of chloride secretion induced by cyclic AMP. This effect of bombesin to inhibit chloride secretion at a site distal to the generation of cyclic AMP is reminiscent of that previously demonstrated for somatostatin, also present in the nerves within the gland. In the present series of experiments we examined the mechanism of action of bombesin. Rectal glands were perfused with a continuous infusion of VIP 1.5×10^{-9}M to obtain sustained chloride secretion. Bombesin, at a final concentration 8×10^{-7}M, reversibly reduced the secretion of chloride by 56.5.±9.7% (n=7, p<0.01). Bombesin evoked a ten fold increase in somatostatin in the venous effluent of the gland. The effect of bombesin to reduce chloride secretion was completely supressed by procaine and nifedipine that inhibit neurotransmitter release. The effect was partially supressed by cysteamine that inactivates somatostatin.

These results indicate that bombesin inhibits the effect of VIP to stimulate chloride secretion by the release of somatostatin from nerve fibers within the gland. These observations indicate that rectal gland activity is normally regulated by the release of endogenous neurotransmitters.

COMPARISON OF PARTICLE ARRAYS AND AGGREGATES IN TOAD BLADDERS. N.L. Shinowara*, T.A. Palaia* and V.A. DiScala, Winthrop-Univ. Hosp., Mineola, N.Y.

Aggregates, associated with water transport, appear and remain in luminal membranes of granular cells (GC) upon ADH-stimulation without a hydro-osmotic gradient. Particle arrays (PA), morphologically indistinguishable from aggregates, may be in basal membranes of GC and apical and basal membranes of underlying basal cells (BC) of control and ADH-stimulated bladders. Cytoplasmic tubules with aggregates can exist in close proximity to GC basolateral membranes and may be associated with PA. A few were identified in BC. Six pairs of hemibladders, with and without ADH for 30 min. and no gradient, were examined for PA densities in 3 plasma membranes. Respective mean PA densities ±S.E.M./100um^2 (mean range=r) for controls vs. ADH groups are as follows: GC basal membranes had 2.41±0.95 (r=0.29-5.48) vs. 3.01±0.92(r=0.25-6.45); BC apical membranes had 1.31±0.91 (r=0-5.80) vs. 5.41±4.67 (r=0-28.47); BC basal membranes had 7.26±5.52 (r=0-34.22) vs. 18.46±9.35 (r=0-57.39). Large variations in values exist within and among groups, especially in BC. No significant differences were among control and ADH groups. However, there tended to be more PA in basal than apical BC membranes and more in ADH than control BC basal membranes. Small gap junction-like structures were in some BC apical membranes. Mean size was 12.06±1.98 particles and densities were 1.51±0.48/100um^2 for controls and 0.65±0.33/100um^2 for ADH bladders. In conclusion, PA are structurally similar to aggregates. Large variations in PA densities in control and ADH-stimulated bladders suggest that PA are associated with a dynamic cell activity, independent of ADH.

BIOCHEMICAL CHARACTERIZATION OF THE NA-D-GLUCOSE COTRANSPORT SYSTEM FROM RENAL BRUSH BORDER MEMBRANE. M. Silverman, M. Behar* and P. Speight*, Membrane Biology Group, Univ. of Toronto, Toronto, Ontario.

Selective deoxycholate (DOC) solubilization and chromatofocussing yields ~ 140 x enrichment of the Na-dependent phlorizin receptor from renal brush border membranes (BBM) (J.B.C. 261:13820,1986). Mice were then immunized and hybridomas produced by standard techniques (Nature 256:495,1975). Sequential screening was carried out using purified material in a dot blot assay and then indirect immunofluorescence on whole kidney cortex. Positive cells were doubly cloned, expanded by production of ascites in mice and the resulting monoclonals purified. One specific IgG_1 monoclonal called H4 was used for further studies. Reaction of H4 with DOC solubilized lactoperoxidase ^{125}I labelled BBM, resulted in immunoprecipitation of a single ~130Kd polypeptide. After DOC solubilized BBM were loaded to an H4 affinity column, a single ~130 Kd protein was eluted. Reduction yielded two peptides of ~72 and ~55 Kd. The eluate from the H4 column was then loaded to a phlorizin affinity matrix (PAM). In the presence of Na the H4 eluate was totally bound to the PAM. Elution from the PAM by D-glucose alone or D-glucose + Na revealed that only the ~74 and 130 Kd polypeptides exhibit Na and D-glucose binding sites while the ~55Kd fragment has a D-glucose site. It is concluded that the Na dependent phlorizin receptor is a ~130 Kd heterodimer consisting of ~72 and ~55 Kd subunits linked by a disulphide bond.

GENTAMICIN INHIBITS CARRIER-MEDIATED DIPEPTIDE TRANSPORT IN THE PROXIMAL NEPHRON, Hal A. Skopicki Demetrios Zikos, Kenneth A. Fisher, Robert D. Bloch, and Darryl R. Peterson. University of Health Sciences/ The Chicago Medical School, Departments of Physiology and Medicine, 3333 Green Bay Road, North Chicago, IL 60064

To extend our knowledge regarding the mechanism of aminoglycoside-induced defects in carrier-mediated transport processes in the proximal nephron, we examined the effects of gentamicin on the luminal transport of the dipeptide pGLU-HIS. This dipeptide has been previously shown to be reabsorbed intact via carrier-mediated transport. Transport was characterized in rabbit renal brush border membrane vesicles prepared one hour after intravenous injection of gentamicin (100 mg/kg) and compared to that in vesicles from control animals. The *in vivo* administration of gentamicin and the *in vitro* addition to control membrane vesicles caused the Vmax of pGLU-HIS transport to decrease by 49% and 44% in the *in vivo* and *in vitro* groups, respectively (control=9.4×10^{-12}, *in vivo*= 4.8×10^{-12}, *in vitro*= 5.3×10^{-12}) without altering Km. Vesicles treated *in vitro* showed a complete reversal of inhibition in the presence of 5mM Mg^{++} (a competitor of gentamicin binding to membrane) while the *in vivo* treated vesicles were unaffected by Mg^{++}. These data suggest that gentamicin reduces the reabsorptive capacity of proximal tubules for dipeptides by a mechanism which influences the number or activity of membrane carriers.

THE TUBULAR REABSORPTION OF PEPSINOGEN A (PGA) AND C (PGC) IN MAN. Reinier W. ten Kate*, Gerard Pals*, Ab J.M. Donker*, Jan C. Pronk*, Stephan G.M. Meuwissen* (intr. by L.W. Statius van Eps). Dept. of Int. Med. and Gastroent. and Inst. of Hum. Gen. Free University Hospital, Amsterdam, The Netherlands.

PGA and PGC are circulating low molecular weight (LMW) proteins (mol.wt. 43 kD) synthesized by the gastric mucosa. We have shown that their renal extraction in man is similar to the extraction of creatinine and in the isolated rat kidney model both PG's are almost freely filtered through the glomerular basement membrane. Interestingly, only PGA is present in the urine. We investigated the tubular reabsorption (TR) of PGA and PGC. A 24 h urine specimen and a serum sample were obtained from 21 healthy volunteers. From 5 of them a second 24 h urine and serum sample, and from another 8, three more urine and blood samples were obtained. To achieve a large filtered load (FL) a second 24 h urine and serum sample were obtained from 8 subjects after rising serum pepsinogen levels by oral administration of omeprazole. Fractional excretions (FE) were calculated. Results comprise 58 observations.

	PGA	PGC
Serum (µg/l)	53.8 (10-209)	17.2 (4-56)
FL (mg/24h):	8.5 (1.5-28.5)	2.9 (0.5-10.3)
Urine (mg/24h):	2.8 (0.2-19.0)	0.02 (0.003-0.137)
TR (mg/24h):	5.7 (0.3-14.7)	2.8 (0.5-9.0)
FE (%)	31 (4-93)	1.0 (0.03-11.0)

With increasing filtered loads more PGA was reabsorbed by the tubules. At filtered loads above 8-10 mg/24h a T_m (10.6 ±3.5 mg/24h) was reached. Despite a two to threefold increase in serum PGC levels hardly any PGC was detected in the urine. When measured 4 times with several weeks interval, changes in TR of PGA were observed, while TR of PGC remained high (n=8). We conclude that the presence of PGA in the urine of healthy volunteers demonstrates the presence of a low affinity system for TR of LMW proteins in man. Tubular affinity changes in time and factors affecting these changes have to be investigated. For PGC a high affinity, high capacity system exists in man.

ACIDOSIS INDUCES A REVERSIBLE CELL VOLUME INCREASE IN RABBIT PROXIMAL TUBULAR S2 SEGMENTS. L. Sullivan, R. Clancy*, D. Wallace* and J. Grantham. Univ. of Kansas Med. Ctr., Depts. of Physiology and Medicine, Kansas City, Kansas.

We have initiated a study of the effect of pH on tubular cell volume and its regulation. We measured the cell pH and volume response of lumen-collapsed, proximal S2 segments from the rabbit kidney to isosmotic bathing medium in which the pH was reduced either by raising CO_2 from 5% to 15% or by reducing $[HCO_3]$ from 25 to 5 mM. Cell pH was determined by measurement of the fluorescence emission of BCECF. 15% CO_2 reduced cell pH 0.25-0.28 units (n=3) within 20 sec with little evidence of subsequent regulation. Reduction of $[HCO_3]$ to 5 mM reduced cell pH 0.36-0.48 units (n=3) within 70 sec again with little evidence of regulation. Cell volume was calculated from measurements of tubular diameter. 15% CO_2 caused the volume to increase 8.6±1.4% (n=6, $p<.005$) in 3 mins; it was still elevated 8.6% at 10 mins and returned to control 1 min after re-exposure to 5% CO_2 (vol. = 102±2% of control). There was no evidence of a volume regulatory solute loss. The volume response to a reduction of $[HCO_3]$ to 5 mM was much slower. It began to rise at 4 mins and increased gradually to 6.5±9% (n=5, $p<.005$) at 8 mins. Upon re-exposure to 25 mM HCO_3 the volume immediately increased to 7.9±1.1% above control and then fell slowly to the control level in 6 mins. Tubules exposed to 5 mM HCO_3 for 30 mins remained swollen with little evidence of a volume regulatory decrease. We suggest that a fall in cell pH induced by changes in PCO_2 or $[HCO_3]$ causes a net increase in cell solute and water content which does not trigger cell volume regulation.

VASOPRESSIN AND OXYTOCIN STIMULATE FORMATION OF COATED PITS IN RAT COLLECTING DUCT IN AN *IN VITRO* SYSTEM. Patrick Weyer*, Aline Coffey, Dan O'Sullivan*, Dennis Brown & Heinz Valtin. Renal Unit, Mass. General Hospital, Boston, MA and Dept. of Physiology, Dartmouth Medical School, Hanover, NH.

The increase in water permeability of collecting ducts induced by the antidiuretic hormone vasopressin is associated with an increased number of clathrin coated pits on the apical membrane of principal cells. These coated pits are the site where vasopressin-induced intramembranous particle clusters, the proposed sites of transmembrane bulk flow, are located and eventually endocytosed. We describe here a system that uses quantification of coated pits as an assay of this cellular action of antidiuretic hormone *in vitro*. Strips containing 3-5 collecting ducts were excised from Brattleboro rat kidney papilla immediately after sacrifice and incubated in oxygenated Krebs-Ringer's solution containing either arginine-vasopressin (2 ng/ml) or oxytocin (50 pg/ml), or both. Some strips were fixed immediately after removal from the animal. Morphometric analysis of thin sections by electron microscopy shows that stimulation by vasopressin induces an increase in the number of coated pits per micron of apical membrane length (0.139 ± 0.027 against 0.045 ± 0.003 in controls incubated without hormone). A similar effect was found with oxytocin (0.163 ± 0.008) or with both hormones together (0.173 ± 0.025). These results indicate that oxytocin can stimulate membrane changes that have been associated with increased membrane water permeability in principal cells. The effect of oxytocin does not appear to be additive with that of vasopressin. This *in vitro* system can, therefore, be used to study related morphologic changes induced by other hormones or pharmacologic agents in the kidney collecting duct.

BATH COLLOID PREVENTS VACUOLIZATION INDUCED BY OSMOTIC WATER FLOW IN PROXIMAL TUBULE. James C. Williams, Jr.*, Dale R. Abrahamson and James A. Schafer. Dept. of Anatomy & Cell Biology, Med. Univ. of S.C., Charleston, South Carolina, and Depts. of Cell Biology & Anatomy and Physiology & Biophysics, UAB, Birmingham, Alabama.

When an osmotic difference was imposed across the epithelium of perfused rabbit proximal straight tubules (270 mosmol/kg H_2O in the lumen and 290 in the bath) in the absence of bath colloid, a severe vacuolization developed within the epithelium such that view of the lumen border was obscured within 5.2±1.2 min (n=13 tubules at 23°C). Such vacuolization was less severe if the bath was hypotonic to the lumen or if the magnitude of the osmotic difference was reduced. If colloid (6% w/v of either bovine serum albumin or 70,000 MW dextran) was included in the bathing medium, this vacuolization was either not observed or was minimal, but the vacuolization became severe upon removal of the colloid and obscured the lumen within 6.1±1.6 min (n=8 albumin and 4 dextran at 23°C). At 38°C, vacuolization obscured the lumen within 4.2±0.9 min following the removal of albumin (n=5). ANOVA suggests that none of the times to vacuolization differ. The rate of passive volume flow due to the osmotic difference was unaffected by vacuolization (0.92±0.10 $nl \cdot min^{-1} \cdot mm^{-1}$ with albumin to 0.79±0.11 without albumin and vacuolated, n=8, $p>0.2$ using paired t-test, 23°C). Electron microscopic examination of tubules fixed after vacuolization showed clear, empty spaces—apparently membrane-bound—within the cytoplasm. These results suggest that the presence of serosal colloid protects the epithelial cells from injury during transepithelial water flow. The mechanism for this protective effect is not apparent, but may be related to effects of colloid in maintaining normal volume absorption in the proximal nephron.

CYCLOSPORINE-INDUCED TUBULAR CYTOTOXICITY IN BSC-1 PRIMATE CELL LINE. Lilly Barba, Harry Ward, and Jay Vadgama*. Renal Transplant Section, Harbor - UCLA Med Ctr, Torrance CA.

The molecular mechanisms of cyclosporine (CsA)-induced renal injury are poorly understood. Recent literature suggests that the tubular epithelium is the primary site of CsA injury. We employed an in vitro model of CsA tubular toxicity using the primate BSC-1 cell line. BSC suspensions were incubated with CsA concentrations of 100, 250, or 500 ng/ml for 5 days, followed by washout of CsA to restore cell morphology and function. Parameters of % growth inhibition, total protein(TP), and DNA synthesis were evaluated in the G1 phase of the cell cycle.

Day	% growth inhib	TP (mg/dl)	DNA synthesis[1]
	100/250ng ml^{-1}	100/250	100/500ng ml^{-1}
2	82.4 / 64.0	0.30/0.23	83.5 / 55.1
3	92.0 / 53.0	0.98/0.57	103.7 / 25.3
5	95.7 / 63.4	2.20/1.50	

1. % cpm of controls; all numbers are mean values.

CsA concentrations of 250ng/ml were found to induc significant increments in vacuole formation, cell swelling, and subcellular membrane damage. Removal or washout of CsA restored cell morphology after 3 additional days in culture. DNA and RNA synthesis measured by ^{3}H-Thymidine and ^{3}H-Uridine, respectively, is significantly reduced in tubular cells receiving CsA above 100ng/ml. ^{3}H-leucine incorporation did not change significantly, while a 24 hr exposure of tubular cells to CsA concentrations ranging from 0 to 250 ng/ml showed an increase in Na-dependent methyl-amino isobutyrate.

Transplantation: Basic or experimental

REJECTION REDUCTION: TRANSFUSION FOR ACUTE AR; HLA MATCHING FOR CHRONIC REJECTION REACTIONS: M.A. Baltzan, R.B. Baltzan, and R.F. Dyck. Univ of Sask, Dept of Med, Saskatoon, SK.

Between 1965 and 1988, forty 3 and 4 antigen-match living donor renal transplants were done. The most common cause of renal failure was chronic glomerulonephritis, but in 6 it was diabetes. Thirty-two were 1st, and 8 were 2nd transplants. Initial steroid dose 100 mgms prednisone equivalent daily, then tapered to 50 mgms by 14 days, then down to 12 mgms at 1 year and thereafter. In addition to steroids, 37 were given azathioprine, 3 cyclosporine, and 0 both these drugs.

Acute rejection (AR) occurred in 58% of those (11/19) who had less than six pre-transplant blood transfusions (NTx), and in 9.5% (2/21) of those with more than five (Tx) (chi-sq 8.877, p<0.01). Pulse steroid therapy reversed all but one AR. It was in the only patient with a second AR, and patient was NTx.

Ten kidneys have functioned more than 10 years, 8 being NTx. and the 10 year actuarial survival rate is 83%. Four have failed, 3 due to death: 2 with AR and drug related infections, and 1 with a pulmonary embolus at 2 years. The other failure, a transplant artery thrombosis at 5 years, was in a diabetic with advanced atherosclerosis. Chronic rejection (CR) is nil in both NTx and Tx. Difference in AR and CR rate in NTx is 11/19 vs 0/8, (chi-sq 7.816. p<0.01).

Thus with conventional prophylactic immunosuppressive therapy and HLA matching: (1) The AR incidence is high, although severity may be reduced, yet the CR incidence is low or even nil. This bifid behavior of the HLA locus is unexplained. (2) More than 5 pre-transplant transfusions (Tx) reduce the AR incidence.

EFFECT OF CYCLOSPORIN A ON ENDOTHELIUM-DEPENDENT AND ENDOTHELIUM-INDEPENDENT VASCULAR RELAXATION. C.Bossaller*, C.J.Olbricht, V.Reschke*, E.Gutjahr*, K.Burgwitz*. Nephrology, Med.School Hannover and German Heart Institute,Berlin, FRG

The mechanism of cyclosporine A (Cyc) induced hypertension is unknown. To evaluate possible effects of Cyc on endothelium-dependent and endothelium-independent vascular relaxation pairfed rats received 30mg/kg/day CYC (n=10) or solvent only (n=10) by gavage. After 6 weeks aortic rings were mounted in oxygenated Krebs buffer for the recording of isometric tension. Following precontraction of the rings by 0.1 µM phenylephrine (Phe), relaxation was induced by acetylcholin (Ach) or nitroglycerin (TNG). Ach-induced relaxation needs an intact endothelium. Relaxation by TNG is endothelium-independent. Phe-induced tension was 1.1±0.06 gm in controls and 1.26±0.05 gm in Cyc ($p < 0.05$). Relaxation is expressed in percent reduction of Phe-induced tension. Values are means±SE of 36-40 rings.

	Control	Cyclosporin	p
Ach,0.01µmol/L	40.3±2.8 %	15.7±2.0 %	<0.001
1.0 µmol/L	92.6±1.0 %	72.2±2.9 %	<0.001
NTG,0.01µmol/L	58.9±2.0 %	19.7±2.1 %	<0.001
1.0 µmol/L	95.3±0.9 %	86.3±1.4 %	<0.001

The higher Phe-induced tension in Cyc indicates increased alpha1-adrenergic vasoconstriction.The decreased relaxation by NTG in Cyc rats indicates reduced endothelium-independent vascular relaxation. The decreased relaxation by Ach shows that endothelium-dependent relaxation is also impaired by Cyc. We conclude that decreased endothelium-dependent as well as decreased endothelium-independent vascular relaxation may contribute to cyclosporin induced hypertension.

ADMINISTRATION OF A THROMBOXANE (TX) RECEPTOR ANTAGONIST IMPROVES RENAL FUNCTION IN RATS WITH CYCLOSPORINE (CyA) NEPHROTOXICITY. TM Coffman, SM Mavros*, DM Collins*, P Ruiz*, WE Yarger and PE Klotman. Duke University and Durham VA Medical Centers, Durham, NC

CyA nephrotoxicity is associated with increased renal production of the vasoconstrictor eicosanoid TX. To specifically examine the functional importance of increased TX production in this disorder, we evaluated the effects of acute administration of the TX receptor antagonist GR32191 in rats with CyA nephrotoxicity. To simulate conditions associated with transplantation, uni-nephrectomized ACI rats underwent renal denervation followed by cross-clamping of the left renal artery for 30 minutes. Animals were then given CyA 50 mg/kg or vehicle by daily IP injection. Urines were collected in metabolic cages and concentrations of TXB2 were measured by RIA following HPLC separation. After 14 days renal hemodynamic studies were performed. Urinary TXB2 excretion was significantly increased in CyA treated rats (103±18 pg/hr) compared to controls which received only vehicle (60±16 pg/hr; $p<0.05$). Inulin clearance and renal blood flow were reduced significantly in rats treated with CyA (2.43±0.38 and 22.06±3.89 ml/min/kg) compared to controls (5.95±0.56 and 31.87±3.60 ml/min/kg; $p<0.005$) although renal histomorphology was essentially normal in both groups. Administration of GR32191 (10µg/kg/min by IA infusion) resulted in a 33.7% increase in GFR ($p<0.01$) and a 17.9% increase in RBF ($p<0.05$) in the CyA group but had no significant effect on renal hemodynamics in control animals. In summary, acute administration of a specific TX receptor antagonist improves renal function in rats with CyA nephrotoxicity. These data suggest that TX is an important mediator of reversible renal dysfunction associated CyA administration.

DISSOCIATION BETWEEN IMMUNOSUPPRESSIVE ACTIVITY OF CYCLOSPORINE (Cs) DERIVATIVES AND THEIR EFFECTS ON INTRACELLULAR CALCIUM (Ca) SIGNALLING IN MESANGIAL CELLS (M). H. Goldberg*, P. Wong*, E. Cole, G. Levy*, and K. Skorecki, Univ. of Toronto, Canada.

A condition for development of improved Cs derivatives is that immunosuppressive activity be dissociated from nephrotoxicity. The latter has been attributed to enhanced vasoconstrictor-mediated Ca release in vascular smooth muscle and mesangial cells with a consequent reduction in ultrafiltration. Therefore, we examined the effect of CsA, its analogs (ANA) and metabolites (MET) on vasopressin (VP)-stimulated Ca release in cultured rat M. Fura-2 loaded cells were incubated with vehicle (veh), CsA, ANA, or MET for 90 min prior to determination of peak Ca response to 30nM VP. Results are expressed as a percent± SE of peak Ca response in veh treated cells (peak Ca=268±20nM;n=42). CsA markedly enhanced this response with a max increase of 305±47% (n=9) above veh at 4ug/ml and an EC50 of 450ng/ml. ANA provided by SANDOZ and MET purified by gradient HPLC from bile of CsA treated patients were tested at 4ug/ml. Peak Ca responses were markedly enhanced ($p<.05$) by CsG (380±34%) and CsH (416± 90%). In contrast, MET OL17 (85±12%) and OL1, (95±15%) did not increase peak Ca. Since CsH which is not immunosuppressive caused a large increase in peak Ca, while OL17 which has been reported to have immunosuppressive activity, had no effect, we conclude that immunosuppressive activity appears to be dissociated from the Ca signalling perturbations responsible for toxicity.

IN VIVO GENERATED ALLOGENEIC CD4+ T CELL LINE WITH SUPPRESSIVE ACTIVITY IN THE RAT A.H.Frankel*, M.H.Sayegh*, D. Rothstein*, C.Kwok*, E.L.Milford* and C.B.Carpenter. Brigham and Women's Hospital. Boston, MA.

Both CD8+ and CD4+ T suppressor cells have been described in the rat. It has been very difficult to propagate and maintain rat suppressor cell lines in culture. Using the autoimmune encephalomyelitis model in the rat Ellerman established a CD4+ T suppressor cell line (Nature 331: 265).

Using a rat cardiac allograft model we have generated a CD4+ T cell line with suppressive activity. Splenocytes from WF strain rats acutely rejecting a LEW strain cardiac allograft were cultured with irradiated* (3000Rads) LEW splenocytes in the presence of 300ng/ml CsA for 7 days. The resulting lymphoblasts were isolated and cultured in Con A supernatent for 4 days. Subsequently the cell line was serially passaged through periods of antigen restimulation and growth in Con A supernatent. The cell line proliferated to LEW* but not to third party BN* lymphocytes. It exhibited no cytotoxic activity by the chromium release assay. Initially the cell line was functionally helper as assessed by increased proliferation of a WF anti LEW* MLC upon addition of 20% irradiated (1000 Rads) cell line. The phenotypic characteristics of the cell line were: >90% OX19+ (pan T cell marker); ASGM-ve (NK marker); 26% ART18+ (IL2 receptor marker); 75% W3/25+(CD4+) and 40% OX8+(CD8+). After 4 weeks functional activity changed to become increasingly suppressive and the phenotypic characteristics were >90% W3/25+ and <5% OX8+; their growth slowed with this change.

We conclude that it is possible to generate a CD4+ suppressor cell line from an in-vivo allogeneic transplant model.

RENAL PROSTAGLANDIN (pg) PRODUCTION BY HYPO-THERMICALLY PRESERVED KIDNEYS. Margery Halstead*, Martin Mangino*, and Charles Anderson. Washington Univ. School of Medicine, St. Louis, MO 63110

Prolonged cold storage of kidneys (>48 H) prior to transplantation results in reflow injury, ATN, and loss of function. The aim of this study was to determine if aberrations in renal pg production are associated with cold ischemia-reflow injury. Dog kidneys were flushed with Collins solution, stored for 48 H at 4°C, autotransplanted into the donor, and allowed to reperfuse for various times. Renal pgs and function were subsequently monitored in the outer cortex (OC), inner cortex (IC), and medulla (M). Thromboxane B2 (TXB2) production was unaltered in the OC and M after cold ischemia and either 60 min or 2 days of reperfusion. In the IC, TXB2 production increased 9 fold after cold ischemia and 60 min of reperfusion but returned to normal values after 2 days. prostacyclin and prostaglandin E2 production was significantly higher (3 fold and 6 fold, respectively) in the medulla after cold ischemia and 2 days of reperfusion and was not altered in any other layer of the kidney at any other times of reperfusion. After cold ischemia, renal blood flow fell during the first 60 min of reperfusion and remained at this level for 2 days. GFR was only 11% of nonischemic kidneys during the 2 days after reperfusion. Conclusion: Renal pg production is altered by cold ischemia and reperfusion and is dependent on species of pg, area of the kidney, and time of reperfusion. These changes may play a role in the loss of renal function after prolonged cold storage or may act as compensatory mechanisms in response to the damage.

PROLONGATION OF MURINE SKIN GRAFTS BY SELECTIVE INHIBITION OF LEUKOTRIENE PRODUCTION.

A. Harford, J. Goodwin, B. Baack, G. Shopp, L. Gibel, A. Smith, W. Sterling, University of New Mexico, Albuquerque, New Mexico.

The role of arachidonic acid metabolites, prostaglandins and leukotrienes, in transplant rejection has not been fully established. This project compares AA861 a selective 5' lipoxygenase inhibitor of leukotriene production, to hydrocortisone in their abilities to prolong murine skin graft survival across a major histocompatibility barrier (H-2). Tail skin grafts from AJ mice 8-12 weeks old were transplanted onto the dorsum of C57BL/6 mice utilizing suture grafting. There were 4 treatment groups each comprised of 15 animals. Group A was injected with hydrocortisone 20mg/kilo subcutaneously daily from the day of transplant onwards. Group B was injected with 5mcg/kilo intraperitoneally daily. Group C was injected intraperitoneally with 12.5mcg/kilo daily. Group D vehicle control received ethanol 10^{-5}M. intraperitoneally daily.

50% survival was prolonged from 13 days for Group D (ethanol control) to 16 days for Group B (AA861 100 mcg). In the higher dose AA861 Group (C) 50% survival was prolonged to 20 days a survival time equivalent to the 19.5 days achieved with hydrocortisone. Analysis of the survival curves indicated all treatments were significantly different from the ethanol control.

These data suggest an immunosuppressive effect of AA861 resulting in murine skin graft survival comparable to that achieved with hydrocortisone.

CYCLOSPORINE A (CsA), PREDNISOLONE AND AZATHIOPRINE INDUCE THE FORMATION OF A SHOCK PROTEIN IN CULTURED MYOCYTES.(introduced by R.A.K. Stahl)

Iris Löw, Thomas Friedrich* and Wilhelm Schoeppe Department of Medicine, Division of Nephrology, University of Frankfurt, F.R.G. and *Department of Medical Chemistry, Kyoto University, Japan

The formation of shock proteins is a cellular response to cell damaging stress. We investigated the ability of several immunosuppressants on their potential effects to induce de novo-synthesis of shock proteins. As a model system served isolated and cultivated cardiac myocytes. Cells were prepared by trypsin digestion from 18 days old fetal mice. These cells synthesize shock-proteins in a concentration-dependant manner after exposition to toxins. Prednisolone and azathioprine at concentrations of > 0.5 mg/ml and > 50 ug/ml induce the de novo-synthesis of a 30 kd polypeptide, a characteristic shock protein of mice myocytes. Cyclosporine A induces the formation of the same protein at concentrations > 10 ng/ml. This effect is due to the substance itself and not to the solvent (poly(oxyethylene)-40-ricin) which does not influence cellular protein-synthesis. Antibody solutions like anti-human-T-lymphocyte globulin do not induce shock protein synthesis.

The data demonstrate that CsA, prednisolone and azathioprine induce the production of a shock protein in cardiac myocytes, which might have a significant role in the response to cardiotoxins.

BENEFICIAL IMPACT OF ATRIAL NATRIURETIC PEPTIDE (ANP) ON RECOVERY OF CANINE RENAL AUTOGRAFT FUNCTION FOLLOWING 24 HR. OF COLD ISCHEMIA.

RM Lewis*, RP Janney*, RW Osgood*, JD McAndrew*, JP Martin* and TR Fried. Univ. Texas Health Sci. Centers Houston and San Antonio, Texas.

The impact of ANP on renal function following cold ischemic injury was studied in a canine autotransplant (autoTx) model. A baseline inulin clearance (Cin) was obtained from the left kidney which was then excised and cold-stored for 24 hr prior to autoTx. A 20' Cin determination was initiated 10' after reflow to the autograft (T10'-30'). Exp. animals (n=7) then received a lug/kg ANP bolus IV followed by continuous infusion at 0.3 ug/kg/min x 30' (αhANP, Penninsula Lab) with Cin measured throughout the entire infusion period (T30'-60'). Saline was substituted for ANP in control animals (n=9).

Cin (ml/min) increased from 0.3±.1 prior to ANP (T10'-30') to 3.3±.8 during ANP infusion (p=.009). Urine flow rate ($\dot{V}$) increased from 0.1±.5 ml/min to 1.1±.3 ml/min (p=.02). Mean syst. BPs (mmHg) prior to and during ANP infusion were 144±14 and 123±24, respectively (p=NS). Cin determined 24 hr after autoTx in 4 ANP-treated animals was equal to or exceeded values determined during ANP infusion at T30'-60' post-reflow in all 4 animals. Neither Cin nor $\dot{V}$ increased significantly in controls (0.4±.1 to 0.7±.2 ml/min and 0.2±.08 to 0.2±.05 ml/min, respectively).

In summary, ANP produced sustained enhancement of initial renal autograft function without causing significant hypotension. These results suggest a potential role for ANP in the amelioration of initial dysfunction of human renal allografts.

LOW DOSE ANTI-CD3, MEDIATED IMMUNOSUPPRESSION IS COMPLEMENT INDEPENDENT AND PRESERVES T-CELL RESPONSIVENESS

JD Mackie*, OG Pankewycz*, MG Bastos*, VE Kelley, TB Strom, Dept of Medicine, Beth Israel Hospital, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

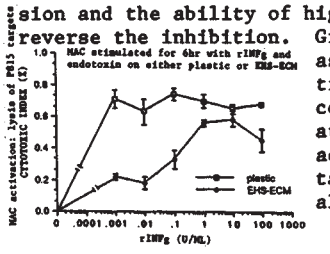
Anti-CD3 Mabs, potent mitogens in vitro, effectively abrogate transplant rejection in vivo. In this study we examine this apparent dichotomy by investigating the effects of a mitogenic murine anti-CD3 MoAb 145-2C11 in allograft rejection and delayed-type hypersensitivity (DTH). Crude pancreatic islets, prepared by collagenase digestion from DBA/2 ($H\text{-}2^d$) mice were transplanted into diabetic $B6AF_1$ ($H\text{-}2^{b/k,d}$) mice. Antibody treatment (5 ug/ip/qdx15) prolongs allograft survival as evaluated by a return of hyperglycemia. Even 0.5 ug of antibody given ip x 7 days profoundly suppresses DTH in BALB/c mice. DTH in complement deficient strains DBA/2J and A/J mice is similarily reduced. Purified T-cells from these animals proliferate normally in vitro in response to 145-2C11. These results suggest the action of anti-CD3 Mabs is not complement dependent and that in these lower doses is still an effective immunosuppressive agent while not causing profound inhibition of T cell function.

IMMUNOPHARMACODYNAMIC STUDIES OF CYCLOSPORINE A IN NORMAL VOLUNTEERS. Manfro RC,* Gupta S,* Tomlanovich S,* Pohanka E,* Benet LZ,* and Garovoy MR. Immunogenetics and Transplantation Laboratory, University of California, San Francisco. San Francisco, California.

The serum of patients on Cyclosporine A (CyA) has been shown to produce four different patterns of inhibition when added to third-party mixed lymphocyte reactions (MLR). (Rogers A.J. et al. Transplantation 38: 657-664, 1984). Pattern I shows a second peak of inhibition 8 to 12 hours after CyA administration, in pattern II this second peak does not occur. Pattern III shows a continuous high level of inhibition and, in pattern IV the inhibition is very poor. Patterns I and III cannot be explained by a second peak or continuous high levels of the parent compound, so we speculate that CyA metabolites could be causing these responses. Oral (10mg/kg) and intravenous (4mg/kg) CyA was administered one week apart to 8 normal volunteers. These individuals were drawn once before and after the admnistration of CyA at regular intervals over a 24 hour period. CyA and metabolites 1, 17, 18, and 21 were measured by high performance liquid chromatography and the effect of these individuals' sera on MLRs with their own cells or third-party cells, against a pool of cells of unrelated individuals was studied. Four patterns of response were observed. Pattern I in 4 experiments, pattern II in 8, pattern III in 2 and pattern IV in 4. Patterns I and III could not be explained by a second peak of CyA or by a concomitant elevated concentration of any of the evaluated metabolites. In conclusion, we observed in normal volunteers the patterns described in renal transplant recipients. Additionally, the patterns I and III cannot be atributed to a second CyA peak or to a concomitant elevated level of these metabolites. The cause of these patterns remains to be established and an active unidentified metabolite is still an attractive possibility.

EXTRACELLULAR MATRIX AS A REGULATOR OF MACROPHAGE FUNCTION: IMPLICATIONS FOR TRANSPLANT TOLERANCE. Dianne B. McKay* and Christopher Y. Lu. Renal Division, Brigham and Women's Hosp., and Harvard Medical School, Boston, MA.

A goal of transplant nephrology is to induce host unresponsiveness to donor alloantigens. This occurs naturally during pregnancy and understanding how this occurs may ultimately improve renal allograft survival. In the decidua basalis (DB) of the murine placenta, maternal and fetal cells are intermixed. Yet, there is no maternal immune response against fetal/paternal alloantigens. We have previously shown that local immunoregulatory forces prevent macrophages (MAC) from functioning in this region (JI 140:3947,1988). To further characterize these local forces, we now examine the ability of the extracellular matrix (ECM) of the DB to regulate MAC activation. Because of the biochemical similarity to DB-ECM, we used ECM produced by the more readily available Englebreth-Holm-Swarm (EHS) murine tumor. MAC cultured on EHS-ECM surfaces required at least 1000x more recombinant interferon gamma (rINFg) to be activated than MAC cultured on plastic (see FIG) or on fibronectin surfaces. The EHS matrix is non-toxic by trypan blue exclusion and the ability of high dose interferon to reverse the inhibition. Given that MAC function as both antigen-presenting and as effector cells, this novel regulatory pathway of MAC activation may be important in preventing fetal allograft rejection.



CYCLOSPORINE (CYA) INHIBITS THE PROLIFERATION OF LLC-PK1 CELLS IN CULTURE. Jerry McCauley*, Richard Ptachcinski*, and Sandra A. Murray*. (Intr. by M.I. Sorkin.) Univ. of Pittsburgh and VAMC, Pgh. PA

Nephrotoxicity is the major side effect limiting the use of cyclosporine in organ transplantation. Although acute and chronic renal failure, have been regularly seen with the use of this agent, the mechanism of its toxicity is poorly understood. Delayed recovery from acute tubular necrosis in CYA treated renal allografts suggests that inhibition of renal cell growth might play a role in CYA nephrotoxicity. We, therefore, studied the effects of CYA upon cell proliferation of an established proximal tubular cell line, the LLC-PK1 cell. These cells were cultured in the presence of increasing doses of CYA, and DNA was measured at intervals of 1,3,6,13, and 16 days. Concentrations of 0.01-0.1 µg/ml had no effect but cells exposed to between 1 and 50 µg/ml experienced a significant reduction in cell proliferation. After 16 days in culture the DNA determinations (mean ± SD) were:

CONTROL	1µ	10µ	50µ
0.627±0.01	0.389±0.02	0.486±0.01	0.463±0.01

In summary, CYA inhibits renal cell proliferation in culture. These findings may suggest an additional mechanism of CYA nephrotoxicity and the LLC-PK1 cell line provides a useful model for further study.

ANTI-CD4 MONOCLONAL ANTIBODY(BWH-4) PROLONGS RENAL ALLOGRAFT SURVIVAL IN THE RAT. E.L.Milford*, M.Sayegh*, J.Kut*, C.Kwok* and C.B. Carpenter. Brigham and Women's Hosp., Boston, MA.

We produced an IgG2a Balb/c anti WF rat CD4 monoclonal antibody (BWH-4 MoAb). This antibody precipitates a monomorphic 53kD polypeptide on rat cells, marks the same cell population as the W3/25 (CD4) MoAb, and inhibits allogeneic mixed lymphocyte proliferation. When one 5-7mg dose of 50% ammonium sulfate purified BWH-4 is injected i.v. into naive rats, antibody is present in serum at 6 but not 24hrs. Spleen, lymph node, thymic and peripheral lymphocytes had normal CD4 phenotype.

We studied the effect of BWH-4 on renal allograft survival in a high responder strain combination. LEW rats had bilateral nephrectomy and received a BN renal graft on day 0. Eight controls were unmodified, 8 received 7mg BWH-4 i.v. on days 0-4 and 7-11, and 2 controls received a similar course of mouse anti human (HB145) MoAb.

TREATMENT	SURVIVAL(DAYS)	MEAN SERUM CR(MG/DL) DAY7	DAY14	DAY21
UNMODIFIED	8,6,7,7,6,5*,5*,3*	8.2		
HB145	8,8	---		
BWH-4	34*,28,24*,23*, 22*,16,9,6*	2.2	2.2	2.5

(*=Sacrificed for in vitro studies)

Control animals rejected their grafts and died at day 6-8 of uremia. Animals treated with BWH-4 had stable renal function and prolonged survival. Graft pathology on 5 BWH-4 treated animals showed only mild to moderate mononuclear interstitial infiltrate. Immunoperoxidase staining showed both CD4+ and CD8+ cells. Lymph nodes and spleen showed a 50-80% decrease in CD4+ cells which normalized after the 2nd week.

We conclude that BWH-4 MoAb prevents acute allograft rejection and may provide an approach to long term tolerance.

ALTERED RENAL PROSTAGLANDIN (PG) AND THROMBOXANE (TX) PRODUCTION IN CYCLOSPORINE NEPHROTOXICITY. L. Persan*, F. Kaskel, R. Barnett, T. Wilson*, L. Moore, L. Arbeit, and D. Schlondorff. Depts. Pediatrics and Medicine, SUNY, Stony Brook, NY, and Dept. Med., Albert Einstein Coll. Med., NY.

Cyclosporine A (CYA) often causes renal insufficiency owing to renal vasoconstriction. To elucidate the role of renal vasoactive substances in CYA nephrotoxicity (CNTX), we measured plasma renin activity (PRA), aldosterone, and urinary and glomerular PG. Rats given 10 mg/kg CYA in olive oil i.m. for 1 week had higher PRA (4.9±1.1 ngAI/ml/hr vs 2.2±0.2 in controls, n=10/group, p<0.05), while aldosterone levels were not different (320±53 pg/ml vs 186±38, respectively). Renal function and PG were measured in pair-fed rats given 20 mg/kg CYA s.c. daily for 1 week.

		Urinary		Glomerular PGE2	
	GFR	PGE2	TXB2	basal	A23187
	ml/min	ng/min		ng/(mg prot. 10min)	
CONT	1.00	56.0	7.5	2.95	4.80
SE	±0.06	±11.7	±1.9	±0.51	±0.55
n	6	6	5	6	6
CNTX	0.79*	18.6*	16.5*	1.38*	2.28*
SE	±0.08	±3.4	±3.8	±0.14	±0.27
n	9	9	7	6	6

*p<0.05 CONT vs CNTX. Acute receptor blockade of TX with SKF 95587 (10 mg/kg i.v.) tended to improve renal function in chronic CNTX, but the effect was not significant. These findings are consistent with disturbances in the renin, aldosterone, prostaglandin axis in CNTX. The vasoconstrictor-vasodilator ratio is altered in CNTX, but this may not fully account for the renal vasoconstriction.

FURTHER CHARACTERIZATION OF SUPPRESSOR T CELL INDUCTION AND THE MECHANISMS OF IMMUNOSUPPRESSIVE DRUGS. DR Salomon, LL Pickard*, and MO Downs*. University of Florida, Gainesville, FL.

Understanding the nature of suppressor T cell (Ts) induction and the effect of CyA and Methylprednisolone (MP) on this process is a key step in unraveling the role of Ts in successful transplantation. We developed an assay for a unique MLR supernatant activity required for Ts activity called suppressor inducer factor (SIF). SIF has an apparent MW of 50-55 kd by gel filtration. A single band at 55kd was demonstrated by SDS-PAGE in a protein-free supernatant.

The SIF assay was used to probe the Ts induction circuit in the primary MLR. SIF is a CD4+ cell-product and is not constitutively produced by primed CD4+ but requires restimulation with alloantigen or mitogen. Primed Ts incubated in SIF incorporate little thymidine and do not proliferate. Thus, the SIF signal is Ts inductive not proliferative. Naive CD8+ cells cultured with SIF do not have Ts activity unless primed first with alloantigen or mitogen.

The addition of CyA (125-250 ng/ml) to the MLR abrogates proliferation yet permits Ts induction. However, the Ts activity generated is approximately 70% of an equal number of control primed CD8+ cells (n=9). The quantity of SIF generated in the CyA-modified MLR is at least twice that of the control. The CyA-modified Ts when moved to fresh media are less responsive than control Ts to a stock SIF. Thus, CyA appears to permit Ts induction while inhibiting Ts amplification. The addition of MP (10^{-7} M) to the MLR, also abrogated proliferation and Ts activity was approximately 30% of the control. In contrast to CyA, SIF activity was reduced by almost 50% as compared to control. Thus CyA and MP reduce the generation of Ts activity by two separate mechanisms, MP resulting in inhibition of Ts induction and CyA inhibiting Ts amplification. The significance of these insights into the way we use these drugs to prevent or treat acute rejection must be considered carefully.

THROMBOXANE (Tx) RECEPTOR ANTAGONIST ATTENUATES RENAL FUNCTION DETERIORATION IN ISOGRAFTED RATS GIVEN CYCLOSPORINE (CYA). Magda Rossini*, Aldo Belloni*, Norberto Perico*, Giuseppe Remuzzi*. Mario Negri Institute for Pharmacological Research, Bergamo, Italy (intr. by R.J. Glassock).

The mechanism by which CyA injures the kidney remains poorly understood. We investigated whether pharmacological inhibition of TxA2 activity reduces CyA nephrotoxicity in a rat model of renal isograft free of rejection processes. Four groups of renal isografted Lewis rats were considered. Group 1 and 2 received a daily oral dose of CyA (20 mg/kg) or CyA plus the Tx receptor antagonist GR32191 (Glaxo, 3 mg/kg b.d.) for 30 days. Group 3 and 4 were given GR32191 alone (3 mg/kg b.d.) or the vehicle for 30 days. No significant difference on body weight, urine output and Na excretion between groups throughout the 30-day experimental period was found. Serum creatinine and urinary excretion of TxB2 are reported in the table.

	S. creatinine (mg/dl)		TxB2 (mg/d)	
	Basal	day 30	Basal	day 30
CyA	0.53±0.06	1.03±0.08*	6.61±1.46	28.41±4.45*
CyA+GR32191	0.50±0.07	0.69±0.08**	7.05±1.47	28.12±3.37*
GR32191	0.52±0.06	0.54±0.05	6.81±1.23	7.57±0.81
Vehicle	0.51±0.05	0.53±0.07	6.52±0.92	8.05±1.93

Mean ± SD; n=8; **p < 0.05; *p <0.01 vs basal; p <0.01 vs CyA+GR32191.

We conclude that pharmacological suppression of TxA2 biological activity improves renal function in isografted rats given CyA, and suggests that drugs blocking TxA2 receptor may be of benefit in reducing CyA nephrotoxicity in transplant patients.

IN VITRO INDUCTION OF RAT CD8+ T SUPPRESSOR CELLS REQUIRES CD4+ CELLS AND DIRECT ANTIGEN CONTACT M.H.Sayegh*, A.H.Frankel*, D. Rothstein*, C.Kwok*, E.L.Milford* and C.B.Carpenter. Brigham and Women's Hospital. Boston, MA.

Incubation of WF strain T lymphocytes with irradiated* LEW strain stimulator cells generates at day 5 OX8+(CD8+) antigen specific suppressor cells which when irradiated (1000 Rads) and added (at 20% of responder population) to a fresh WF anti LEW* mixed lymphocyte culture (MLC) suppresses the proliferative response (J. Immunol 131:1065).

We now report on the interactions involved in the induction of MLC generated suppressor cells. Depletion of W3/25+ (CD4+) cells from the responding population (<1% CD4+ contamination) at day 0 of the generating MLC prevents suppressor cell induction. Reconstitution of this CD4+ depleted MLC with at least 10% naive WF CD4+ cells at day 0 restores suppressor cell induction; irradiation (1000Rads) of these CD4+ cells abrogates this restorative capacity.

GENERATING MLC	% SUPPRESSION (±SD)
WFxLEW*(n=16)	72 ± 26
CD4+ DEPLETED (n=10)	-21 ± 42
" + >10% CD4+(n=4)	48 ± 15

We next used a transwell culture system with two chambers separated by a semipermeable membrane allowing cells to be co-incubated without direct cell to cell contact. WF anti LEW* MLC in one chamber releases a soluble factor which is capable of inducing naive CD8+ cells in the other chamber to become active T suppressor cells, but only if these CD8+ cells are in direct contact with LEW* stimulator cells.

We conclude that there is a suppressor inducer cell within the CD4+ T lymphocytes which on contact with alloantigen releases a suppressor inducer factor. Induction of CD8+ suppressor cells requires both the presence of this factor and direct antigen contact.

EFFECT OF CYCLOSPORINE A (CsA) ON MESANGIAL CELL (MC) GROWTH AND ADHESION IN CULTURE. K. Servilla, T. Cook*. University of Utah School of Medicine, Salt Lake City, UT.

A marked decline in GFR is recognized as a major problem of CsA. Since the glomerular ultrafiltration area, a major determinant of GFR, is felt to be governed by mesangial cell tonicity, we studied the effects of CsA on rat MC in culture using phase contrast and electron microscopy (EM), tritiated thymidine (^{3}HT) labeling assays to measure proliferation and metabolic labeling of MC to analyze proteoglycan production by SDS PAGE. MC were isolated and characterized according to accepted techniques. Quiescent MC were induced to cycle at the beginning of each study. Experimental groups consisted of MC incubated with 12.5µg/ml, 25µg/ml or 50µg/ml of CsA for 24 or 48 hr, and MC exposed to the same CsA schedule followed by removal of CsA and a 24 or 48 hr recovery period. Media containing 20% or 0.5% serum were used as + and - controls. Vehicle was used in each control group in equivalent volumes. MC viability was ascertained by dye exclusion. CsA in a dose response fashion by phase contrast microscopy resulted in alteration of attachment and rounding of MC bodies without cell death. At doses <50µg/ml this was reversible. By EM CsA caused a decrease in number and orientation of microfilaments. ^{3}HT labeling of MC was significantly depressed at doses >12.5µg/ml CsA. Removal of CsA resulted in incorporation of ^{3}HT. Proteoglycan synthesis by MC appeared to be impaired by CsA. These findings indicate that CsA may damage MC, alter MC cytoskeletal function and matrix composition resulting in changes in MC tonicity and ultimately GFR. Thus alteration in MC growth capacity and matrix may be another mechanism of CsA nephrotoxicity.

CD4+ KILLER CELLS: A NOVEL LYMPHOCYTE SUBSET INVOLVED IN ALLOGEN RESPONSE. G.Sunder-Plaßmann, L.Wagner, F.Stockenhuber, and P.Balcke. University of Vienna, 1rst Dept.of Medicine, Austria.

Infiltrating CD4+ lymphocytes are frequently found in rejected human kidney allografts. However, their effector function is not yet clear. Today it is generally assumed that lymphocytes, capable of mediating target lysis, have to be equipped with cytoplasmic granules containing a substrat specific serine esterase (SE) in order to initiate the lethal hit. Using a novel cytochemical staining method for this cytotoxicity linked SE (BLT as substrate and Fast Blue BB salt as capture agent) the peripheral blood CD4+ T cells of 100 graft recipients were investigated for being equipped with a cytotoxic apparatus. When stable graft function was present, the SE+CD4+ T cells accounted for about 8% of the CD4+ lymphocytes. During acute rejection episodes this values increased up to 60%, being strikingly increased in comparison to healthy volunteers, in whom only 2% of the CD4+ T cells are SE+. Obviously this cells are involved in the immune respnse in organ transplantation. It is most likely, that CD4+SE+ T cells display in situ cytolytic activities and represent a great deal of the CD4+ lymphocytes found in surprisingly high numbers in transplant biopsy specimens of patients with rejection episodes.

THE ADDITION OF ATRIAL NATRIURETIC FACTOR DURING COLD STORAGE PRESERVATION OF CANINE KIDNEYS WITH COLLINS SOLUTION. Nicholas T. Stowe, Mark Moran, Emmanuel L. Bravo, Erdal Erturk*, Arturo Martinez*, David Goldfarb*, Magnus O. Magnusson*, Andrew C. Novick. Cleveland Clinic Foundation, Cleveland, OH, and G.D. Searle and Co, Skokie, IL.

Atrial natriuretic factor (ANF) is known to increase glomerular filtration rate especially in vasoconstricted kidneys. This property makes ANF attractive as a protective agent in kidney preservation and transplantation. The purpose of this study was to test the effect of ANF given during cold storage renal preservation on renal function following autotransplantation in a canine model. In a control group (n=8), kidneys were harvested from anesthetized dogs, flushed with Collins C-2 solution and stored for 24 hrs at 5°C and then autotransplanted with a contralateral nephrectomy. In the ANF treated group (n=9), the kidney at the time of harvest was immediately flushed with 60 mcg of atriopeptin 24 (AP24). Three animals died in each group from uremia. Serum creatinine levels peaked in the control group at day 3 at 3.4±1.5 mg% vs 5.3±3.3 mg% in the AP24 group (NS). At day 22 serum creatinine levels returned to control values in both groups. Renal venous ANF levels were measured in both groups by radioimmunoassay.

	ANF Levels (pg/ml) After Reperfusion			
	0 min	5 min	15 min	45 min
Control (n=4)	12±3	11±3	7±2	9±3
AP24 (n=6)	20±3	2495±1706	166±54	107±46

Following 24 hr storage with ANF, ANF was still present in the kidney and was released into the circulation upon reperfusion.

SYNERGISTIC SIGNALING VIA THE T-CELL ANTIGEN RECEPTOR AND CD2 ANTIGEN. M. Suthanthiran. The Rogosin Institute, CUMC, New York, New York.

The role of the T-cell antigen receptor (TCR-1) and differentiation antigens CD2, CD4 and CD5 in transmembrane signaling was investigated with monoclonal antibodies (mAbs) and highly purified T-cells (>98% CD2+). Here we report that mAbs directed at a framework determinant of TCR-1 and mAbs directed at the SRBC-binding epitope of the CD2 antigen are synergistic in promoting the expression of interleukin-2 receptors (CD25) and proliferation of T-cells. The proliferative response is strictly dependent upon the presence of both anti-TCR-1 and anti-CD2 and upon crosslinking of mAbs and exhibits a high degree of specificity in that additional combinations of mAbs ([anti-TCR-1 + anti-CD5 or anti-CD4] or [anti-CD2 + anti-CD5 or anti-CD4]) fail to activate T-cells. T-cell proliferation induced with crosslinked anti-TCR-1 and anti-CD2 is IL-2 dependent since mAb anti-CD25 inhibits such proliferation and recombinant IL-2 reverses anti-CD25 associated inhibition. Moreover, T-cell activation accomplished with anti-TCR-1 and anti-CD2 is inhibitable with EGTA and with three different inhibitors of protein kinase C. Collectively, these observations indicate a novel pathway for the antigen-dependent activation of T-cells in which complementary signals initiated via the TCR-1 and CD2 antigens promote the activation of immunocompetent T-cells. Abrogation of generation and/or transduction of signals via the TCR-1 and/or CD2 antigen therefore has the potential to mitigate anti-allograft responses. Indeed, cyclosporine inhibits (half-maximal inhibitory concentration: 37 ng/ml) T-cell proliferation accomplished by transmembrane signaling via TCR-1 and CD2 antigen.

INFLUENCE OF CYCLOSPORINE ASSAY METHOD IN THE ACUTE, POST-TRANSPLANT PERIOD. J. Joseph Walshe, Mark T. Holdsworth*, and Gene D. Morse*. SUNY at Buffalo, Depts. of Pharmacy and Medicine, Buffalo, N.Y.

The use of high performance liquid chromatography (HPLC) for identifying the parent compound versus the radioimmunoassay (RIA) which measures certain cross-reactive metabolites is controversial. To examine the influence of assay method in the acute, post-operative period, eight renal transplant recipients were studied after intravenous and oral cyclosporine on up to four occasions. Whole blood was analyzed by HPLC and RIA. Intravenous kinetic analysis yielded a mean total body clearance of 0.24 ± 0.2 L/min (RIA) and 0.31 ± 0.1 L/min (HPLC) ($p > 0.05$), a mean volume of distribution of 2.17 ± 0.6 L/kg (RIA) and 2.75 ± 1.2 L/kg (HPLC) ($p > 0.05$) and a mean half-life of 11.7 ± 4.4 h (RIA) and 12.8 ± 3.8 h (HPLC) ($p > 0.05$). The mean bioavailability was 0.36 ± 0.23 (RIA) and 0.28 ± 0.15 (HPLC) ($p > 0.05$). Regression of the 12 h cyclosporine (RIA vs HPLC) concentration was described as follows; RIA = 72 + 1.6 (HPLC). The mean ratio (RIA/HPLC) of the area under the blood cyclosporine concentration versus time curve was 1.6, 1.5 and 1.7 during the oral study periods, and was poorly correlated with the serum creatinine. Overall, the two assay methods provided similar pharmacokinetic estimates. However, RIA cyclosporine levels yielded an overestimation of the 24 h AUC by HPLC. Therapeutic monitoring of the parent drug in the acute, post-operative period may best be accomplished by HPLC analysis.

CYCLOSPORINE-INDUCED HYPERTENSION (CIH) IN THE CONSCIOUS SHEEP IS MODIFIED BY THROMBOXANE SYNTHESIS INHIBITION (TSI). Judith A. Whitworth*, Janette Tresham*, John F. McDougall*, Bruce A. Scoggins*, William M. Bennett. Howard Florey Inst of Exper Physiol & Med. Royal Melbourne Hosp., Parkville, Victoria, Australia.

CIH is frequent clinically but only inconsistently occurs in anesthetized animal models. Whitworth et al reported increased mean arterial pressure (MAP) in conscious sheep given CSA, 12 mg/kg IV for 3-5 days without reduced glomerular filtration rate (GFR), effective renal plasma flow (ERPF) or increased plasma renin activity (Clin Exp Physiol Pharm 14:573,1987). Since thromboxane production increases in CSA treated patients and rodents, we studied U63,577A (Upjohn), a selective TSI, before and after CIH. Merino-cross ewes (35-45 kg) with externalized carotid loops for hemodynamic measurements were maintained on constant Na and K diets. U63,577A, 30 mg/kg q 12h was given to control animals and after 3 days of CIH produced by 12 mg/kg/day i.v. CSA. MAP, cardiac output (CO), calculated peripheral resistance (PVR), GFR and ERPF were measured:

	PreCSA	CSA	PreTSI	TSI	CSA + TSI
MAP (mmHg)	73	90*	68	69	79
GFR (ml/min)	52	59	59	56	58
ERPF (ml/min)	429	560	420	379	369
PVR (mmHg.min/L)	16	21*	12	11	14

n=4 or 5 for each experiment. *$p<0.05$ vs PreCSA, and CSA + TSI. CSA and TSI had no effect on CO. CONCLUSION: Under resting conditions, TSI has no physiological effect however, with CSA it may modify MAP and PVR, suggesting a role for thomboxane in CIH.

THE IMMUNOSUPPRESSIVE AND B-CELL LYMPHOMA PROMOTING PROPERTIES OF CYCLOSPORINE MAY BE LINKED. G Walz*, B Zanker*, E Hadro*, KH Wieder*, TB Strom, Department of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA

The properties of interleukin-6 (IL-6) as a growth and differentiation factor for T- and B-cells have been documented. IL-6 also facilitates IL-2 release and restores the ability of macrophage-depleted T-cells to proliferate in response to PHA or antibodies against the T-cell receptor complex. We showed that cyclosporine (CsA) blocks the IL-6 mediated T-cell proliferation. However, CsA (0.5-2.0 µg/ml) or verapamil (Vera) (30-250µM), two agents known to inhibit IL-2 gene expression, actually increased IL-6 transcription in PHA/phorbol ester stimulated peripheral blood mononuclear cells in a dose dependent fashion, and prolonged the duration of detectable IL-6 mRNA. Moreover, increased IL-6 bioactivity was detectable in supernatants of cell cultures treated with Vera/CsA, thereby excluding a post-transcriptional blockade of IL- 6 expression. Increased IL-6 expression in CsA treated graft recipients could contribute to the increased incidence of B-cell lymphomas by supporting the growth of EBV-transformed B-cells. Though CsA abrogates IL-6 mediated T-cell proliferation, it may be prudent to use CsA in combination with a drug that prevents CsA induced IL-6 gene expression.

SANDIMMUNE® AND PLATELET AGGREGATION IN VITRO
D. Wiesinger*, J. Mason®
(introduced by L.C. Moore)
Preclinical and Clinical Research, Sandoz, Basle.

Sandimmune® has been suggested to enhance platelet aggregability in transplant patients in vivo. To investigate this possibility further, platelet aggregation to ADP, that depends mostly on an increase in Ca influx, was examined in vitro with and without Sandimmune®. Platelet-rich plasma from varying species was preincubated with Sandimmune and platelet aggregation was assessed with an aggregometer.

An enhancement of the aggregatory response after incubation with Sandimmune® was not seen in platelets from rats, was present but variable in those from man and dogs and extremely constant and reproducible in those from guinea pigs.

In guinea pig platelets the height of the submaximal aggregatory response to 0.2 µmol/l ADP increased after 60 min incubation at 37 °C with 0.1 and 1.0 µg/ml Sandimmune® from 75 ± 5 mm to 99 ± 10 and 167 ± 7 mm, respectively.

It is concluded that in guinea pig platelets acute exposure to Sandimmune® at therapeutically relevant concentrations leads to an enhancement in platelet aggregation that may reflect the increased passive and receptor-operated Ca influxes reported in smooth muscle cells (1,2).

1. Pfeilschifter et al. Biochem. J. 248: 883, '87
2. Meyer-Lehnert et al. Kidney Int. 31: 464, '87

CYCLOSPORIN THERAPY IMPAIRS ENDOTHELIUM-DEPENDENT RELAXATIONS IN THE RENAL CIRCULATION. Zhihong Yang*, Dennis Diederich, Fritz R. Bühler* and Thomas F. Lüscher. Dept. of Research and Medicine, University Hospital, Basel/Switzerland

Cyclosporin A (CyA) is an immunosuppressive substance which causes structural and functional changes of endothelial cells. The endothelium can modulate vascular tone by the release of endothelium-derived relaxing (EDRF) and constricting factors (EDCF). Male Wistar Kyoto rats (20-24 weeks) were treated with CyA 50 mg or 30 mg s.c./day, the solvent or saline for 1 or 2 weeks, respectively. CyA did not cause significant increases in blood pressure. Renal artery rings were suspended in organ chambers filled with physiological salt solution (37°C; 95% O_2/5% CO_2); isometric tension was recorded. In saline treated rats, acetylcholine (ACh) induced relaxations in rings with, but not in those without endothelium (71±7%; n=9). The sensitivity and maximal response to ACh was impaired in rats receiving either 30 mg CyA for 2 weeks or 50 mg for 1 week (13±10% and 31±11%, respectively; $p<0.05$; n=9 and 22, respectively). The solvent also tended to reduce the relaxations. Inhibition of cyclooxygenase with either indomethacin or meclofenamate (10^{-5}M) augmented the response in CyA rats. Thus, (1) ACh releases EDRF in the rat renal artery; (2) chronic CyA therapy blunts endothelium-dependent relaxations in the renal circulation and (3) an endothelium-derived cyclooxygenase product interferes with the action of EDRF in CyA treated rats.

ATRIAL NATRIURETIC FACTOR (ANF) PROTECTS COLD ISCHEMIC INJURY IN THE RAT KIDNEY. H.Z. Zhou*, J.I. Shapiro, R.W. Schrier, L. Chan. Dept. of Med. Univ. of Colorado Med. Sch. Denver, CO.

We have previously shown that ANF was effective in ameliorating ischemic acute renal failure in the isolated perfused kidney (IPK) and in vivo in the rat (J Clin Invest 80:698, 1988). To assess the effect of ANF in a transplant setting, the IPK model was used to investigate the effect of ANF on renal function following cold ischemia. 3 groups of kidneys were studied: Group I was a control in which the kidney was flushed in situ with Collins solution at 0°C, removed and stored at 0°C for 4 hr and then perfused under constant pressure (100 mmHg at cannula tip) at 37°C for 1 hr. In group II, ANF was added to the flush-solution at a concentration of 1 µg/30 ml. In group III, ANF was added both to the flush-solution (1 µg/30 ml) and to the perfusion medium (1 µg/100 ml). Results are summarized as follow:

	C_{In}(µl/min/g)	T_{Na}(µmol/min/g)	V(µl/min/g)
I (n=6)	173 ± 16	19 ± 2	40 ± 7
II (n=6)	365 ± 26**	45 ± 7*	66 ± 5
III(n=6)	385 ± 33**	42 ± 7*	103 ± 6**

Both ANF groups II and III showed significantly higher inulin clearance (C_{In}) and net tubular Na^+ reabsorption (T_{Na}) than the control group I. These data were confirmed in vivo with a corresponding improvement in C_{In} (26±4 vs 100±10µl/min/g, n=6 and $P<.05$) and T_{Na} (3±1 vs 13±2 µmol/min/g, $P<.05$). ANF appears to exert a protective effect on renal function in models of cold ischemia. Better protection from ischemic injury might be therefore afforded by inclusion of ANF in a flushing-solution for preservation in human kidney transplantation.

DEXAMETHASONE BLOCKS INTERLEUKIN-6 (IL-6) GENE EXPRESSION B. Zanker*, G. Walz*, K. Wieder*, L. Melton, T.B. Strom, Dept. of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA

Interleukin-6 is a powerful mediator of proliferation and differentiation of immunocompetent lymphocytes. IL-6 is predominantly produced by macrophages and substitutes for accessory cell functions in PHA and anti-CD3 induced T-cell proliferation. On the level of gene expression, anti-proliferative doses of Cyclosporine A (CsA) inhibit IL-2 gene transcription, but not IL-6 gene expression and IL-6 protein synthesis. Hence, CsA blocks T-cell function by blocking the signals transduced by IL-6/macrophages. We have determined that dexamethasone (DM), a powerful immunosuppressive drug, mainly acts on macrophages. DM blocks transcription of IL-6 mRNA in vitro using clinically achieved drug concentrations. Hence, DM blocks T-cell proliferation at a step proximal to CsA's action. These findings provide a rationale for the combined use of CsA with glucocorticoids in order to achieve and maintain immunosuppression in renal transplant recipients.

SUCCESSFUL, NON-NEPHROTOXIC ANTIBIOTIC PROPHYLAXIS FOR PERIRENAL TRANSPLANT INFECTIONS. M. Bomba*, A. Sagalowsky*, I. Dawidson*, R. Toto, J. Thompson, and J.H. Helderman. UT Southwestern and Parkland Memorial Hospital (PMH), Dallas, TX.

Prior to the widespread use of perigraft prophylactic antibiosis, post-surgical wound infections were serious complications occurring frequently. From 1970-1977 wound infection rates averaged 18% at PMH after which a prophylactic protocol was instituted. Thereafter, wound infection rates have been rare, from 0-4%/yr. Post-surgical acute renal failure (ARF) in the cyclosporine era has become an important, negative predictor. For 1987, 1 yr graft survival at PMH was 80% but was 95% with initial function and 52% with delayed function. One strategy to delimit the consequences of primary nonfunction has been to minimize potential nephrotoxic insults when cyclosporine is employed. Our study was designed to examine the capacity to substitute non-nephrotoxic gram negative antibiosis with the monobactam aztreonam for the more nephrotoxic aminoglycosides as part of a prophylactic antibiotic regimen. All consenting adult renal transplant recipients at PMH were prophylaxed with a combination of vancomycin, one gm IV x1, and aztreonam, 1 gm Q8H x3d. During this period we observed a reduction in ARF from 15% to 8%. We have observed two wound infections in 1987 and 1988.

We conclude that it is possible to successfully prevent post-surgical wound infections with non-nephrotoxic antibiotics, an important clinical management advance in the cyclosporine era.

GRAFT SURVIVAL DIFFERENCES WITH AND WITHOUT CYCLOSPORINE TREATMENT IN LIVING RELATED RENAL TRANSPLANT RECIPIENTS. Randall R. Bovbjerg*, The Urban Institute, Washington, D.C.*, Robert A. Wolfe, University of Michigan*, Philip J. Held, The Urban Institute*, Nathan W. Levin, Henry Ford Hospital, Detroit, MI, Friedrich K. Port University of Michigan.

Using the Cox proportional hazards model and data from the Medicare system, we analyzed the impact of cyclosporine (CSA) on graft survival for 5,457 living-related renal allografts performed between 1982 and 1986. Covariates included age, sex, diagnoses, HLA and DR matches, pretransplant blood transfusions, donor and recipient race, immunosuppressive therapy, and a measure of the transplant center's past success (center effect). The analysis was performed separately for the 1982-83 (n=2,985) and the 1984-86 (n=2,472) period.

In the 1982-83 period (pre-licensure of CSA), only 8.6 percent of the sample received cyclosporine, and the relative risk of graft failure was 2.01 ($p<0.01$) compared to patients with other immunosuppressive therapies and with other covariates held constant. In the 1984-86 period, 41 percent of the sample received cyclosporine, and the relative risk of graft failure was 1.29 ($p<0.05$). These findings reflect increasing experience in the use of a nephrotoxic agent and indicate that LRT patients receiving CSA have a higher failure rate. It will be important to determine whether this difference is due to unmeasured patient characteristics or due to CSA.

OKT3 PROPHYLAXIS (OKT3-P) IN RENAL TRANSPLANT (TXP) PATIENTS (PTS) WITH DELAYED GRAFT FUNCTION (ATN). DJ Cohen, J Cianci*, AI Benvenisty*, MA Hardy*. Columbia Univ, NY, NY

ATN may be associated with decreased allograft survival. Early use of cyclosporine (CYA) in ATN pts may lead to prolonged ATN and difficulty in diagnosis of rejection. In 1985-6, all cadaver kidney recipients received CYA on d1-2 post-txp. In 1987, only pts with immediate graft function (non-ATN) began CYA on d1, while ATN pts began OKT3-P (5 mg/d, iv x 10-14 d) 24-48 h post txpl. CYA was held until ATN resolved, with 3 d overlap CYA/OKT3. All pts began prednisone + azathioprine immediately pre-txp. We compared allograft survival, duration ATN, and time to 1st rejection in 1987 ATN (OKT3-P) pts (n=18) to 1985-6 ATN pts (early CYA) (n=40). One yr allograft survival in OKT3-P pts was 78% vs 55% for 1985-6 ATN (=early CYA) pts ($p=0.057$). Mean $\pm$ SD duration ATN was shorter (8.5d$\pm$3.7d vs 14.9$\pm$10.2d ($p=0.001$) and median time to first rejection episode longer (28.5d vs 11.0d, $p=0.003$) for OKT3-P vs non OKT3-P pts. There was no significant difference in 1 yr allograft survival for non-ATN pts 1985 vs 1986 vs 1987 (combined = 78%). We conclude that compared to early CYA in ATN pts, OKT3-P leads to decreased duration ATN, increased time to first rejection, and increased allograft survival.

CYCLOSPORINE A & PREDNISONE VS. CYCLOSPORINE A & AZATHIOPRINE & PREDNISONE IN FIRST CADAVERIC RENAL TRANSPLANTS. K. Brinker, *R. Dickerman, P. Vergne, R. Velez, D. Nesser, *J. Langley, *G. Trevino, D. Long, A. Hull, and T. Gonwa, Methodist Medical Center, Dallas, Texas.

This prospective study continues to compare 2 immunosuppressive regimens in 1st cadaveric renal transplants. Patients randomly receive cyclosporine (CsA) & prednisone (P) (Group A) or CsA & azathioprine (AZA) & P (Group B). We report on the 1st 161 patients entered (A85,B76)

The two groups have not differed as to age, sex, HLA matching, or cold ischemia time. Table I shows that 12 mo. graft survival, graft function, incidence of rejection, and rehospitalization rate are not different.

	Graft Surv.	Serum Cr	I125 Clear.	1st Rej.	2nd Rej.	Rehosp. Rate
Table 1						
Group A	83%	1.6±0.1	49±3	61%	20%	62%
Group B	77%	1.7±0.1	48±3	68%	20%	68%
p value	.81	.44	.72	.34	.97	.61

The incidence of primary non-function (1°NF) has been similar (A 18/85, B 20/76, p .80). Significantly more diabetics (D) have been entered into Group B (A 14/85, B 24/76 p=.02). However, Table 2 shows that the two groups have similar graft survival in these patient subsets.

Table 2 (12 mos. Graft Survival)

	Non-D	D	No 1° NF	1° NF
Group A	81%	86%	86%	72%
Group B	84%	61%	82%	65%
p value	.50	.21	.81	.80

To date, this study shows no advantage of triple therapy over double therapy in 1st cadaver renal transplants.

NIFEDIPINE IS A MORE EFFECTIVE RENAL VASODILATOR THAN CAPTOPRIL IN CYCLOSPORINE (CSA) TRANSPLANT (TX) HYPERTENSION. Curtis JJ, Laskow DA*, Julian BA, Luke RG, Jones P*, Diethelm AG*.University of Alabama Medical Center, Birmingham, AL

We measured renal blood flow (ERPF) and blood pressure (MAP) in nine hypertensive CSA treated renal tx patients (study patients [S pts]) and six normotensive tx pts (controls [C pts]). Renal vascular resistance (RVR=MAP/ERPF) and total peripheral resistance (TPR=MAP/Cardiac Output [CO]) were calculated. S pts baseline MAP was 114±5 mm Hg (mean±sem), on 377±55 mg/day of CSA, with a whole blood level (WBL) on CSA of 430±76 ng/ml and elevated RVR (55±5 units). C pts baseline MAP was 102±2 mm Hg on 273 mg/day of CSA, with a WBL of CSA of 280±88 ng/ml and low RVR (34±1.5 units). Both groups were studied for three 2 day study periods: off antihypertensives (baseline) on captopril 25 mg tid and on Nifedipine 10 mg tid. The drug intervention was randomized in a crossover fashion. At the completion of each period the patients had measurement of ERPF (I^{131} orthoiodohippurate), MAP (mean of 5 measurements), GFR (technetium DPTA), and cardiac output (by Ultracom™). Neither drug significantly changed blood pressure, RVR, or TPR in C pts.

Change from baseline	RVR	TPR	GFR	CO	ERPF	MAP
Nifedipine	-13%**	-19%**	-3%	+9%	0%	-13%**
Captopril	0%	-5%	-8.5%*	-5%	-10%	-9%**

**= p<0.01 *= p<0.05, others p=ns

Both drugs significantly decreased MAP from baseline in the S pts. Only Nifedipine, however, significantly decreased RVR and TPR from baseline in the S pts.

We conclude: (1) in CSA treated tx pts with elevated RVR, Nifedipine lowers MAP and RVR while captopril lowers MAP without changing RVR. (2) Unlike its effects on ERPF in essential hypertension and non CSA tx hypertension, captopril decreased ERPF and GFR in CSA treated hypertensive tx pts.

EVALUATION OF FINE NEEDLE BIOPSY (FNAB) IN THE DIAGNOSIS OF RENAL TRANSPLANT DYSFUNCTION. G. Danovitch, C.C. Nast, T. Rosenthal*, A. Wilkinson*. Division of Nephrology and Division of Urology; UCLA School of Medicine, Los Angeles, CA, and Department of Pathology, Harbor UCLA Medical Center, Torrance, CA.

Sixty-two patients in their early post-transplant course underwent FNAB during 107 episodes of graft dysfunction in order to assess the diagnostic value of this new technique. For each episode a clinical diagnosis was made, frequently with histological confirmation and compared to the FNAB result which was made independently. Twenty-three (21%) of the aspirates provided inadequate material and were discarded. Of 35 episodes diagnosed as rejection (16 biopsy-proven), 26 (74%) showed confirmatory immune activation on FNAB with a mean total corrected increment (TCI) of 5.6 ± 0.7 (SEM). Of 49 episodes diagnosed as 'ATN' or cyclosporine toxicity (16 biopsy-proven), 46 (94%) showed absence of immune activation frequently with evidence of tubular damage or cyclosporine effect (mean TCI 1.6 ± 0.2). Comparison between FNAB and core biopsy diagnoses yielded a 75% concordance. Thirteen aspirates yielded diagnoses that were discrepant from those made clinically. Vascular rejection, aspiration of medullary samples and non-rejection mediated lymphocytosis were considered as some potential causes of this discrepancy. FNAB should not be used as a sole diagnostic arbiter. It is a valuable adjunct to the management of renal transplant patients as long as its accuracy is not overestimated and its pitfalls are recognized.

OKT3-INDUCED PULMONARY EDEMA IS NOT AN IDIOSYNCRATIC REACTION AND DOES NOT PRECLUDE RETREATMENT WITH OKT3. John J. Dillon ,* Clara H. Danziger,* and William F. Finn. Univ. of North Carolina, Dept. of Medicine, Chapel Hill, N.C.

Severe pulmonary edema is a side effect of treating allograft rejection with the monoclonal antibody OKT3. We present here a case in which a renal transplant patient who developed first-course pulmonary edema was subsequently treated with a second course of OKT3.

A 19 year old male developed acute rejection and received his first dose of OKT3 16 days after receiving a cadaveric renal transplant. He was euvolemic on exam and was within 0.4 kg. of his admission weight, but his chest xray showed a small left pleural effusion and slight pulmonary vascular redistribution. Within 24 hrs. of receiving OKT3 he developed pulmonary edema and required intubation, but recovered after fluid removal by ultrafiltration and completed the OKT3 course. Five and one half months post-transplant he developed acute rejection and failed to respond to methylprednisolone 500 mg. i.v. q.d. x 4. Although euvolemic on exam, his chest xray demonstrated small bilateral pleural effusions. After fluid removal by ultrafiltration he was treated with a course of OKT3, did not develop pulmonary edema and had resolution of rejection.

The failure of pulmonary edema to develop with the second course of OKT3 argues against an idiosyncratic patient-drug interaction as the cause of OKT3-induced pulmonary edema and supports the current hypothesis linking this complication to fluid overload.

INTERACTION OF DILTIAZEM (DIL) AND NIFEDIPINE (NIF) WITH CYCLOSPORINE (CS) IN RENAL TRANSPLANT RECIPIENTS. C. Diaz, and D.M. Gillum,Dept. of Medicine,Baylor College of Medicine,Houston,Tx.

Recent evidence supports a variety of interactions between calcium channel blocking (CCB) agents and CS including enhanced immunosuppression, elevated CS blood levels, and protection against CS nephrotoxicity. We performed a retrospective review of renal transplant recipients receiving concurrent CS and CCB therapy. Thirty nine patients were included in the study all of whom were treated with CS and low dose prednisone. Fifteen patients were receiving concurrent NIF for hypertension while seven were treated with DIL, and the other seventeen were age and sex matched controls. Mean followup was 11.2±5.1 months and no data was included from the first month post transplant. Parameters evaluated over the period of followup included mean CS dose and whole blood levels, serum creatinine (Scr), episodes of acute CS nephrotoxicity, and incidence of acute allograft rejection (AR).

	CS Dose (mg/d)	CS Level (ng/ml)	Scr(mg/dl)
NIF	297.41±44.78	434.57±40.04+	1.91±.24
DIL	282.63±25.27	344.41±41.64+	1.94±.3
CON	286.69±33.63	250.30±12.96	1.89±.4

The incidence of acute CS nephrotoxicity was 0% in the NIF group, 14.2% for the DIL group and 29.4% in controls. The annual incidence of acute rejection was not different among the three groups.

We conclude that both NIF and DIL raise CS blood levels, and that both agents protect against the development of acute CS nephrotoxicity. Despite the elevated CS blood levels, there was no apparent effect of the CCBs on the incidence of acute rejection. + $p < .01$

THE USE OF ELDERLY LIVING DONORS. Per Fauchald*, Halvard Holdaas,Dagfinn Albrechtsen* and Audun Flatmark*.Univ.Hospital,Dept.of Medicine and Surgery,Rikshospitalet,Oslo,Norway.

Age greater than 55 years has been given as guideline for exclusion as living donor in kidney transplantation(Ann Int Med,1987,106,719-27).The aim of this study was to evaluate the use of elderly living donors.

Between 01.03.85 and 01.01.87 235 living donor transplantations were performed in Norway and 70 (30%) of these donors were 60 years or older(mean age 66.2 years,range 60-81 years).The donors and recipients were followed till 01.03.88 and none were lost to follow-up.One donor suffered from a non-fatal myocardial infarction postoperatively. No other serious postoperative complications occured.One year actuarial graft survival in the group of 1 haplotype mismatched grafts was 90% (n=101) with donors under the age of 60 years compared to 89%(n=49) with donors above 60 years. Mean serum creatinine(µmol/l) at 1 year was 153.7 in recipients of grafts from donors under the age of 60 years compared to 218.8 in recipients of grafts from elderly donors($p<0.01$).Similar results were found in the smaller groups of HLA-identical and 2 haplotype mismatched grafts.

The use of elderly living donors is safe and 1 year graft survival is equal to younger donor grafts.Recipients of grafts from elderly donors have higher serum creatinine at 1 year.

IMMUNOLOGIC MONITORING DURING RETREATMENT WITH OKT3. M.R. First, T.J. Schroeder,* P.E. Hurtubise,* M.E. Monsour,* Univ. of Cincinnati Medical Center, Cincinnati, Ohio.

Treatment of 142 patients with 168 courses of OKT3 resulted in the development of antimurine antibody in 28% of the patients. Development of antimurine antibody was more frequent in renal transplant recipients (33%) than in hepatic (12%) or cardiac transplant recipients (18%). Twenty-six patients (16 kidney, 6 liver, 3 heart, 1 pancreas) were retreated with OKT3. Eighteen patients had no antimurine antibodies and the rejection reversal rate was 83% (15/18). Six patients had a low titer antimurine antibody and rejection reversal occurred in 5 (83%). Two patients had a high titer antibody and in neither was rejection reversed. Retreatment of patients with no anti-OKT3 antibody resulted in depletion of CD3+ cells from the peripheral blood, but it took longer than in patients being treated for the first time. Similarly, serum OKT3 levels rose more slowly in retreated patients compared to first treatment. In retreating patients with a low titer antimurine antibody, it was often necessary to increase the dose of OKT3 in order to achieve adequate serum OKT3 levels and to deplete CD3+ cells. De novo antimurine antibody developed in 4 of the 18 (22%) antibody negative patients who were retreated. Retreatment with OKT3 should not be considered unless the antibody status of the patient is known. Development of low titer antibodies does not preclude successful retreatment; however, alternate antirejection therapy should be used in patients with high titer antimurine responses.

DECLINE OF MICROSOMAL AND CYTOSOLIC LIVER FUNCTION IN STABLE RENAL ALLOGRAFT RECIPIENTS. Felix J. Frey*, Brigitte M. Frey* and Ruedi Preisig* (Introd. by P. Weidmann). Univ. of Berne, Medical Policlinic and Dept.of Clinical Pharmacology, Berne, Switzerland.

Hepatic failure is a leading cause of death in stable renal allograft recipients. In order to assess the magnitude and the natural history of the hepatic functional derangement, the kinetics of xenobiotics which are metabolized by cytosolic (galactose) or microsomal (prednisolone, cyclosporine A) enzymes were determined in 28 consecutive stable kidney transplant patients one month and one year after transplantation. Renal transplant patients had a decreased mean (± SD) galactose elimination capacity at one month (6.26 ± 0.94 mg/minxkg) and at one year (5.93 ± 0.96 mg/minxkg), when compared with a different group of 28 healthy control subjects (7.52 ± 0.78 mg/minxkg, $p < 0.001$). When compared with the controls (2.71 ± 0.43 ml/minxkg), renal transplant patients had a lower total body clearance of prednisolone at one month (2.13 ± 0.34 ml/minxkg, $p < 0.001$), which further decreased over the following year to 1.76 ± 0.32 ml/minxkg ($p < 0.001$). The clearance of cyclosporine A declined significantly during the first year of successful transplantation from 5.9 ± 2.1 ml/minxkg to 4.9 ± 1.2 ml/minxkg ($p < 0.05$). Conclusion: 1) A substantial proportion of stable renal transplant recipients have decreased cytosolic and microsomal liver functions despite the absence of clinical and laboratory evidence of significant liver disease. 2) In the course of the first year of successful transplantation, the decline in microsomal liver function is more pronounced than that in cytosolic function.

CYCLOSPORINE KINETICS IN RENAL TRANSPLANT PATIENTS AS ASSESSED BY HPLC AND RIA USING MONOCLONAL AND POLYCLONAL ANTIBODIES. Brigitte M. Frey*, Robert F. Speck*, Felix J. Frey*. (Introd. by P. Weidmann). Univ. of Berne, Medical Policlinic, Berne, Switzerland.

Recently a RIA with a monoclonal antibody against cyclosporine A (CyA) was marketed (Sandoz) and said to measure specifically CyA. The purpose of the present investigation was to compare the areas under the blood concentration vs. time curve of CyA (24 hrs), measured by that monoclonal RIA with those obtained by HPLC or by the commonly used polyclonal RIA in 10 renal transplant patients. The mean blood concentrations determined by monoclonal RIA were 10-20% higher than those measured by HPLC, whereas the concentrations assessed by polyclonal RIA were > 100% higher than those determined by HPLC. As a corollary, the pharmacokinetic parameters (clearance, volume of distribution and systemic availability) differed when the results from the three methods were compared. The RIA/HPLC concentration ratio of CyA was higher after oral than after i.v. dosing when RIA measurements were performed by the polyclonal but not by the monoclonal RIA. These ratios changed continuously during the first 12 hours after the administration when the polyclonal but not when the monoclonal RIA was used. In conclusion, blood concentrations assessed by the three methods are not identical and, when compared with the polyclonal RIA, the monoclonal RIA exhibits three advantages, 1) much less crossreactivity with metabolites, 2) a constant RIA/HPLC concentration ratio after the third hour after administration of CyA and, 3) a RIA/HPLC concentration ratio which is independent of the route of administration.

HEPATITIS BS-ANTIGENEMIA IS NOT A CONTRAINDICATION TO KIDNEY TRANSPLANTATION. M.M.Friedlaender* D.I. Rubinger,* J.Silver,* Z. Katzir,* M.M. Popovzer. Hadassah Univ. Hospital, Jerusalem, Israel.

Recent reports showed that the presence of positive tests for HBS Ag is associated with a prohibitively high morbidity and mortality and thus suggest that such patients should not be considered for kidney transplantation. However, in most of these reports, no distinction was made between the patients who were positive before transplantation and those who became positive after transplantation. The present study evaluates the clinical outcome in 10 patients out of a total number of 200 transplantations who were HBS Ag positive before transplantation. There were 7 females and 3 males with average age 25.2±2.6 (mean ±SE, range 22 to 46) years. Nine received cadaveric kidneys and one from a living donor. The follow-up period ranged from 1 to 10 years (6.0±1.1 years). All were treated with immunosuppressive therapy consisting of Azathioprine and Prednisone, one also with Cyclosporin A. No patient died of liver failure and in none of the patients was there clinical or laboratory evidence suggesting a change in liver function. Out of 200 transplants, 2 patients died of acute liver failure due to hepatitis B but both were HBS Ag negative before transplantation. These findings suggest that patients with a positive test for HBS Ag should not be excluded from kidney transplantation programs. The previous papers which report poor patient prognosis may in fact represent patients who became infected after transplantation.

THE SIGNIFICANCE OF NEUTROPHIL MARGINATION IN RENAL PERITUBULAR CAPILLARIES IN POST REVASCULARIZATION ALLOGRAFT BIOPSIES. Lillian W. Gaber*, William M. Murphy, and A. Osama Gaber*. Univ. of Tennessee, Dept. of Path. & Surgery, Memphis, Tennessee

Neutrophils (PMN) are important factors in immune mediated renal disease, yet their role in early rejection remains to be established. We examined 32 core needle biopsies of renal allografts obtained 30 minutes after implantation for the presence and distribution of PMN. PMN aggregated or diffusely marginated in peritubular capillaries (PTC) with more than 10 PMN in 10 consecutive HPF occurred in 7 of the 32 cases. Of the 7 patients, 5 were recipients of a first allograft (71%) and only one recipient was sensitized to > 50% of a random panel of lymphocytes (PRA). Biopsy-proven allograft rejection occurred within 20 days in 6 of 7 (85%). In contrast, only 3 of 25 (12%) patients whose biopsies lacked PMN aggregation in PTC had biopsy proven rejection ($p<0.001$). Of 9 patients with rejection, 6 (67%) had aggregates of PMN in PTC. Prominent PMN margination in glomeruli did not occur in the absence of aggregation in PTC. The results indicate that post reperfusion changes might have predictive value for rejection, perhaps by identifying sensitized PTC endothelial cells. Immune mechanisms involving endothelial cell antigens may be operative in early post transplant rejection.

ABNORMAL LEFT VENTRICULAR(LV) FUNCTION DURING EXERCISE IN PEDIATRIC RENAL TRANSPLANT(TX) RECIPIENTS. **Coral D. Hanevold, Margaret A. Gainey*, Marie A. Capitanio*, H. Jorge Baluarte. Temple Univ. Sch. of Med., St. Christopher's Hospital for Children, Sections of Nephrology and Nuclear Medicine, Philadelphia, Pa.**

Abnormal LV function is a complication of renal failure that typically resolves after a successful TX. However, studies are usually performed with patients(pts) at rest and do not include an exercise phase. Using rest and exercise equilibrium gated nuclear angiocardiograms we evaluated LV function in children post TX. Pts exercised to fatigue following a modified Bruce protocol. Five children, ages 13.7 ± 3.6 ($\bar{X}\pm$sd) years(yrs), were studied at $1.1\pm.5$ yrs post TX. Mean creatinine and hemoglobin values were $1.1\pm.2$mg/dl and 12 ± 1gm%. All pts had normal shortening fractions by Echocardiogram pre and post TX. No pt had a patent arteriovenous fistula or uncontrolled hypertension. During exercise systolic(S) blood pressure(BP) increased by 30 ± 3% and diastolic(D) BP by 41 ± 37%. Maximum SBP and DBP were 150 ± 18 and 94 ± 11mmHg. Peak heart rate was 170 ± 12. Resting ejection fraction(EF) was normal in all pts. In 3 pts exercise response was normal, with EF increasing by 15 ± 5% during exercise, and by 10 ± 2% during recovery. In 2 pts exercise response was abnormal; EF fell by 8% in one and did not change in the other. During recovery, EF increased in both pts by 23 ± 1%. We conclude that subtle abnormalities of LV function may go undetected if studies are limited to the resting state. Inclusion of exercise testing should be considered when evaluating LV function in children with end stage renal disease.

REVERSIBLE INCREASES IN EXCRETION OF ADENOSINE DEAMINASE BINDING PROTEIN (ABP) DURING CYCLOSPORINE A (CSA) THERAPY IN CARDIAC TRANSPLANT PATIENTS. Marshall P. Goren, Mary J. Viar,* Jody L. Lerner,* Jay M. Sullivan.* St. Jude Children's Research Hosp., and Univ. of Tennessee, Memphis, Tennessee.

We studied the acute and chronic effect of CSA, azathioprine, and prednisone therapy on shedding of ABP by the proximal renal tubule in 10 patients after cardiac transplantation. CSA dosage was adjusted to maintain trough serum concentrations, determined by RIA, of >350 ng/ml for 30 days, 250-350 ng/ml for the next 60 days, and 75-125 ng/ml thereafter. Proximal renal tubular damage was judged by increased urinary concentrations (>0.2 A.U.) of the brush border glycoprotein ABP, measured with a dual monoclonal antibody enzyme immunoassay (Cambridge Research Laboratories). ABP excretion increased in every patient by day 3 after surgery, declining to normal levels within 9 to 35 days as the CSA dosage was reduced. Peak urinary ABP and serum CSA levels coincided in 9 patients. During long term immunotherapy (6 mths. to 3 yrs.), ABP exceeded the normal range in 4 instances, 3 of which were associated with CSA conc'ns. >750 ng/ml. ABP increased during 2 of 17 episodes of potential transplant rejection. Serum creatinine transiently exceeded 2 mg/dL in 1 patient; the creatinine clearance rate did not decline in 7 patients given CSA >1 year. Thus, blood CSA conc'ns. >750 ng/ml were acutely toxic to the renal tubule, but the long-term use of this triple-agent immunosuppressive regimen was not associated with persistent effects on the brush border of the renal tubule or with clinically overt nephrotoxicity.

PRE-TRANSPLANT INTERLEUKIN-2 RECEPTOR (IL-2R) LEVELS IN LIVING RELATED DONOR (LRD) KIDNEY RECIPIENTS RECEIVING DONOR SPECIFIC BLOOD TRANSFUSIONS (DST). EA Harden, TJ Schroeder, R Munda, ME Brunson, PE Hurtubise, AJ Pesce, K Balakrishanan, JW Alexander, MR First, Dept. Path./Surg./ Int. Med. Univ. Cincinnati, Cinti., OH.

Activation of T lymphocytes is accompanied by the stimulation of IL-2 and generation of IL-2R. 162 soluble IL-2R serum levels were measured by ELISA in 55 LRD recipients. There was no significant difference in IL-2R levels between those patients who had no rejection episodes post-transplant (X=1174 U/mL) and those who did (X=1117 U/mL). The pre-transplant IL-2R value was not predictive of the timing of rejection; days 1-30 (1111 u/mL), days 31-180 (1098 u/mL), days 181-365 (1091 u/mL). However, the pre-transplant IL-2R level was found to be indicative of response to antirejection therapy. Patients responding to antirejection therapy had a X IL-2R of 970 U/mL. In comparison, those who experienced a graft loss due to rejection had a X IL-2R of 1543 U/mL ($p\leq .05$). A similar trend was observed in patients with two rejection episodes. The pre-transplant IL-2R X values were 820 U/mL in patients responding to a second course of antirejection therapy compared to 1278 U/mL in patients who lost their graft following the second rejection. In conclusion, pre-transplant IL-2R levels in LRD recipients receiving DST did not predict who would reject their graft. However, pre-transplant IL-2R levels did indicate which rejecting patients would best respond to therapy.

INTERRACIAL OUTCOME DIFFERENCES BETWEEN BLACK AND WHITE RENAL ALLOGRAFTS. Philip J. Held, The Urban Institute, Washington, D.C.*, Nathan W. Levin, Henry Ford Hospital, Detroit, MI, Jose R. Garcia, The Urban Institute*, Randall R. Bovbjerg, The Urban Institute*, and Mark V. Pauly, Univ. of Pennsylvania, Philadelphia, PA*.

Using the Cox proportional hazards model, we analyzed graft and patient survival for 16,103 cadaver and 5,457 living-related allografts performed between 1982 and 1986. Covariates included age, sex, diagnoses, HLA and DR matches, pretransplant blood transfusions, donor and recipient race, immunosuppressive therapy, and a measure of the transplant center's past success (center effect).

Black kidney transplant patients have markedly poorer outcomes than do whites. While black patients tend to receive older organs, with fewer HLA and DR matches and generally worse levels of all risk factors associated with renal transplantation, their outcomes are worse than that of whites even when all these factors are considered.

Using white donor to white recipient as the reference group, the relative risks of cadaver allograft failure (RR) for white to black and black to white were 1.16 ($p<0.01$) and 1.21 ($p<0.04$), respectively. For black to black, the RR was 1.49 ($p<0.01$). For living related, the RR again with white to white as the reference group, was 1.90 ($p<0.01$). These results apply holding constant an extensive list of covariates, including one for center experience.

OMEGA-3 POLYUNSATURATED FATTY ACIDS IMPROVE RENAL FUNCTION IN RENAL TRANSPLANT RECIPIENTS TREATED WITH CYCLOSPORIN-A. J.J. Homan van der Heide*, H.J.G. Bilo*, A.M. Tegzess*, A.J.M. Donker* (intr. by L.N. Statius van Eps). Univ. of Groningen and Free Univ. of Amsterdam, The Netherlands.

Cyclosporin A (Csa)-induced renal dysfunction is the most frequent and clinically important adverse effect in renal transplantation. Animal studies have shown that dietary fish oil supplements containing omega-3 poly-unsaturated fatty acids (Ω-3 PUFA's) protect against Csa nephrotoxicity. In a double blind controlled study we investigate the effect of Super EPA^R (Pharmcaps UK; daily 6 grams containing 30% 20:5 Ω-3 and 20% 22:6 Ω-3) versus corn oil (daily 6 grams containing 50% 18:2 Ω-6) in renal transplant recipients, all treated with 10 mg prednisolone and Csa. Patients are admitted at least 9 months after grafting, renal function being stable for 3 months prior to the start of the trial. Before fish- or corn oil supplementation is started glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) are measured as are blood pressure (MAP), whole blood Csa through levels (CsaL; monoclonal RIA Sandoz, Basle, Switzerland) and lipid profile. These variables are measured again after 3 months, during which period diet and medication, including the Csa dose (4.6 mg/kg/day; n=14), are kept constant.

	FISH OIL (n=10)		CORN OIL (n=4)	
	PRE	POST	PRE	POST
GFR (ml/min/1.73m^2)	57±4.7	69±5.5*	66±7.8	65±8.0
ERPF (ml/min/1.73m^2)	240±16.4	272±23.8	245±25.9	243±27.5
MAP (mmHg)	107±2.6	98±2.7*	113±3.9	112±4.1
CsaL (ng/ml)	96±6.1	98±5.3	100±7.6	81±3.4

* $p < 0.05$ (Wilcoxon); all values are reported as mean ± SEM.

In contrast to corn oil-treated patients, all fish oil-treated patients till now showed an increase in GFR and a decrease in MAP. Fish oil-treated patients showed no changes in total cholesterol and HDL-chol while triglyceride levels decreased significantly. Further studies are warranted to investigate whether Ω-3 PUFA supplementation protects against acute postoperative and long term irreversible Csa-induced renal dysfunction.

MORPHOLOGICAL COMPARISON OF TRANSPLANT NEPHRECTOMY SPECIMENS FROM PATIENTS IMMUNOSUPPRESSED WITH CYCLOSPORIN VS. NON-CYCLOSPORIN PROTOCOLS. GA Herrera*, RW Alexander*, S Soong*, JJ Curtis, WH Barber, BA Julian, MH Deierhoi, AG Diethelm, RG Luke, Palm Beach Path, WPB, Fla, UAB, Bgham,Ala, Univ. Cinc. Med Center, Oh.

Two pathologists (GAH/RWA) examined in a blinded and independent fashion one hundred fourteen kidneys removed for irreversible allograft failure. In sixty-nine patients, immunosuppression was with Cyclosporin (CY) and in forty-five conventional immunosuppression (Prednisone and Azathioprine; NC) was used. Thirty-two morphological criteria were analyzed.

Statistically significant findings were: 1) more frequent and severe chronic vasculopathy and glomerulopathy in NC, 2) increased acute vascular rejection in renal arteries and parenchymal infarcts in CY, 3) increased frequency and severity of acute tubular necrosis and oxalate crystals in CY.

The increased occurrence of the chronic vascular and glomerular changes was most marked when the duration from transplant to nephrectomy was between one and twelve months. We conclude that chronic vasculopathy (so-called Cyclosporin-vasculopathy) does not occur more commonly in kidneys with acute or chronic rejection in whom Cyclosporin has been used for immunosuppression than in kidneys from patients treated with Prednisone and Azathioprine.

COMPARISON OF TWO RIA METHODS FOR MEASURING CYCLOSPORINE (CsA) LEVELS IN CHILDREN. Leonard C. Hymes and Barry L. Warshaw. Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322.

We compared two RIA methods for measuring CsA in whole blood: a polyclonal antibody (poly A) that detects parent compound + metabolites, and a monoclonal antibody (mono A) that measures only parent compound. 58 trough measurements were performed in 25 children (mean age 11 ± 5 years) who were ≥ 2 months post-transplant (mean 12 ± 9 mo). CsA doses were adjusted to maintain poly A levels between 400 to 600 mcg/L. Poly A levels were significantly higher than mono A levels (mean poly A = 499 ± 226 mcg/L; mean mono A = 253 ± 136 mcg/L, $p<.0005$). There was excellent correlation between the two assays ($R=.93$, $p<.0001$) as defined by the regression equation $y = 0.55x - 24$. Therapeutic CsA doses ranged from 4.3 to 16.9 mg/kg (mean 9 ± 3 mg/kg) and varied inversely with age ($R = -.61$, $p<.005$), but did not correlate with either poly A ($p>.25$) or mono A ($p>.10$) levels or with months post-transplant ($p>.25$). We conclude that CsA levels, measured by either RIA method, are essential in the management of children because therapeutic CsA doses will vary widely and must be individualized for each patient.

ATTENUATION OF RENAL TRANSPLANT-INDUCED OSTEOPENIA BY TRANSPLANTATION (TX) PRE-(EARLY) DIALYSIS AND TRIPLE IMMUNOSUPPRESSIVE DRUG THERAPY. Julian BA, Laskow DA*, Curtis JJ, Jones P*, Patterson T*, Dubovsky EV*, Deierhoi MH*, Barber WH*, Diethelm AG*, Quarles LD. University of Alabama at Birmingham, Birmingham, AL and Duke University, Durham, NC.

Osteopenia due to accelerated bone loss is a common sequela of renal tx. Factors responsible include pre-tx bone disease, the dosage of glucocorticoids in the early post-tx period, and aluminum-associated bone disease. This study evaluated changes in bone when these factors were minimized. We measured bone density in the lumbar spine by dual photon densitometry (DPA) and performed transiliac bone biopsies at the time of living-related renal tx and 6 months later. Triple-drug immunosuppressive therapy included cyclosporine, azathioprine, and prednisone. To date, 6 nondiabetic patients without pre-tx exposure to glucocorticoids have been studied. Four patients required hemodialysis before tx (1 to 8 mos). Mild hyperparathyroid bone disease was found pre-tx (mildly increased resorptive activity, 6.9±2.0% [mean±SEM]; mild peritrabecular fibrosis, 2.0±1.1%; and normal bone formation rate, 52±16%/yr). Aluminum staining was negative in all biopsies. Six mos post-tx neither trabecular bone density nor histologic trabecular bone volume (BV) decreased significantly.

	Pre-tx	6 mos post-tx	
DPA g/cm^2	0.93±0.04	0.89±0.03	p=NS
BV %	17.2±2.0	16.7±6.6	p=NS

One patient had acute rejection reversed with monoclonal antibody OKT3; no patient received a methylprednisolone bolus for rejection. Mean creatinine clearance 6 mos post-tx was 74 ml/min. Mean daily prednisone dose in the 6 mos was 21 mg. This preservation of bone mass dramatically differs from other series and, we speculate, may reflect (1) low rate of bone turnover because of mild hyperparathyroidism pre-tx or (2) the steroid-sparing effect of cyclosporine.

CYCLOSPORINE INDUCED RENAL DYSFUNCTION IN RENAL ALLOGRAFT RECIPIENTS, B. Kiberd, Dept. of Med, Queen's Univ., Kingston, ONT, Canada

These studies were conducted to determine whether acute changes in renal function could be detected in renal allograft recipients receiving cyclosporine (CyA). We postulated that increases in CyA levels post dose might transiently impair renal function. DTPA (GFR) and PAH (Cpah) clearances were made on three seperate occasions: pre CyA dose (Trough CyA), 2 hrs post dose (Peak CyA), and 2 hrs post CyA dose and Nifedipine PA 20 mg (CyA+Nif). Results (mean+SE) in seven patients were as follows:

	Trough CyA	Peak CyA	CyA+Nif
MAP (mmHg)	100+4	106+5	94+4 t
Scr (umol/L)	123+19	120+17	117+14
CyA (ng/ml)	104+20 t	363+70	377+75
GFR (ml/min)	57+6 **	47+6	57+7 *
Cpah (ml/min)	173+18	147+24	182+32

* P<0.05 ** P<0.02 t P<0.005 vs Peak CyA

These results show that renal function deteriorates transiently post CyA dose. Acute changes occurred both in recent (1 month) and long term (3 yr) recipients. Patients with the lower trough CyA levels developed the greater falls in GFR post CyA dose. Additionally Nifedipine prevented the acute deterioration without affecting polyclonal serum RIA CyA levels. It remains to be determined whether long term Nifedipine treatment prevents chronic CyA nephrotoxicity. This methodology is useful for examining the acute mechanisms of CyA induced renal dysfunction without discontinuation of the drug.

THE EFFECT OF PROPHYLACTIC IMMUNE GLOBULIN (IG) ON CYTOMEGALOVIRUS (CMV) INFECTION IN RENAL TRANSPLANT (TX) PATIENTS (PTS). B.L. Kasiske, K.L Heim-Duthoy*, K.V. Rao, Henn. Co. Med. Ctr., U. of Minn., Mpls, MN.

CMV is the most common and serious infection in the early post TX period. Recent studies have suggested that IG may reduce the severity of CMV in renal TX PTS. In this randomized controlled trial, IG (Gamimmune-N, 500 mg/kg) was administered weekly for 12 weeks to 28 cadaver renal TX PTS. All PTS were treated with Minnesota ALG, azathioprine and corticosteroids. The 13 controls (C), and 15 IG treated PTS did not differ with respect to age, sex, AB mismatches, cytotoxicity, percent diabetics, donor CMV status (68% positive), or no. of acute rejections. With more than 7 mo follow-up, symptomatic CMV infection was observed in 10/13 C and in 9/15 IG treated PTS. Results:

	Onset of CMV (Weeks)	Maximum Temperature (°F)	Total Hospital Days	WBC Nadir (X1000)	Maximum SGOT (IU/ml)
C	6.5 ±2.1	101.2 ± 2.1	27 ±16	3.6 ±1.8	49 ±28
IG	6.1 ±1.7	101.5 ± 1.4	30 ±24	3.5 ±0.5	45 ±25

The duration of fever was similar in IG treated PTS and C. There were no deaths. One graft was lost in each group, and renal function was not affected by IG. Thus, prophylactic IG had no effect on the incidence or severity of CMV infection in the cadaver renal TX recipients at our institution.

INCREASED URINARY LIPOPEROXIDE LEVELS IN RENAL TRANSPLANT (RTX) PATIENTS. Joseph A. Knight*, Alfred K. Cheung, Robert K. Pieper*, Karen Servilla. VA Med. Ctr. and Univ. of Utah, Salt Lake City, Utah.

Lipid peroxidation (LP) of cell membranes is a common feature of free radical-mediated cell injury. RTX are frequently exposed to immunologic and/or toxic insults. We wished to investigate if such insults would lead to chronic cell damage and increased LP. We therefore measured the plasma and urinary concentration of a LP product, malondialdehyde (MDA), in RTX patients.

Thirty clinically stable outpatients (2-44 months post-RTX) without intercurrent illness or overt clinical evidence of rejection were studied. Mean serum creatinine (Cr) concentration was 1.7 mg/dl. All patients were receiving maintenance oral steroid. Group I (n=21) were receiving daily cyclosporine (CsA) with or without azathioprine. Plasma CsA levels were below the presumed toxic range. Group II (n=9) were receiving azathioprine without CsA. MDA was measured by high performance liquid chromatography. The mean plasma and urinary values (±SD) were compared to those obtained from normal subjects (n=121):

	Plasma MDA (µmol/L)	Urine MDA (nmole/mg Cr)	
Group I	0.62(0.23)	3.32*(1.57)	*p<0.001
Group II	0.58(0.16)	4.15*(3.03)	vs normal
Normal	0.58(0.23)	0.89 (0.35)	

Conclusions: (1) Urinary MDA is increased in RTX patients probably secondary to increased LP in the kidneys. (2) The mechanism(s) of the increased LP is unknown. It is, however, unlikely to be due to CsA toxicity alone, and may be caused by low grade immunologic response of the host to the RTX. (3) Urinary MDA measurements may have wide application in monitoring patients with various types of renal diseases.

PROSPECTIVE EVALUATION OF RENAL TRANSPLANT BONE DISEASE. Jeffrey B. Kopp*, Nancy A. Meissner*, Dennis L. Andress, Norma A. Maloney*, Susan M. Ott, Bruce A. Porter* and Donald J. Sherrard. NIDR, NIH, Bethesda MD, First Hill Diagnostic Imaging Center, Seattle WA and the University of Washington, Seattle WA.

We have entered 31 patients (pts) into a prospective study of living related donor renal transplant (tx). At the time of tx and 18-24 months later, we perform biochemical testing, bone densitometry, magnetic resonance imaging (MRI) of the hips, and iliac crest bone biopsy. We report here on the first 16 pts. Fifteen pts had abnormal bone histology at the time of tx and 14 showed marked improvement at follow up. Five pts (31%) developed symptomatic bone disease: 2 with pathologic fractures and 3 with symptomatic osteonecrosis (ON). Three pts developed asymptomatic ON, diagnosed by both abnormal MRI and bone scan. The total incidence of ON was 38%. Five pts had normal MRI at follow up and 3 pts did not undergo MRI. Compared to those with normal MRI, pts with ON or fracture had lower follow up serum 25-OH vitamin D (19±9 vs 38±14 ng/ml, mean±SD, P<.02) and trends which did not reach statistical significance toward lower bone formation rate at the time of tx and greater glucocorticoid dose in the first month after tx.

We conclude (1) symptomatic bone disease remains a common complication of tx despite the use of cyclosporine; (2) 50% of pts with ON are asymptomatic; this high prevalence of asymptomatic ON has not been previously recognized and the clinical course remains to be defined; (3) low serum 25-OH vitamin D after tx may be a risk factor for bone disease.

COMPLEMENT DEPENDENT CYTOTOXICITY BY ^{51}CR RELEASE (CDC-CR) PREDICTS DESENSITISATION BY DONOR SPECIFIC TRANSFUSION (DSBT). T. Kovithavongs, R. Sharma‡ J. Schlaut‡ F. Pazderka*and J.B. Dossetor. University of Alberta, Edmonton, Alberta, Canada.

In this review of 50 patients (pts) who received DSBT (200ml biweekly x3) with no concomitant immunosuppression, sensitisation by standard dye exclusion microcytotoxicity (CDC-de) occured in 16. Five of these 16 were grafted from a different source: 3 did well (2-6 years), 2 rejected, twice. Eight received more DSBT in an effort to desensitise; 3 await cadaveric graft with no further DSBT.

Of the 8 pts with more DSBT, 4 remained sensitised in CDC-de. All 4 were transplanted with kidneys from a different source and did well (upto 30 months). The remaining 4 became CDC-de crossmatch negative: 3 were transplanted with the blood donor kidney and did well (3-46 months) while the fourth is still waiting to be grafted. In these 8 pts a comparison was made of CDC-de with CDC-CR. It was found that a) CDC-CR was either negative (2) or disappeared (2) before CDC-de by a matter of weeks to months in the 4 pts who were desensitised with more DSBT. In contrast, CDC-CR was persistent in the other 4 pts in whom further DSBT had no effect. Thus, the disappearance of CDC-CR was an early sign of desensitisation; b) CDC activity of early positive sera could be blocked (upto 80 %) by late negative sera, indicating the development of a blocking factor(s) with more DSBT being responsible for desensitisation. Blocking activity was not donor specific, in this study, and was not an immunoglobulin as it separated out with the non-immunoglobulin fraction by ammonium sulfate precipitation.

HEMOSTATIC AND THROMBOLYTIC ACTIVITY IN RENAL TRANSPLANT RECIPIENTS. I B Kovacs,* B Tucker,* and L R I Baker* (intr by H H Malluche) St Bartholomew's Hospital, London, UK.

We have used the new in-vitro technique of hemostatometry to measure hemostatic plug formation and dislocation in non-anticoagulated blood samples from renal transplant recipients treated with cyclosporin and prednisolone (Group 1, n=16), azathioprine and prednisolone (Group 2, n=12) and triple therapy (Group 3, n=12).

Time to 30 per cent primary hemostasis was normal in Groups 1 and 2 (89 ± 70 and 119 ± 63 seconds, mean ± SD) and shortened in Group 3 (48 ± 32 seconds; $p < 0.03$, Group 1 v Group 3; $p < 0.002$, Group 2 v Group 3).

Time to plug dislocation was prolonged in each group (Group 1 : 2586 ± 954; Group 2 : 2117 ± 967; Group 3 : 2497 ± 816 seconds, mean ± SD;normal range 1100-1700).

Hemostatic plug formation time is shortened in renal transplant recipients on triple therapy. Plug dislocation time is prolonged in renal transplant recipients irrespective of immunosuppressive regimen.

The methods we have used appear more likely than those employed hitherto to reflect events in vitro and to have implications in clinical transplantation.

THE INFLUENCE OF CYCLOSPORINE A USE AND RENAL ALLOGRAFT AND PATIENT SURVIVAL IN THE MARYLAND END STAGE RENAL DISEASE (ESRD) NETWORK:1982 TO 1986. F.L. Kriger*, P.K. Whelton, A.J. Seidler*, J.F. Burdick*, J.H. Sadler*, M.J. Klag*. Johns Hopkins Univ. and Univ. of Md, Baltimore, Md.

Cyclosporine A (cyA) use has increased markedly over the past 5 years. To study the influence of cyA on renal transplant graft and patient survival, all patients (n=453) undergoing renal transplantation in the Maryland ESRD Network between 1/1/82 and 12/31/86 were followed prospectively until 12/31/87. Demographic data, primary cause of renal failure, dates of initiation of dialysis and transplantation, immunosuppressive therapy, and outcome were analyzed. Data were verified using the Southeast Organ Procurement Foundation database. CyA use increased progressively from 1982 (22% of transplants) to 1986 (90%; p<.001). Overall, 1-year graft survival increased from 63% in 1982 to 80% in 1986 (p<.1). One year cadaveric graft survival increased from 62% in 1982 to 76% in 1986 (p=.025). Cadaveric graft recipients not receiving cyA (N=66) had a 1-year graft survival of 62% compared with 77% amongst those (n=255) that did receive cyA (p=.025). Among cadaveric, but not living-related transplants, Cox proportional hazards analysis demonstrated a positive relationship between cyA and graft survival (p<.015), independent of all other variables studied. Patient survival was not affected by cyA use. Our data strongly suggests that cyA has contributed to the marked improvement in cadaveric graft survival and confirms the clinical impression that cyA should be used in cadaveric transplantation, unless contraindicated.

CHANGES OF MEDULLARY SOMATO-VISCERAL INTEGRATION MECHANISMS IN KIDNEY GRAFT RECIPIENTS TREATED WITH CYCLOSPORINE.
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Well documented side-effects of cyclosporine (CsA) include hypertension, tremor, and loss of alertness. Sympathetic tone, originating in a multifunctional center in the lower brainstem, is responsible for cardiovascular and respiratory regulation as well as for CNS basic activity, muscle tone and alertness. CsA-treated kidney graft recipients were surveyed for the following parameters: blood pressure, heart rate, respiratory rate, EEG, EMG, and the blood volume of the first finger. Power spectra and multivariance analysis of these parameters revealed an increase of muscle tone, sympathic and basic CNS activity, and correlated with an increase of respiratory frequency and alertness. These findings support the hypothesis that hypertension in CsA treated patients is caused by a permanently elevated activity of the multifunctional brainstem center. Other CsA side-effects could be related to the longlasting increase of CNS activity and alteration of the somato-visceral integration by the neurons of the common brainstem system.

ON THE WISH FOR RENAL TRANSPLANTATION
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The distribution of renal transplants to patients on chronic dialysis is markedly dependent on age (old patients get less transplantation than young) and sex (women get less transplant than men).

We investigated whether differences in wishes for renal transplantation was responsible for this by interviewing 268 patients on chronic dialysis.

140 patients (66%) wished for a transplant, 71 (34% did not). The staff did not discuss transplantation with 57 (22%) of the patients. 65% of the women and 67% of the men wanted to be transplanted (PNS). In-center patients tended to wish for transplants more often than self dialysis patients (PNS) (68% vs 59%). The wish to be transplanted was strongly negatively correlated to age. Thus, 80% of the patients below age 50 wished for a transplant compared to only 37% of those over 70 years ($p < 0.01$). Also, the older the patient, the less often did physicians discuss transplantation (5% below age 50 had not discussed transplant vs 50% above 70 years [$p<0.001$]). We calculated the chance of fulfillment of the wish of transplantation by dividing the incidence of transplantation in different age groups by the wish for transplant. While 90% of the patients below age 50, who wished for transplant, could expect that wish to be fulfilled, only 41% of the patients over age 70 who wished for a transplant could expect to receive one.

Differences in wish for transplantation do not explain why women get less transplants than men and only partially explain why old patients get less transplants than young. Physicians often avoid discussing transplantation with old patients.

THE EFFECT OF GRAFT FUNCTION, DONOR SPECIFIC TRANSFUSION AND THE MODE OF IMMUNOSUPPRESSION IN 80 CASES OF LIVING DONOR RENAL TRANSPLANTATION

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The effect of donor specific transfusion(DST) in living renal transplantation and mode of immunosuppressive therapy between Prednisolone(P) + Azathioprine(Aza) and Prednisolone(P) + Cyclosporine-A(CsA) in post-DST living related and unrelated renal transplantation was not settled. From Dec. 1984 and Dec. 1987 80 cases of renal transplantaion were perfomed, of which 15 cases were HLA-identical(HLA-ID), 52 cases were HLA -haploidentical(HLA-HID), and 8 cases were living unrelated(LUR). The effect of CsA + P and Aza + P as immunosuppressive agents on renal allograft function were compared according to the HLA-ID, HLA-HID and LUR group. Excluding 4 cases of graft failure due to nonimmunological causes, the acturial graft survival in 2 years was 100% in the HLA -ID group, 88.6% in the HLA-HID group and 84.6% in the LUR group. Numbers of patients with serum creatinine above 2mg/dl were 4 of 16 cases(25.0%) in the Aza + P treated HLA-HID group during mean observation of 19.3 months and 4 of 36 cases(11.1%) in the CsA + P treated HLA-HID group during mean observation of 10.5 months and 2 of 8 cases(25.0%) in the CsA + P treated LUR group. However the difference was not statistically significant. Above results suggest that the effect of CsA + P on renal allograft seemed to be better than that of Aza + P even after post-DST related and unrelated living renal transplantation.

RISK OF EARLY POST-TRANSPLANT HYPERLIPIDEMIA IN CYCLOSPORINE TREATED RENAL TRANSPLANT PATIENTS IS RELATED TO PRE-TRANSPLANT LDL LEVEL
Mariana S. Markell*, Clinton D. Brown* and Eli A. Friedman. Dept. of Med. SUNY Health Science Center at Brooklyn, Brooklyn, New York.

Hyperlipidemia has been noted in renal transplant patients both in the early transplant period and at late (greater than 1 year) follow up. This study followed patients at our center who received a transplant between May and Nov. 1987, all of whom were treated with azathiaprine plus cyclosporine (CSA) and prednisone (Pred) or CSA and rapidly tapered steroids, in an attempt to define the patients at risk for post-transplant hyperlipidemia. Fasting bloods for total cholesterol (TC), LDL, HDL, VLDL and triglycerides (TG) were drawn prior to transplantation and at monthly intervals post-transplant. Pretransplant the average value for total cholesterol (TC) was 192±47, with LDL 103±50, HDL 41±18, VLDL 32±18 and TG 203±127. By 1 to 3 months post-transplant, the average TC was 259±48 ($p<.00005$) with LDL 145±55 ($p<0.00005$), HDL 63±28 ($p<0.00005$), VLDL 40±20 ($p<0.05$) and TG 231±128 (NS). Of the 43 patients studied, 40 showed an increase in TC with an average increase of 66±43. The patients were assigned to 2 groups, those whose TC post-transplant was greater than 240 (HC), and those who remained below 240 (LC). There were no significant differences between these two groups, when analyzed by a 2-tailed t-test, for age, sex, weight gain, cadaveric or living related transplant, prednisone, cyclosporine and azathiaprine dose, presence or absence of hypertension, treatment with beta blocker or diuretic, number of rejection episodes, BUN or creatinine.

The only significant differences were found for pretransplant TC, LDL and VLDL ($p<0.001$, $p<0.0005$ and $p<0.05$ respectively). In addition, when the same factors were studied by discriminant analysis, pretransplant LDL appeared to be the most important factor for successfully predicting whether a patient would fall into the HC group, with an 80% success rate and $p<0.0001$. We conclude that the majority of renal transplant recipients develop elevation of lipids post-transplant and that those patients who are at risk for clinically significant elevations may be predicted by their pre-transplant lipid levels, specifically the LDL fraction.

DIFFICULTIES IN THE DIAGNOSIS OF RENAL ALLOGRAFT REJECTION IN THE SMALL CHILD RECEIVING ADULT KIDNEYS

S. Michael Mauer, Timothy E. Bunchman, David S. Fryd,* and Richard K. Sibley, Univ. of Minnesota, Minneapolis, Mn. and Stanford Univ., Stanford, Calif.

Serum creatinine (Cr) elevation may be a late sign of allograft rejection in small pediatric recipients of adult grafts. To evaluate this we looked at 58 children less than 4 years of age undergoing living related donor transplantation (tx) at the U of M from 1980 to 1987. Biopsy (bx) proven acute rejection episodes (R) occured in 22 children who were pair matched with the 36 (C) who did not have rejection. We matched for (1) age (R-23.7 ± 2.3 months [x ± SEM], C-18.8 ± 1.4 months), (2) weight (R-9.4 ± 0.4 kg, C-9.3 ± 0.3 kg), (3) date of tx (± 12 months), and (4) immunosuppression. Fourteen R patients each had 2 and 8 R patients each had 1 matched C patient. The time of bx in the R patient and the identical time from tx in the pair matched C patient is considered time zero (0). We reviewed the clinical data at time intervals of 8, 4, 3, 2, and 1 week(s) prior to and at 0. We found that 18/22 (82%) in the R group had hypertension for 3.3 ± 0.6 weeks prior to 0 vs 3/36 (8%) of the C group. Two of 3 of these C patients had recurrent oxalosis on bx. Ten of 22 (45%) in the R group vs 0% in the C group had intermittent daily temperatures > 101° F of unknown cause for more than 7 days prior to 0. Only 10/22 (45%) in the R group had increased Cr at 0 and only 3/22 (14%) prior to 0. Fifty nine % of the R group had moderate to severe rejection on bx yet we found no relationship between the clinical presentation and severity of rejection. Actuarial allograft survival at 3 years was less in the R (65%) group than in the C (95%) group ($p < 0.01$). We conclude that small children transplanted with large kidneys often have subtle clinical signs of serious rejection. Thus, a high index of suspicion and liberal allograft bx criteria are indicated for these patients.

INDUCTION OF ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY AGAINST ENDOTHELIAL CELLS BY RENAL TRANSPLANTATION.

André M.M. Miltenburg*, Marjon E. Meijer-Paape*, Jan J. Weening*, Mohamed R. Daha*, Bob L.A. van Es* and Fokko J. van der Woude*, Departments of Nephrology & Pathology, University Hospital Leiden, Leiden, The Netherlands (introduced by G.A. Andres).

Using the serum of a renal transplant patient who had experienced a severe vascular rejection episode after receiving a HLA-identical kidney graft from a living related donor, antibody dependent cellular cytotoxicity (ADCC) against human umbilical vein endothelial cells (EC) was detected. This reactivity was absent in serum obtained before transplantation. We characterized the ADCC-inducing substance as antibodies of the IgG1 subclass. The specificity of the anti-EC serum was shown in panelstudies using EC-lines obtained from different individuals. We examined sera of nine additional renal transplant patients with rejection. Sera of two of these patients were found positive in the ADCC assay whereas 20 normal serum donors were negative. Donor monocytes did not selectively absorb the anti-EC antibodies and no staining of peritubular capillaries in standard immunofluorescence tests on kidney tissue slides occurred, indicating that anti-EC ADCC was not a result of classical anti-endothelial-monocyte (EM) antibodies. Furthermore, anti-EM sera did not induce ADCC of EC. The effector cell responsible for EC-lysis in the ADCC assay was demonstrated to be of natural killer/ killer (NK/K) cell origin. These findings support the idea that IgG1 antibodies directed against polymorphic non-HLA, non-EM antigens on EC can be induced by renal transplantation.

EFFECT OF CYCLOSPORINE(CYA) VERSUS ANTILYMPHOCYTE GLOBULIN(ALG) ON DELAYED GRAFT FUNCTION(DGF) IN RENAL TRANSPLANT PATIENTS.

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CYA has been associated with prolongation of DGF, longer hospitalization and impaired long term renal function. Between 12/85 and 3/88, all patients with DGF of 24 hrs. after an initial 12-24 hrs. of CYA were randomized to either 1.) daily Minnesota ALG, prednisone with CYA(10mg/kg/day) begun only after resolution of DGF or 2.) low dose CYA(10mg/kg/day) and prednisone. Resolution of DGF was defined as lack of dialysis dependency and a 25% fall in the serum creatinine (CR). Data was analyzed via nonparametric methods. A $p \geq 0.05$ was considered not significant.

Of the 45 patients who recovered graft function, 19 received ALG and 26 received CYA. Cold ischemia time(CIT), mean hospitalization, and mean duration of DGF for ALG was not significantly different from that of CYA(CIT ALG 29.8 hrs., CYA 30.7 hrs.;Mean hospitalization ALG 16.89 days, CYA 18.46 days;Mean DGF ALG 9.74 days, CYA 13.69 days). No difference in CR was found between the two groups at 1 mo., 3 mos., or 12 mos. Mean CR at 12 mos. was 1.98 mg/dl for ALG vs. 1.96 mg/dl for CYA.

Unlike other investigators we have demonstrated that CYA can be used in patients with DGF without significantly prolonging the duration of DGF, length of hospitalization or adversely affecting graft function. Thus, monoclonal and/or polyclonal antibody therapy can be saved for rejection episodes.

MISOPROSTOL (M) IMPROVES RENAL FUNCTION AND REDUCES THE FREQUENCY OF ACUTE REJECTION (AR) IN CYCLOSPORINE-TREATED (CsA) RENAL TRANSPLANT RECIPIENTS.

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The acute nephrotoxicity of CsA is reversed in rodents by M, a PGE1 analog. To investigate whether M would improve renal function in CsA-treated patients, we performed a prospective study of 65 renal transplant recipients continuously immunosuppressed with CsA and prednisone. In this randomized, double-blinded trial, subjects also received either M (N=33) or placebo (PL, N=32) each day for 3 months posttransplantation. We compared serum creatinine (Cr, mg/dl), creatinine clearance (CCr, ml/min/1.73 m2), and the frequency of AR (episodes/month). [Results are expressed as mean ± SEM.]

		1 mo	2 mos	3 mos
Cr	PL	2.2±0.2	1.9±0.2	1.8±0.1
	M	1.6±0.2*	1.4±0.1*	1.4±0.1*
CCr	PL	60±5	66±5	64±5
	M	73±5@	77±6	81±5#
AR	PL	13	7	1
	M	3	3	0

*$p < 0.05$, # $p < 0.06$, @ $p < 0.08$ vs. placebo

AR was significantly reduced during the 3 mos of treatment with M ($p < 0.007$). We conclude that misoprostol improves renal function in CsA-treated renal transplant recipients and reduces the frequency of acute transplant rejection.

EVALUATION OF CYCLOSPORINE (CY) TOXICITY (T) BY RENAL TRANSPLANT FINE NEEDLE ASPIRATION BIOPSY (FNAB). C.C.Nast, C. Blifeld, G.M. Danovitch, R.N. Fine, R.B. Ettenger. Harbor-UCLA Med Ctr, Torrance, CA and UCLA Sch of Med, Los Angeles, CA.

FNAB is a currently used method for morphologic assessment of renal transplants. In FNAB from some patients receiving Cy, tubular cells contain isometric vacuoles (IV), which have been previously attributed to CyT rather than Cy administration alone. To determine the specificity of IV for CyT, we identified FNAB with and without IV. Another reported Cy effect, lymphocytosis without IV or blast response, was also assessed. Forty-eight adequate FNAB from 18 patients receiving CyA were evaluated by the method of von Willebrand. FNAB with IV were divided into those with $\geq$ 50% (Group A, n=8) and < 50% (Group B, n=15) tubular cell population containing IV. Ten FNAB had graft lymphocytosis as the only alteration (Group C) and 15 had no abnormalities (Group D). Clinically diagnosed CyT which improved with reduced Cy doses was present in 7/8 from Group A and 1/15 from Group B ($p < .001$). In Group C, 1/10 had CyT with the remainder exhibiting multiple disease processes. None from Group D had CyT. Serum creatinine and Cy levels did not correlate with IV or lymphocytosis. We conclude that not all Cy treated patients have IV. When IV occur, CyT should be strongly considered only when $\geq$ 50% of the tubular cell population is involved. Graft lymphocytosis is not specifically related to CyT or administration.

PROTEIN LOADING (PrL) IN RENAL TRANSPLANTATION (RT). Julia Nunley,* Anne King, Domenic A. Sica, Thomas Comstock,* Carol Marshall,* Marc Posner.* Depts of Med and Surgery, Med. Coll. of Virginia, Richmond, VA.

Acute and/or chronic administration of a high Pr diet is associated with an increase in glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and natriuresis (NA). This study was designed to evaluate renal functional reserve (RFR) by determining the influence of PrL (1 gm/kg TBW) on GFR, ERPF, NA and plasma renin activity (PRA) in RT maintained on either CyA (n=5) or conventional therapy (CT) (n=6). Inulin/PAH clearances (cc/min), PRA (ng/ml/hr) and NA (mEq/hr) were determined prior to, and hourly for 5 hours after PrL. Baseline (B) and maximal response at 3 hrs (MR) are reported as mean $\pm$ S.D. (* = $P < 0.05$ between groups):

	CyA		CT	
	B	MR	B	MR
GFR	47 $\pm$ 14	38 $\pm$ 10*	49 $\pm$ 13	69 $\pm$ 27*
ERPF	216 $\pm$ 97	161 $\pm$ 53	196 $\pm$ 37	293 $\pm$ 142
NA	64 $\pm$ 13	70 $\pm$ 45	52 $\pm$ 27	94 $\pm$ 49
PRA	5.3 $\pm$ 4.2	5.3 $\pm$ 4.9	2.2 $\pm$ 1.1	6.7 $\pm$ 6.4

The GFR AUC_{0-5hr} are 241 $\pm$ 48 and 275 $\pm$ 80 for CyA and CT patients (pts), respectively.

We conclude that: (1) acute PrL increases maximal GFR and ERPF in CT (pts) but not in CyA pts; (2) NA follows PrL in CT pts and to a lesser extent in CyA pts; (3) PRA increases after PrL in CT pts with little effect in CyA pts. Thus, CyA pts would appear to have a diminished RFR and blunted NA in response to PrL.

DEMOGRAPHICS, IMMUNOTHERAPY AND OUTCOME OF PEDIATRIC RENAL TRANSPLANTS IN NORTH AMERICA: A REPORT OF THE NORTH AMERICAN PEDIATRIC RENAL TRANSPLANT COOPERATIVE STUDY (NAPRTCS).

From Jan. 1987 thru June 1988 486 renal transplants in pediatric recipients <18 yrs have been reported to NAPRTCS registry from 69 participating pediatric centers. Data of first 390 children has been analyzed and forms the basis of this report. 20% of transplants were in recipients <5 yrs (5.7% in infants <2 yr); 36% and 43% were in the 6-12 and 13-18 yr age groups respectively. Frequent original diagnoses were Aplastic/dyslastic kidneys (18.9%), obstructive uropathy (17.4%), and focal segmental glomerulosclerosis (11.5%). 23% received renal allografts as the primary therapy (without previous dialysis). Pretransplant bilateral nephrectomy was reported in 28% of recipients. Donor source was cadaver (C) in 56% and live related (LR) in 44%. During the 1st 30 days post transplant, prednisone (P), cyclosporine (CS) and azathioprine (Aza) was used in 90%, 83% and 78% of recipients respectively. Of 199 patients with a functioning graft and a six months follow up, 69% were receiving triple therapy (P/CS/Aza), 15% P&CS; 10% P&Aza and 4% (CS&Aza). Rehospitalizations during the 1 to 6 month period occurred in 64% of these patients; rejection 29% and infection 23% were the main causes. 9 of the 12 fatalities were in recipients of C grafts; overall patient survival at 6 mos was 96.6%. 54 grafts failed; the 6 month actuarial graft survival rate is 81% for C grafts and 92% for LR grafts. This report is the first multicenter analysis of pediatric renal transplantation in U.S. and Canada. Long term follow up will address special issues in children receiving renal transplants.

THALLIUM SCINTIGRAPHY IN POTENTIAL RENAL TRANSPLANT RECIPIENTS. Diane Oathes*, Louis Cotterell*, Douglas Norman, Richard Wilson*, John Barry, William Bennett. Div of Nephrology & Hyperten-sion, Oregon Hlth Sci Univ, Portland, Oregon.

Atherosclerotic heart disease (AHD) is the most common cause of mortality following renal transplantation (RT). To identify patients who may be at increased risk for cardiovascular events, we prospectively studied all high risk patients referred for RT. All patients with diabetes; over the age of 50; a history suggestive of AHD or an abnormal ECG other than LVH underwent exercise stress testing (EST) with thallium^{201} imaging (TI). Of 100 patients evaluated for RT between 6/87 and 2/88, 43 met the criteria for EST, and 39 completed ESTTI (21 males, 18 females; 24-68 yr of age). There were 22 diabetics. Fifteen of these and 9 of the 17 non-diabetics had abnormal scans. All patients with abnormal ESTTI were given the options of further cardiac evaluation or not pursuing RT. Of the 24 patients with abnormal ESTTI, 7 decided to have further cardiac evaluations. Five had coronary arteriography. Two had normal arteriograms and 3 had significant stenoses. Of these 7 patients, 2 have had successful RT (1 following coronary surgery bypass grafting), 4 are awaiting RT, and 1 was turned down for RT. None of the remaining 17 patients are pursuing RT. Of the 15 patients with normal scans, 2 have been RT and 7 are awaiting RT. There have been no cardiac complications in the patients transplanted or awaiting RT. There have been 2 deaths in patients not pursuing RT. ESTTI is of value in selecting high risk patients for full cardiac evaluation prior to RT.

FAILURE OF DONOR SPECIFIC TRANSFUSION IN IMPROVING THE GRAFT SURVIVAL IN KIDNEY TRANSPLANTATION. Alvaro Pacheco-Silva, José O.A.Pestana,Liz M.Kuschnaroff,Claudio S. Melaragno, Paulo S. Luconi, Sergio A. Draibe, Oswaldo L.Ramos (Intr. by Roberto Zatz).Nephrology Division. Escola Paulista de Medicina. São Paulo, SP, Brazil.

Since 1980 DST has been used in haploidentical (HP) transplantation to increase graft survival; indeed many centers have observed a graft survival rate similar to HLA identical. This study includes 107 recipients of HP grafts(First Transplant), who underwent kidney transplantation at our institution between Feb 83 and April 88; 54 patients received HP grafts without DST (group I) and 53 patients received HP grafts after the administration of 200 ml of whole blood on three ocasions at two week intervals (group II). The groups were not diferent concerning sex, age, and kidney disease. All patients received prednisone and azatioprine. All rejections crisis were treated with methylprednisolone 3 to 5 grams.No patient received antilymphocyte antibodies; cyclosporine A (CSA) was never used as first immunesupressive terapy. The actuarial 1 year patient and graft survival was 92% and 74% respectively for group I and 91% and 79% for group II.There were 12 and 9 grafts lost by rejection respectively in group I and II with 4 deaths in each group,2 after the rejection and 2 due to infection with good kidney function. Our results do not show a significant improvement in the graft survival of receptors of HP kidneys after DST. These results are significantly worst then those reported in the literature for transplantation in HP subjects with CSA. The use of CSA seems to be mandatory in these patients.

DEVELOPMENT OF "ANTI-IDIOTYPIC" ANTIBODIES TO HLA IN DST-PATIENTS. E. Pohanka*, R.C. Manfro*, B.W. Colombe*, O. Salvatierra Jr.* and M.R. Garovoy. Immunogenetics and Transplantation Laboratory, Dept. of Surgery, University of California, San Francisco, CA

The aim of our study was to investigate, whether the disappearance of temporary, specific anti-HLA Ab (Ab1), developed after DST, was caused by "anti-idiotypic" antibodies (Ab2). Nine potential kidney graft recipients, who became transiently sensitized after DST were selected for the investigation. A lymphocytoxicity assay was used to evaluate the capacity of various post DST sera to inhibit the anti-HLA activity of Ab1 sera. T cells obtained from volunteers that matched the HLA type of the transfusion donors, served as target cells. The inhibiting or augmenting capacity of the post DST sera was determined by its comparison with pre DST sera. By the nine patients investigated 12 specific anti-HLA Ab were transiently developed; to 4 of them inhibiting post DST serum activity was observed indicating the presence of "anti-idiotypic" antibodies. Enhancement was found to 6 anti-HLA Ab and no serum activity was detected against two Ab1. In one patient we could confirm the "anti-idiotypic" activity measured with the complement dependent cytotoxicity assay (inhibition of Ab1 function) by flow cytometry (inhibition of Ab1 binding). 3 of the 4 patients presenting with inhibition had been randomly transfused previously. None of the 4 previously non-transfused patients showed blocking activity but 3 of them demonstrated enhancement. These findings indicate an influence of random blood transfusions to the development of "anti-idiotypic" Ab. Although the total number of cases is small our observation could offer an explanation for our earlier finding that panel low-reactive patients with previously high PRA and a history of third party transfusions had a low incidence of sensitization after DST. In conclusion we believe that "anti-idiotypic" Ab play a role in the disappearance of anti-HLA Ab; however, as Ab2 could not be found in all cases, other mechanisms might additionally contribute to the loss of sensitization.

RETROVIRAL INFECTION IN RENAL TRANSPLANTATION. G. Perez, C. Ortiz,* D. Roth, J.J. Bourgoignie, M. Milgrom,* V. Esquenazi,* J. Miller. Univ. of Miami and VAMC, Miami, FL.

To evaluate the prevalence of retroviral infection among patients transplanted before HIV screening was introduced, we retrospectively tested sera on 224 of 331 patients transplanted between 1979 and 1985. The presence of viral IgG antibodies to human immunodeficiency virus (HIV-1) and human T-cell leukemia virus (HTLV-I) was screened by EIA and confirmed by Western blot. Seven patients (3.1%) were infected, 5 with HIV-1, and 2 with HTLV-I. All 5 HIV-1 infected patients seroconverted following transplantation. Transient HIV-1 antigenemia preceding seroconversion was demonstrated in 4. One patient died of Kaposi's sarcoma after 2 years with a functioning graft, 2 are asymptomatic with a functioning graft after 4 and 5 years and 2 rejected their grafts and are HIV-1 carriers on maintenance dialysis 3 and 6 years after transplantation. Six patients tested positive for HTLV-I by EIA. But, only 2 were confirmed positive by Western blot, RIPA, p24 antigen RIA and lymphocyte culture. One patient rejected and the other has poor graft function. Both are free of retroviral symptoms after 3 years of follow-up. Intravenous drug use was a risk factor but in one patient. Most patients probably became infected from transfusions, but donor organs could not be ruled out. The incidence of renal graft loss in patients with retroviral infection is high although maintenance of adequate graft function can occur.

DISCREPANCIES IN THE DISTRIBUTION OF RENAL ALLOGRAFTS CAUSE PROLONGED WAITING TIMES FOR BLOOD TYPE O PATIENTS. FK Port, PJ Held#, RA Wolfe#, J Garcia#, LL Rocher. The Urban Institute, Washington DC and Univ. of Michigan, Ann Arbor, MI

The waiting period for blood type O transplant candidates is markedly longer than that for other blood types. We examined the utilization of first cadaveric grafts by blood type during 1982-1986 in a national (HCFA) sample of 14,597 recipients. Of 6.060 type O grafts, 14.7% were transplanted to non-O recipients. Of 4,794 type A grafts, 275 were transplanted to type AB recipients. Type A patients received 5,036 allografts (517 from type O) giving them a 5% advantage over strict intra blood group transplantation. Similarly, 1,206 type B donor kidneys were available whereas 1,477 type B patients were transplanted (22.5% advantage). Type AB patients received more than twice as many kidneys as the number of available AB organs. Blood type distribution differences by race played comparatively minor roles. The national practice of transplanting kidneys across blood types unfairly disadvantages type O patients by cumulatively prolonging their waiting times to transplantation. At the observed rates, an extra year of waiting is added to type O patients every seven years. To make access to transplantation more equal, renal allografts should be distributed within the same blood type whenever possible.

LATE MORBIDITY AND MORTALITY ASSOCIATED WITH RENAL TRANSPLANTATION (TX): FOLLOW-UP OBSERVATIONS BEYOND THE FIRST DECADE. K.V. Rao, B.L. Kasiske, R.C. Andersen, Henn. Co. Med. Ctr., U of Minn., Minneapolis, MN.

We analyzed the clinical data of 79 patients (pts) who received kidney Txs at our center between 1-1-68 and 6-30-78 and followed with a functioning allograft for 10 years or longer. There were 44 males. Twenty-seven received live donor Txs. Mean age at Tx was 35.6 years. The mean Azathioprine dose was 128mg and prednisone dose was 9.8mg. Results: In the second decade, during a mean follow up of 3 yrs (range 0.1-10.2 years) 7 pts (9%) suffered graft loss (chronic rejection - 6, irreversible ATN 1). Eleven others (14%) died with a functioning kidney. Causes: Sepsis- 2, hepatic failure- 4, cardiac infarction- 2 and malignancy- 3. The pt and graft survival rates at 15 years were 85% and 72% respectively. The problems observed in the second decade were: 1) Infection 40 pts (51%)-minor 28, major 12; 2) Hypertension 32 pts (40%); 3) Musculoskeletal problems 37 pts (47%); 4) Graft rejection 16 pts (20%)-clinical 11, histological 10; 5) Liver disease 27 pts (34%); 6) Cataracts 27 pts (34%); 7) Vascular disease 18 pts (23%), (coronary 9, cerebral 6, peripheral 3); 8) Malignancy 20 pts (25%)-visceral 6, cutaneous 14; 9) G.I. bleeding 6 pts (8%); 10) other problems 30 pts (38%). At the last assessment, 84% were fully rehabilitated, 13% were partially rehabilitated and 3% were medically disabled. Continued medical follow up at regular intervals is essential for the detection and management of these late complications which inturn should improve the long term success of renal transplantation.

BETTER PROGNOSIS IN CHILDREN WITH TRANSPLANTS AND FOCAL SEGMENTAL GLOMERULOSCLEROSIS. Seth Schulman, Bruce Kaiser*, JoAnn Palmer*, Martin Polinsky*, H. Jorge Baluarte. Temple Univ. Sch. of Med., St. Christopher's Hosp. for Children, Philadelphia, PA.

We reviewed 244 transplants (Txs) in 173 children between 1971-1988. 98 Txs were performed in 84 children with nephritides reported to recur. Focal segmental glomerulosclerosis (FSGS), the most common classified nephritis to cause end stage renal disease in children, was biopsy proven in 24 Txs (17 cadaveric, 7 live related) representing 10% and 24% of total Txs and Txs secondary to nephritis respectively. 19 children age 11.0 $\pm$ 4.4 ($\bar{x}\pm sd$) years represented 11% and 27% of all children and children with Txs secondary to nephritis respectively. The population consisted of 12 males and 7 females. Patients time from onset of disease to dialysis was 5.3 $\pm$ 4.1 years. Time on dialysis was 0.90 $\pm$ 0.67 years. Recurrence was defined on the basis of a biopsy and/or presumptively if nephrotic range proteinuria was present. 4/24 Tx and 4/19 children showed evidence of recurrence of FSGS. No child showed recurrence on a subsequent Tx. In 2 of the 4 cases FSGS was evident with nephrotic syndrome but resolved within one year. 1 child had FSGS superimposed on acute rejection with graft loss in 9 months. The last child had FSGS only on biopsy with demise of the graft in 7 months. Our data for recurrence and graft loss compares favorably to Streigal JE et. al. (KI 30:S44-S50) where 12/24 children had recurrence ($p=.05$) and 10/24 children lost their grafts ($p<.01$). We conclude that in our population FSGS recurred at a lower rate and rarely caused graft loss.

RENAL TRANSPLANTATION WITHIN A YEAR OF ESRD LESSENS THE MORTALITY RATE OF RECIPIENTS WITH PRE-EXISTING HYPERTENSION BUT NOT THOSE WITH A HISTORY OF LEFT VENTRICULAR FAILURE. Robert R. Riggio, Rudy Haschemeyer*, Jhoong Cheigh, M. Suthanthiran, Luis Tapia, William Stubenbord*, Kurt Stenzel, Albert Rubin, and C. Chinea. The Rogosin Institute, The New York Hospital-Cornell Medical Center, New York, New York.

The presence of Hypertension (HBP) and/or Left Ventricular Failure (LVF) prior to renal transplantation results in a relative shortened life expectancy afterwards. In a retrospective analysis of data gathered from 407 recipients of a first cadaver transplant performed between 1976 and 1987, we found Recipient Survival Rates (RSR) significantly diminished, compared to respective Norms (N), among those with a history of HBP and/or LVF:

Condition(C)	Ratio of C:N	2 yr CSR C:N	p
HBP	196:185 pts	81% vs 88%	0.017
LVF	55:328 "	79% vs 85%	0.045
HBP + LVF	40:166 "	79% vs 89%	0.003

Given these differences, we examined what impact a shortening of the transplant waiting time had on the survival rate of recipients with these pre-existing conditions:

Condition	CRT < 1 YR (#)	CRT $\geq$ 1 YR (#)	p
HBP	88% (119)	77% (76)	0.09
LVF	83% (40)	80% (15)	0.56
HBP + LVF	80% (13)	78% (27)	0.29

We conclude that transplant RSRs are adversely affected by the pre-existing conditions of LVF and/or HBP. Therapeutic measures to lessen the impact of the latter should include early transplantation.

SOLUBLE INTERLEUKIN - 2 RECEPTOR PLASMA LEVEL AS A METHOD TO MONITOR TRANSPLANT REJECTION. Ahmed Shoker,* Sarita Angra,* Paul Daly,* Chris Feindel*, Carl J. Cardella. Univ. of Toronto, Dept of Med., Toronto Western Hospital, Toronto Ontario.

Soluble interleukin 2 receptor (SIL-2R) production is increased after immune activation. Plasma levels of S IL-2R may reflect this event and may be useful as a non invasive method of detecting organ rejection.

To evaluate this possibility plasma SIL-2R was measured using an ELISA assay (T cell science) in 34 heart Tx patients prior to and at regular intervals post Tx. Rejection episodes were determined by the presence of a lymphocyte infiltrate and cell necrosis on endomyocardial biopsy and were correlated to plasma SIL-2R.

The mean pre Tx SIL-2R was 519 $\pm$ 286 units/ml & increased to 954 $\pm$ 413 units/ml ($P = < 0.001$) 5 days post Tx without evidence of rejection. Therefore a two fold increase or greater of SIL-2R above pre Tx levels within 3 days of a positive endomyocardial biopsy was considered a positive correlation. 16 of the 189 assays were a true positive; 45 a false positive, 115 a true negative and 13 a false negative. The sensitivity of this test is 55% with a specifity 72%. A positive test predicts rejection 26% of the time and a negative test is correct 90% of the time.

Measurement of SIL-2 plasma levels correlates very poorly with rejection and is not an adequate screening test for rejection in Tx patients.

EVALUATION OF KIDNEY FUNCTION IN RENAL TRANSPLANT PATIENTS RECEIVING LONG-TERM CYCLOSPORINE. L. Slomowitz*, A. Wilkinson*, R. Hawkins*, G. Danovitch. Division of Nephrology, Department of Medicine, and Division of Nuclear Medicine, Department of Radiology; UCLA School of Medicine, Los Angeles, CA.

We assessed renal function in a group of 37 renal transplant patients receiving cyclosporine over a prolonged period of follow-up. Renal function was measured using urinary albumin excretion (UAlb), serum creatinine concentration (SCr), creatinine clearance (CCr) and Tc99m DTPA clearance (CDTPA) as a measure of true GFR. Group 1 consisted of 24 patients who were studied repeatedly over 32 ± 1 (SEM) months. Their initial CDTPA (46 ± 2 ml/min), CCr (63 ± 3 ml/min) and SCr (1.6 ± 1 ml/dl) remained constant over the follow-up period. Group 2 consisted of 5 patients with progressive graft dysfunction and return to dialysis. Their initial values for CDTPA (29 ± 3 ml/min), CCr (38 ml/min) and SCr (2.4 mg/dl) were significantly lower than in group 1. In all group 2 patients CDTPA at 12 ± 1 months was less than 40 ml/min. 50% of all patients studied with an initial CDTPA of less than 40 ml/min lost their grafts, whereas no graft loss has occurred in patients whose initial CDTPA was greater than 40 ml/min. In group 1 the log of UAlb varied inversely with CDTPA and directly with SCr, but showed no change with time. Thus, patients with early impairment of graft function are at a high risk for eventual graft loss. The majority of patients, however, have shown remarkable stability of renal function over a prolonged period of follow-up and despite continued use of cyclosporine.

A DISTINCT VARIETY OF ACCLERATED REJECTION MEDIATED BY ANTI CLASS I ANTIBODIES. N.S. Srinivasa, A. Wadgymar, S. Ritchie, J. Falk, K. Solez, P.F. Halloran, Univ. of Alb.Hosp., Edmonton, and Toronto Gen. Hosp. Toronto, Canada.

Anti HLA class I antibodies cause hyperacute rejection but their role in the pathogenesis of other forms of rejection is unknown. In the course of 270 cadaver renal transplants (Tx), we have observed 7 who developed an oliguric acute tubular necrosis (ATN) like syndrome within 7 days of Tx. usually after a period of good function, associated with anti class I. All 7 pts had panel reactive antibodies (20-60%), 5 due to previous Txs. Fever, graft tenderness and enlargement were absent. Renal biopsies in 2 showed typical acute rejection, but in the other 5, a distinctive pattern of endothelial injury was seen with polymorphs in glomeruli and peritubular capillaries and minimal mononuclear cell infiltration, 4 showed widening of the subendothelial space in the absense of cyclosporine. Retrospective testing revealed weak positive T cell cross matches (CM) on the day of Tx in 3 of 7, and strongly positive CM in all pts in association with ATN. 5 of 7 grafts were lost without regaining function; in 2, anti class I disappeared after OKT3 and the function recovered. We conclude that presensitized pts are at risk for early development of an uncommon but distinctive ATN-like syndrome, different from hyperacute rejection, caused by anti class I mediated injury to the microvasculature. Early diagnosis by testing for anti class I and treatment with OKT3 may improve the prognosis in this condition.

UNCONVENTIONAL LIVING KIDNEY DONORS (LD): ATTITUDES AND USE AMONG TRANSPLANT CENTERS. Aaron Spital, Francis Scott Key Md. Ctr., Johns Hopkins University School of Medicine, Baltimore, MD.

The organ shortage remains a major obstacle to renal transplantation. One way to increase the supply of organs would be to use unconventional LD, i.e., minors and genetically unrelated adults. To determine current practice regarding such donors, we surveyed all 169 U.S. transplant centers (Tx Ctr) by mail. 59% responded; these 99 Tx Ctr had performed 5,918 Tx in the past year. Donor preference was: living related 36%, cadaver 20%, and none 44%. 82% of Tx Ctr would allow spouses to donate and 51% would accept adult friends. However, among responding Tx Ctr, only 44 spouses served as donors in the previous year and only 11 friends donated in the past 5 years. 91% of Tx Ctr opposed using strangers.

66% of responding Tx Ctr would allow twin minors (<18 yrs) to donate and 45% would accept non-twin related minors. However, at the responding Tx Ctr, in the past five yrs only 9 twin and 29 non-twin minors served as donors. 94% opposed using friends <18 yrs. The minimum donor age accepted was 16.1±2.8 yrs (5-21).

Despite a generally accepting attitude among Tx Ctr toward allowing spouses, friends and related minors to serve as LD, these unconventional LD are seldom used. This discrepancy could in part relate to a fear of stepping outside the presumed standard of practice. By revealing and pooling Tx Ctr ideas regarding unconventional LD, our data may lessen anxiety over their use and help to increase the donor pool.

ERYTHROCYTOSIS (ECY) AND HYPOFERRITINEMIA IN RENAL TRANSPLANT (Tx) PATIENTS: ERYTHROPOIETIN (EPO) PROFILES BASED ON RECOMBINANT HUMAN EPO RADIOIMMUNOASSAYS. **C.H. Sun*, H.J. Ward, W. Paul*, M.A. Koyle* and D.B.N. Lee. VA Med. Ctr., Sepulveda, Harbor-UCLA Med. Ctr., Torrence, SmithKline Bio-Science, Vay Nuys and UCLA Sch. of Med, Los Angeles.**

Serial EPO measurements in 31 early Tx patients demonstrated parallel increases in EPO and Hct following recovery of graft function. EPO rose from 14±2 mU/ml (pre-Tx) to peak at 52±5 and then dropped to preTx level as Hct rose above 32%. Thereafter Hct rose to normal without further elevation in EPO. In patients with low serum ferritin (FER) and patients who ultimately developed erythrocytosis (ECY, Hct>55%) this anticipated "turn-off" of EPO was not observed and EPO elevation persisted up to 3M after Tx. In 46 long-term (13.8±1.6 M) Tx patients with stable serum creatinine (2.1±0.1 mg/dl), EPO in 8 ECY patients was not different from 28 non-ECY, normal FER patients (16±4 vs 17±3). However, EPO in 10 non-ECY, low FER patients was significantly higher (P<0.001) than, normal FER patients. Because Hct was not different between the normal and the low FER groups (40±1 vs 37±2%), the persistently elevated EPO in low FER patients suggests an association between iron store depletion and increase EPO production. The apparent time-dependent shift in EPO-dependency in ECY patients raises additional questions on the mechanism of Tx ECY. Recently, an association between HLA-A2 and Tx ECY has been reported. We tabulated the frequency of several HLA antigens in 12 ECY vs 75 non-ECY patients: A2 60% vs 44%; C3 50% vs 13%, C7 50% vs 28% and DR4 50% vs 31%. The frequency of C3 was different (P<0.01%) between the two populations, raising the possibility of a genetic influence in the development of post Tx ECY.

PLACEBO CONTROLLED TRIAL OF CALCITONIN (CT) IN THE PREVENTION OF PRIMARY NON-FUNCTION (PNF) IN RENAL ALLOGRAFT TRANSPLANTATION (RTX). P.Sweny*, S.F.Lui*, Z.Varghese*, O.N.Fernando*, J.F. Moorhead. Royal Free Hospital, London, England.

Reperfusion injury due to renal vasoconstriction has been recognised as one of the cause of post-tx PNF. There is some evidence to suggest that CT is a potent renal vasodilator. We have conducted a trial of CT infusion vs placebo in the prevention and/or limitation of PNF in RTX. 13 recipients were randomised for CT infusion (5 iu/hr in albumin solution), commenced 2 hr before, and continued for 48 hr post-operation. 15 recipients were randomised to the control group (given only albumin solution). The two groups were similar in their age, ischaemia time, and HLA matching. 6 of the 15 recipients in the control group were given peri-operative DP because of obvious poor immediate graft perfusion. None of the CT group required DP. The incidence of PNF was similar in the two groups (30% vs 27%). Of the 4 grafts with PNF in the CT group, the duration of PNF was 15 and 27 days in 2 grafts, 2 grafts had persistent PNF due to persistent rejection (histological diagnosis). The duration of PNF was 6, 7, 11, and 13 days for the 4 grafts with PNF in the control group. There was no difference in the serum creatinine or the rate of fall of creatinine for the first 3 days post-transplantation. The perfusion index (^{99m}Tc DTPA renogram) was lower in the CT group (277 vs 447, p=0.14). Although CT has been shown to be a potent renal vasodilator, it did not prevent or limit PNF in this small study.

DONOR-SPECIFIC LYSIS OF HUMAN KIDNEY EPITHELIAL CELLS OF PROXIMAL TUBULUS ORIGIN BY RENAL ALLOGRAFT-INFILTRATED LYMPHOCYTES. Fokko J. van der Woude*, Marjon E. Meijer-Paape*, Mohamed R. Daha*, Bob L.A. van Es* and André M.M. Miltenburg*, Department of Nephrology, University Hospital Leiden, Leiden, The Netherlands (introduced by G.A. Andres).

We deviced a culture procedure in which graft infiltrating cells (GIC) as well as kidney epithelial cells of proximal tubulus origin (PTEC) were selectively expanded from biopsies taken from human renal allografts. PTEC were characterized by morphology and the presence of epithelial membrane antigen and adenosin deaminase complexing protein (ADCP). GIC were tested for lytic acitivity against donor and third party PTEC and against K562 and OKT-3 hybridoma cells. GIC cultured from biopsy material of 2 patients with cellular rejection were shown to lyse, in a dose dependent manner, PTEC of donor origin and were functionally characterized as T cells. GIC cultured from a biopsy specimen of a patient with glomerulonephritis did not show lytic activity against PTEC. GIC cultured from the two renal transplant patients with rejection also lysed phytohaemagglutinin-blasts derived from donor spleen cells. Monoclonal antibody inhibition studies showed inhibition of the anti PTEC lytic activity by anti-CD3 and W6/32 (anti-class I). Anti-CD4, CD8, CD2, class II and leucocyte function associated antigen-1 antibodies had no effect. The results suggest that PTEC may be an important target for the efferent cellular immune response during rejection after renal allotransplantation.

OPEN HEART SURGERY IN PATIENTS UNDERGOING RENAL TRANSPLANTATION: COMPARISON OF SURGERY PRE VS POST TRANSPLANTATION. Claudia Swift,* Donald R. Steinmuller, Andrew C. Novick, Stevan B. Streem,* Ernest Hodge,* Robert Hobbs,* Barbara A. Dlugosz.*

Cardiovascular disease remains one of the most significant causes of morbidity and mortality for end-stage renal disease (ESRD) patients being considered for renal transplantation. These patients are at high risk for complications when undergoing open heart surgery (OHS). We reviewed the records of patients who underwent OHS from 1978 to 1987 and who either had previously received a renal transplant (RT) or who subsequently underwent RT.

	OHS pre RT (N=19)	OHS after Successful RT (N=9)	p
Valvular replacement:	4	4	
CABG:	15	5	
Moderate-Severe Myocardial Dysfunction:	4/16	2/7	
Diabetes Mellitus	6	3/8	
Transfusion ≥5 units:	11/18	1/7	<.01
Reoperation for bleeding:	9 (5 pts)	0	=.14
Infectious complications:	2	4	<.01

In conclusion, OHS can be successfully performed on patients who are candidates for RT or who have undergone successful RT. There is a higher incidence of complications in these patients with bleeding complications being more common in the dialysis recipient and infectious complications more common in the patient who has already received an RT and is on immunosuppressive therapy.

ENALAPRIL-ASSOCIATED ACUTE RENAL FAILURE IN RENAL TRANSPLANTS WITHOUT RENAL ARTERY STENOSIS: POSSIBLE ROLE OF CYCLOSPORINE. Rocco C. Venuto, Brian M. Murray, Romesh Kohli and Eugene Cunningham, SUNY at Buffalo, Department of Medicine, Buffalo, New York.

Acute renal failure (ARF) in renal transplants secondary to the use of angiotensin converting enzyme (ACE) inhibitors has been described previously but almost always in association with either transplant artery stenosis or chronic rejection. We present two cases in which ARF requiring dialysis occured following administration of the ACE inhibitor, enalapril, to patients receiving cyclosporine. Prior to introduction of enalapril both patients had had stable serum creatinine (SCr) for several months (1.1-1.3 and 2.6-2.8 mg/dl respectively). Renal function deteriorated rapidly over a period of 1-2 weeks when the dose of enalapril was increased from 5-20 mg/day. Dialysis was instituted when the SCr reached 7.2 and 9.4 respectively. Discontinuation of both cyclosporine and enalapril in one case and enalapril alone in the second resulted in the return of renal function essentially to baseline (SCr 1.4 and 3.3) after 43 and 20 days respectively. Neither patient was receiving any other nephrotoxic agents. Coexistent rejection and transplant renal artery stenosis were ruled out in both cases by renal biopsy and digital subtraction angiography. Why ARF developed during ACE inhibition in these two patients is therefore not clear but we believe that cyclosporine-induced intrarenal vasoconstriction may have been the predisposing factor. ACE inhibitors should be used with caution in patients receiving cyclosporine and further studies are needed to study the interaction of these two types of agents on renal function.

NEOPTERIN AND CREATININE RELATIONSHIPS IN RENAL TRANSPLANTATION. Harry J. Ward, Martin Koyle* Chao Sun, Takeshi Kita*, and Mitsuo Takasugi*. Renal Transplant Section and Dept of Surgery, Harbor-UCLA Med Ctr, Torrance CA and Westwood, CA.

The relationship between the activated T-cell product neopterin, and plasma creatinine was investigated in a serial study of 74 renal transplnt patients engrafted at Harbor-UCLA between 1984 and 1987. Plasma creatinine and neopterin levels were drawn simultaneously, the latter being measured by radioimmunoassay. Mean neopterin/creatinine scores were determined in patients with complications e.g rejection, CMV infection or allograft ATN and then compared to those with normally functioning grafts.

Patients	N	Neopt	Creat	Neop/Creat	# failed
Functioning graft	63	52.1±4.2	3.3	18.2±0.99	0
Failed	11	201±42.8$^{\alpha}$	6.7^{a}	41.6±7.75$^{\alpha}$	11
Highest Neopterin	11	233±34.9^{a}	7.2^{a}	38.9±4.11$^{\alpha}$	7
Highest Creatinine	11	202±42.2^{a}	7.95$^{\alpha}$	29.3±4.76$^{\alpha}$	6
Highest Neop/Creat	11	222±38.5^{a}	6.4$^{\alpha}$	49..2±6.0$^{\alpha}$	7

all values $\bar{x}$±SEM; $\alpha \geq p < 0.01$ compared to patients with functioning grafts receiving cyclosporine. Mean neopterin and creatinine levels and the neop/creat molar ratios were signigicantly higher for 11 recipients with graft failure in comparison to 63 with functioning grafts by study's end. The molar ratio we employed placed emphasis on immune stimulation and less on renal function. Neop/creatinine ratios are of value in monitoring renal grafts.

EFFICACY AND COST OF THE VARIOUS TESTS USED IN THE DIAGNOSIS OF REJECTION IN CADAVERIC RENAL TRANSPLANT RECIPIENTS. J.D. Welchel,* V.B. Delaney D. O'Brien,* B. Ling,* P.Fekete,* W.G. Campbell. Emory University School of Medicine, Departments of Medicine, Surgery, and Pathology, Atlanta, Georgia.

A clinical diagnosis of acute (AR) and acute complicating chronic rejection (ACR) was confirmed histologically in 40 and 34 patients respectively. Fine needle aspirates (FNAB), renal scans (T_cDTPA, PAH), and Doppler ultrasounds (US) were each performed within 24 hours of biopsy and read blindly. A corrected increment (CI) of >3.5 (FNAB), a decreased, delayed T_cDTPA appearance with preserved PAH elimination, and a resistive index (RI) of >72% (US) were each considered diagnostic of acute rejection.

	BIOPSY	FNAB CI >3.5	DOPPLER US RI >72%	RENAL SCAN
# Patients	40	40	40	40
AR	40	22	22	31
-Humoral	5	0	5	4
-Cellular	35	22	17	27
False negatives	0	18	18	9
Sensitivity	100%	55%	55%	78%
# Patients	34	34	34	34
ACR	34	20	10	28
False negatives	0	14	24	6
Sensitivity	100%	59%	29%	82%
Approx. $ cost	476	296	354	436

Conclusions: At our institution, 1) The most sensitive (78-82%), but least specific (52%) test short of renal biopsy for the diagnosis of acute rejection is renal scan; 2) Doppler ultrasound is 100% sensitive in the diagnosis of acute humoral rejection but becomes progressively less helpful as cellular rejection and/or chronicity intervenes; 3) FNAB was not helpful in the diagnosis of acute humoral rejection and only 50-60% sensitive in detecting cellular rejection.

RESPONSE OF ANTI IgM ACTIVATED B CELLS FROM RENAL TRANSPLANT PATIENTS TO EXOGENOUS LYMPHOKINES. Joshua Weissgarten,* Chang Ng,* Eva Freidman,* Carl J. Cardella. Univ.of Toronto Dept. of Med., Toronto Western Hospital., Toronto

Ig production from SAC activated B cells of Tx pts is below normal even in the presence of exogenous lymphokines. To further evaluate this abnormality B cells were activated by a different pathway (anti IgM) in the presence or absence of B cell lymphokines.

PBL from 8 Tx pts & 10 controls were activated with (FAb)2 anti-IgM and incubated with or without BCGF, recombinant IL-2, Interleukin 1B (IL-1), interferon (IFN), interleukin 4 (IL-4) and interleukin 6 (IL-6). IgG was measured in the supernatant by ELISA. IgG production after anti IgM activation was 191 ± 63 ng/ml in controls & 26 ± 8 ng/ml in Tx pts. (p =<0.01) and was not significantly increased by the addition of IL-1, IL-2, IFN, and IL-6 in either controls or pts. However, Ig production after anti IgM and BCGF was 691 ± 82 ng/ml in controls and 499 ± 36 ng/ml in Tx pts. (p=N.S.) The addition of IL1, IL-2, IFN and IL-6 did not enhance this response. The total IgG levels were not increased by IL-4 in controls or pts but the level of IgG1 without IL-4 was 55 ± 18 ng/ml in controls & 3 ± 1 ng/ml in pts and with IL-4 increased to 115 ± 35 in controls & 8.7 ± 1.8 in pts.

Anti IgM activated B cells from Tx pts do not produce normal amounts of IgG. BCGF but not IL-1, IL-2, IFN, IL-6 and IL-4 corrected this response. IL-4 increased IgG1 production. In contrast to SAC activation BCGF can normalize the response of anti IgM activated B cells.

EVIDENCE FOR THE EXISTENCE OF A Na^+-$2Cl^-$-K^+ CO-TRANSPORT IN THE LUMINAL MEMBRANE OF THE MACULA DENSA (MD) CELLS. A.E.G. Persson*, E. Schlatter,* M. Salomonsson* and R. Greger* (intr. by F.S. Wright). Department of Physiology, University of Lund, Sweden and Department of Physiology, University of Freiburg, FRG.

In the tubuloglomerular feedback mechanism the NaCl concentration is sensed at the MD site. The mechanism for this sensing is not completely known and one possible step in this process may involve the coupled uptake of Na^+ and Cl^-. The present experiments were designed to evaluate if a Na^+-$2Cl^-$-K^+ co-transport existed in the apical membrane of the MD cells like the one in c-TAL. From 115 rabbits 166 glomeruli with attached c-TAL segments were dissected. These segments were perfused in vitro. The MD cells were punctured with microelectrodes at 400X with DIC contrast microscopy. Some 2500 to 3000 attempts to impale these cells were made. Mostly only unstable recordings were obtained. The basolateral membrane voltage (PDbl) of 8 stable impalements were -56±4 mV. With 10^{-5} M furosemide added to the luminal perfusate the PDbl hyperpolarized from -55±5 to -79±4 mV (n=7). A reduction in luminal NaCl concentration with 120 mM hyperpolarized the PDbl from -48±3 mV to -66±5 mV (n=4) while a reduction in bath Cl^--concentration by 120 mM depolarized the PDbl from -57±8 mV to -33±9 mV (n=4). Furosemide, a known blocker of the Na^+-$2Cl^-$-K^+ co-transport, in our experiments hyperpolarized the MD cells. This probably depends on a reduced Cl^- activity in the MD cells. Thus the present experiments indicate the existence of such a co-transport. We propose that this co-transport is the first step in sensing the luminal NaCl concentration by the MD cells.

EFFECTS OF ANTI-TNF ANTISERUM ON PROTEINURIA IN AUTOLOGOUS NEPHROTOXIC NEPHRITIS. Zbigniew Hruby and Robin P. Lowry, McGill University, Dept. of Medicine, Montreal Canada.

The pathogenesis of glomerular injury in nephritic states with prominent glomerular macrophage infiltration is uncertain. We have reported isolated glomeruli of rats with autologous nephrotoxic nephritis release cytotoxins inhibitable both by antibody to recombinant TNF (rMuTNF) as well as by protease inhibitors. These data have been clarified by recent experiments. Cytotoxins released by glomeruli of nephritic rats elute on gell chromatography in a single peak MW 40 KD fully inhibitably by anti-TNF antiserum. Further, the cytolytic activity of rMuTNF is also inhibited by protease inhibitors. Accordingly we have assessed the effect of administering anti-TNF antiserum to nephritic rats. In three seperate experiments anti-TNF antiserum caused a 30-80% reduction in albuminuria on days 2-4 post induction. In parallel studies we have noted equivalent (additive/synergistic) inhibition of proteinuria with administration of proteinase inhibitor BABIM. We have not as yet determined if BABIM inhibits TNF cytotoxicity. Our results highlight the probable importance of TNF in the physiopathology of glomerular injury in experimental nephritis and underline the potential difficulties of critically defining the roles of proteases/protease inhibitors in the pathogenesis/treatment of immune glomerular disease.

ABNORMAL LOW DENSITY LIPOPROTEIN (LDL) RECEPTOR FUNCTION IN CHILDREN WITH END STAGE RENAL DISEASE (ESRD). Ronald J. Portman, Richard Weinberg*, Susan B. Conley and Jacques M. Lemire. Univ. of Texas Med. Sch., Dept. of Peds., Houston, Texas.

LDL receptor function, a critical determinant of serum lipid levels, was investigated in seven hyperlipidemic children with ESRD and 19 controls. LDL receptor function using peripheral blood lymphocytes (PBLs) was assayed as per Cuthbert JA et al (NEJM 1986;314:879). Lovastatin incubated with PBLs prevents endogenous cholesterol synthesis making lectin stimulated cell proliferation dependent upon LDL receptor function. Small amounts of LDL added to media can normally reverse this inhibition by receptor mediated uptake. Results are presented as the percent inhibition of proliferation.

	LDS (ug/ml) per well						*p	.05
	0	1	3	5	10	20	40	80	mevinolate
Cont.	89	61	22	9	-.6	-2	-1	8	-12
SEM	1.5	4.6	4.2	3.1	3.8	4.3	4.8	7.8	5.4
Pts.	86	67	40*	32*	26*	17*	22*	20	-9
SEM	2.7	5.7	8.9	8.6	7.9	9.2	8	8.3	5.3

Thus the addition of even large amounts of LDL did not overcome lovastatin inhibition due to a dysfunctional receptor. 5/7 pts had abnl receptor function. Analysis of the severity of renal failure, serum cholesterol, triglycerides, Ca or PO4 could not explain the difference between pts. In addition, 3 pts with chronic renal failure not yet on dialysis had defective receptor function. One pt had normalization of receptor assay after transplantation; conversely, a nephrotic pt developed defective function after nephrectomy. These data suggest an abnormality in LDL receptor function in uremia. Further, it is possible that this defect may be acquired in the uremic state.

INCREASED RENAL PELVIC PRESSURE (PP) AFTER THROMBOXANE SYNTHESIS INHIBITION (TXI) IN HYDRONEPROTIC RATS (HR) DURING VOLUME EXPANSION. Peter Morsing*, Arne Stenberg* and A Erik G Persson*, Dpt. of Physiol. & Biophys., Lund University, and Dpt. of Ped. Surg., Uppsala University, Sweden. (intr. by Fred S. Wright)

In a previous study on HR, an increased sensitivity and activity of the tubuloglomerular feedback system (TGF) was found during volume expansion (VE) (Morsing et al., Kidney Int 32: 212-218, 1987). After TXI, however, the TGF resetted in a normal way during VE, i.e. to a lower sensitivity. (Morsing et al. Acta Physiol Scand 129:57, 1987). It was proposed that the increased TGF sensitivity served to control PP in HR during VE.

The aim of this study was therefore to measure PP during VE in HR, with and without TXI. To measure PP a catheter was introduced through the parenchyma into the renal pelvis. TXI was performed by i.v. bolus injection of 20 mg/kg BW of UK 38.485 (Pfizer, UK), 30 min before VE was commenced.

In the control period PP was approx 3.5 mm Hg (range 1.5-6 mm Hg), which was not altered by either TXI or vehicle infusion. After VE there was no, or very low pressure increase in the vehicle treated group (5 mm Hg, range 2-6.5 mm Hg), while an increase to a mean value of 14.5 mm Hg, range 8.5-24 mm Hg, was found after TXI.

It is concluded that the pelvic pressure in HR are low during VE, and also that TXI causes the pelvic pressure to increase severalfold during VE. The present results indicates that the increased sensitivity of the TGF protects the hydronephrotic kidney from high PP and that this is mediated through thromboxane release.

PATHOGENESIS AND RECURRENCE OF PERITONITIS DURING CAPD MAY BE ORGANISM SPECIFIC. R. Swartz, B. Starmann*, J. Reynolds*, L. Rocher. University of Michigan Med Ctr, Ann Arbor MI.

Surveillance data show that coagulase positive Staphylococcus (CPS) peritonitis during CAPD is increasing in frequency, and that CPS is the most common pathogen cultured in extraperitoneal catheter infection and in recurring peritonitis.

In a prospective study over 18 months, we have compared cultures of peritoneal effluent with cultures of the catheter exit site taken at the time of presentation in all available cases of peritonitis, noting whether the exit site appeared infected. Cultures from 87 peritonitis episodes (over 70% of all episodes) show that in 56 cases (65%) organisms from the effluent and exit site were concordant. Active infection of the extraperitoneal catheter was detected clinically in 66% of cases with concordant culture results, but in only 35% of cases with discordant cultures, $p \leq .02$. The simultaneous occurrence of peritonitis and culture-concordant active tunnel or exit infection was associated more often with CPS (63%) than with gram negative rods (32%) or coagulase negative Staphylococcus (CNS) (37%), $p \leq .02$.

These results suggest that peritonitis during CAPD is associated commonly with pathogens which colonize the external catheter, particularly in the presence of active infection at the tunnel or skin exit site. This is especially true for simultaneous peritonitis and extraperitoneal catheter infection due to CPS. We conclude that the mechanism for recurring or refractory peritonitis during CAPD may be organism specific (extraperitoneal catheter infection for CPS, some other mechanism for CNS), the approach to eradication may also be organism specific, and prophylaxis for CPS carriers should be considered.