

Abstracts

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ABSTRACTS

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CELL BIOLOGY

HUMAN MESANGIAL CELLS IN CULTURE EXPRESS THE GENES FOR THE A CHAIN AND B CHAIN (c-SIS) OF PLATELET-DERIVED GROWTH FACTOR (PDGF): REGULATION BY THROMBIN. Hanna E. Abboud, Bernard Silver,* Paul DiCorleto* and Pamela Shultz*. VA Medical Center, Case Western Reserve University, and Cleveland Clinic Foundation, Cleveland, Ohio.

PDGF, a major mitogen for cells of mesenchymal origin, consists of two similar but not identical polypeptide chains referred to as A and B chains. Each chain is encoded by a separate gene. We have recently reported that mesangial cells release a PDGF-like protein that competes with authentic PDGF for binding to receptors on fibroblasts. We now analyzed polyadenylated RNA isolated from human mesangial cells by Northern blotting and hybridization to radiolabeled v-sis cDNA that codes for the B chain of PDGF as well as a cDNA that codes for PDGF-A chain. Mesangial cells expressed mRNA that hybridizes with both probes, with the levels of the A chain mRNA being much higher than the B chain mRNA. Since some forms of glomerulonephritis are associated with intraglomerular coagulation and since thrombin is a mitogen for mesangial cells, we studied the effect of thrombin on PDGF gene expression and secretion of PDGF-like protein by human mesangial cells. Utilizing total RNA and Northern blotting, thrombin (0.1-10 U/ml) increased A chain mRNA levels with maximal effect at 4 to 8 hrs returning to basal by 24 hrs. In addition, thrombin stimulated the production of the PDGF-like protein, in a dose (0.1-10 U/ml) and time dependent manner (8-24 hrs). Five U/ml thrombin elicited over five fold increase in A chain mRNA levels and similar increase of the secreted protein. These data suggest a possible mechanism by which the PDGF genes and their secreted product may be involved in the pathogenesis of glomerular hypercellularity.

PROTEIN KINASE C-ACTIVATING PHORBOL ESTERS INHIBIT PROSTAGLANDIN E (PGE)-INDUCED ELEVATION IN CYTOSOLIC FREE Ca^{2+} CONCENTRATION $[Ca^{2+}]_i$ IN MDCK CELLS.

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PGE-induced stimulation of cAMP accumulation in MDCK cells has been well described. Whether the PGE receptor might be linked to the phosphoinositol (PI) pathway in these cells has not been elucidated. Confluent cultures of MDCK cells were loaded with the Ca^{2+} -sensitive fluorescent probe FURA-2, and changes in $[Ca^{2+}]_i$ monitored as a measure of activation of the PI pathway. Acute exposure of cells to PGE₁ and PGE₂ but not PGF_{2α} evoked a transient increment in $[Ca^{2+}]_i$ that was independent of extracellular Ca^{2+} and the $K_{0.5}$ for the process was $7.5 \times 10^{-7}M$. Brief incubation of cells with either phorbol 12-myristate 13-acetate (PMA), phorbol-12,13-dibutyrate (PDB) or phorbol-12,13-diacetate (PDA) (all $10^{-6}M$) completely blocked the PGE-induced increment in $[Ca^{2+}]_i$. Neither 4α- nor 4β-phorbol blocked the PGE-induced Ca^{2+} transient. When PMA, PDB or PDA-treated cells were acutely exposed to bradykinin ($10^{-9}M$), the previously reported acute increment in $[Ca^{2+}]_i$ was not blocked. **Conclusions:** 1. The PGE receptor of MDCK cells is linked to the PI pathway. 2. Protein kinase C appears to modulate transduction of PGE activation of the PI pathway at the level of the PGE receptor.

ONTOGENIC HETEROGENEITY OF LAMININ WITHIN DEVELOPING GBM OF NEWBORN MOUSE KIDNEYS. Dale R. Abrahamson and Patricia L. St. John,* Univ. of Alabama at Birmingham, Birmingham, Alabama.

The GBM originates in development from the fusion of endothelial basement membranes with those beneath podocytes in S-shaped and early capillary loop stage glomeruli. Subsequently, additional basement membrane synthesized by podocytes is spliced into existing GBM during capillary loop expansion. To examine these processes further, we prepared and characterized 9 different rat monoclonal IgGs against mouse laminin, a glycoprotein abundantly present within all basement membranes. These IgGs reacted only with laminin, and not with any other known basement membrane component. Many of these IgGs bound to areas of mature mouse TBM, GBM, and mesangial matrix as shown by immunoelectron microscopy. Several monoclonals, however, which bound to TBM, failed to react with mature GBM indicating that the relevant epitopes at this site were unexposed or missing. To determine whether the availability of these specific epitopes was developmentally related, cryostat sections of newborn mouse kidney were labeled with appropriate monoclonal anti-laminin IgGs and examined by immunofluorescence microscopy. Rat IgG bound in linear patterns to developing GBMs of all comma, S-shaped, and early capillary loop stage glomeruli but the GBMs of more mature nephrons were unlabeled. This suggests that some laminin epitopes become masked or removed, perhaps as a result of basement membrane fusion or splicing, during GBM assembly. Alternatively, glomerular laminin may undergo conformational changes as the GBM matures.

ORGANELLE INHERITANCE DURING CELL DIVISION. Robert L. Bacallao* and William Wickner.* Division of Nephrology, Molecular Biology Institute and Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA. (Intro. by L.G. Fine).

During the process of cell division, the daughter cells inherit all the genetic material necessary for the survival of the cell. It is not yet known how a daughter cell inherits organelles during cell division. It is possible that they are inherited in a semiconservative manner analogous to DNA inheritance. Alternatively the daughter cell may inherit a small aliquot of maternal organelles which serves as a template for further *de novo* synthesis.

To study the division and inheritance of organelles we used *Saccharomyces cerevisiae* as a model system. Growing yeast were covalently bound to polystyrene beads. The tethered yeast grew at a normal rate and released daughter cells. The bound cells were pulse labeled with [³⁵S]-methionine, and inheritance of labeled parental components by successive generations of daughter cells were assayed. Approximately 50% each of the parental vacuolar carboxypeptidase Y, mitochondrial matrix protein B and cytoplasmic hexokinase were inherited by the daughter cells in each successive generation. Conclusion: The daughter cell inherits 50% of the total of each of the maternal cell's organelles.

ANALYSIS OF FUNCTIONAL DIFFERENCES IN THE GROWTH AND SYNTHESIS OF TUBULOINTERSTITIAL FIBROBLASTS (TF) AND TUBULAR EPITHELIUM (MCT) IN MICE. R.J. Alvarez*, T.P. Haverty*, and E.G. Neilson, Renal Section, Univ. of Penna., Phila. PA.

Fibroblasts and tubular epithelium play an important role in the interstitial fibrogenesis of progressive renal failure. In analyzing these cells we wished to examine the hypothesis that syngeneic mouse TF cells may be functionally distinct from fibroblasts in other anatomic locations, like the skin (SF), and that TF cells may be biologically unique when compared to MCT cells. We, therefore, analyzed TF, MCT, SF cells by their synthesis of procollagen molecules, and for their proliferative response to growth factors and immune modulators. Secretion of procollagen molecules in the naive, confluent state by radioimmunoassay using collagen-specific antibodies demonstrates that:

Types	Procollagen Secretion (ng/10 ⁵ cells)		
	I	III	IV
TF	75	3	250
SF	1250	225	50
MCT	21	45	1651

Using ³H-TdR uptake as a measure of growth in serum-free media, we observed that various factors differentially altered DNA synthesis:

	³ H-TdR incorporation (Stimulation Indices)			
	TGF _β (1 ng/ml)	EGF (2 ng/ml)	IL-2 (100 U/ml)	IFN (10 ³ U/ml)
TF	3.4	2.3	0	0.6
SF	15.0	14.4	0	0
MCT	0.6	1.4	0.9	0.2

These findings indicate that TF cells are functionally unique when compared to tubular epithelium and fibroblasts from other anatomic sources, and their cell biology may be differentially modulated by local environmental events.

GLOMERULAR ENDOTHELIAL CELL (GEC)-DERIVED FACTOR(S) STIMULATE MESANGIAL CELL (GMC) DNA SYNTHESIS. Barbara J. Ballermann, and Mary M. McNamara*. Brigham and Women's Hospital and Harvard Medical School, Boston, MA

To investigate a potential influence of GEC on GMC growth, bovine GEC were established in culture. GEC were separated from epithelial and GMC by fluorescence-activated cell sorting using indocarbocyanine-conjugated acetylated LDL (ac-LDL). GEC were propagated on gelatin-coated plates in heparin- and endothelial cell growth factor-supplemented media. GEC cultures were positive for angiotensin I converting enzyme activity, uptake of ac-LDL, and Factor VIII, characteristics not expressed by GMC. Angiotensin II receptors were present in GMC but not in GEC.

GEC and GMC were rendered non-proliferative by replacing serum in the medium with 2% plasma-derived serum (PDS) for 72 hrs. GMC were then co-cultured with GEC or more GMC on 0.4 μm membranes for 18 hrs. Alternatively, GMC were fed with GEC conditioned medium (GEC-CM) containing 2% PDS for 18 hrs. ³H-thymidine incorporation was stimulated 2.0 ± 0.3 fold (p < 0.05, n = 3) in bovine GMC when co-cultured with GEC, whereas no stimulation was observed in GMC co-cultured with GMC. Also, ³H-thymidine incorporation in bovine and rat GMC was stimulated 4.9 ± 1.2 (n = 4) and 2.2 ± 0.6 fold (n = 3), respectively, by GEC-CM (p < 0.05) for both, whereas GMC-CM had no effect. Conclusions: Bovine GEC can be successfully cultured in vitro (15 passages, 3 separate cell lines to date). These cells express gene products similar to bovine aortic endothelial cells. Finally, bovine GEC produce factor(s) that stimulate DNA synthesis in bovine and rat GMC.

MACULA Densa CYTOSOLIC CALCIUM CONCENTRATION DURING CHANGES IN LUMINAL FLUID OSMOLALITY. P. Darwin Bell, Andrew Krause,* and Martha Franco. University of Alabama at Birmingham, Birmingham, AL, and Johns Hopkins University, Baltimore, MD.

Previous *in vivo* micropuncture studies, suggested that the macula densa cells may detect changes in luminal fluid composition through a cytosolic calcium system. To evaluate this proposal directly, thick ascending limbs with attached glomeruli were dissected from rabbit kidney in a media containing 5 μ M Fura 2AM. After one hour of incubation in Fura 2AM, the tubule was transferred to a chamber mounted on a fluorescent microscope. The thick ascending limb was cannulated and the tubule warmed to 37 C. Thirty min were allowed to elapse before measurements were obtained. Fura 2 fluorescence was measured at excitation wavelengths of 345 nM and 386 nM and emission wavelength was set at 510 nM. Using a diaphragm, a window was placed over the macula densa plaque and photon counting was performed within this window with a photometer system. The tubule was initially perfused with a 100 mOsm solution and the bath remained constant at 290 mOsm. Under these conditions, macula densa cytosolic calcium concentration averaged 47 ± 14 nM (n=8). In response to an increase in luminal fluid osmolality to 290 mOsm, macula densa cytosolic calcium increased to 153 ± 24 nM (p<0.001). This increase was reversible, with macula densa cytosolic calcium returning to 54 ± 8 nM during reperfusion with the 100 mOsm perfusate. Thus, these results directly support the notion that an increase in the composition of tubular fluid results in an activation of a macula densa cytosolic calcium system.

NaKATPase ACTIVITY IN PROXIMAL CONVOLUTED TUBULE (PCT) SEGMENTS IS MODULATED BY A G-PROTEIN WHICH IS Na DEPENDENT AND INHIBITABLE WITH DOPAMINE (DA). Alejandro Bertorello,* Anita Aperia. Karolinska Institute, Stockholm, Sweden.

To examine whether a G-protein is involved in the shortterm regulation of NaKATPase activity, single PCT segments were made permeable and preincubated with nucleotides or vehicle (=control). NaKATPase was determined as ouabain sensitive 32 P-ATP hydrolysis during Vmax conditions for K and ATP. Na concentration in medium (Na_m , mM) varied. Intracellular Na concentration was approximately the same as Na_m in the permeabilized PCT. In control PCT NaKATPase (pmol Pi/mm tubule/h) was 296 ± 29 (SE) at $Na_m 20$ and reached Vmax, 1535 ± 88 at $Na_m 70$ (mean \pm SE, n=3-6 rats, 10-12 tubules analyzed from each rat). An inhibitor of GTP dependent activation of G-protein, GDP β S (400 μ M) promptly decreased NaKATPase activity to $33 \pm 4\%$, (GDP β S vs control) at $Na_m 70$. Inhibition was attenuated at $Na_m 50$, $52 \pm 8\%$ and $Na_m 30$, $68 \pm 8\%$ and not significant at $Na_m 20$. A nonhydrolysable GTP analogue, GppNHp (100 μ M) significantly increased NaKATPase activity at $Na_m 30$, $179 \pm 22\%$ (GppNHp vs control), but not at $Na_m 70$. DA inhibits PCT NaKATPase activity at $Na_m 70$ (AJP 232:F32). In this study we found that DA inhibition of NaKATPase was attenuated at $Na_m 30$ and no longer significant at $Na_m 20$. DA inhibition at $Na_m 70$ was competitively abolished by GppNHp, $K_i = 24$ μ M. Conclusion: NaKATPase activity is modulated by a G-protein that is activated when intracellular Na concentration is increased. DA inhibits NaKATPase in Na loaded PCT by inactivating this G-protein.

ISOLATION OF cDNA ENCODING ANGIOTENSIN-CONVERTING ENZYME (ACE). K. E. Bernstein, B. M. Martin, E. Bernstein, L. Striker, and G. Striker. NIDDK and NIMH, NIH, Bethesda, Maryland.

ACE, an enzyme produced in large amounts by endothelium and tubular epithelium, converts Angiotensin I to Angiotensin II. We purified enzymatically active ACE from renal lysates on an affinity column constructed by linking an ACE inhibitor, Lisinopril, to Affi-Gel 15. The molecular weights of ACE were 144 kDa (mouse) and 149 kDa (bovine). N-terminal amino acid sequence analysis revealed:

	1	5	10	15	20
Mouse	L	D	P	G	L
Bovine	E	L	D	P	A

Though bovine ACE has 1 additional N-terminal amino acid, these 2 partial sequences are homologous at 16 of 20 positions. Amino acid sequence analysis of tryptic fragments of mouse ACE revealed the following N-terminal sequences: TLGPA, ELYES, KYEEL, VSEEF, WYESP, FHVPA, GLYES, and ALLEY. cDNA was prepared in lambda gt10 from mouse kidney mRNA enriched for species encoding large peptides. Screening this library with 5 oligonucleotide probes identified 26 plaques hybridizing with 3 of the oligonucleotides and 5 plaques hybridizing with the other 2 oligonucleotides (including 1 encoding ACE N-terminal sequence). cDNA in the first group are as large as 4500 base pairs while 2 cDNA from the second group are 1000 base pairs. Northern analysis of mouse kidney mRNA with probes from both groups of putative ACE cDNA gave an identical pattern, two bands of 4600 and 3850 bases. Thus cDNA has been isolated encoding almost the entire ACE mRNA.

NOVEL AND ENHANCED INTERLEUKIN 1 GENE EXPRESSION IN LUPUS NEPHRITIS. J. Boswell*, M. Yui*, D. Burt*, V.E. Kelley. Brigham & Women's Hosp., Boston, MA

Interleukin 1 (IL-1) is a pleiotropic factor encoded for by at least 2 genes α and β , and capable of eliciting a broad set of immunologic and inflammatory events. Infiltration of macrophages (macs) and the proliferation of the glomerular mesangial cells are prominent features of autoimmune MRL-*lpr* mice with lupus nephritis. Since mesangial cells and macs can synthesize IL-1, the purpose of this study was to determine whether enhanced IL-1 gene expression occurs in lupus nephritis. Glomerular macs, abundant in the kidneys of MRL-*lpr* mice, but rarely present in congenic MRL-*+* mice, were isolated and cultured and found to express a 10 fold increase in both IL-1 α and IL-1 β mRNA transcripts as compared to MRL-*+* and MRL-*lpr* mesangial cells. IL-1 α was not detected in the total RNA extracted from freshly excised kidney, while IL-1 β transcripts were in both the renal cortex and medulla. MRL-*lpr* as well as MRL-*+* mice. A previously undetected truncated 1,200 nucleotide IL-1 β transcript together with the conventional 1,600 nucleotide IL-1 β transcript was in kidneys from MRL-*lpr* and MRL-*+* mice but was abundantly expressed only in glomeruli of MRL-*lpr* mice with nephritis. These studies indicated that IL-1 β gene expression is increased in lupus nephritis and is generated, at least in part, by glomerular macs. We speculate that a specific defect in IL-1 β gene expression is responsible for causing a cascade of events leading to acute and chronic renal injury.

ENDOTOXIN STIMULATES MYRISTYL ACYLATION IN MESANGIAL CELLS & MONOCYTES: MYRISTYLIZATION OF THE IL-1 PRECURSOR. S.L. Bursten, R.M. Locksley,* J.L. Ryan,* & D.H. Lovett. U. of Washington, Seattle, WA.; U. of California, San Francisco, CA.

Acylation of cellular proteins with the fatty acid myristate represents an important mechanism for the co-translational modification of proteins. Lipid A, the active component of bacterial endotoxin is known to stimulate a number of cell types including mesangial cells (MC). We have previously shown that MC are stimulated by Lipid A to secrete prostaglandins & interleukin 1 (IL-1), as well as to undergo acute shape changes characteristic of cellular activation. In this study, Lipid A stimulated the rapid synthesis & myristyl acylation of several discrete proteins in both monocytes & cultured MC. The synthesis & acylation of a 33 kD, pI 7.2 protein was particularly enhanced in Lipid A-treated monocytes & MC. This protein was identified as the IL-1 precursor on the basis of specific immunoprecipitation. The 17 kD, extracellular form of IL-1 did not contain covalently-linked myristate. Myristyl acylation of the IL-1 precursor, which lacks a hydrophobic signal sequence, may provide a mechanism whereby this protein can insert into cellular membranes. Proteolytic processing may then yield a non-acylated, secretory form of IL-1.

These findings demonstrate the similarities in the post-transcriptional processing of IL-1 by monocytes & MC, and provide further insight into the mechanisms whereby bacterial components induce cellular activation & secretion of inflammatory mediators.

MECHANISMS OF ARGININE VASOPRESSIN (AVP) AND ANGIOTENSIN II (AII) DESENSITIZATION IN CULTURED RAT VASCULAR SMOOTH MUSCLE CELLS (VSMC). C. Caramelo,* P.H. Tsai and R.W. Schrier. Univ. Colorado Sch. Med., Denver, CO.

The role of protein kinase C (PKC) in the AVP (10^{-7} M) and AII (10^{-7} M) desensitization in VSMC was studied by examining the effect of phorbol-12-myristate-13-acetate (PMA) (10^{-9} to 10^{-7} M) on these hormones' capacity to increase 1) inositol trisphosphate (IP_3) as measured by Dowex chromatography, 2) cytosolic Ca^{2+} ($[Ca^{2+}]_i$) by fura 2 and 3) Ca^{2+} efflux. Pretreatment with PMA (10^{-7} M) was associated with a decrease in IP_3 concentration as compared to hormone alone, both for AVP (423±96 vs 896±112 counts, $p < .005$) and AII (620±81 vs 1063±144 counts, $p < .01$). PMA pretreatment also decreased the hormone-mediated increment in $[Ca^{2+}]_i$, both for AVP ($\Delta 123 \pm 18$ vs $\Delta 30 \pm 6$ nM, $p < .005$) and AII ($\Delta 131 \pm 12$ vs $\Delta 29 \pm 2$ nM, $p < .005$). Ca^{2+} efflux was also decreased by PMA pretreatment for AVP and AII by 75% and 71%, respectively, both $p < .005$. These effects of PMA were partially reversed by the specific PKC inhibitor H7 (5×10^{-5} M) and were not mimicked by the inactive 4- α -phorbol. PMA enhanced the effect of the Ca^{2+} ionophore, ionomycin (10^{-7} M), to increase Ca^{2+} efflux (2.7 ± 0.1 vs $4.2 \pm 0.2\%$ total counts/30 sec, $p < .01$). We therefore conclude that activation of PKC with subsequent substrate phosphorylation is involved at a common site in the process of desensitization of AVP and AII. This effect may involve receptor or regulatory subunit phosphorylation with subsequent diminution of IP_3 generation, $[Ca^{2+}]_i$ release, and Ca^{2+} efflux, effects which may obscure a separate action of PMA to enhance Ca^{2+} efflux.

CHARACTERISTICS OF SIMIAN VIRUS 40 (SV40) - TRANSFORMED RABBIT KIDNEY PROXIMAL CELLS (PC). R. Cassingena*, A. Vandewalle,* S. Estrade,* B. Baudouin,* M. Geniteau*, P. Verroust*, P. Ronco*, INSERM U246, U64 and IRSC CNRS ER278, Paris, Villejuif, France (Intr. by C. Le Grimellec).

Isolated PC from rabbit kidney were grown in either fetal calf serum (CS) or serum free hormonally defined medium (DM), infected 24 h after the first subculture with wild type SV40 (100 PFU/cell) and then cultured in newborn CS and DM respectively. All the infected cells (IC) examined at different passages (P) expressed SV40 large T antigen. Studies were performed between P8 and P19. Basal (B) and 10^{-6} M forskolin (FK) stimulated cAMP were higher in IC than in uninfected cells (UC) grown in CS (B: 31 ± 2 vs 20 ± 3 ; FK: 1022 ± 102 vs 252 ± 14 pmol/mg) or DM (B: 37 ± 8 vs 9 ± 1 ; FK: 1430 ± 47 vs 194 ± 44). Definite PTH stimulation of cAMP production was observed in IC (x 2.0) although to a lesser extent than in UC grown in CS (x 5.1) or DM (x 21.9). Hydrolase activities (2-10 times lower than in UC) were similar in CS and DM grown IC but the enzymes exhibited a contrasting distribution akin to that observed in the parental UC with antibodies to dipeptidylpeptidase IV, endopeptidase and AII converting enzyme: they were detected in cytoplasmic vesicles in all CS grown IC whereas they showed strong membrane expression in the DM IC. When injected to nude mice at P13, both IC populations induced tumors. In conclusion: 1. Transformed PC with several differentiated functions can be obtained after infection with SV40. 2. DM seems more suitable than CS to generate immunomorphologically differentiated IC. 3. These cells may provide a useful model to study SV40 gene control of differentiation and tumor growth.

PROTEIN KINASE C DEPENDENT PATHWAYS OF STIMULATED RENAL GROWTH. K.V. Chacko* and M.K. Hise. Univ. of Maryland Hosp., Dept. of Medicine, Baltimore, MD.

New polyamine synthesis is an essential requirement for cell growth. Ornithine decarboxylase (ODC) is the first and rate limiting step in polyamine synthesis. The ability of protein kinase C (PKC) to regulate ODC was examined using the rat model. Four hours following the intraperitoneal (IP) injection of 2.5 nmol/g body weight of 4 α -phorbol, an inactive phorbol, kidney ODC activity averaged 93.1 ± 27.4 pmol mg^{-1} min^{-1} while ODC activity following the IP injection of 2.5 nmol/g phorbol 12-myristate 13-acetate (PMA), a potent stimulator of PKC, averaged 284.1 ± 10.9 pmol mg^{-1} min^{-1} ($n=4$, $p < .001$). PMA stimulated ODC activity was dose related in the kidney and maximal at 1.25 nmol/g. Mezerein, a chemically distinct stimulator of PKC had an identical effect on ODC activity. Stimulated ODC activity was dependent upon new mRNA and new protein synthesis.

Four hours following sham nephrectomy, ODC activity averaged 112.9 ± 15.6 pmol mg^{-1} min^{-1} while activity in the contralateral kidney 4 hours following unilateral nephrectomy (UNx) averaged 319 ± 30.0 pmol mg^{-1} min^{-1} ($n=4$, $p < .01$). Activity of ODC stimulated by PMA was not additive to that observed following unilateral nephrectomy. When renal growth was stimulated by folic acid administration, ODC activity stimulated by UNx and PMA was down regulated.

Taken together, the above data indicate that: 1) PKC dependent pathways of renal growth are present in rat kidney, 2) the PKC dependent pathway is saturated following unilateral nephrectomy, and 3) both PMA and UNx pathways are similarly down regulated following a potent stimulus for cell growth.

INSULIN, INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND EPIDERMAL GROWTH FACTOR (EGF) STIMULATE AND TRANSFORMING GROWTH FACTOR β (TGF β) INHIBITS PROLIFERATION OF CULTURED RAT MESANGIAL CELLS. Hangil Chang* and Kiyoshi Kurokawa. IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo, Japan

Mesangial cell proliferation is an important feature of certain glomerulonephritis. In order to clarify the mechanisms underlying mesangial cell proliferation, we examined the effects of fetal calf serum (FCS) and of well characterized growth factors (insulin, IGF-I, EGF, and TGF β) using thymidine incorporation as a marker for cell proliferation. Cultured rat mesangial cells of 4-10th passages were used. Growth arrested mesangial cells, cultured for prior 2 days in 1g μ (0.5%) FCS medium, were incubated with 1 μ Ci/ml 3 H-thymidine and test agents for 2 days and 3 H-thymidine incorporation to 5% TCA precipitate was measured. FCS (2-20%), insulin (16 nM-16 μ M), IGF-I (0.1-5 nM) and EGF (0.1-10 nM) all stimulated thymidine incorporation dose-dependently with the maximum stimulation being 6-8, 1.22, 1.22 and 1.20 fold of controls (0.5% FCS), respectively. The dose response curves of the effects of insulin and IGF-I suggest that both peptides act through IGF-I receptors. A combination of maximum doses of IGF-I and EGF led to 1.70 fold stimulation of thymidine incorporation, thus the effect was synergistic. TGF β dose-dependently suppressed FCS-stimulated thymidine incorporation, the maximum inhibition being 40-60% at 1 ng/ml. These results show that FCS as well as IGF-I and EGF stimulate and TGF β inhibits mesangial cell proliferation and suggest that their interaction may be important in the regulation of glomerular physiology and in the pathogenesis of glomerulonephritis.

MECHANISM OF ACTION OF ERYTHROPOIETIN (EPO): A SINGLE CELL STUDY. Joseph Y. Cheung, Russell C. Scaduto, Jr.,* Douglas L. Tillotson,* and Barbara A. Miller.* The Pennsylvania State Univ., Depts. of Medicine, Physiology & Pediatrics, Hershey, PA.

EPO has recently been used successfully in the correction of anemia of end-stage renal disease. The mechanism of action of EPO remains unknown. Using fluorescence microscopy coupled to digital video imaging, we measured changes in cytosolic free calcium concentration ($[Ca_C]$) in single human erythroblast in response to recombinant EPO and granulocyte-macrophage colony stimulating factor (GM-CSF). Erythroblasts were derived from human cord blood erythroid progenitors and cultured for 8 days. Cells were loaded with fura-2 acetyl-methoxy ester (6 μ M) for 90 min. Paired epifluorescence (505nm) video images of single erythroblasts excited at 350 and 380nm were obtained and digitized (512 x 240 pixels) on line. $[Ca_C]$ of single erythroblasts was obtained from the mean pixel intensity of the ratioed (350/380nm) image compared to that obtained by in vitro calibration of fura-2 using identical hardware. Basal $[Ca_C]$ (nM) was 33 \pm 7, increased to a peak of 137 \pm 23 at 5 min post EPO (2 U/ml) addition, and decreased to 93 \pm 15 at 10 min (n=6). Further addition of GM-CSF (50ng/ml) resulted in a second increase in $[Ca_C]$ to 163 \pm 13. Extracellular Ca was not required for the observed $[Ca_C]$ increase. Two control groups of cells treated with mock Cos cell supernatant (inactive) showed no change in $[Ca_C]$ after 10 min. Our observation is the first measurement of $[Ca_C]$ in human erythroblasts and indicates that EPO and GM-CSF mobilize intracellular Ca. The resultant transient rise in $[Ca_C]$ may be the intracellular signal mediating the proliferative/differentiating effects of hematopoietic growth factors.

THE INTRACELLULAR DISTRIBUTION OF COBALT AND ITS EFFECT ON MITOCHONDRIAL RESPIRATION AND MORPHOLOGY IN RAT MYOCARDIUM AND SKELETAL MUSCLE. N. Clyne, R. Wibom, N. Havu, E. Hultman, L-E Lins, S. K. Pehrsson, B. Persson, J. Rydström (intr: C. M. Kjellstrand). Dpts Med, Path, Chem, Karolinska Institute, Dpt Biochem, Sthlm Univ, Stockholm, Sweden.

Cobalt (Co) has been suggested as a myocardial toxin and accumulates in the myocardium of uremic humans. In order to study its cellular effects 3 groups of rats (n = 12/group) were fed a diet containing 12 % protein, 12 % protein supplemented with 20 and 40 mg CoSO $_4$ x 7 H $_2$ O/kg bw/day respectively. After 8 weeks the hearts and soleus muscles were removed. The ultrastructure was analysed by light and electron microscopy. Co in tissues and in four cell fractions was analysed with neutron activation analysis (ng/gww). Mitochondrial respiration was analysed as ATP production rate (mmol/g protein/min) using pyruvate-malate (pyr) and palmitoyl-carnitine-malate (pal) as substrates.

	control	20 mg	40 mg
Co heart	31.5 \pm 5.6	1753 \pm 133***	2480 \pm 279***
Co soleus	15.5 \pm 15.6	445 \pm 60 ***	587 \pm 73***
Pyr heart	0.790 \pm 0.142	0.699 \pm 0.146	0.852 \pm 0.152*
Pyr soleus	0.553 \pm 0.108	0.535 \pm 0.085	0.553 \pm 0.080
Pal heart	0.489 \pm 0.167	0.360 \pm 0.122*	0.386 \pm 0.101
Pal soleus	0.454 \pm 0.092	0.045 \pm 0.078	0.459 \pm 0.068

* < 0.05, *** < 0.001

Co exposed hearts showed slight hyperemia.

The microsomal fraction contained 80 and 68 % of the total Co in unexposed and exposed myocardial cells respectively.

In conclusion, Co shows a marked accumulation in myocardial cells, particularly the microsomal fraction, causes only minor microscopical changes, only slightly affects MR and probably exerts its toxic effect by damaging microsomal functions.

ELEVATED PROTO-ONCOGENE EXPRESSION FOLLOWING ACUTE RENAL INJURY. B.D. Cowley, Jr.*, J.J. Grantham, & J.P. Calvet*. Univ. of Ks. Med. Ctr., Depts. of Med. & Biochem., Kansas City, KS.

Ischemic and nephrotoxic acute renal failure are characterized by a recovery phase in which surviving tubule epithelial cells proliferate and reline the tubule. We have previously demonstrated elevated *c-myc* oncogene levels 24 hours after a single dose of folic acid, which causes tubular injury followed by cellular proliferation during the reparative phase. In the current study we included other oncogenes and determined the time dependence of oncogene expression. Adult CF-1 mice were injected intraperitoneally with 250 mg/kg folic acid in 150 mM NaHCO $_3$; controls received only diluent. Acute azotemia was confirmed by mean BUN levels of 111 mg/dl 24 hours after injection. Mice were sacrificed at various times after injection, whole kidney poly(A) $^+$ RNA was isolated, and steady state oncogene levels were determined by Northern blot analysis. Increased levels of the oncogenes *c-fos*, *c-myc*, and *c-ras* were detected relative to controls. *c-fos* levels were increased as early as 3 hours post injection and rapidly fell, *c-myc* levels rose later and peaked near 12 hours post injection, and *c-ras* levels rose even later and peaked near 36 hours post injection. To investigate whether the renal response was drug specific, we induced renal injury with 5mg/kg intravenous HgCl $_2$ and sacrificed animals 24 hours later. *c-myc* levels were clearly increased, suggesting that the increased oncogene levels following renal injury are not specific for the cause of injury, but are related principally to reparative renal cell proliferation. The pattern of oncogene expression following folic acid induced renal injury is very similar to that seen following CCl $_4$ induced liver injury, and suggests that the mechanisms of cellular proliferation following acute injury *in vivo* are similar in these organs.

BRADYKININ (BK) Ca^{2+} TRANSIENTS IS DEPENDENT ON Na^+/Ca^{2+} EXCHANGE AND NOT BY THE Na^+/H^+ ANTIPORT IN MDCK CELLS. Daniel Coyne, E.J. Crague, D. Portilla, and A.R. Morrison, Washington Univ., Depts. of Pharmacology and Medicine, St. Louis, MO.

In some cell systems, activation of the Na/H antiport is required for stimulus induced activation of phospholipase C and subsequent Ca^{2+} release. To investigate whether cell pH and Na/H antiport modulate $[Ca^{2+}]_i$ release in response to BK in MDCK cells, we used $0 [Na]_0$ and an amiloride analogue, L651, to inhibit Na/H exchange, and nigericin (N) and monensin (M) to fix pH_i at various levels. $10^{-8}M$ BK stimulation led to a rapid rise in $[Ca^{2+}]_i$ in Fura-2 loaded cells, from 116 ± 23 nM to 972 ± 238 nM with return to baseline at 2 min. At $10^{-8}M$ BK, nominally $0 [Na^+]_0$ reduced $[Ca^{2+}]_i$ transient to 720 ± 221 nM ($N=6$, $P=0.11$). L651 exhibited a K_i of $9 \mu M$ in 10 mM $[Na]_0$, and at $25 \mu M$ inhibited Ca^{2+} release by $82 \pm 6\%$. However, in 145 mM K^+ , 10 mM Na^+ , pH 7.4, $1 \mu M$ N failed to reverse L651 inhibition. Fura-2 loaded cells suspended in 70 mM K^+ , 70 mM Na^+ buffers at pH 7.1, 7.4, and 7.6 were treated with $0.5 \mu M$ N or $10 \mu M$ M, then stimulated with $10^{-8}M$ BK. N caused $33 \pm 5\%$ inhibition of Ca^{2+} release, but this was not pH dependent. M did not affect Ca^{2+} release at any pH. Neither N nor M reversed the inhibition by L651. BCECF loaded cells, in NMG buffer, pH 7.4 were acidified by addition of $2 \mu M$ N. The normal recovery in pH_i with 40 mM Na^+ was completely blocked at $1 \mu M$ L651, and partially blocked at $0.1 \mu M$. We conclude that Na/H antiport activation is not required for BK induced Ca^{2+} transients. The inhibition of Ca^{2+} transients by L651 is likely mediated through inhibition of Na^+/Ca^{2+} exchange.

TRANSDUCTION MECHANISMS INDEPENDENT OF PHOSPHOINOSITIDE TURNOVER MEDIATE AGONIST-INDUCED CHANGES IN RENAL MICROVASCULAR ENDOTHELIAL CELL (RMEC) PDGF mRNA TRANSCRIPTION. TO Daniel*, E Yang* and Z Fen*. Departments of Medicine, Pharmacology, and Cell Biology, Vanderbilt University, Nashville, TN. (Intr by M Bryer)

The regulation of endothelial growth factor expression may play an important role in mediating destructive perivascular proliferation in many renal diseases. We have shown previously that the level of RMEC *c-sis* mRNA, encoding the B chain of platelet-derived growth factor (PDGF), is induced by thrombin (T) and transforming growth factor- β (TGF- β) and attenuated by agents which increase cellular cAMP (J Biol Chem, in press). The mechanism(s) mediating these bidirectional changes in *c-sis* mRNA levels are yet undefined. In these experiments, stimulation of 3H -myoinositol labeled RMEC's with T in the presence of LiCl increased phosphoinositide turnover 2.3 fold over basal levels, suggesting that T might be acting through activation of kinase C. However, TGF- β induced *c-sis* mRNA levels without effect on PI turnover. Increasing cellular cAMP levels with forskolin attenuates basal *c-sis* expression and blocks induction responses to T and TGF- β . Forskolin had no effect on either basal or T-stimulated PI turnover, showing that cAMP-mediated attenuation acts distal to PI turnover. Moreover, bradykinin increased RMEC PI turnover 2.0 fold without effect on RMEC *c-sis* mRNA levels, suggesting that kinase C may be activated without subsequent induction of *c-sis* mRNA.

To discriminate between changes in mRNA stability and primary effects on transcription of the *c-sis* gene, we measured the rate of *c-sis* mRNA degradation after treatment with actinomycin D. The half life of *c-sis* mRNA was 60 min in untreated cells, and 90 min following stimulation with T in the presence or absence of forskolin, changes in degradation rate insufficient to account for the effects on *c-sis* mRNA expression. These data indicate that both T and forskolin affect RMEC *c-sis* mRNA levels through transcriptional regulation, and that mechanisms independent of PI turnover mediate signalling of these nuclear events.

"ACTIVATION" OF GLOMERULAR EPITHELIAL CELLS (GEC) BY THE MEMBRANE ATTACK COMPLEX (MAC) OF COMPLEMENT (C). A. V. Cybulsky, D. J. Salant, R. J. Quigg, J. Badalamenti*, J. V. Bonventre. Boston University Med. Ctr. and Mass. Genl. Hosp., Harvard Med. Sch., Boston, MA.

In rat membranous nephropathy, proteinuria is due to antibody-directed formation of the C5b-9 MAC and is associated with morphological evidence of GEC injury. We investigated if antibody-directed insertion of the MAC into the GEC plasma membrane in vitro leads to the production of intracellular "messengers" that might act to alter GEC function and morphology. The effect of C5b-9 on the intracellular Ca concentration ($[Ca]_i$) was measured with fura-2. GEC were sensitized with anti-Fx1A and with C8-deficient serum (C8DS) to form C5b-7. Completion of the MAC with a sublytic amount of C8 and C9 in buffer containing 0.5 mM Ca resulted in a $[Ca]_i$ increase from 81 ± 14 to 161 ± 52 nM within 40 sec, persisting for 2 min and then declining ($n=9$, $p<0.001$ by paired analysis). No change was observed in control experiments, where C8DS was substituted with heat-inactivated serum ($n=9$). Completion of the MAC in the presence of 4 mM EGTA also increased the $[Ca]_i$, although to a lesser extent (72 ± 11 to 92 ± 16 nM, $n=6$, $p<0.02$). Activation of phospholipases (PL) by C5b-9 was monitored by changes in 3H -arachidonic acid (AA)-labelled lipids. Addition of a sublytic amount of C8 and C9 to C5b-7-sensitized GEC induced a rise above control in 1,2-diacylglycerol (10.5 ± 3.2 vs $6.0 \pm 2.8\%$ of total 3H cpm, $n=3$, $p<0.05$), phosphatidic acid (7.5 ± 1.7 vs $4.2 \pm 1.6\%$, $n=3$, $p<0.01$) and AA (1.8 ± 0.6 vs $0.6 \pm 0.4\%$, $n=3$, $p<0.05$) at 5 min. Similar increases above control were evident after 20 min. Thus, in GEC, the MAC induces Ca influx, Ca release from intracellular stores, and PL activation. The release of Ca from intracellular stores may be secondary to the hydrolysis of inositol phospholipids by C5b-9-activated PLC.

INVOLVEMENT OF RECEPTOR AND CATALYTIC SUBUNIT IN PROTEIN KINASE C (PKC)-MEDIATED INHIBITION OF VASOPRESSIN-STIMULATED ADENYLATE CYCLASE ACTIVITY (VP-ACA). Bradley S. Dixon*, Ruth Breckon*, Carolyn Burke*, Robert J. Anderson. UCHSC and VAMC, Denver, CO.

Recently, we have shown that the phorbol ester, 4 β -phorbol 12-myristate 13-acetate (PMA) rapidly activates PKC and following a 4 hour delay inhibits VP-ACA in cultured rabbit cortical collecting tubular cells (cRCCT). The inhibition appears to occur at the level of the VP receptor as PMA does not affect cholera toxin- or forskolin (FSK)-stimulated ACA. To further examine these results we studied the effects of two different activators of PKC, the diacylglycerol 1-oleyl-2-acetyl-glycerol (OAG) and the diacylglycerol kinase inhibitor, R59022 on VP-ACA in cRCCT. R59022 increases endogenous diacylglycerol levels by preventing their metabolism to phosphatidic acid. Both OAG ($10 \mu g/ml$) and R59022 ($5 \mu M$) activate PKC as shown by translocation of PKC from cytosol to a membrane bound fraction (% membrane bound PKC: control= $9.6 \pm 6\%$, OAG= $49 \pm 7\%$, R59022= $25 \pm 4\%$, $*p<0.05$) and inhibit VP-ACA. In contrast to PMA, inhibition occurs after only 15 minutes exposure to R59022 (% of control= $53 \pm 10\%$) and after 2 hours exposure to OAG (% of control= $68 \pm 15\%$ $\dagger p<0.01$). To determine whether the catalytic subunit of adenylate cyclase was involved in this inhibition we studied the effect of OAG and R59022 on FSK-ACA. FSK-ACA was not affected by pretreatment with $1 \mu M$ PMA for 6 hours, but was inhibited by both R59022 and OAG (% of control: PMA= $103 \pm 15\%$, OAG= $66 \pm 10\%$ \dagger , R59022= $60 \pm 15\%$). The results demonstrate that PKC-mediated inhibition of VP-ACA can involve the receptor and catalytic subunits of adenylate cyclase depending upon the type of PKC-activator employed.

NA/CA EXCHANGE AND THE FALL OF CYTOSOLIC CALCIUM CAUSED BY REMOVAL OF EXTRACELLULAR CALCIUM IN RAT PROXIMAL TUBULE. J. Dominguez and J. Rothrock*. V.A. Med Ctr & Ind Univ Med Sch, Indianapolis, IN.

We tested the hypothesis that in rat proximal tubules (PT), cytosolic calcium (Ca_i) is regulated by exchange of extracellular Na^+ (Na_o) for Ca_i (Na_o/Ca_i). Ca_i was measured in aequorin loaded PT perfused with Krebs-bicarbonate buffer at 37°C. We also measured ^{45}Ca efflux expressed as fractional efflux ratio of PT (FER). In 4 PT, equimolar substitution of 1.5 mM Ca_o with Sr^{2+} decreased Ca_i from 99 ± 18 to 51 ± 8 (nM, Mean \pm S.E.). The fall in Ca_i may be due to decreased Ca^{2+} influx in which case FER may also decrease. However, in 5 experiments FER did not fall. Alternatively, Ca_i may decrease due to enhanced Na_o/Ca_i . If so, substitution of Na_o from 150 mM to 15 mM with TMA and choline, may prevent the fall of Ca_i when Ca_o is substituted. In 19 PT, substitution of Na_o , 2 minutes after Ca_o substitution, increased Ca_i from 100 ± 7 to 201 ± 14 , which fell to 62 ± 6 when Na_o was restituted. In the same PT repeated Ca_o substitution, while Na_o was 150 mM, lowered Ca_i from 98 ± 6 to 60 ± 7 . In 5 PT Ca_o was substituted for 20 minutes, after which Na_o was substituted, Ca_o substitution reduced Ca_i from 100 ± 8 to 64 ± 4 , which increased to 113 ± 31 when Na_o was substituted and again fell to 59 ± 16 when Na_o was restituted. We propose that the fall in Ca_i when Ca_o is substituted is partly dependent on Na_o , which may be expected if Na_o/Ca_i is activated, and that Na_o/Ca_i has a significant role in Ca_i homeostasis in rat PT.

ANGIOTENSIN EFFECTS ON PHOSPHOLIPASE A_2 IN PROXIMAL TUBULAR EPITHELIUM ARE MEDIATED BY A GTP BINDING PROTEIN. Janice G. Douglas, University Hospitals, Case Western Reserve University, Cleveland, Ohio.

Recent studies from this laboratory support the existence of a unique class of angiotensin II (AII) receptors on rabbit proximal tubular epithelial cells. AII is coupled to adenylate cyclase through an inhibitory GTP binding protein (ED_{50} of 1 pM), an action that may mediate antinatriuresis. The present studies were designed to test the hypothesis that AII is coupled, at higher concentrations, to phospholipase A_2 . Rabbit proximal tubular cells maintained in tissue culture were labelled with [3H] arachidonic acid and product formation monitored by reverse-phase HPLC. AII (10 nM) stimulated formation of very non-polar products which migrate with epoxyeicosatrienoic acids (epoxides). Product formation was inhibited by glucocorticoid consistent with an action mediated through phospholipase A_2 . In addition, pertussis toxin pretreatment blocked AII's effect. These observations support the hypothesis that AII is coupled to phospholipase A_2 , proximally through a pertussis toxin sensitive GTP binding protein. This represents a mechanism of signal transduction alternative to adenylate cyclase. These findings suggest that AII-induced epoxide formation in proximal tubule may contribute to natriuresis analogous to PGE_2 production by distal tubular and collecting duct epithelium.

EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ ON CYTOSOLIC pH OF CULTURED RAT GLOMERULAR MESANGIAL CELLS. George R. Dubyak*, Paolo Mene*, Antonio Scarpa*, and Michael J. Dunn. Depts. of Medicine and Physiology, Case Western Reserve University and University Hospitals, Cleveland, Ohio.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a major product of arachidonate cyclooxygenation in the kidney. Exposure of quiescent cultures of rat mesangial cells to $PGF_{2\alpha}$ results in a rapid elevation of cytosolic calcium ($[Ca^{2+}]_i$) followed by cell proliferation. We asked whether rapid $[Ca^{2+}]_i$ movements stimulated by $PGF_{2\alpha}$ are accompanied by changes of intracellular H^+ concentration ($[pH]_i$), as observed with other mitogens or activators of protein kinase C. $[pH]_i$ was monitored spectrofluorometrically at 510/535 nm excitation/emission wavelengths in BCEFC-loaded confluent monolayers of rat mesangial cells grown on plastic coverslips. Signals were calibrated by varying extracellular pH in the presence of 130 mM K^+ and the K^+/H^+ exchanger, nigericin. $PGF_{2\alpha}$ stimulated a rapid cytosolic acidification, from a basal $[pH]_i$ of 7.28 ± 0.04 to 7.18 ± 0.16 (0.1 uM) and 7.10 ± 0.06 (1 uM) within 120 sec, followed by partial or complete reversal in <10 min, with net alkalization detectable only in multiple passaged cells. $PGF_{2\alpha}$ also further acidified cells whose $[pH]_i$ was preset to 6.95 ± 0.07 by isosmotic replacement of Na^+ with choline, thereby inactivating Na^+/H^+ exchange. Rapid net alkalization followed addition of 20 mM Na^+ , an effect enhanced by 1 uM PGF_2 (0.95 ± 0.17 vs. 0.43 ± 0.12 pH units/min, $p < 0.05$), suggesting differential activation of a Na^+/H^+ exchanger. Arginine vasopressin (0.1 uM) and the Ca^{2+} ionophore, ionomycin (10 uM) also induced rapid cytosolic acidification (to 6.92 ± 0.16 and 7.05 ± 0.10 , respectively), suggesting Ca^{2+}/H^+ exchange as a mechanism underlying early $[pH]_i$ responses. Sustained changes of $[pH]_i$ may reinforce $[Ca^{2+}]_i$ as an intracellular signal for certain prostanoids and mediate their long-term effects on the glomerular mesangium.

FIBRONECTIN (FN) AND ADHESIVE GLYCOPROTEINS (AGP) STIMULATE PROLIFERATION OF QUIESCENT MESANGIAL CELLS. Michael J. Dunn and Michael S. Simonson*. Dept. of Medicine, Case Western Reserve Univ. and University Hospitals, Cleveland, OH

Immunocytochemical localization of FN and AGP suggests their participation in glomerular inflammation. We investigated whether FN and AGP would stimulate proliferation of quiescent (G_0) rat glomerular mesangial cells in culture. When added exogenously to serum-free, defined media, FN and AGP stimulated a dose-dependent increase in 3H thymidine uptake. Increments above control at 10 ug/ml for 48 h: FN, 149%; laminin, 157%; fibrinogen, 145%; type 1 collagen, 233%; n=5-12 independent experiments. Denatured FN and equivalent concentrations of bovine serum albumin had no effect. Moreover peptide fragments from FN's cell-binding domain, which inhibit FN binding to cell receptors, blocked FN-induced proliferation whereas nonspecific fragments did not. The ability of FN and AGP to promote adhesion of quiescent mesangial cells, assessed by measuring adhesion of radiolabeled cells to FN- and AGP-coated substrata, roughly paralleled their ability to stimulate proliferation. In addition, we demonstrated *de novo* synthesis of FN by mesangial cells. Immunoprecipitates of biosynthetically labeled (^{35}S methionine) FN showed that newly synthesized FN was both associated with the monolayer and secreted into the culture supernate. In cells stimulated to proliferate with 5% FBS, FN secretion increased 1.20 fold over quiescent cells.

Our data suggest FN and AGP, synthesized by glomerular mesangial cells or by cells infiltrating the glomerulus, might contribute to the mesangial proliferation and accumulation of mesangial matrix associated with glomerular disease.

REGULATION OF GLUCOCORTICOID RECEPTORS (GR) AND NA-K ATPase ACTIVITY BY CORTISOL (C) IN PROXIMAL TUBULAR EPITHELIAL CELLS (PTEC). Demetrius Ellis, Tran Dang Sothi*, Byron Ballou*, and Ellis D. Avner. University of Pittsburgh School of Medicine, Pgh, PA.

GR modulation of Na-K ATPase activity has recently been demonstrated in primary cultures of a mixed population of murine tubular epithelial cells. The current studies were designed to localize these phenomena to isolated PTEC.

High yield immunoseparation was achieved prior to primary cell culture using monoclonal antibody against stage-specific embryonic antigen which attaches exclusively to PTEC. Immunostaining showed that 99.4% of the cultured cells were PTEC. This was confirmed by measurement of PTEC-specific enzymes. After reaching confluent growth, the cells were grown for 48 hrs in serum-free medium containing 0 (control); or 50 nM C; or 50 nM C plus 20 nM of the antiglucocorticoid RU38486. GR binding capacity and Na-K ATPase activity were then determined. GR binding decreased to $51 \pm 9\%$ of control values after treatment with 50 nM C and $\leq 5\%$ after treatment with RU38486 ($p < 0.01$). Na-K ATPase activity increased to $182 \pm 20\%$ of controls with 50 nM C ($p < 0.01$), but was similar to controls in cells grown in C plus RU38486. Brief incubations of C-treated cells in 1 mM ouabain resulted in a fall in GR without stimulation of Na-K ATPase activity.

These data indicate that in PTEC, C down-regulates GR binding and enhances Na-K ATPase activity. Ouabain inhibits this biological response occurring after hormone-receptor interaction. The abrogation of the C-induced stimulation of Na-K ATPase activity by RU38486 suggests a direct link between GR binding and Na-K ATPase activity.

MOLECULAR CLONING OF A FUNCTIONAL HUMAN GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE GENE.

L. Ercolani, B. Florence*, M. Denaro*, N. Nasrin*, and M. Alexander*. Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme which catalyzes the conversion of glyceraldehyde 3-phosphate to 1,3 diphosphoglycerate. GAPDH has been recently found to perform non-glycolytic functions related to membrane transport processes. In erythrocytes, GAPDH is tightly associated with band 3, the anion transport protein. In skeletal muscle, GAPDH exhibits a protein kinase-like activity resulting in the phosphorylation of transverse tubule proteins which mediate the assembly of junctional triads. GAPDH gene expression is also regulated by differentiation and hormonal signals. In an attempt to further define the activities and regulation of this protein we have isolated a genomic clone for the entire human GAPDH gene. A human genomic library was screened with a full length cDNA for human GAPDH. A 10 kb BamHI fragment was isolated. Subsequent restriction mapping and nucleotide sequencing of this DNA segment revealed the human GAPDH gene consists of 9 exons separated by 8 introns. 5' intron donors were GT whereas 3' intron acceptors were AG. The exon sequence confirmed previous cDNA sequences for human GAPDH in muscle, liver, and erythrocytes. Typical eukaryotic signals for the RNA polymerase entry site TATAA and for the polyadenylation site ATAAA were found. A long promoter region spanned by 203 bases revealed a 5' conserved sequence GGCCAATCT the CAAT box found in virtually all globin genes. The 5' flank region harbored many repetitive sequences. Eleven direct repeats from 7-19 bases long are noted as well as the inverted repetitive element CCGCCC - GGCGGG associated with upstream enhancer elements for human metallothionein genes. The 5' flank revealed an HTF-like island associated with 41 potential methylation sites which may be involved in selective gene expression. Fragments of the GAPDH 5' flanking sequence were subcloned into a vector containing the chloramphenicol acetyl transferase (CAT) reporter gene and transfected into L cells for transient expression. CAT activity was demonstrated only in constructs which fully spanned promoter sequences. Stable cotransfection of the entire GAPDH gene with a plasmid-neomycin resistance gene into H35 cells revealed the production of a complete human GAPDH message by Northern analysis and a complete human GAPDH polypeptide by Western analysis. Numerous GAPDH pseudogenes exist, however Southern blots of human genomic restriction fragments utilizing an intron segment probe revealed only one copy of the isolated gene to be present. We have therefore isolated a functional unique human GAPDH gene. Further studies to understand the regulation and activities of GAPDH in various human tissues may now be approached.

ADAPTATION OF Na-DEPENDENT PHOSPHATE (P) UPTAKE TO P DEPRIVATION IS NOT SPECIFIC OF PROXIMAL TUBULAR CELLS. Brigitte Escoubet* and Claude Amiel (intr. by Christian Le Grimellec). INSERM U. 251 and Université Paris 7, France.

Stimulation of renal P reabsorption with an increase in V_{max} of Na/P cotransport is the adaptive mechanism to dietary P deprivation in the kidney proximal tubule and in the kidney cell line LLCPK1 when exposed to P-free medium (P-).

We show that adaptation of a Na-dependent P uptake is not specific for proximal tubular cells, as a kidney cell line which retains distal tubular properties (MDCK), a differentiated hepatoma cell line (Fao), and cardiac myocytes in primary culture exhibit a Na-dependent P uptake, i.e. dependent on transmembrane Na gradient and on energy, and specific for P. P uptake was stimulated by cell exposure to P- (significant increase in V_{max}):

	Km/Vmax ($\mu\text{M}/\text{pmoles.mg protein; mean} \pm \text{SD}$)	
	Control	P-free medium
LLCPK1	196 \pm 96 / 14 \pm 4	168 \pm 63 / 33 \pm 13
MDCK	374 \pm 106 / 9 \pm 2	350 \pm 116 / 59 \pm 13
Fao	235 \pm 45 / 11 \pm 1	202 \pm 26 / 37 \pm 7
Myocytes	115 \pm 29 / 18 \pm 2	104 \pm 21 / 30 \pm 6

The adaptation was dependent on de novo gene transcription, as it was abolished by cycloheximide and 3-deoxyadenosine treatment.

Thus, a depletion-stimulated, Na-dependent P transport appears to be a pathway for entry of P into both epithelial and non-epithelial cells of non renal tissues. It is suggested that the impaired renal Na-dependent P reabsorption documented in hypophosphatemic diseases could be one among the expressions of a wide-spread alteration of cell P regulation.

ISOLATION OF PRINCIPAL (PC) AND INTERCALATED CELLS (ICC) FROM RABBIT CORTICAL COLLECTING TUBULE BY FLUORESCENCE-ACTIVATED CELL SORTING (FACS). C. Fejes-Tóth*, A. Náray-Fejes-Tóth*, P. Schmiedlin-Renar* (intr. by O.A. Carretero), Hypertension Research Div., Henry Ford Hospital, Detroit, MI

To explore possible biochemical and functional differences between PC and ICC, we developed a method to separate them from rabbit kidney. Partially digested tubular fragments, containing both PC and ICC were isolated by immunodissection, with a monoclonal antibody (MCAB) directed against PC. Single cell suspensions, obtained from these cell clusters, were stained with a fluorescein-conjugated MCAB (ST.48) reacting with an antigen expressed on both PC and ICC, but being present at ~20 fold greater density on PC than on ICC. PC and ICC were separated by FACS based on differences in their green fluorescences and scatter signals. Identity of the isolated cells was confirmed by staining with MCABs specific for each cell type. Flow cytometric reanalysis of the sorted cells revealed purities $>97\%$ for ICC and $>95\%$ for PC. Viability was $>95\%$. ICC responded to isoproterenol (10^{-5}M) with an 7 fold increase in cAMP production, whereas PC did not. Vasopressin (AVP) increased cAMP levels in both PC and ICC. Maximal stimulation was 11 fold in PC and 3.2 fold in ICC, and was observed at 20 nM AVP. Half-maximal stimulation occurred at 60 pM in both cells. Scatchard analysis of ^3H -ouabain binding revealed a single class of binding sites with a K_d of 300 nM on PC and 80 nM on ICC and with a density of 2.9×10^6 on PC and 7.8×10^6 on ICC. We conclude that differences in AVP-responsiveness and in Na^+/K^+ -ATPase content between PC and ICC are quantitative rather than qualitative in nature.

OLIGONUCLEOTIDE PROBES OF THE BETA SUBUNIT OF RENAL NaK-ATPase HYBRIDIZE WITH A LIVER ISOFORM. DS Friedenber*, AS Pollock, VJ Yee, and DG Warnock. Dept. Med., VA Med. Ctr., UCSF, San Francisco, CA.

Multiple forms of beta subunit mRNA exist in brain and kidney (JBC 262:4905, 1987). Liver beta subunit is not detected with kidney cDNA probes while alpha subunit mRNA is detected. Liver isoforms of the beta subunit are worth identifying because of the distinct hormonal responsiveness of liver NaK-ATPase. We have used oligonucleotides to probe kidney and liver mRNA; probes were complementary to highly conserved regions of the beta subunit. An 800 bp coding region probe from sheep kidney cDNA (Nco I digest) hybridized to rat kidney but not liver mRNA on Northern or dot-blot analyses. Several oligonucleotide probes hybridized to both; the best was a 21 bp probe complementary to the 5' end of the coding region. The Nco I probe was used to identify kidney isoforms of the beta subunit in a cDNA library prepared from kidney mRNA. A liver library was negative on Nco I screening, but the oligonucleotide probes have identified plaques in the liver library which are presumed to be the liver isoform of the beta subunit.

Conclusions: 1). The liver beta subunit mRNA of the NaK-ATPase appears to be distinct from the kidney and brain isoforms. 2). Conserved regions in the kidney isoforms appear to be expressed in the liver forms. 3). Tissue specific expression of beta subunits may account for the different hormonal responsiveness of kidney and liver NaK-ATPase.

ALBUMIN GENE TRANSCRIPTION IS ENHANCED IN THE LIVER OF NEPHROTIC RATS. Y. Fukuhara*, A. Yamauchi*, E. Imai*, T. Noguchi*, T. Tanaka*, S. Yamamoto*, M. Fujii*, H. Mikami*, Y. Orita*, and T. Kamada*. Osaka Univ. Med. Sch., Osaka. (intr. by J. S. Handler).

The level of albumin mRNA (A-mRNA) and the transcription rate of the albumin gene (A-trans) were studied in the livers of control rats (C) and nephrotic rats (N) induced by injection of the aminonucleoside of puromycin. Total cellular RNA was extracted from liver by the guanidium thiocyanate method. A-mRNA was measured by cDNA-RNA dot blot hybridization, and A-trans was measured by a "run on" transcription assay in isolated nuclei. Urinary protein excretion in N was significantly higher than in C (258 ± 132 vs 12 ± 2 mg/day) and serum albumin concentration in N was significantly lower than in C (2.5 ± 0.1 vs 3.0 ± 0.1 g/dl). There was no difference in body weight, liver weight, serum creatinine or urea nitrogen between the two groups. The results were as follows (Mean \pm SD):

Group(n)	Total RNA (mg/g liver)	A-mRNA (cpm/ μ g RNA)	A-trans (ppm)
C (6)	3.4 ± 1.0	1506 ± 583	713 ± 235
N (6)	5.0 ± 0.5	2644 ± 783	1674 ± 524
T-test(p)	<0.01	<0.05	<0.01

Northern analysis showed that both the putative precursor species and the mature form of albumin mRNA were increased in N. We conclude that hepatic synthesis of albumin in nephrotic rats is the result of increased albumin gene transcription and mRNA accumulation.

OPENING OF TIGHT JUNCTIONS (TJ) AND INCREASE IN MEMBRANE FLUIDITY (MF) HAVE SIMILAR EFFECTS ON Na-COUPLED UPTAKES IN RENAL EPITHELIAL CELLS. G. Friedlander*, M. Shahedi*, C. Le Grimellec and C. Amiel*. INSERM U 251 and Dept. Physiol., Fac. X. Bichat, Univ. Paris 7, France.

In epithelial cells, TJ maintain differences in lipid composition and MF of apical vs basolateral domains of the plasma membrane (van Meer et al., Embo J., 5:1455, 1986; Le Grimellec et al., Am. J. Physiol., 245:F227, 1982). Because MF is known to modulate the activity of membrane-bound proteins, we hypothesized that opening TJ, or increasing MF with a fluidizing agent should induce similar changes in the activity of transport proteins located in the apical membrane of renal epithelial cells. Na-dependent phosphate (Pi) and α -methyl D-glucoside (MG) uptakes were measured in confluent LLCPK1 and MDCK cells, grown in serum-free medium. Opening TJ by a 15-min. preincubation in calcium-free medium (no Ca), or increasing MF with benzyl alcohol (BA) increased Pi uptake and decreased MG uptake, without affecting Km values:

Vmax (μ moles/mg protein/10 min, mean \pm SE; *p<.05)			
Preincub. [BA]	LLCPK1/Pi	MDCK/Pi	LLCPK1/MG
with Ca	0	6.4 ± 0.50	5.6 ± 0.29
no Ca	0	$9.5 \pm 0.24^*$	$7.7 \pm 0.54^*$
with Ca	10 mM	$9.1 \pm 1.19^*$	$8.6 \pm 0.73^*$
			$6.1 \pm 0.15^*$

We conclude that: i) opening TJ, or increasing MF with BA modify similarly the activities of transport proteins of the apical membrane; ii) changes in MF have different effects on Na-Pi and Na-MG transport proteins, which suggests differences in their lipid environment; iii) these data support a physiological role for MF in the modulation of carrier-mediated transport.

EFFECTS OF PDGF ON GROWTH, INTRACELLULAR pH (pH_i) AND CALCIUM (Ca_i) OF CULTURED RAT MESANGIAL CELLS (MCs). M.B. Ganz* and R.B. Sterzel, VAMC-Yale Univ School of Med, New Haven, CT.

Changes of pH_i and Ca_i are thought important for cellular signal transduction leading to mitogenesis. Since PDGF is a mitogen of MCs, we examined how PDGF affects growth, pH_i and Ca_i of cultured rat MCs (passage 2-5). MC replication was determined using 3H -thymidine uptake at 24 & 48h. Acute effects of PDGF on pH_i and Ca_i of quiescent MCs were assessed by spectrofluorometry using the dyes BCECF and FURA-2, respectively. PDGF (1.5 & 5.0 ng/ml) stimulated replication of quiescent MCs in Hepes free, HCO_3^- -buffered medium (pH 7.4) at 24h (by 293 ± 31 and $571 \pm 74\%$ over baseline) and at 48h (by 123 ± 17 and $189 \pm 25\%$). In HCO_3^- -bath, PDGF induced a small and gradual acidification of MCs (max: -0.03 and -0.05 pH_i unit change at 5 through 30 min). In Hepes, PDGF induced a brief initial acidification followed by alkalinization for 30 min. This pH_i increase over baseline (max: $+0.05$ and $+1.1$ pH_i unit change, $p < .01$) was Na-dependent and EIPamiloride sensitive. PDGF induced an acute, transient rise of Ca_i in Hepes (baseline Ca_i : 90.3 nM; 1.83 and 3.09 fold increase at 20s, $p < .005$) and in HCO_3^- (2.13 and 2.84 fold, $p < .01$). Conclusions: 1) the mitogenic effect of PDGF on MCs in HCO_3^- -containing medium is not associated with early intracellular alkalinization but mild gradual acidification and an initial transient Ca_i rise. 2) The findings support the interpretation that activation of Na-H exchange and subsequent alkalinization are not primary events for transduction of PDGF-induced signals. 3) The buffer and pH_i -independent rise of Ca_i may be important in mediating the mitogenic signal in MCs.

VASOACTIVE AGENTS THAT AFFECT MESANGIAL (MS) CELL ADHESION AND SHAPE CHANGE ALTER PLASMINOGEN ACTIVATORS (PA) LOCATED IN CELLULAR ADHESION PLAQUES (AP). W. Glass,* R. Radnik, M. Venkatachalam and J. Kreisberg. Univ Texas HSC, San Antonio, Texas.

Cultured MS cells changed shape and became less adhesive in response to cAMP elevating agents (e.g., chlorphenylthio cAMP and isoproterenol plus isobutylmethylxanthine [I-MIX]). Forty-five mins. following cAMP elevation MS cells maximally changed shape to a highly arborized configuration with rounded cell bodies and long thin processes; cell adhesion was decreased as assessed by interference reflexion microscopy and a trypsin assay. Shape change and adhesion loss were prevented by the contractile agent vasopressin (V) and by serine protease inhibitors. AP isolated from MS cells were run on 11% SDS-polyacrylamide gels containing gelatin and plasminogen. AP contained nearly all of the PA. Four zones of lysis (ZL) were evident on plasminogen gels from control AP: One inconspicuous zone with an apparent molecular weight \approx 150 Kd, another \approx 115 Kd and a doublet at \approx 32 Kd. Five mins. following I-MIX treatment all ZL were increased; the zone at 150 Kd increased the most. Maximum activity (zone intensity) was reached by 20 mins. V, which prevented shape change and adhesion loss when added along with I-MIX, inhibited the 150 Kd PA and decreased the activity of the other PA. Activation of plasminogen throughout the gels revealed multiple protease resistant bands which markedly increased with I-MIX (maximal at 45 min.). These may represent focal control mechanisms. The shape change in response to cAMP-elevating agents involves PA located in AP. PA may mediate focal proteolysis which results in shape change and decreased adhesion.

NH₄Cl IN THE ABSENCE OF EXTRACELLULAR ACIDEMIA INDUCES HYPERTROPHY AND INCREASED Na⁺/H⁺ ANTIPORT ACTIVITY IN MONKEY PROXIMAL TUBULE CELLS (JTC CELLS). K. Golchini*, J. Norman, I. Kurtz. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

In vivo renal hypertrophy is associated with increased ammoniogenesis. The direct effect of NH₄Cl exposure on cell growth and Na⁺/H⁺ antiport activity was examined in vitro on confluent JTC cells maintained in DME media, pH 7.4. Hypertrophy was assessed as increased protein/cell and DNA synthesis measured by tritiated thymidine (³H-TdR) incorporation. Exposure to NH₄Cl for up to 96 hrs caused a dose dependent increase in cell protein with no increase in ³H-TdR. In cells exposed to 20mM NH₄Cl, cell protein increased by 35% at 72 hrs; 103% at 96 hrs. Steady state intracellular pH (pH_i) measured with BCECF in 72 hr NH₄Cl treated cells was 6.87 ± 0.03 vs. 6.95 ± 0.01 in control, $p < 0.05$. Na⁺/H⁺ antiport activity at 72 hrs was studied by measuring the amiloride inhibitable Na⁺-dependent rate of H⁺ efflux (mM/min) following acute intracellular acidification. H⁺ efflux in the NH₄Cl treated cells was 12.4 ± 2.2 vs. 3.87 ± 0.26 in control $p < 0.01$. Conclusion: Exposure of proximal tubule cells to NH₄Cl at an extracellular pH of 7.4 results in 1) cell hypertrophy without increased DNA synthesis. 2) increased Na⁺/H⁺ antiport activity. 3) intracellular acidosis.

CHANGES IN CYTOSOLIC pH AND Ca²⁺ IN OXIDATIVE STRESS. M. Goligorsky and E. Cragoe*. Jewish Hosp. of St. Louis, MO, and Merck Sharp & Dohme, W. Point, PA.

We have previously demonstrated a precipitous cell acidification (CA) and Ca²⁺ transient coincident with reoxygenation of proximal tubular cells (PTC). Single cell microfluorometry following application of H₂O₂, gentamicin, and cyclosporin A was performed to monitor changes in pH_i and Ca²⁺_i in cultured PTC. All agents caused comparable degree of CA (0.15-0.2 pH units) within 2-3min. H₂O₂ resulted in an increase in Ca²⁺_i. However, this was not the sole cause of CA, since CA was not prevented by treatment of PTC with an intracellular Ca²⁺ chelator Maptam. Selective amiloride derivatives, 5-(N,N-hexamethylene)- or 5-(N-ethyl-N-isopropyl)-amiloride ($1-4 \times 10^{-6}$ M) resulted in CA, but failed to prevent completely H₂O₂-induced acidification. In cells loaded with FITC-Dextran (pH of endocytic/lysosomal compartment), application of the above agents caused brisk alkalinization and accelerated fusion of vesicles with the plasma membrane. Repeated application of the substances did not produce CA and was of no effect on pH of endocytic/lysosomal compartment. The described events coincided with a decrease of the mitochondrial membrane potential, reorganization of F-actin, and clustering of decentralized endoplasmic reticulum (Con.A staining). In conclusion: 1) H₂O₂, gentamicin, and cyclosporin A cause profound CA. 2) This event is not solely precipitated by an increase in Ca²⁺_i. 3) H₂O₂-induced CA is not solely due to an inhibition of Na/H exchange. 4) CA caused by these agents seems to be primarily due to a discharge of H⁺-ions from endocytic and/or lysosomal compartment, which is unaffected by a repeated exposure to these substances.

ACETAZOLAMIDE (ACZL) BLOCKADE OF PROTON TRANSPORT IS INDEPENDENT OF CELL pH M.L. Graber, T. Dixon, D. Coachman,* and P. Devine. VAMC, Northport, and SUNY at Stony Brook, New York.

ACZL inhibits turtle bladder proton secretion (JH), presumably by alkalinizing the cell. This inhibits proton pumping or retrieves pumps by endocytosis. To test this hypothesis we measured JH, endocytosis rates by dextran uptake, and single cell pH by 4MU fluorescence in response to 0.5 mM ACZL. In control bladders ACZL inhibited JH 80±6%, and alkalinized cell pH. 50 mM DMO effectively clamped cell pH in response to ACZL, but JH still fell 74±2%. In bladders exposed simultaneously to ACZL and the permeant acid propionate, 7.5 mM, cell pH acidified by $.17 \pm .05$ units and JH fell 100±12%. ACZL at 10 μM also inhibited JH in propionate-acidified bladders. The changes in JH from ACZL were prevented by 1 mM NaN₃, a dose known to block endocytosis. ACZL increased endocytosis rates in propionate bladders relative to controls ($1.16 \pm .12$ vs. $.68 \pm .16$ nl/min/mg protein, $p = .03$). Bladder cells alkalinized by 50 mM NH₃ showed endocytosis rates unchanged from controls. We conclude that cell alkalinization is not a signal for endocytosis, and that ACZL stimulates endocytosis and blocks proton transport independent of changes in cell pH.

CULTURED RAT KIDNEY MESANGIAL CELLS SYNTHESIZE PREDOMINANTLY THE TRIMERIC FORM OF TYPE I COLLAGEN. Michael A. Haraldson*, Richard L. Hoover*, and Harry R. Jacobson. Vanderbilt Univ. Med. School, Dept. of Pathology and Div. of Nephrology, Nashville, TN.

Progressive glomerular sclerosis results from the accumulation of abnormal amounts and possibly different types of matrix components. Mesangial cells are likely to contribute to this pathological process; thus, studies of mesangial cell matrix synthesis and its control are important. We have evaluated both the amounts and molecular forms of the collagens synthesized by cultured rat kidney mesangial (RKM) cells. The collagens secreted into the culture medium and extracted from RKM cell layers were isolated after pepsin digestion. Gel electrophoresis under denaturing conditions indicated components which correspond to the chains present in types I, III, IV and V collagen. CM-trisacryl chromatographic analysis revealed that ~95% of the collagen synthesized by cultured RKM cells was type I collagen. Greater than 50% of this collagen type was recovered as the type I-trimer, the molecular form of type I collagen reported to accumulate in hepatic cirrhosis and scirrhous carcinoma of the breast. The type IV molecules recovered from RKM cells exhibited the molecular structure $[a1(IV)]_3$, whereas the type V molecules had the molecular composition $[a1(V)]_2a2(V)$. These data establish the relative proportions of the collagens synthesized by RKM cells and suggest that the process of cell culture may induce a wound-healing or sclerosing phenotype in the glomerular mesangial cell.

PRESENCE OF VOLTAGE-SENSITIVE CALCIUM CHANNELS IN SUBCULTURED MESANGIAL CELLS. Aviv Hassid*, Poonam Lall* and Kevin Westbrook* (intr. by Michael J. Dunn). Department of Pharmacology, University of Tennessee, Memphis, TN.

We have investigated voltage-sensitive Ca channels in primary and subcultured rat mesangial cells by determining the effects of elevated extracellular potassium (high K+) on cytosolic free Ca levels. Cells grown on coverslips were loaded with fura-2 and superfused continuously in a fluorescence spectrophotometer. Free Ca levels were determined by dual-wavelength fluorescence spectroscopy. Free Ca levels in primary cultured mesangial cells were unresponsive to high K+ (up to 100 mM). On the other hand, 10 to 100 mM extracellular K+ dose-dependently increased free Ca in subcultured mesangial cells. Resting free Ca levels in these cells were in the range of 100 to 160 nM. At maximally effective doses of 30 mM to 100 mM, K+ increased free Ca by 20 to 25%. Moreover, 100 mM K+ increased the uptake of ^{45}Ca by about 30% after 10 min of incubation. Nifedipine and verapamil (both at 10 μM) decreased resting Ca levels by 10 to 20% and also inhibited 100 mM K+-induced increase of Ca by 50 to 75%. A Ca-channel agonist Bay K 8644 (10 μM) significantly increased resting Ca levels by 10 to 20%, but did not have a further effect on Ca levels that were increased by high K+. These results demonstrate that voltage-sensitive Ca channel activity is not expressed in primary mesangial cultures. On the other hand, Ca channels are partially activated in resting subcultured mesangial cells and the Ca agonist Bay K 8644 or high K+ further activate these channels. Ca channel antagonists decrease free Ca, both in the resting condition and in cells stimulated by high K+. The reason(s) for the difference between primary and subcultured cells remain to be determined.

IMMUNOREGULATION OF CLASS II MHC GENE EXPRESSION BY TUBULAR EPITHELIUM INFLUENCES SUSCEPTIBILITY TO MURINE INTERSTITIAL NEPHRITIS (TIN). T. Haverty*, C.J. Kelly, M. Watanabe*, and E.G. Neilson; Renal Section, U. of Pa., Phila., PA.

Susceptible mice (S) express $\alpha\text{TBM-Ab}$ and class II MHC-restricted, tubular antigen-reactive, Lyt-2^+ effector cells which easily produce TIN. Non-susceptible mice (NS) also produce $\alpha\text{TBM-Ab}$, but do not develop TIN. When their class II MHC-restricted, L3T4^+ effector cells are adoptively transferred into recipients pretreated with λ interferon (IFN), however, they develop interstitial injury. Postulating that marginal renal class II MHC expression, modulated by $\alpha\text{TBM-Ab}$, may impair the nephritogenic potential of NS mice, we measured MHC mRNA levels in mouse tubular cells (MCT) that also secrete the target antigen of TIN. Using ^{32}P -cDNA probes we found lower class II mRNA in cells co-cultured with monoclonal $\alpha\text{TBM-Ab}$; 0.51 ± 0.04 relative units (ru) vs. 2.86 ± 0.71 ru with control Ab ($p < 0.01$), while class I mRNA was 3.3 ± 1.7 ru for control Ab vs. 4.6 ± 1.1 ru for $\alpha\text{TBM-Ab}$ ($\alpha\text{TBM-Ab}$ eluted from NS or S kidney had same effects). Class I and II gene products were detected on MCT cell surface by cytofluorography and were increased by IFN. Class II mRNA by nuclear-runoff transcription of MCT treated with $\alpha\text{TBM-Ab}$ was 52% of control Ab, while class I rates were equivalent. Kidneys from mice immunized to produce TIN and hybridized with ^{35}S -cDNA probes in situ showed class II expression = 16.7 ± 4.5 grains/hpf vs. control = 51.2 ± 4.4 ($p < 0.001$) and class I expression = 142.7 ± 20 vs. control = 110.4 ± 4.4 ($p = \text{NS}$). Our findings indicate that $\alpha\text{TBM-Ab}$ can down-regulate class II MHC gene expression by decreasing gene activation in cultured tubular cells and in vivo in mice immunized to produce TIN. This protective effect in NS mice can preserve their non-susceptibility status by decreasing the visibility of their target epithelium.

DESMIN AS A MARKER FOR MESANGIAL CELLS IN RAT GLOMERULAR CULTURE. R.G. Hegel, A. Katz and M. Silverman, Membrane Biology Group and Depts. of Pathology and Medicine, University of Toronto, Toronto, Ontario.

Characterization of cells in glomerular tissue culture has been an ongoing concern (Striker et al., *Adv Nephrol* 16: 169-186, 1987). We studied immunocytochemical staining profiles of primary rat glomerular culture systems grown in K1, K1-Nu/3T3 and RPMI/3T3 (Harper et al., *Kidney Int* 26: 875-880, 1984). In each culture system, two distinct cell populations could be discerned: a polygonal cell type which was desmin-negative and a spindle cell type which was desmin-positive. Electron microscopic evaluation of the spindle cell type showed evidence of smooth muscle differentiation, consistent with mesangial origin. We applied a commercially available anti-desmin antibody (Dako M 472) to primary cultures of rat glomeruli at different stages post-explantation. In all instances, proliferating colonies of desmin positive cells were identified in early primary culture, a phenomenon not previously appreciated using other techniques. Previous data (Norgaard, *Lab Invest* 48: 526-542, 1983) have apparently underestimated the proliferation of mesangial-like cells in early primary glomerular culture. Our findings suggest that phenomena observed in such systems may be attributable to a heterogeneous population of cells. Desmin represents a sensitive and specific morphological marker for mesangial-like cells in rat glomerular culture.

Cl⁻-DEPENDENT H⁺ ATPase IN RABBIT RENAL CORTICAL ENDOSOMES. S.A. Hilden,* C. Johns,* and N.E. Madias. Division of Nephrology, New England Medical Center, Boston, Massachusetts.

We have isolated an endosomal fraction enriched in H⁺ ATPase activity (acridine orange assay) from rabbit renal cortex by a novel, fast and simple procedure involving differential centrifugation and Mg²⁺ precipitation. ATP-driven proton pumping was oligomycin-insensitive, but was inhibited by DCCD, NEM, and other inhibitors such as Zn²⁺, Hg²⁺ and NBD-Cl. The diuretic mer-salyl also exerted a potent inhibitory effect on the H⁺ ATPase. No substantial Na⁺/H⁺ exchange was detected in these endosomes. In addition, these membranes featured an ATP-dependent Cl⁻ flux. The ATP-driven proton pumping had an absolute requirement for Cl⁻: an inside negative membrane potential was not a substitute. The protonophore FCCP dramatically inhibited ATP-driven Cl⁻ uptake; this uptake was also inhibited by DCCD and NEM. Both ATP-driven proton pumping and ATP-dependent Cl⁻ flux were inhibited by several Cl⁻-channel inhibitors, including 5-nitro-2-(3-phenylpropyl-amino)-benzoic acid, 5-nitro-2-(2-phenylethyl-amino)-benzoic acid (gifts of R. Greger), diphenyl-amine-2-carboxylic acid and anthracene-9-carboxylic acid. The order of effectiveness of these agents in inhibiting the two endosomal processes (H⁺ and Cl⁻ transport) was identical to that reported in inhibiting Cl⁻ conductance in various Cl⁻-transporting epithelia. We suggest that the absolute dependence on Cl⁻ of the endosomal H⁺ ATPase derives from an acid pH-activated Cl⁻ channel whose function is intimately related to that of H⁺ pumping by the ATPase.

CLONING AND SEQUENCING OF cDNA FOR 31K SUBUNIT OF VACUOLAR H⁺ATPase. S.Hirsch*, K.Masood*, V.Sukathme*, A.Strauss*, S.Gluck. U. of Chicago, Chi., Ill., & Washington U., St.Louis, Mo.

Western blots of various rat tissue microsomes demonstrate that expression of the 31K subunit of vacuolar H⁺ATPase(P.P.) is enhanced in kidney and brain, suggesting an important role for the 31K in P.P.function in these tissues. To study further the 31K subunit we screened a bovine kidney medulla cDNA library with polyclonal antibodies(PAb) to the P.P. Fusion proteins from 2 isolated clones, expressed in lysogenic bacteria, were recognized by PAb to 31K. One clone was chosen for further study. Protein sequences from 4 tryptic peptides of the 31K matched with sequence predicted from the cDNA clone. The cDNA sequence, 1213bp long, includes the entire coding region. It is largely hydrophilic, suggesting that the subunit is part of the enzyme's cytoplasmic head. Northern analysis demonstrates the highest levels of 31K mRNA in kidney medulla and brain, consistent with protein blots.

A N terminal hydrophilic sequence previously described in Na-K α ATPase and gastric H-K ATPase is conserved in the 31K subunit. Another sequence similar to a phosphorylation domain sequence of Ca ATPase was also found. Further study of the function of these sequences should aid in understanding how ion translocating ATPases function.

In summary, we have cloned and sequenced a cDNA for the 31K subunit of the vacuolar P.P. and have identified sequences conserved in other ATPases.

PROTEIN PHOSPHORYLATION PATTERN DIFFERS DURING RENAL ONTOGENY BUT NOT COMPENSATORY RENAL GROWTH (CRG) IN RATS. T. Hoang* and M. Bergeron. Dept. physiology, Univ. Montréal, Montréal, Québec.

Polypeptide hormone stimulation of metabolic responses is thought to be mediated through protein phosphorylation. In order to solve the controversy on the extent of the hormonal effect, we monitored changes occurring during renal ontogeny and CRG in cytosolic extracts by measuring cAMP-dependent protein kinase (cAMP-PK) activity, [³H]-cAMP binding and protein phosphorylation.

Activity of cAMP-PK and [³H]-cAMP binding varied with age, being high at birth but decreasing within the first two weeks; later, a slight increase occurred. A slight and transient increase was observed only 24 hours post-nephrectomy.

Phosphorylation of proteins was analyzed by high resolution two dimensional electrophoresis followed by radioautography. Extracts from newborn and young rats showed a greater number of phosphorylated proteins migrating between the origin and Mr = 30,000. The one co-migrating with phosphorylase B (Mr = 97,000) was highly regulated during ontogeny. The phosphorylation of most of these proteins was gradually reduced with age. In mature rats, other proteins of higher mobility became phosphorylated, in particular the Mr = 44,000. The number of bands and the staining intensity in extracts from normal and uninephrectomized rats did not differ. Protein phosphokinase system and protein phosphorylation are markedly altered during renal post-natal development indicating that polypeptide hormones are involved during renal ontogeny but not in CRG.

IDENTIFICATION OF CULTURED GLOMERULAR CELLS BY LECTIN AND ANTIBODY STAINING. H. Holthofer*, S. DeCandido*, and D. Schlondorff, Dept. of Medicine, Albert Einstein Coll of Med, Bronx, NY.

To better identify specific cells in glomerular culture, we correlated the pattern of lectin binding (10 lectins tested) to that of antigenic determinants and morphology. Both rat kidney sections, early (3-7 days) and late (3-5 wks) glomerular cultures and "mesangial" subcultures were studied. In culture four morphologic types of cells were observed: Type I, small cells in cobblestone pattern predominated early. They were negative for all lectin and antibody markers and may represent undifferentiated cells. Type II, stellate cells predominated late in culture, were positive for Con A, WGA and RCA I lectins and Thy 1.1 antibody and phalloidin (for F-actin). The same staining pattern was uniformly observed in subcultured "mesangial" cells and in the mesangium of kidney slices. We propose this pattern identifies mesangial cells. Type III, large, round cells accounted for 10-20% of early cellular outgrowth, but were virtually absent in late cultures. They bound only BSI-B4 lectin and factor VIII antibody, a pattern typical for endothelial cells. Type IV, elongated cells in groups, constituted 5-10% of outgrowth. They bound LFA and MPA strongly, but faintly also WGA, Con A, Heymann antibody and Thy 1.1. This pattern was reminiscent of glomerular epithelial cells. No common leucocyte or Ia antigen positive cells were observed in culture. The results show that patterns of lectin and surface antigen binding can be helpful in identifying cultured glomerular cells and especially mesangial cells in subculture.

SOLUBILIZATION OF THE PHOSPHONOFORMIC ACID (PFA) BINDING PROTEIN FROM RENAL CORTICAL BRUSH BORDER MEMBRANE (BBM). Anzelm Hoppe*, Mirosława Szczepanska-Konkel, and Thomas P. Dousa. Neph. Res. Unit, Mayo Clinic, Rochester, Minnesota.

In our previous studies, we documented that PFA is a specific competitive inhibitor of Na⁺/Pi cotransport in BBM of renal cortical tubules. ¹⁴C-PFA apparently binds on Na⁺/Pi cotransporter at the equifacial site. In the present study, we explored whether ¹⁴C-PFA can serve as a marker for isolation and purification of Na⁺/Pi cotransporter from rat renal cortical BBM. We found that ¹⁴C-PFA binding proteins can be solubilized from BBM in beta-octylglucoside; the other detergents were less suitable. The binding of ¹⁴C-PFA on octylglucosides solubilized BBM extract was determined by filtration on Sephadex G 50 column. ¹⁴C-PFA activity eluted from column in protein fraction served as probe attached to Na⁺/Pi cotransporter. ¹⁴C-PFA binding on solubilized protein was markedly enhanced in a medium containing sodium instead of potassium. Binding of ¹⁴C-PFA on solubilized BBM was suppressed by phosphate but not by D-glucose. No Na⁺-dependent PFA binding was found in solubilized fractions of basolateral membrane or mitochondria from renal cortex. Further fractionation by chromatofocusing suggests that Na⁺-dependent PFA binding protein appears as a specific fraction in pH range 5.7 to 6.4. These results indicate that Na⁺-dependent binding of ¹⁴C-PFA can be employed for tracing Na⁺/Pi cotransporter or at least its Pi binding moiety in course of purification and its isolation.

DISTINCT G PROTEINS MEDIATE ACTIVATION OF PHOSPHOLIPASE C BY THROMBIN AND BY PLATELET DERIVED GROWTH FACTOR. C.-L. Huang* and H.E. Ives. Div. Nephrol., CVRI, Univ. Cal., S.F., CA.

Thrombin and platelet derived growth factor (PDGF), like many mitogens and vasoactive peptides, induce a series of common biochemical events. However, the signal transduction mechanisms which generate these responses may differ. Pertussis toxin and phorbol myristate acetate (PMA) impair G protein function in some systems. In cultured vascular smooth muscle cells, pertussis toxin (100 ng/ml) inhibited thrombin (1 U/ml)-induced inositol phosphate release by 63% and Ca²⁺_i spike by 54%. Likewise, PMA (200 ng/ml) inhibited thrombin-induced inositol phosphate release by 82% and Ca²⁺_i spike by 59%. PMA did not affect thrombin binding. In contrast, neither the PDGF (7.5 nM)-induced inositol phosphate release nor the Ca²⁺_i spike were affected by pertussis toxin or PMA. To ask whether this pertussis toxin- and PMA-insensitive signalling mechanism for PDGF involves a G protein or not, we introduced GTPγS, a stable GTP analog, into saponin-permeabilized cells. GTPγS (1 μM) increased inositol phosphate release synergistically with both thrombin and PDGF.

³ H-inositol phosphate release (cpm/dish)			
Control	74 ± 5	Control	363 ± 31
THR	94 ± 8	PDGF	732 ± 66
GTPγS	72 ± 4	GTPγS	1066 ± 71
THR+GTPγS	225 ± 16	PDGF+GTPγS	2895 ± 286

This synergism indicates that both mitogens require G proteins for signal transduction. Thus, different G proteins, as distinguished by pertussis toxin and PMA sensitivity, mediate signal transduction for thrombin and PDGF.

NOVEL ROLE FOR PROTEIN KINASE C: GROWTH INHIBITION VIA BLOCKADE OF A LATE EVENT IN MITOGENESIS. H.E. Ives and C.-L. Huang, Div. Nephrology and CVRI, Univ. of Cal., S.F., CA.

α-thrombin, activated during coagulation, promotes vasoconstriction, growth of vascular smooth muscle (VSM), and may play a role in glomerular or renal vascular diseases. Phorbol-myristate-acetate (PMA), acting via kinase C, modulates various cellular events associated with mitogenesis. In cultured rat VSM, 10 min exposure to 200 ng/ml PMA inhibited thrombin-, but not platelet derived growth factor (PDGF)-induced Ca²⁺ spike by 59% and inositol trisphosphate release by 82%. This was not due to interference with thrombin binding. PMA also inhibited thrombin-, but not PDGF-, induced DNA synthesis by 61%. Most importantly, PMA (> 3 ng/ml) maximally inhibited thrombin-induced DNA synthesis when added 4 h after, and not before, thrombin.

³ H-thymidine incorp., cpm/dish	
Control	2903 ± 162
Thrombin	25555 ± 1480
+ PMA (10 min before Thr)	11663 ± 723
+ PMA (4 h after Thr)	2970 ± 223

Inhibition of thrombin-induced mitogenesis by PMA requires kinase C activity, since inhibition was eradicated in kinase C-deficient cells. Thrombin-, but not PDGF-induced mitogenesis requires prolonged receptor occupancy, since removal of thrombin up to 8 h after initial exposure prevented DNA synthesis. Since maximal growth inhibition by PMA occurs well after the completion of "early" events associated with mitogenesis, phorbol esters probe a critical, perhaps receptor-mediated, event late in the G₁ phase of the growth cycle.

POSSIBLE LIGAND FOR THE AUTOANTIGEN (GP600) OF HEYMANN NEPHRITIS (HN). John J. Kanalas* and Sudesh P. Makker. Univ. of Texas Health Sci. Ctr., Dept. of Pediatric Nephrology, San Antonio, Texas.

The HN antigen has been shown to be present in the coated pits of the plasma membrane and in multivesicular bodies of glomerular epithelial cells (GEC). Based on these locations, it has been suggested that the HN antigen in GEC may be a receptor (Karjaschki & Farquhar, J. Exp. Med., 1983). However, a ligand for this glycoprotein has not been established. We now report the identification of a possible ligand. Purified HN antigen was used as a probe on Western blots of normal rat serum separated by sodium dodecylsulfate polyacrylamide gel electrophoresis under non-reducing conditions. Reaction was revealed directly by ¹²⁵I labelled gp600 and indirectly by preincubation with gp600 followed by anti-gp600 nephritogenic autoantibody and biotinylated anti-rat IgG/avidin-peroxidase complex. Reaction with a 76kd polypeptide was seen by both techniques. No reaction was seen with an irrelevant antigen or normal serum antibody controls. Results were confirmed by enzyme-linked immunoassay using a partially purified serum protein preparation. Interestingly, the same 76kd protein also reacted with anti-gp600 nephritogenic autoantibody eluted from glomeruli of diseased animals. Preincubation of electrophoresed normal rat sera with gp600 enhanced the reactivity of anti-gp600 during Western analysis. The ligand was also present in sera of diseased HN rats. Reactivity of both gp600 and the nephritogenic autoantibody towards the 76kd band was lost when sera was electrophoresed under reducing conditions. We conclude that the 76kd polypeptide present in serum is a possible ligand for gp600 and since it reacts with both gp600 and anti-gp600, it may play a role in the pathogenesis of HN.

STIMULATION OF PHOSPHOINOSITIDE (PI) HYDROLYSIS IN LLC-PK₁ CELLS BY VASOPRESSIN (VP). Elzbieta Kapturczak*, Lal C. Garg, Martin Steiner* and M. Ian Phillips*. Univ. of Florida Coll. of Med., Gainesville, FL.

LLC-PK₁ cells have been shown to possess VP receptors (V₂ type) coupled to adenylyl cyclase to generate cyclic AMP as a second messenger. In order to determine if LLC-PK₁ cells also have V₁ type receptors (coupled to phospholipase C to hydrolyze PI), we measured the release of inositol phosphates (IP) in the LLC-PK₁ cells in the absence and presence of various concentrations of VP. The method involved the incubation of LLC-PK₁ cells with [³H]-inositol (for 3 hours) for its incorporation into the PI and the measurement of the release of IP in the presence of LiCl which prevents the dephosphorylation of IP. The results of [³H]-IP formation are expressed as % of total [³H]-inositol incorporation into LLC-PK₁ cells.

Control	VP concentrations (M)			
	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶
1.21±0.31	0.84±0.50	2.79±0.43*	13.04±1.59*	26.51±0.58*

* P < 0.01 vs control.

10⁻⁸ M concentration of VP produced a 100% increase in PI hydrolysis in the LLC-PK₁ cells. The effect was much greater at higher concentrations of VP. Our data suggest that VP receptors (V₁ type), coupled to phospholipase C to hydrolyze PI, are present in LLC-PK₁ cells.

EFFECT OF DEXAMETHASONE (DEXA) ON HEPARAN SULFATE PROTEOGLYCAN CORE PROTEIN (HSPG-CP) OF CULTURED GLOMERULAR EPITHELIAL CELLS (GEC). B.S.Kasinath, A.K.Singh, Y.S.Kanwar, and E.J.Lewis. Rush Medical College, Chicago, IL.

HSPG is an important contributing polyanion in the glomerular capillary wall which is believed to play a role in the maintenance of the charge barrier. It is also believed that HSPG is synthesized by the GEC. As glucocorticoids are known to exert important therapeutic effects in proteinuric states, the effect of dexamethasone on the synthesis of HSPG was examined. HSPG was detected on the GEC by the use of an antibody directed against its core protein (CP). Specificity was shown by the ability of the antibody to immunoprecipitate ³⁵S₀₄-labeled macromolecules which were sensitive to HSPG degrading treatments. GEC were incubated with various doses of dexamethasone for 24 and 72 hrs and HSPG-CP was measured by quantitative immunoperoxidase. Data are presented as percentage of control.

Dexa (ug/ml)	24 hr (X±SEM)	72 hr (X±SEM)	P*
0.1	113±4.7 (n=16)	114±3.3 (n=8)	<.02
1.0	114±4.7 (n=16)	119±4.4 (n=11)	<.05
5.0	125±5.5 (n=12)	150±6.1 (n=11)	<.001
10	125±3.4 (n=12)	140±5.1 (n=11)	<.001

*compared to control

There was no change in ³H-leucine incorporation into the total cellular protein pool in the presence of dexamethasone indicating that dexamethasone did not induce non-specific alteration in protein metabolism. We concluded that dexamethasone selectively increases HSPG-CP content of GEC. This metabolic effect could be reflected *in vivo* as an increase in capillary wall charge barrier to anionic proteins.

ENDOCYTOSIS AND Na⁺/SOLUTE COTRANSPORT IN LLC-PK₁ CELLS. SA Kempson, AL Ying, JA McAteer, and H Murer. Indiana University, USA, and University of Zurich, Switzerland

Confluent cell monolayers of the kidney cell line LLC-PK₁ were used to determine if membrane internalisation by endocytosis (EDC) plays a role in regulating the co-transport systems present in the plasma membrane of these cells. EDC was determined as cell uptake of ¹⁴C-sucrose. Treatment for 5 hr with concanavalin A (con A) at 0.5-3.0 mg/ml caused dose-dependent inhibition of EDC. Inhibition was 66% at 1 mg/ml. This dose of con A stimulated Na⁺-dependent uptake of alanine by 358% and uptake of phosphate by 145%. There was no increase in Na⁺-independent uptake. Con A stimulation of Na⁺/solute cotransport was dose-dependent and was due to an increase in V_{max} with no change in K_m. Gentamicin, another inhibitor of EDC, also increased the V_{max} for Na⁺-dependent uptake of alanine and phosphate. Neither con A nor gentamicin had a direct effect on solute uptake. These initial studies support the idea that an increase in the capacity of the cell for Na⁺/solute cotransport may be achieved by decreasing the rate of EDC, thereby decreasing the rate of removal of solute transporters from the plasma membrane. Membrane recycling may provide a mechanism for rapid adaptive responses of Na⁺/solute cotransport systems in kidney cells.

EVIDENCE FOR MULTIPLE SIGNALING PATHWAYS FOR PLATELET-ACTIVATING FACTOR IN CULTURED RAT MESANGIAL CELLS. M. Kester*, J. Wang*, J. Orehek*, P. Mene*, and M.J. Dunn. Depts. of Medicine and Physiology. Case Western Reserve Univ. and University Hospitals of Cleveland, Ohio.

We have recently reported that platelet-activating factor (PAF) elevates cytosolic free calcium concentration ([Ca²⁺]_i) in a dose-dependent manner in fura-2 loaded rat glomerular mesangial cells (MC). Therefore, we investigated the sources of secondary messengers derived from PAF-stimulated phospholipase C in MC. Anion exchange chromatography of the ³H-myo-inositol phosphates extracted from 0.1 uM PAF-treated MC revealed rapid (15 sec), transient formation of inositol trisphosphate (169±5 % of control, n=5) and inositol bisphosphate (132±7%) while sustained inositol phosphate production was observed only after 5 min. In concomitant experiments, 0.1 uM PAF stimulated accumulation of ³H-arachidonate-labeled 1,2-diacylglycerol (DAG) with an initial peak at 30 sec followed by a sustained rise after 5 min. The secondary rise in DAG concentration was not a consequence of phosphatidylcholine (PC) hydrolysis as increments of ³²P-choline phosphate or ³H-choline were not observed. Yet, a 5 minute incubation with 0.1 uM PAF elevated ³H-lysophosphatidylcholine concentration 138±8% compared with controls. Arachidonic acid released from PC may be a source of PGE₂ stimulated by PAF (2.29±0.31 ng PGE₂/mg prot⁻¹·20 min⁻¹ vs 1.23±0.24; 1 uM PAF vs control, respectively). Pertussis toxin (3-100 ng/ml) inhibited nearly 100% of the PAF-induced PGE₂ stimulation while having only minimal effects (-28±3%) upon PAF-induced [Ca²⁺]_i transients. These data suggest hydrolysis of PC by a pertussis toxin sensitive PAF-stimulated phospholipase A₂, independent of polyphosphoinositide turnover by phospholipase(s) C. PAF may modulate glomerular hemodynamics and inflammation through combined activation of C and A₂ phosphodiesterases.

EGF AND PDGF STIMULATE MESANGIAL CELL PROSTANOID SYNTHESIS BY DIFFERENT MECHANISMS. TC Knauss, P Mene, M Kester* and HE Abboud. Cleveland VAMC and Case Western Reserve University. Cleveland, OH.

The protein growth factors epidermal growth factor (EGF) and platelet derived growth factor (PDGF) have diverse biologic effects in cultured glomerular cells. We have assessed the effect of incubations of EGF and PDGF upon PGE₂ synthesis by rat mesangial cells.

	pg PGE ₂	30 min	6 hr	24 hr
CONTROL	4.7 ±0.9	12.7±1.8	15.8±1.7	
EGF 1ng/ml	11.5 ±4.9	24.4±4.5	28.2±4.0	
EGF 10	13.5 ±4.1	48.7±9.2	47.2±7.7	
PDGF 1ng	18.3 ±5.4	47.5±12.6	52.3±11.7	
PDGF 10	25.5 ±6.7	70.7±12.4	106.0±14	

Both proteins stimulated PGE₂ synthesis in a dose and time dependent manner. While EGF plateaus by 6 hrs, PDGF continues to stimulate PGE₂ accumulation. Studies with exogenous arachidonate and calcium ionophore suggest that the stimulation by growth factors is at both phospholipase and cyclo-oxygenase levels. To study the mechanisms of signal transduction, we measured growth factor induced changes in inositol phosphates, diacylglycerol and cytosolic calcium. While PDGF stimulated each parameter, EGF failed to increase any measurement above baseline. Thus unlike PDGF, EGF stimulates PGE₂ production without activation of phospholipase C or measurable changes in cytosolic calcium. As has been suggested in other systems, EGF induction of lipocortin proteins may be critical to stimulation of PGE₂ synthesis in cultured rat mesangial cells.

EFFECTS OF CHLORIDE CHANNEL ACTIVATION ON CALCIUM SIGNALLING IN GLOMERULAR MESANGIAL CELLS. S. Kremer*, W. Breuer* and K. Skorecki. Toronto General Hospital and Univ. of Toronto, CANADA.

The relationship of membrane depolarization (dep) to vasoconstrictor hormone induced Ca signalling was investigated in cultured rat renal glomerular mesangial cells (MC). MC were loaded with the Ca sensitive fluorescent probe Indo-1, or equilibrated with the membrane potential (E_m) sensitive dye bis-oxonol. Basal cytosolic free Ca ([Ca]_i) was 227±4nM and transiently increased 4-6 fold upon stimulation with maximal concentrations of either vasopressin (VP) or angiotensin II (AII). Resting E_m was 45.8±0.9mV and vasoconstrictor hormones caused a dep of 14-18mV. External medium ion substitutions indicated that the observed dep was due to Cl efflux. Exposure of MC to the calcium ionophore ionomycin in Ca deplete medium, or loading the cells with the intracellular Ca buffer BAPTA prevented a hormone induced rise in [Ca]_i. However a dep persisted under these conditions, indicating that a rise in [Ca]_i is not required for vasoconstrictor hormone induced dep. In contrast, graded dep of MC using KCl substituted medium, resulted in a corresponding prolongation of the Ca response to VP and AII. We conclude that the vasoconstrictor hormones VP and AII depolarize MC by activating Cl channels and that this dep does not require a rise in [Ca]_i. In addition, vasoconstrictor hormone induced activation of Cl channels with resulting dep may serve as a critical mechanism for modulating the Ca signal.

CO-EXPRESSION OF CYTOCHROME P-450 AND METABOLISM OF ARACHIDONIC ACID TO NOVEL PRODUCTS BY EARLY PROXIMAL TUBULAR EPITHELIAL CELLS. Dennis Koop, Aubrey Morrison and Janice Douglas, Case Western Reserve Univ., Cleveland, OH and Washington Univ. Med. School, St. Louis, MO.

Early proximal tubular cells are relatively deficient in cyclooxygenase, thereby, accounting for low PGE₂ biosynthetic capacity. The present studies were designed to test the hypothesis that an isozyme of cytochrome P450 (mixed function oxidase) might be responsible for metabolism of arachidonic acid (AA) in this nephron segment. Acutely isolated rabbit cortical epithelial cells separated into regions of origin by Percoll density gradient centrifugation were evaluated for the distribution of P450 isozymes by immunoblots with polyclonal antisera following SDS-polyacrylamide gel electrophoresis. The concentration of isozyme 6 was found to be highest in early proximal tubule (PT) as compared to proximal straight (PS) and distal tubule (DT). Other isozymes (2,3a,3b,3c, and 4) were not detectable at cellular concentrations employed. Cell homogenates from the same regions were incubated with [¹⁴C]arachidonic acid (AA) and NADPH. The primary products of early PT migrated in the region of epoxides and/or ω/ω-1 OH products. Significantly fewer products were formed in PS tubule. DT yielded products that were more polar. These findings suggest that isozyme 6 of cytochrome P450 may be responsible for metabolism of AA to ω and ω-1 OH products and/or epoxides. Such products may represent important modulators of early proximal tubular transport.

INDUCTION OF TRANSIENTLY EXPRESSED GENES IN PC-12 CELLS. D. Kujubu*, R. Lim*, B. Varnum*, and H. Herschman* (Intro. by L.G. Fine). Div. of Nephrology and Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA.

To study the early transcriptional events following exposure of cells to mitogenic stimuli, a cDNA library from density arrested Swiss 3T3 cells following a three hour exposure to tetradecanoyl phorbol acetate (TPA) and the protein synthesis inhibitor cycloheximide (CHX) was constructed. Seven distinct clones were isolated from this library; all were rapidly and transiently induced by TPA and CHX. These TPA induced sequences (TIS) were also rapidly and transiently induced in density arrested 3T3 cells by other mitogens, ie. FGF, EGF, and serum. In 3T3 cells, these genes may be involved in mediating the mitogenic response. On the other hand, rat pheochromocytoma (PC-12) cells, in response to nerve growth factor (NGF), stop proliferating and differentiate into sympathetic neuron-like cells. Six of the seven TIS genes were rapidly and transiently induced in response to NGF as well as EGF, TPA, and a depolarizing stimulus (elevated K⁺). Conclusion: The induction of a family of TPA-induced sequences may be involved in the general activation of a cell response to both mitogenic and differentiative stimuli or in the transduction of extracellular signals into biological responses.

GENE EXPRESSION OF ANGIOTENSINOGEN AND RENIN IN THE HUMAN HEART: IN SITU HYBRIDIZATION STUDY. S. Kunapuli*, K. Graves*, and S. Rajaraman. Univ. of Texas Med. Branch, Div. of Renal Immunopathology, Galveston, TX.

Renin-angiotensin system plays a major role in the regulation of blood pressure. Angiotensinogen, precursor of the biologically active peptide angiotensin II, is generally believed to be synthesized in the liver and secreted into the blood. Others and we have recently demonstrated its synthesis in several extrahepatic tissues in the rat. The kidneys are the only known source of circulating active renin in normal humans. In this study, we investigated the synthesis of angiotensinogen and renin, in the various cellular compartments of the human heart. The expression of the respective mRNA species was evaluated by in situ hybridization, using nick translated human angiotensinogen cDNA and rat renin cDNA as molecular probes, under appropriate hybridization conditions. Both the angiotensinogen and renin mRNA species were strongly expressed by the atrial and ventricular myocytes, moderately by the vascular smooth muscle and with negligible signals in the endothelium or the interstitium. We conclude that: 1) the human cardiac muscle is a rich source of angiotensinogen and renin; and 2) these may play a crucial role in the regulation of systemic and/or local hemodynamics.

EXTRACELLULAR CHLORIDE ION CONCENTRATION IS AN IMPORTANT DETERMINANT OF MESANGIAL CELL FUNCTION: MODULATION VIA INOSITOL TRIPHOSPHATE (IP₃), CELL Ca⁺⁺, AND PGE₂ METABOLISM. Kiyoshi Kurokawa, Itaru Kojima*, and Toshihiro Okuda.* IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo, Japan

We reported angiotensin II (AII) and vasopressin (VP) stimulate Ca⁺⁺-activated Cl⁻ conductance of mesangial cell plasma membrane, leading to membrane depolarization, a phenomenon which coincides with cell contraction (JCI 78: 1443, 1986). This observation raises the possibility that mesangial cell function may be modified by extracellular Cl⁻ concentration ([Cl⁻]_o). Thus, we examined in cultured rat mesangial cells, how changes in [Cl⁻]_o modulate the effects of AII and VP. Both cell contraction and Ca⁺⁺-transients in response to AII and VP were suppressed in Cl⁻-free medium. A reduction in [Cl⁻]_o resulted in increased PGE₂ synthesis of mesangial cells and addition of PGE₂ also suppressed both cell contraction and Ca⁺⁺-transients in response to AII and VP. Based on these findings, we postulate that suppression of mesangial cell contraction and Ca⁺⁺-transients by low [Cl⁻]_o may be mediated by enhanced PGE₂ production (Clin Res 35: 636A, 1987). Indeed, the effects of lowering [Cl⁻]_o on cell contraction and Ca⁺⁺-transients were reversed by 50 nM indomethacin, results consistent with our postulate. In addition, we found that when [Cl⁻]_o was reduced, basal and AII- and VP-stimulated IP₃ content of mesangial cells both decreased. These results indicate that [Cl⁻]_o modulates mesangial cell contraction by altering PGE₂ production thereby affecting IP₃ metabolism thus Ca⁺⁺-transients in response to AII and VP.

DETERMINATION OF PH_i IN SINGLE PROXIMAL TUBULAR CELLS (PTC) FROM 2 DAY PRIMARY CULTURES.

Stefan H Larsson*, Yutaka Y Fukuda* and Anita Aperia. Dept of Pediatr, Karolinska Institutet, St.Göran's Children's Hospital, Stockholm, Sweden.

Rat PTC retain differentiated function after short time in primary culture (AJP, 251: C455-) and might therefore be a suitable model for studies of how changes in pHi relate to epithelial growth and differentiation. We have established a sensitive method to determine pHi in single PTC cultured for only 2 days. PTC from adolescent rats were grown in monolayer colonies on coverslips. The cells were loaded with BCECF-AM (2µM). In an inverted microscope light emitted at 520-560 nm was measured. With low intensity excitation light (485/436nm) dye bleaching was minimized. Thereby experiments and calibration could be performed on the same cells. The signal to BG ratio was >10 and (E485-BG485 / E436-BG436) used as an index of pHi. Proliferating cells (peripheral cells in colony, cultured w/FBS) show a larger variation in pHi than quiescent cells (central cells, no FBS 24H) pHi=7.0-7.8 SD=.3 n=29 vs. 7.5-7.8 SD=.1 n=16. Kinetics of pHi changes were determined in 3-5 central cells simultaneously. Cells were acidified to pHi 6.4 after NH₄Cl incubation. Cells regained pHi via Na/H exchange with a J₀ of .4±.1 pH/min, which is faster than for fibroblasts. Conclusion: PTC in short primary culture display growth dependent variations in pHi and express rapid H-transport via Na/H exchange.

MEMBRANE FLUIDITY ASYMMETRY IN LIVING MDCK CELLS. Christian Le Grimelec, Gérard Friedlander* and Marie-Cécile Giocondi*, INSERM U.251, Faculté de Médecine Xavier-Bichat, Paris, France.

Lipid order of the plasma membrane of living MDCK cells has been determined from fluorescence anisotropy (r) and lifetime measurements of 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene (TMA-DPH) which specifically labels the plasma membrane of intact cells. Fluorescence microscopy, chemical quenching by trinitro benzene sulfonate and back-exchange experiments using soybean phospholipids liposomes revealed that TMA-DPH was exclusively localized in the apical plasma membrane domain of intact MDCK cells monolayers. Accordingly, lipid order of the apical domain was determined from these monolayers whereas the physical properties of the basolateral domain were estimated by comparison with the data obtained from monolayers with opened tight-junctions or MDCK cells suspensions. Steady state anisotropy of intact monolayers at 37°C (r=.325 ± 0.008, N=6) significantly exceeded that of monolayers with opened tight-junctions (r=.285 ± 0.006, N=6) which was similar to that of cells suspensions. Fluorescence lifetime (τm=6.89 ± 0.35 nsec) was identical for the three experimental conditions. Addition of calcium to MDCK monolayers with opened tight-junctions had, if any, a very limited effect on TMA-DPH anisotropy. These experiments demonstrate that the lipids of the apical plasma membrane domain of polarized MDCK cells are much more ordered, i.e. less fluid, than those of the corresponding basolateral domain.

CYCLIC AMP, pH_i , AND CELL-TO-CELL COMMUNICATION IN CULTURED PROXIMAL TUBULAR CELLS. D. Loftus*, M. Hirsch*, and M. Goligorsky. Jewish Hosp. St. Louis, U.S.A. and Ctr. Natl. de la Rech. Sci. Paris, France.

Gap junctional type of intercellular communication exists in the proximal nephron. We attempted to study cell-to-cell communication (CTCC) in primary cultures of proximal tubular cells (PTC) using microinjection of lucifer yellow (LY) and electron microscopy (EM) of freeze-fractured (FF) monolayers. In subconfluent cultures, the transfer of LY is abundant: 4-6 contacts. In confluent monolayers, contacts are limited to 1-2 cells and EM of FF monolayers failed to reveal intramembranous particles (IMP's). Pretreatment of these PTC with 8-br-cAMP for 30 min. resulted in an appearance of IMP's. 8-br-cAMP also resulted in an increase in the number of cell contacts (4-5) as assessed by LY transfer from the site of microinjection. Exposure of these contact-rich monolayers, as well as subconfluent monolayers to H_2O_2 caused uncoupling of the cells. This was associated with an increased leakiness of occluding junctions. Both perfusion with 8-br-cAMP and microinjection of cAMP produced cell acidification by 0.15 pH units. (BCECF fluorescence at dual excitation wavelength). H_2O_2 produced similar degree of cell acidification. Conclusions: 1) cAMP-treated confluent primaries of PTC exhibit abundant CTCC and characteristic IMP's. 2) Although cell acidification is known to uncouple the cells, the specific effect of cAMP overrides its acidifying effect. 3) H_2O_2 causes an uncoupling effect. This event may serve as a stimulus for regeneration following oxidative insult.

GLYCOPROTEINS SIMILAR TO THE HEYMANN NEPHRITIS (HN) ANTIGEN OF RAT (GP600) ARE PRESENT IN HUMAN KIDNEY. Sudesh P. Makker, and John J. Kanalas,* Univ. of Texas Health Sci. Ctr., Dept. of Pediatric Nephrology, San Antonio, Texas.

Crude renal cortical fraction (Fx1A) of human kidney was purified similarly to the HN antigen (gp600) by deoxycholate solubilization and *Lens culinaris* agglutinin affinity chromatography in the same manner used for rat (Makker and Singh, Lab Invest 50:287, 1987). Anti-gp600 nephritogenic autoantibody eluted from glomeruli of HN rats was used to analyze reactivity with purified rat and human antigens by enzyme linked immunoassay (ELISA), immunodetection on Western blots and immunoprecipitation of ^{125}I labelled antigens. The human kidney glycoprotein was reactive in ELISA with anti-gp600 nephritogenic autoantibody. The silver stained polypeptide patterns of both rat and human purified proteins on non-reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were similar in the high molecular weight (MW) region with a prominent band at 330kd in both preparations. Patterns of immunoreactive bands on Western blots using nephritogenic autoantibody were also similar in the high MW regions. The binding of antibody was completely lost when both glycoproteins were electrophoresed under reducing conditions. Immunoprecipitation patterns for both rat and human preparations were essentially identical when compared to their respective Western blot patterns. These results demonstrate that human kidney contains a glycoprotein(s) which is similar to the HN antigen (gp600) in both physicochemical characteristics and immunoreactivity with the nephritogenic autoantibody of the HN rat.

OSMOTIC WATER PERMEABILITY AND MEMBRANE FLUIDITY OF ISOLATED TOAD BLADDER GRANULES IS EXTREMELY LOW. S.K. Masur & A.S. Verkman. Dept. Phys. & Biophys., Mt. Sinai Sch. of Med. & CVRI, UCSF.

Osmotic water permeability in the apical membrane of the toad urinary epithelium is increased greatly by ADH and is associated with exocytic addition of granules and aggregophores at the membrane surface. The osmotic water permeability and membrane fluidity of isolated granules, surface membranes and microsomes prepared from toad bladders in the presence and absence of ADH (JMB 89:39-51, 1986) were studied using stopped-flow light scattering and steady-state diphenylhexatriene (DPH) anisotropy. In response to a 90 mM inward osmotic gradient, granule size decreased with a single time constant of 2.3 ± 0.1 s (SEM, 7 preps), corresponding to a P_f of 5×10^{-4} cm/s at 23°C; the activation energy (E_a) for P_f was 17.2 kcal/mole. Under the same conditions, the volume of surface membrane vesicles decreased with time constants of 0.13s (~70% of signal) and 1.9s (~30%); E_a for the fast component was 3 kcal/mole. Granule, microsomal and surface membrane time constants were unaffected by ADH, however in surface membranes, there was a small decrease (6±2%) in the fraction of surface membranes with fast time constant. DPH anisotropies were 0.253 (granules), 0.224 (surface membranes) and 0.190 (microsomes), and unaffected by ADH. We conclude: 1) granules have among the lowest water permeabilities of biological membranes, 2) granule membrane fluidity is remarkably lower than that of surface and microsomal membranes, and 3) rapid water transport occurs in surface membrane vesicles. The unique physical properties of the granule membrane may be important for apical insertion of membrane with low basal water permeability.

ANALYSIS OF SINGLE CHANNEL ACTIVITIES OF CULTURED RAT MESANGIAL CELLS: THE PRESENCE OF NON-SELECTIVE CATION CHANNELS AND Ca^{++} -ACTIVATED K^+ CHANNELS. H. Matsunaga*, T. Okuda*, H. Chang* and K. Kurokawa. IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo

We reported that angiotensin II (AII) and vasopressin (VP) stimulate Ca^{++} -activated Cl^- conductance of the plasma membrane leading to membrane depolarization of mesangial cells (JCI 78: 1443, 1986). Our data also suggested the presence of additional ion channels regulated by AII and VP. To examine further the hormonal regulation of mesangial cells, we analyzed the membrane channel activity of primary culture of rat mesangial cells using patch-clamp techniques. In cell-attached membrane patches with NaCl-Ringer in the pipet, little channel activity was observed at basal condition. In response to AII or VP, bursts of inward currents and voltage-dependent outward currents appeared. Inside-out membrane patch studies revealed two distinct channel activities. One is a voltage-independent cation channel, non-selective to Na and K ions with a unit conductance of about 40 pS and activated by 1 μ M intracellular Ca^{++} . This non-selective cation channel activity explains the AII-evoked inward currents. The other is a voltage-dependent and Ca^{++} -activated K^+ channel, which explains the outward currents. These data show the presence of distinct channel activities in mesangial cells, and suggest that an increase in cell Ca^{++} by AII and VP activates Cl^- channels as we reported previously and also non-selective cation channels, both leading to membrane depolarization. A rise in cell Ca^{++} and membrane depolarization activate K^+ channels which then modulate membrane potential changes of mesangial cells by these peptides.

STIMULATION OF PHOSPHOINOSITIDE HYDROLYSIS BY CARBACHOL IN INNER MEDULLARY COLLECTING DUCT CELLS. Shari McArdle* and Lal C. Garg. Univ. of Florida Coll. of Med., Gainesville, FL.

We have shown that carbachol, a cholinergic agonist, stimulates the hydrolysis of phosphoinositides (PI) in inner medullary (IM) slices from rabbit kidney (Fed. Proc. 45:427, 1986). In order to localize the effects of carbachol in the IM, we measured PI hydrolysis in isolated IM collecting duct (IMCD) cells. The cells were prepared from IM slices treated with collagenase followed by the addition of water to lyse cells other than the IMCD cells (Am. J. Physiol. 241:F94-F104, 1981). To measure PI hydrolysis, the cells were incubated with [³H]-inositol for its incorporation into PI prior to the measurement of inositol phosphates (IPs) released in the presence of 10 mM lithium which prevents the dephosphorylation of IPs. The amount of [³H]-IPs released is expressed as % of total [³H]-inositol incorporated into the cells.

CON	CAR	ATR	CAR +ATR	HEX	CAR +HEX	DMPP
1.53 ±1.34	26.26* ± 4.59	3.42 ±0.99	5.14 ±1.27	3.54 ±1.24	22.81* ± 4.98	2.80 ±0.91

Values are mean ± S.E.M. of 3 animals.

* P < 0.05 vs control (CON).

Carbachol (CAR) stimulated the formation of IPs from IMCD cells (an increase of >1600% over control values). This response was blocked completely by 1 μM atropine (ATR), a muscarinic antagonist and not by 1 μM hexamethonium (HEX), a nicotinic antagonist. The nicotinic agonist, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, 1 mM) had no significant effect on PI hydrolysis in IMCD cells. We conclude that stimulation of PI hydrolysis occurs through muscarinic receptors in IMCD cells may play a role in the diuretic and natriuretic effects of cholinergic agents.

VARIABILITY OF C4 GENE NUMBER IN THE NEPHROTIC SYNDROME. Robert H. McLean, Wilma B. Bias,* Leticia Tina, Jerry Ruley, Glenn H. Bock. Dept. Ped. & Med., Johns Hopkins Hosp., Baltimore, MD; Georgetown U. Hosp., and Children's Hosp. Nat. Med. Cntr., Wash., DC, Chesapeake Ped. Neph. Study Group.

We have investigated whether patients with the idiopathic nephrotic syndrome (INS) have genetically determined deficiencies of the complement (C) system. C phenotypes for 57 patients with INS (50 with steroid-responsive INS, 6 with focal segmental glomerulosclerosis), and 257 local controls were determined. Haplotypes for MHC Class I, II, and III genes were determined for 11 patients (22 haplotypes) and 15 controls (30 haplotypes). TaqI digested DNA was electrophoresed in agarose, transferred to nylon membranes and hybridized with a 5'-end cDNA probe for C4 (pAT-A). Previously established C4 restriction fragments are: 7.7 kilobase (kb)-C4A, 6.5kb-"long" C4B, 5.7kb-"short" C4B and 7.0 kb-C4B and deleted C4A (on HLA-A1, B8, DR3, BFS, C2C, C4AQ0, C4B1).

C protein phenotypes showed an increased frequency of C4A null (Q0) phenotypes (INS=22/57 controls=46/268, p=.003) with no differences in the frequency of C4BQ0, BF, or C2 phenotypes. However, by C4 gene analysis, the unique 7.0 kb TaqI restriction fragment, linked to deleted C4A gene, was not increased, whereas the combination of TaqI 7.7 kb C4A gene and 5.7 kb "short"- C4B gene restriction fragments was increased in both random INS patients (INS=9/21, controls=6/39, p<.02) and on INS haplotypes (INS=13/22, controls=9/30, p=.04). In the 11 INS pedigrees, C4B duplication, C4A deletion or C4B deletion was present in 4 patients and possible C4B duplication in 5/7 remaining probands. We conclude that variability in C4 gene number or expression occurs more frequently in INS than controls.

CALCIUM UPTAKE BY RAT KIDNEY MICROSOMES IS STIMULATED BY MALEATE. J.M. McLeese* and M. Bergeron. Dept. physiology, Univ. Montréal, Montréal, Québec.

In maleate treated rats, a generalized renal dysfunction resembling the Fanconi syndrome is noted; the endoplasmic reticulum (ER) is poorly stained with osmium impregnation, suggesting that calcium transfer mechanisms of the ER membrane may be disturbed. Rats were injected with Na maleate (400 mg/kg) and were sacrificed after 45 min, 2h, 24h or 96h. Microsomes were prepared from whole kidney by column separation (Pyykkö, Acta Pharmacol. Toxicol. 52: 39, 1983); NADPH cytochrome c reductase activities were enriched 5-9 times compared to homogenate. Ca⁴⁵ uptake was measured by the rapid filtration technique. Ca uptake was higher in treated rats than in controls (15-30%). Vanadate (2 mM) decreased Ca uptake by microsomes, but *in vivo* treatment with maleate appeared to provide protection against this inhibition. Microsomes from 96h-treated rats showed the least inhibition (30%) while those from controls showed the most (60%). In controls, 45 min and 2h-treated rats, *in vitro* addition of 4 mM maleate resulted in a greater increase in Ca uptake compared to addition of the same volume of water. Maleate had no effect on, or slightly inhibited, the increase in 24h and 96h treated rat microsomes. These data suggest that maleate modifies microsomal Ca uptake through action at the ER membrane or on the Ca-ATPase. The different response of 24h and 96h microsomes to *in vitro* maleate addition indicates that organelle reorganization has occurred following maleate exposure.

GP330 ACTS AS A MATRIX RECEPTOR FOR PROXIMAL TUBULE EPITHELIAL CELLS. Donna L. Mendrick, Daniel C. Chung,* and Helmut G. Rennke. Brigham & Women's Hospital, Department of Pathology, Boston, MA.

GP330 is a glycoprotein present in glomerular (GEC) and proximal tubule (PT) epithelial cells of the rat and is a target antigen in Heymann nephritis. We have tentatively identified GP330 as a matrix receptor of rat PT cells since a monoclonal antibody, K35/9, directed against this antigen blocks attachment and proliferation of such cells to various extracellular matrix molecules. K35/4, a monoclonal antibody directed against a 107 kd brush border glycoprotein does not inhibit PT cell attachment and growth. GP330 binds preferentially to fibronectin, as demonstrated by solid phase radioimmunoassay, using detergent-solubilized brush border membranes. K35/9 mimics a matrix protein by binding to the cell membrane receptor and promoting PT cell growth as shown below in a proliferation assay performed with isolated PT cells cultured on wells coated with fibronectin (17ug/ml), purified K35/9 (10-100ug/ml), or purified K35/4 (10-100ug/ml).

	Fibronectin	K35/9	K35/4
CPM±S.E.	4993±500	6200±281	897±53

These studies indicate that GP330 acts as a receptor for fibronectin and possibly other matrix components, and that K35/9 recognizes the fibronectin-binding epitope of this molecule. We propose that GEC abnormalities observed in Heymann nephritis may result, in part, from the interaction between this matrix protein receptor and epitope-specific antibodies as demonstrated for similar receptors on other epithelial cells.

TWO POINT MUTATIONS IN $G_{s\alpha}$ DEFINE DOMAINS REQUIRED FOR COUPLING TO RECEPTOR OR TO ADENYL CYCLASE (AC). R.T. Miller*, K.A. Sullivan*, S.B. Masters*, and H.R. Bourne* (Intr. M.G. Cogan). Depts. Med., Pharm. & CVRI, UCSF, CA.

Structural and functional similarities among the heterotrimeric G proteins indicate that their interactions with receptors and effectors utilize a common molecular mechanism and homologous structural elements. To identify regions of $G_{s\alpha}$ that interact with receptors and AC, we cloned and sequenced cDNAs from two S49 lymphoma mutants. In the unc mutant, $G_{s\alpha}$ cannot couple to receptors, while in the H21a mutant, $G_{s\alpha}$ cannot stimulate AC, but couples to receptors normally. cDNA sequences indicate that the unc mutation encodes proline in place of arginine at the sixth position from the carboxy terminus. This mutation would result in a charge change and a kink in a predicted alpha helix, and identifies the carboxy terminus as a region that interacts with receptors. In the H21a mutation, alanine is substituted for glycine at position 208, a site in the presumptive GTP-binding region predicted to be a hinge required for GTP-dependent changes in conformation. By immunoblot we show that trypsin cleaves GDP-bound $G_{s\alpha}$ near Gly 208, but that this cleavage is prevented by binding of GTP. $G_{s\alpha}$ from H21a is cleaved under conditions that protect wild type $G_{s\alpha}$. The H21a mutation prevents GTP from inducing the active conformation of GTP-bound $G_{s\alpha}$. In conclusion, these studies provide the first structure-function analysis of $G_{s\alpha}$ and identify sites in $G_{s\alpha}$ that interact with receptors or are required for the active conformation that stimulates AC.

INTRACELLULAR Ca^{2+} REQUIREMENT FOR ACTIVATION OF THE Na/H EXCHANGER IN VASCULAR SMOOTH MUSCLE CELLS (VSM). T. Mitsuhashi* and H.E. Ives. Div. Nephrology and CVRI, Univ. of Cal., S.F., CA.

Na/H exchange is increased in VSM from spontaneously hypertensive rats and in cultured VSM by vasoconstrictors like thrombin and phorbol myristate acetate (PMA). Ca^{2+} is a 2nd messenger for vasoconstrictors, but a role for cell Ca^{2+} (Ca^{2+}_i) in regulating Na/H exchange is controversial. We varied Ca^{2+}_i from 40-200 nM by varying extracellular Ca^{2+} from 0-5 mM. Na/H exchange (maximal amiloride inhibitable Δ pH) was first measured following stimulation by osmotic shrinkage (Osm) or NH_4Cl -induced acid load (Acid) and was unaffected by variations in Ca^{2+} . Thus, the exchanger itself requires <40 nM Ca^{2+} . However, kinase C-dependent (PMA) and -independent (thrombin) activation of Na/H exchange does require Ca^{2+} .

[Ca^{2+}] _i	PERCENT OF BASAL Na/H EXCHANGE			
	200	90	50	40
PMA	112±8	95±11	46±13	30±9
Thrombin	102±19	40±3	24±8	21±10

Simple elevation of Ca^{2+}_i above basal (150 nM) by ionomycin did not activate Na/H exchange.

Conclusions: In VSM, increased Ca^{2+}_i is not necessary or sufficient to activate Na/H exchange. Activation by PMA is blunted by reduced Ca^{2+}_i , demonstrating a Ca^{2+} requirement for kinase C in vivo. Maximum kinase C-independent activation of Na/H exchange by thrombin also requires maintenance of normal resting Ca^{2+}_i . Thus, vasoconstrictor-, but not Osm- or Acid-induced activation of Na/H exchange is blunted when Ca^{2+}_i is reduced below the resting level.

THE ROLE OF MICROTUBULES IN MOVEMENT OF PROTEINS AND PHOSPHOLIPIDS TO THE SURFACE MEMBRANE.

B.A. Molitoris and C.A. Hojllien*, VAMC Denver, CO.

Proximal tubule apical and basolateral membranes (BLM) differ markedly with respect to protein and phospholipid (PL) composition yet the development and maintenance of this surface membrane (SM) polarity is little understood. To determine the role microtubules play in movement of proteins and PL to the SM in vivo incorporation of 3H -leucine and $^{32}PO_4$ into rat proximal tubule apical and BLM was used to quantitate protein and PL incorporation rates, respectively. Apical and BLM were isolated as previously described and neither colchicine nor vinblastine altered marker enzyme enrichments. A 1 and 2 hr pulse was used for 3H -leucine and $^{32}PO_4$ incorporation studies, respectively. While cellular 3H -leucine specific activities were not altered by colchicine or vinblastin, colchicine in doses of 1, 2 and 5 mg/Kg (IV) significantly ($p < 0.05$) reduced apical protein incorporation by 11 ± 5 , 40 ± 9 and $71 \pm 6\%$ respectively. Vinblastine (40 mg/Kg, IV) also reduced apical protein incorporation by $42 \pm 16\%$ ($p < 0.01$). In contrast, neither colchicine (2 mg/Kg) nor vinblastine (40 mg/Kg) had any effect on total PL incorporation rates into apical or BLM. For individual PL species only phosphatidylserine (PS) incorporation was altered. PS incorporation into both apical and BLM was reduced by > 50% following either colchicine or vinblastine consistent with the unique golgi - mitochondrial - SM migration of PS. These data indicate microtubules are essential for movement of proteins but not PL to the surface membrane. We propose proteins and PL move independently to the SM and not via a common vesicle shuttle pathway.

CELL PERMEABILITY TO CALCIUM MEASURED WITH FURA 2 IN SINGLE RAT CORTICAL COLLECTING TUBULES (CCT).

François Morel*, Shigeo Taniguchi* and J. Marchetti* (intr. by C. Le Grimellec), Lab. Physiologie Cellulaire, Collège de France, Paris, France.

Single microdissected and Fura 2-loaded CCT were superfused (37°C, 0.4 ml/min) in a chamber fixed on the stage of an epifluorescence microscope, and the Fura 2 fluorescence (450 to 500 nm) emitted from a selected tubular area (about 30 x 100 μ m) was continuously recorded under 40 fold magnification at two (336 and 384 nm) excitation wavelengths alternately (10 cycles per min). Free calcium concentration in cytosol ($[Ca]_i$) was calculated from the 336/384 fluorescence ratio according to the usual equation.

Under control conditions ($[Ca]_e = 1$ mM in superfusate), $[Ca]_i$ averaged 202 ± 19 (SE) nM (N=33). When $[Ca]_e$ was varied from 0.25 to 4 mM, $[Ca]_i$ varied accordingly: The changes in $[Ca]_i$ were rapid (<2 min), sustained and fully reversible. In a series of 7 CCT randomly exposed to at least 4 different $[Ca]_e$ successively, $[Ca]_i$ steady state values were linearly correlated with $[Ca]_e$ in each CCT ($r > 0.97$, average slope value: $62 \pm 9\%$ of $[Ca]_i$ control values per mM $[Ca]_e$ change).

Addition of verapamil (10^{-5} M), ouabain (10^{-4} M), or Na^+ substitution in the perfusate did not alter $[Ca]_i$. Cell depolarization induced by 100 mM $[K]_e$ resulted in a marked and reversible fall in $[Ca]_i$.

It is concluded from the data that the main passive pathways for Ca^{++} in CCT cell membranes are neither voltage-dependent Ca channels nor Na/Ca exchangers, but rather a passive entry permeability to Calcium balanced by an active Calcium extrusion via Ca-ATPase.

Na, K-ATPase CODISTRIBUTES WITH ANKYRIN AND SPECTRIN IN RENAL TUBULAR EPITHELIAL CELLS. J.S. Morrow, AS Mann, C Cianci, T Ardito and M Kashgarian, Yale Univ. School of Med., Dept. of Pathology, New Haven, Connecticut.

Na, K-ATPase in renal tubular epithelial cells is preferentially localized to the lateral and infolded surfaces of cell membranes and excluded from regions where plasmalemma comes in contact with the basement membrane and the apical membrane. Since studies on the erythrocyte have established that segregation and immobilization of membrane proteins results from interaction with the cytoskeletal proteins, spectrin and ankyrin, the distribution of these proteins was studied in renal epithelial cells. Spectrin and ankyrin codistributed with the α -subunit of Na, K-ATPase in intact renal tubular epithelium. Dog kidney membranes bind red blood cell ankyrin *in vitro* and solid phase binding assays show this binding to be the result of a specific interaction of the α -subunit of Na, K-ATPase with an ankyrin analog. The development of cell polarity was studied in MDCK cells. A polar distribution of Na, K-ATPase and spectrin occurred only in confluent cultures. Co-localization of Na, K-ATPase and spectrin *in vivo* pattern was seen only in cells grown on substrata coated with basement membrane components (type IV collagen, type V collagen and laminin). These studies demonstrate that interaction of Na, K-ATPase, ankyrin and spectrin are important in the development and maintenance of renal epithelial cell polarity.

DIFFERENTIATED TRANSPORT FUNCTIONS IN PRIMARY CULTURES OF RABBIT CORTICAL COLLECTING TUBULES (CCT). A. Naray-Fejes-Tóth* and G. Fejes-Tóth* (intr. by O.A. Carretero), Hypertension Research Division, Henry Ford Hospital, Detroit, MI

Using a monoclonal antibody (MCAB) directed against a membrane antigen on principal cells (PC), CCT fragments were immunodissected from rabbit kidney. Flow cytometric analysis of the isolated cells stained with fluorescein-conjugated cell specific MCABs indicated that the ratio of PC to intercalated cells (ICC) was 3:1. In primary culture a spontaneous enrichment for ICC occurred, resulting in a PC/ICC ratio of 1:3 on day 9. Confluent monolayers of CCT cells, grown on permeable supports (0.6 cm²) had a transepithelial voltage (V) of 7.2±1.25 mV (lumen +) and generated pronounced solute gradients. The composition of the luminal (L; 400 μ l) and basolateral (B; 600 μ l) bath after 48 h of incubation was [mM]

	[Na]	[K]	[PO ₄]	[Ca]	[Cl]	[lactate]	pH
L	119	11.9	2.8	0.04	134	1.0	5.52
B	145	2.0	7.3	0.81	117	23.5	7.15

When confluent cultures were transferred to a defined medium (2% Ultrosor G), the % of cells expressing PC-specific antigens increased to 65, and the polarity of V was reversed. At the same time, K⁺ secretion increased ~7 fold and Na⁺ reabsorption ~4 fold, while PO₄ reabsorption was significantly reduced. V was reduced from 43±2 (lumen -) to 3.1±0.7 mV by 10 μ M amiloride and reversed to 8.7±1.2 mV (lumen +) by 100 nM ouabain. These data indicate that differentiated transport functions can be maintained in primary cultures of immunodissected CCT cells, and raise the possibility that ICC and PC, at least *in vitro*, can be transformed one into the other.

THE HIGHER INOSITOL LEVEL IN MDCK CELLS IN HYPEROSMOTIC MEDIUM IS CAUSED BY INCREASED INOSITOL TRANSPORT. Takeshi Nakanishi,* R. James Turner and Maurice B. Burg. NHLBI/NIDR, Bethesda, MD.

During antidiuresis renal inner medullary cells contain a high level of inositol which helps balance the high extracellular osmolality. Having previously found that MDCK cells can grow in hyperosmotic medium (DMEM, 10%FBS, 900mOsm/kg, 300mM NaCl, 300mM urea), we have now studied their handling of inositol. With 40 μ M inositol in the medium, intracellular inositol was 26mM under isosmotic and 250mM under hyperosmotic conditions. The rise occurred only with inositol in the medium, suggesting that transport was involved. ³H-inositol influx was Na-dependent. The kinetics of Na-dependent uptake, assayed at the same osmolality as in the growth medium were:

	mosm/kg	300	900	900
Osmolality				
NaCl in assay	mM	150	150	300
Vmax	pmoles/min/mg	37	86	106
Km	μ M	62	64	55

The significant increase in Vmax without change in Km is consistent with an increased number of transporters. In defined medium containing 120 μ M inositol, ³H-inositol uptake rate reached a maximum within one day after the osmolality was elevated. This enhancement of ³H-inositol flux by high osmolality was greater when inositol was omitted from the growth medium.

CONCLUSIONS: 1) MDCK cells accommodate to elevated osmolality by accumulating high levels of inositol, as do cells in the renal inner medulla. 2) The inositol is accumulated because of increased Na-dependent uptake from the medium.

AMMONIAGENIC PATHWAYS IN CULTURED HUMAN RENAL EPITHELIAL CELLS: STUDY WITH ¹⁵N. Itzhak Nissim and Beatrice States,* Univ. of Pennsylvania Sch. of Medicine, Dept. of Pediatrics, Philadelphia, PA

The major ammoniagenic pathways in cultured human renal cortical epithelial cells have been evaluated. Experiments either at pH 7.4 or 6.8, in the presence and absence of pyruvate, α -ketoglutarate or amino-oxycetate were conducted with phosphate buffered saline supplemented with either 1mM[5-¹⁵N]glutamine, [2-¹⁵N]glutamine, [1⁵N]glutamate or the alkyl substituted glutamine, e.g., L-[2-¹⁵N]- γ -glutamylmethylamide. The results indicate that approximately 75% and 35% of NH₃ was derived from 5-N and 2-N of glutamine respectively, when these amino acids were added as a sole substrate in incubation at pH 7.4. Similarly, 55% and 60% of NH₃ was so derived at pH 6.8. At either pH, ¹⁵NH₃ formation from glutamate was one third of 2-N of glutamine. In each experiment, a balance was observed between ¹⁵N utilization and subsequent appearance of ¹⁵N in NH₃ and amino acids. When L-[2-¹⁵N]- γ -glutamylmethylamide replaced glutamine in the incubation system, a significant amount of ¹⁵NH₃ was formed. Addition of amino-oxycetate, pyruvate or α -ketoglutarate with [2-¹⁵N]glutamine or L-[2-¹⁵N]- γ -glutamylmethylamide significantly effects ¹⁵NH₃ formation regardless of H⁺ concentration.

Comparative studies with human renal cortical slices suggest that human cultured renal epithelial cells appear to possess metabolic properties that the fresh kidney demonstrates *in vitro*. The data indicate that in addition to phosphate dependent glutaminase and glutamate dehydrogenase, glutaminase II pathway does exist in cultured human renal cells and may be an important control mechanism of ammoniogenesis and therefore of acid-base homeostasis in humans.

BRADYKININ AND EGF STIMULATE C-MYC mRNA EXPRESSION IN RABBIT PROXIMAL TUBULE CELLS BUT RESULT IN DISPARATE EFFECTS ON CELL PROLIFERATION. E.P. Nord, J. Schlosser* and J. Norman. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

Increased expression of c-myc mRNA has been correlated with the stimulation of cell proliferation in a number of cell types. The present study investigated the effect of bradykinin and EGF on the expression of c-myc mRNA and cell proliferation in renal epithelial cells. Quiescent, confluent monolayers of rabbit renal proximal tubule (PT) cells in primary culture were treated for up to 24 hrs with either 10^{-9} M bradykinin (previously shown to activate the phosphoinositol pathway in these cells) or 10^{-9} M EGF (a known mitogen). Expression of c-myc mRNA was determined on Northern blots and DNA synthesis (at 24 hrs) measured by incorporation of tritiated thymidine ($^3\text{H-TdR}$). Bradykinin caused a rapid increase in c-myc mRNA expression yet DNA synthesis was not different from control values. In contrast, EGF stimulated the same magnitude of c-myc mRNA expression as bradykinin, yet $^3\text{H-TdR}$ incorporation was increased 10-fold above control. **Conclusions:** 1) Bradykinin and EGF induce a rapid increase in expression of c-myc mRNA in rabbit PT cells. 2) No direct correlation exists between induction of c-myc mRNA and proliferation in PT cells.

FETAL CALF SERUM STIMULATES PLASMA MEMBRANE Cl^- CONDUCTANCE AND DEPOLARIZES MEMBRANE POTENTIAL OF CULTURED RAT MESANGIAL CELLS. Toshihiro Okuda*, Naohide Yamashita*, and Kiyoshi Kurokawa. IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo, Japan

Fetal calf serum (FCS) is essential to maintain mesangial cell proliferation in culture probably due to the presence of necessary growth factors. Since changes in ion permeability of plasma membrane may occur as early events of cell proliferation, we examined the effects of FCS on mesangial cell membrane potential and ion permeability using conventional microelectrode techniques. FCS caused an immediate and sustained membrane potential depolarization. Upon removal of FCS, membrane potential quickly repolarized to the baseline value. During depolarization, input resistance decreased, suggesting an increased permeability to some ions. Reversal potential of the response was about -30 mV and became less negative with decreasing extracellular Cl^- , indicating an increased Cl^- conductance, responses similar to those we reported with angiotensin II (AII) (JCI 78: 1443, 1986). However, there are several distinct differences in the responses to FCS and AII. Membrane potential depolarization by FCS was sustained while that by AII was transient. Further, tachyphylaxis was present in response to repeated exposure to AII but not to FCS. In addition, FCS did not evoke cell contraction while AII consistently evoked mesangial cell contraction. These data indicate FCS, similar to AII, increases Cl^- conductance of mesangial cell plasma membrane leading to membrane depolarization as an early event of proliferation, but the mechanisms involved may differ from those with AII which evokes cell contraction.

EARLY GENE EXPRESSION DIFFERS IN RENAL CORTICAL CELLS UNDERGOING HYPERTROPHY OR HYPERPLASIA. J. Norman, J. Bowen*, A. McDonough*, L. Fine. Division of Nephrology, UCLA School of Medicine and Dept. of Physiology, USC School of Medicine, Los Angeles, CA.

In vivo models were used to compare cell hypertrophy (unilateral nephrectomy, UNI-NX) and hyperplasia (folic acid injection (FA) 250mg/kg) in rabbit renal cortical cells. At 48 hours cell size increased in both groups but DNA synthesis (9-fold) and a shift of cells into $\text{S/G}_2/\text{M}$ of the cell cycle occurred only with FA. mRNA levels of genes which show cell cycle dependent expression were measured on Northern blots (oncogenes: c-fos, c-myc, c-ras^{Ha}; structural proteins: β -actin, vimentin; transport proteins: $\text{Na}^+\text{K}^+\text{ATPase}$, ADP-ATP translocase; calcium binding protein: calyculin). In general FA induced rapid (within 0.5-6 hours), transient increases (2-16 fold) in mRNA levels whereas UNI-NX caused a slow, progressive increase in mRNA levels with transient early peaks (2-4 fold) of c-myc and ADP-ATP translocase mRNA.

Conclusion: Distinct and different patterns of gene expression occur during cell enlargement in renal cortical cells destined to undergo hypertrophy vs. hyperplasia. Failure of cells to undergo mitosis in renal hypertrophy is not due to interruption of the normal cell cycle but is a growth process regulated by a unique pattern of gene expression.

IMMUNOLOGICAL HETEROGENEITY OF KIDNEY BAND 3 ANION EXCHANGER. L. Ostedgaard*, M. Jennings*, R. Anderson*, and V.L. Schuster. Univ. of Iowa, Iowa City, IA and Univ. of Texas, Galveston, TX.

Medullary collecting ducts (MCD) and erythrocytes (E) have functionally similar anion exchange. We showed that a monoclonal antibody (mAb) to human E band 3 membrane domain (anti-hMD) immunocytochemically labels rabbit MCD basolateral cell membranes. Here we analyze the size of the immunoreactive membrane protein(s). A mAb to rabbit E band 3 membrane domain (anti-rMD), a polyclonal Ab to human E band 3 cytoplasmic domain (anti-hCD), and anti-hMD showed identical immunocytochemical labeling of rabbit MCD. However, immunoblots of rabbit or bovine medullary membrane proteins revealed size heterogeneity. Anti-rMD and anti-hCD labeled a band 3-like protein (95 kd) plus bands at ~75 kd and ~50 kd. In contrast, blots of the same membrane preparation with anti-hMD showed a single band at 45 kd. Because a 95 kd protein is present in the membrane preparation, proteolysis cannot explain the absence of a 95 kd band with anti-hMD. Immunoprecipitation of ^{14}C -labeled kidney membranes with anti-hMD also showed no large MW protein (control E membranes yielded the expected 95 kd band). To further study size heterogeneity, a bovine renal medullary $\lambda\text{gt}11$ cDNA library was screened. Three positive clones have been isolated; two react with only anti-rMD and one with both anti-rMD and anti-hMD. **Conclusion:** Though different anti-band 3 Ab's give identical immunocytochemical MCD staining, the size of the immunoreactive protein(s) is variable. Sequencing the cDNA clones should provide further information about the origin of such heterogeneity.

MEDIATION OF PTH EFFECTS ON ACIDIFICATION BY RAT PROXIMAL CONVOLUTED TUBULAR (PCT) CELLS IN SITU E. Pastoriza, R. Harrington, and M. Graber. VAMC, Northport, and SUNY at Stony Brook, NY.

We have previously shown that PTH inhibits luminal acidification in the PCT. To assess whether this effect is mediated by inhibition of the luminal Na/H exchanger, an action that would acidify the cell, the effect of PTH on cell pH was measured in PCT cells in situ loaded with the pH-sensitive fluoroprobe 4MU. In thyroparathyroidectomized (TPTX) rats cell pH rose from 7.14 ± 0.01 to 7.24 ± 0.01 ($p < .01$) after PTH. cAMP did not change cell pH (7.12 ± 0.01 in TPTX control vs 7.14 ± 0.01 after cAMP). The effects of PTH on cell calcium (Ca) were studied in PCT cells of TPTX rats loaded with FURA2 using the Ca-sensitive 334/365 fluorescence ratio. After a 2-3 minute delay PTH caused a rapid and marked increase in cell Ca followed by a partial recovery extending beyond 10 minutes.

We conclude that the impaired luminal acidification by PTH is most likely mediated by inhibiting base exit across the basolateral membrane, an action which is mediated by a cAMP independent pathway. The cell alkalization by PTH may be mediated via activation of phosphatidylinositol metabolism and elevation of cell Ca.

IN-VITRO STIMULATION OF GENE TRANSCRIPTION IN PRIMARY PROXIMAL TUBULE CULTURES BY LOWERED pH. A.S. Pollock, D.D. Horowitz and R.C. Clouse. Univ. of Calif. and VA Med. Cntr. San Francisco.

The renal response to lowered systemic pH includes alterations in membrane transport and intermediary metabolic functions. Gluconeogenesis is known to be increased in the kidney in acidosis and this increase is in part due to increases in renal cytoplasmic phosphoenolpyruvate carboxykinase (PEPCK) activity. The expression of the single copy gene for this enzyme is known to be transcriptionally regulated, and transcription increases in kidney with administration of hormones and systemic acidosis *in-vivo*. In contrast, we have recently demonstrated that liver PEPCK mRNA, also responsive to glucoregulatory hormones, is unaffected by systemic acidosis. To determine whether this effect represents a primary tissue-specific renal response or a secondary hormonal response, we prepared primary cultures of rat proximal tubules and herein demonstrate that lowered extracellular pH *per se* increases the level of mRNA for this acidosis-responsive message *in-vitro*.

Primary cultures of rat cortex were prepared; after 1-2 days of serum and hormone deprivation, they were exposed to normal and reduced HCO_3^- (24, 12 and 6 mM at $\text{pCO}_2=40$), 8Br-cAMP, PTH, insulin or dexamethasone for 2 hours. Dot-blots were hybridized with ^{32}P RNA probes for rat PEPCK and actin mRNA.

Lowering pH by decreasing HCO_3^- concentration increased relative PEPCK mRNA levels to 2.2-2.5 fold control in the absence of extracellular hormones. In addition, 8Br-cAMP and PTH increased relative PEPCK mRNA to 1.7 and 1.5 fold control. Insulin and dexamethasone had an inconsistent effect.

Conclusions: 1) *In-vivo* the kidney demonstrates a tissue-specific increase in transcription of the PEPCK mRNA in response to acidosis. 2) In the *in-vitro* proximal tubule cell culture model, lowered pH alone is sufficient to cause increased expression of the PEPCK message. Further study of the mechanisms of induction of this message in kidney may offer an insight into mechanisms of the unique renal response to acidosis.

ACTIVATION OF PROTEIN KINASE C MODULATES BRADYKININ STIMULATED PHOSPHOLIPASE C ACTIVATION IN MDCK CELLS. Didier Portilla, Daniel Coyne and Aubrey Morrison. Washington Univ. Med. School, Depts. of Med. & Pharmacology, St. Louis, MO.

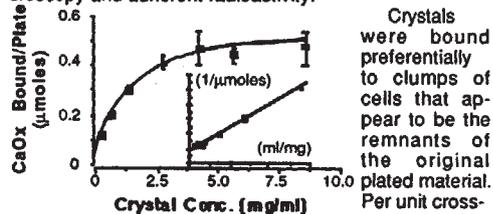
Phorbol myristate acetate (PMA) induces PKC translocation and stimulates the phosphorylation of a 40 and 48 kDa proteins in MDCK cells. 1-(5-isoquinolonyl-sulfonyl)-2-methylpiperazine (H7) an inhibitor of PKC *in vitro* inhibits the PMA stimulated protein phosphorylation in these cells. To assess the modulator role of the diacylglycerol C kinase pathway in the calcium signalling system in bradykinin-stimulated MDCK cells we studied the effect of PMA and H7 upon Ca^{2+} in fura2 loaded MDCK cells and phospholipase C activation monitored by changes in ^3H inositol 1,4,5 trisphosphate formation in BK-stimulated MDCK plasma membranes. BK resulted in a concentration dependent transient rise in Ca^{2+} from a basal level of 10^{-7}M to peak levels of about 10^{-6}M . Pretreatment of MDCK with PMA significantly inhibited this response in a dose-dependent manner with an IC_{50} of 10^{-5}M while 4 alpha didecanoate (inactive phorbol ester) at a doses of 10^{-4}M had no effect. Preincubation of MDCK cells with H7 resulted in a dose dependent reversal of the PMA inhibition with a recovery of BK stimulated Ca^{2+} transients to control levels at a K_i of $12 \mu\text{M}$ similar to that previously reported. HA1004, a more selective cAMP dependent kinase inhibitor was inactive. Pretreatment of MDCK cells with PMA prior to the membrane preparation did not have a significant effect in the guanine nucleotide dependent phospholipase C activation. We conclude that: 1) PMA inhibits the calcium response to bradykinin; 2) H7 a specific PKC inhibitor reverses this inhibition suggesting the PMA inhibition occurs through PKC activation; 3) The inhibition by phorbol ester may occur through the PKC dependent phosphorylation of an IP_3 triphosphatase increasing the rates of degradation of IP_3 and a fall in IP_3 levels.

IDENTIFICATION OF THE SITES OF SYNTHESIS OF ANGIOTENSINOGEN AND RENIN IN THE KIDNEY BY IN-SITU HYBRIDIZATION. S. Rajaraman, K. Graves*, and S. Kunapuli*. Univ. of Texas Med. Branch, Div. of Renal Immunopathology, Galveston, TX.

Several studies suggest the existence of local renin-angiotensin-system (RAS) in various organs, with diverse functional implications. We studied the expression of angiotensinogen, the precursor of the biologically active angiotensin-II and renin in human and rat kidneys. We localized the peptides by immunohistochemistry, using monospecific, polyclonal antibodies and studied the expression of the respective mRNA species by RNA dot blot and *in situ* hybridization, using nick translated human and rat angiotensinogen cDNA and rat renin cDNA as molecular probes. Both the human and rat kidneys revealed similar findings. Co-expression of the peptides was observed predominantly in the juxtaglomerular apparatus and to a lesser extent in the proximal, collecting and distal tubules and the arterial smooth muscle. In contrast, angiotensinogen and renin mRNA were identified in the glomerular mesangial cells, collecting and proximal tubules with variable expression in the distal tubules. Renin mRNA was also present in the major vascular endothelium. We conclude that: 1) the mesangial cells and various tubular epithelial cells synthesize angiotensinogen and renin and 2) they may play a crucial role in the regulation of vascular tone, glomerular filtration and fluid and electrolyte homeostasis, independent of the plasma RAS.

BINDING OF CALCIUM OXALATE CRYSTALS (CAOX) TO RENAL PAPILLARY COLLECTING TUBULE CELLS (RPCT) IN PRIMARY CULTURE. B. Riesa,* J Kleinman, J Wiessner, G Mandel*, and N Mandel*, Dept Medicine VA Medical Center & Med Coll of WI, Milwaukee, WI.

Attachment of microcrystallites within the renal papilla may be an important component of the pathophysiology of nephrolithiasis. We have described attachment of preformed CaOx to RPCT (J. Urol, in press). The purpose of these studies was to characterize this binding so as to provide a basis for its use as an *in vitro* model of nephrolithiasis. Primary cultures of medullary tubule cells pooled from the kidneys of 150-250g male Sprague-Dawley rats were grown in a defined medium on 12 mm diameter circular glass cover slips. Cultures became confluent after 4 days. Cells on coverslips were incubated with ^{14}C -CaOx (average diameter $3.8 \pm 0.43 \mu\text{m}$) for 30 min at 37°C in an artificial urine at pH 6.0. The cells were washed and crystals bound quantified by microscopy and adherent radioactivity.



sectional area, 32 ± 11 times more crystals were bound to clumps than to the simple monolayer. Binding demonstrated concentration dependent saturation: $B_{\text{max}} = 0.51 \mu\text{moles}$; $K_{\text{m}} = 1.56 \text{ mg/ml}$. Coincubation with hydroxyapatite crystals showed inhibition of CaOx adherence with a K_{i} of 1.27 mg/ml .

In summary, CaOx adherence to RPCT demonstrates selectivity for cellular clumps, saturation and inhibition, features suggesting specific binding.

HORMONAL REQUIREMENTS FOR MEMBRANE SORTING OF HYDROLASES IN CULTURED PROXIMAL TUBULE CELLS (PTC). P. Ronco,* M. Geniteau,* M. Antoine,* B. Baudouin,* F. Chatelet,* P. Verroust,* and A. Vandewalle,* INSERM U. 64 and U. 246, Paris, France (Intr. by C. Le Grimellec).

Isolated rabbit PTC can be grown either in DMEM supplemented with 1% foetal calf serum (FCS) or in defined medium (DM) containing $5 \times 10^{-8} \text{ M}$ dexamethasone (Dex) and $5 \mu\text{g/ml}$ insulin (Ins). Brush border hydrolases are detectable under both conditions but there is little information on their subcellular localization. This question was addressed using monoclonal antibodies specific for dipeptidyl peptidase IV (DPP), endopeptidase (EP) and AII converting enzyme (ACE). The 3 hydrolases were expressed in all confluent cells (day 11) in both media but their distributions were dramatically different. When cells were grown in FCS, DPP, EP and ACE were detected in numerous large cytoplasmic vesicles, over rare and short apical microvilli as well as on the basolateral domain. In marked contrast, cells grown in DM exhibited a polarized distribution of hydrolases similar to that observed on rabbit kidney sections: DPP was detected over the entire membrane areas of PTC whereas EP and ACE were restricted to abundant tall microvilli of the apical domain. Furthermore, this membrane sorting process is associated with a twofold increase in enzymatic activities.

	ACE	EP	DPP
FCS (6)	3.7 ± 0.9	1.1 ± 0.9	82.1 ± 7.7
HDM (6)	6.3 ± 1.7	2.6 ± 0.4	155.3 ± 22.3

Values (U/g protein) are means \pm SE.

The results thus show that FCS does not permit polarized membrane expression of hydrolases in cultured PTC, and suggest a role for Dex and/or Ins.

HUMAN GLOMERULAR EPITHELIAL CELLS (GEC) IN CULTURE RELEASE BOTH UROKINASE (u-PA) AND A TYPE I PLASMINOGEN ACTIVATOR INHIBITOR (PAI1): ROLE OF PROTEIN KINASE C (PKC). E. Rondeau, R. Medclaf, F. Delarue, W.D. Schleming, J.D. Sraer. Inserm U 64 Paris and Chuv Lausanne. (intr. by D. Schlondorff).

We studied the fibrinolytic components released by human GEC in culture. After 10% SDS PAGE and zymography on a fibrin-agar underlay containing plasminogen, GEC were found to release a 53 kd form of PA immunoprecipitated by anti-u-PA antibodies. Small amounts of tissue-type PA (t-PA, 70 kd) and of a t-PA-PAI1 complex (110 kd) were also identified. When measured by a spectrophotometric assay using a synthetic chromogenic substrate of plasmin (S 2251), the PA activity of the GEC conditioned medium was quite undetectable suggesting that u-PA was released in its inactive single chain form (SC-u-PA). After activation of SC-u-PA to u-PA by plasmin (1 mU for 2 h at 37°C), the PA activity increased in a time and dose dependent manner when GEC were treated with phorbolmyristic acetate (PMA). By ELISA, both u-PA and PAI1 antigens were also found to increase after stimulation by PMA. Cyclic AMP analogs had no effect and H7, a PKC inhibitor, suppressed the effect of PMA. Using specific cDNA probes for Northern blot analysis both the u-PA and the PAI1 mRNA increased in GEC after a 4 hours stimulation by 16 nM PMA. Thus, the release of both u-PA and PAI1 by GEC is regulated by PKC activity. The persistence of extracapillary fibrin deposits in crescentic GN may be explained by the release of the inactive form of u-PA and the concomitant release of PAI1.

PERTUSSIS TOXIN (PT) AND CYCLIC AMP INHIBIT BRADYKININ (BK) STIMULATION OF PGE2 AND PHOSPHOLIPASE C IN MDCK CELLS. D. Schlondorff, S. DeCandido*, J.A. Satriano*, Albert Einstein Coll. Med., Bronx, NY.

Cyclic AMP inhibits stimulation of PGE2 formation in collecting tubule and toad bladder cells. A GTP-binding N-protein, similar to the inhibitory subunit Ni of adenylate cyclase, and also ADP ribosylated by PT, may be involved in phospholipase C activation and PGE2 formation. We therefore examined the effect of cAMP and PT on PGE2 and inositolphosphate formation in MDCK cells stimulated with BK. Pretreatment for 2 hrs with PT (100ng/ml) 8 Bromo cAMP (0.1mM) or forskolin (1uM) had no effect on basal PGE2 synthesis (30+5ng/10 min), or A23187 stimulation, but inhibited BK effect.

(n=9-10/group)	Contr	PT	8BrcAMP	Forskolin
PGE2 in	BK	205+76	117+76*	88+27*
ng/10 min	A23187	338+193	241+88	322+135
				316+126

In [3H] myoinositol prelabeled cells PT, 8BrcAMP, or forskolin pretreatment abolished the 2-3 fold stimulation of [3H] labeled IP₁, IP₂ and IP₃ (Berridge method) after 15 and 30 sec with BK. [32P] autoradiography of ribosylation by PT on SDS-PAGE showed two bands (39-41kDa). PT pretreatment of cells decreased subsequent [32P] ribosylation indicating prior ribosylation. On immunoblot only one of the bands reacted with antibody against Ni protein. We conclude: PT and cAMP/forskolin inhibit BK stimulation of phospholipase C and PGE2 synthesis. However the PT effect is not mediated by cAMP. PT ribosylates two N-proteins which may be involved with phospholipase C stimulation and PGE2 formation by BK.

GLUCOCORTICOID ISORECEPTOR IB (GR_{IB}) MEDIATES Na⁺ ABSORPTION (abs) IN TRANSPORTING EPITHELIA. G. Schulman and C. Bastl, Temple University Health Science Center, Philadelphia, PA.

Glucocorticoids (G) regulate renal proximal tubular and intestinal Na abs. We found that the activated glucocorticoid receptor (GR) in renal cortex and colon is smaller and less negatively charged (GR_{IB}) than the GR in tissues where G regulate metabolic events (GR_{II}). This suggested that GR_{IB} was the transport receptor. If so GR_{IB} should be present in all epithelia where G mediate Na transport. G stimulate Na abs. in intestine but GR in jejunum (J), ileum (I) and cecum (C) have not been studied. Rat intestinal cytosol was prepared with a combination of protease inhibitors found to eliminate > 90% of protease activity insuring the GR was not proteolyzed. Specific ³H-TA binding was present in all segments with a K_d of 2-4nM and B_{max} of 70±20 (J), 58±18 (I), 189±19 (C) fmol/mg protein. Competitive binding assay showed G specificity. Unactivated GR eluted as a peak at 400mM KCl on DEAE-Sephadex anion exchange chromatography in J, I and C. After activation, measured by increased DNA binding, GR from C and I eluted in the prewash (GR_{IB}). GR from J eluted in 2 peaks, the prewash and at 200mM KCl (GR_{II}). Gel filtration of activated GR revealed a Stokes radius (R_s) of 34 Ang. Elution of activated GR in the prewash on anion exchange chromatography indicating a less negative charge and the smaller R_s than described for GR_{II} demonstrate that J, I, and C contain predominantly GR_{IB}. In all epithelia thus far studied where G regulate Na abs. the unique isoreceptor GR_{IB} is found suggesting GR_{IB} mediates G effect on Na abs.

HOMOLOGOUS AND HETEROLOGOUS DESENSITIZATION OF VASCULAR SMOOTH MUSCLE CELLS (VSMC) TO PLATELET ACTIVATING FACTOR (PAF). Ulrich S. Schwertschlag, Duke Med Ctr, Div. of Nephrology, Durham, N.C.

The effects of PAF on cytosolic calcium concentration (Ca_i), phosphatidyl-inositol (PI)-hydrolysis and diacylglycerol (DG) formation were studied in cultured VSMC from rat aorta. PAF (100 nM) transiently increased Ca_i in VSMC from 73±21 nM to 119 ± 14 nM (P<0.05). A second dose of PAF (1000 nM) was ineffective in changing Ca_i, indicating homologous desensitization. PAF stimulated PI-hydrolysis at a threshold concentration of 1 nM with a lag period of 3 min: IP₂ increased 79 ± 30% and IP₃ 32±7% compared to control (P<0.05). DG increased continuously from 156±15% of control after 5 min of PAF to 190±20% of control within 10 min of PAF stimulation. Angiotensin II (ANG II) and arginine-vasopressin (AVP) increased Ca_i in a dose dependent manner: 673±69 nM Ca_i at 1 uM ANG II and 472±53 nM Ca_i at 1 uM AVP. Pretreatment of VSMC with PAF (100 nM) attenuated the ANG II (1uM) induced rise in Ca_i by 65% (673 ± 69 versus 238±31 nM; P<0.05) indicating heterologous desensitization but AVP-induced changes in Ca_i were not affected. To further characterize the response of Ca_i to PAF and the interaction of PAF and ANG II, VSMC were pretreated with phorbol ester-myristate-acetate (PMA, 10 nM) for 60 min. PMA attenuated the ANG II response and inhibited PAF-induced changes in Ca_i. We conclude that VSMC demonstrate both homologous and heterologous desensitization to PAF, and that the mechanism probably involves DG-induced changes in the ANG II and PAF receptors.

EVIDENCE FOR RECEPTOR OPERATED CA²⁺ CHANNELS IN BASOLATERAL MEMBRANES (BLM) OF PROXIMAL TUBULAR CELLS (PTC): EFFECTS OF INOSITOL 1,4,5-TRISPHOSPHATE (IP₃). John E. Scoble,* Roberto Civitelli, and Keith A. Hruska. Renal Div., Jewish Hospital, St. Louis, Missouri.

BLM vesicles (BLMV) from canine kidneys exhibit stimulation of Ca²⁺ flux by PTH under conditions in which the Na⁺/Ca²⁺ exchange and Ca²⁺ ATPase systems are inactive. The stimulation may represent the opening of receptor operated Ca²⁺ channels. To investigate the modulation of the stimulation of this Ca²⁺ flux, BLMV were prepared from canine renal cortices and suspended in a KCl-mannitol-tris Hepes buffer, pH 7.0. BLMV were equilibrated with 25 μM ⁴⁵CaCl₂ in the suspension buffer containing 2 mM benzamil prior to the initiation of the Ca²⁺ efflux. Various test substances were added to the solutions used to initiate Ca²⁺ efflux. IP₃, 10⁻⁶ M, stimulated the initial rates of Ca²⁺ efflux from 6.1±4 to 10±5 pmol/mg protein/sec, p<.01, N=5. The effect of IP₃ was not inhibited by Dantrolene, an inhibitor of an IP₃-gated Ca²⁺ channel in the endoplasmic reticulum. Inositol tetrakisphosphate, cyclic nucleotides, and guanine nucleotides were ineffective in stimulating the Ca²⁺ efflux. The data indicate that inside-out vesicles with exposed IP₃ receptors, in which Ca²⁺ efflux is directionally the same as Ca²⁺ entry in the cell, exhibit an IP₃ stimulated Ca²⁺ flux. Thus, the stimulation may represent the function of a receptor operated Ca²⁺ channel stimulated to open by IP₃, which in vivo, would be generated by hormonal stimulated phosphatidylinositol bisphosphate hydrolysis.

MYO-INOSITOL TRIS-, TETRAKISPHOSPHATE PATHWAY OF RENAL PAPILLARY COLLECTING TUBULE CELLS. J.A. Shayman, VAMC and Univ. of Michigan, Ann Arbor, Michigan.

The phosphatidyl inositol pathway has been demonstrated to play an important role in signal transduction in papillary collecting tubule cells. Inositol phosphates (IPs) other than inositol 1,4,5 trisphosphate (I_{1,4,5}P₃), however, have been suggested to have important biological effects. In order to identify potentially important products, the metabolic fate of I_{1,4,5}P₃ was investigated with a novel HPLC method. Utilizing ion pair chromatography, 12 distinct IPs including IP₅ and IP₆ could be resolved in an isocratic reverse phase system. Products were identified by (1) coelution with authentic standards, (2) periodate oxidation and borohydride reduction of IPs with separation by descending paper chromatography, (3) migration on high voltage paper chromatography, and (4) product formation following exposure to inositol 5 phosphomonoesterase isolated from cortical basolateral membranes. Rabbit renal papillary collecting tubules were solubilized with saponin in the presence of an ATP regenerating system and the time-dependent product formation after the addition of [3H] I_{1,4,5}P₃ was followed. These experiments demonstrated: (1) Inositol 1,3,4,5 tetrakisphosphate formation occurs rapidly but is ATP dependent; (2) I_{1,4,5}P₃ is directly degraded to inositol 1,4 bisphosphate and inositol 4 monophosphate; (3) inositol 1 monophosphate is not a direct metabolite of I_{1,4,5}P₃; (4) inositol 1,3,4 trisphosphate lags temporally behind IP₄ formation; and (5) IP₅ and IP₆ are not produced under these conditions.

PARTICLE ARRAYS IN TOAD BLADDER GRANULAR AND BASAL CELL MEMBRANES: A FREEZE-FRACTURE STUDY. N.L. Shinowara*, and V.A. DiScala. Winthrop-Univ. Hosp. Division of Nephrology, Mineola, New York

Granular Cell (GC) luminal membrane aggregates occur with ADH stimulation. Particle arrays (PA), structurally similar to aggregates, were seen in GC basal membrane and in apical and basal membranes of the underlying basal cells (BC). Paired hemibladders were studied under a number of experimental conditions with ADH. Quantitation of PA distribution and density were made from replicas of protoplasmic faces of GC and BC basal membranes. PA locations were flat areas, ice-filled structures similar to fusions sites, and particle-free depressions which appear as opened vesicles. In GC crossfractures, aggregophores were often close to basal membrane, but fused images were not found. Particle-free vesicles did underly openings with large diameters ($d=84\pm 18$ nm SD). Of 364 PA, 28% were at these ice-filled structures, 34% bordered particle-free depressions ($d=92\pm 20$ nm) and 38% were in flat membrane. In the BC there were 45% of PA bordering particle-free depressions and large ice-filled sites ($d=98\pm 27$ nm), 22% at flat, round particle-free areas, 24% near the numerous small openings to vesicles ($d=42\pm 10$ nm) and 9% in flat membrane. Functions for arrays are unknown. The PA densities per bladder ranged from 0-20 PA/100 nm² for GC and 0-57 PA/100 nm² for BC. Preliminary studies indicate that PA densities may not be altered by the experimental conditions in BC (consistent with Wade, J.B., 1978, J. Membrane Biol. special issue, 281-296) but may be changed in GC.

THROMBIN INCREASES DNA SYNTHESIS, PHOSPHOINOSITIDE TURNOVER AND CYTOSOLIC CALCIUM IN CULTURED HUMAN MESANGIAL CELLS. P. Shultz*, T. Knauss, P. Mene and H.E. Abboud. Dept. of Medicine, VA Medical Center and University Hospitals, Case Western Reserve University, Cleveland, OH.

Glomerular coagulation with fibrin deposition, as well as mesangial cell proliferation, can be found in a number of acute and chronic glomerular diseases. Thrombin is an enzyme with diverse biologic effects that is generated at sites of vascular coagulation and injury. We studied the effect of bovine thrombin on: 1) DNA synthesis, 2) phosphoinositide turnover, and 3) cytosolic calcium in cultured human mesangial cells (HMC). Cells were made quiescent by placing them in serum-free media prior to the experiments. Thrombin stimulated DNA synthesis in HMC, measured as ³H-thymidine incorporation, in a dose dependent manner at concentrations from 0.1 to 5 U/ml. Half-maximal stimulation occurred at 2 U/ml and elicited a 6 fold increase in cpm incorporated compared to control cells. The peak mitogenic effect of thrombin occurred after 28 hrs of incubation. Thrombin also stimulated inositol phosphate turnover in these cells. Total water soluble inositol phosphates separated by ion exchange chromatography were increased in a dose-dependent manner with 0.1 to 10 U/ml thrombin. Thrombin-stimulated phosphoinositide hydrolysis was rapid; 5 U/ml thrombin increased IP₃ accumulation 15 fold at 10 sec. The addition of thrombin also increased the cytosolic calcium concentration in HMC measured by Fura 2, an intracellular fluorescent probe. Significant increases were detected at concentrations as low as .001 U/ml and cytosolic calcium was increased 10 fold by 0.1 U/ml thrombin. This stimulation was not attenuated by removal of Ca⁺⁺ from the extracellular media.

These data demonstrate that thrombin is mitogenic for cultured mesangial cells and suggest that this effect may be mediated by activation of a phospholipase C pathway that is dependent on intracellular calcium. Thrombin generated locally within the glomerulus may contribute to mesangial cell proliferation seen in glomerular diseases.

DIMINISHED β -ADRENERGIC STIMULATION OF GLOMERULAR CYCLIC AMP (cAMP) IN SPONTANEOUSLY DIABETIC RATS. SR Silbiger* and AJ Cohen. Univ of Mass Med School, Worcester, MA.

Diabetes leads to abnormal vasoregulation of the glomerular microcirculation. Previously, we demonstrated diminished renin secretion from spontaneously diabetic BioBreeding/Worcester rats (BB/W-D) (Cohen et al, Diabetes 35(3):341, 1986) using the β -adrenergic agonist, isoproterenol(I). Since β -adrenergic control of renin release and vascular smooth muscle tone is mediated via cAMP, this was measured in glomerular suspensions from BB/W-D and nondiabetic (BB/W-N) rats. Cyclic AMP accumulation (pM/mg protein) was determined following coincubation with the phosphodiesterase inhibitor, isomethylxanthine and varying doses of agonists. Basal (no agonist) cAMP was lower from BB/W-D glomeruli than from BB/W-N (74.1 ± 6.9 vs 85.7 ± 7.2 , $p < .001$). I (10^{-4} M) also produced lower glomerular cAMP levels in the BB/W-D glomeruli (144 ± 38 vs. 182 ± 33 , $p < .05$). In addition, the dose-response curve for cAMP accumulation was shifted downward and to the right in the BB/W-D group ($p < .05$). Histamine (10^{-3} M) produced equivalent cAMP levels in BB/W-D and BB/W-N glomeruli (266 ± 100 vs. 299 ± 78 , p NS) and identical dose-response curves. Stimulation of adenylate cyclase with forskolin (10^{-4} M) also produced similar peak values of cAMP (567 ± 124 vs. 693 ± 184 , p NS) and identical dose-response curves.

We conclude that β -adrenergically-stimulated cAMP is attenuated in glomeruli from BB/W-D rats. Cyclic AMP formation in response to other agonists, such as histamine, appears to be intact. Since direct stimulation of the catalytic moiety of adenylate cyclase with forskolin produced equivalent cAMP values in BB/W-D and BB/W-N, the defect may reside at the glomerular β -receptor or its coupling to adenylate cyclase via G-proteins.

THE SURFACE ELECTRICAL PROPERTY OF THE GLOMERULAR EPITHELIAL CELL (GEC). A.K. Singh, B.S. Kasinath, E.J. Lewis. Rush Medical College, Chicago, IL

The GEC are endowed with a rich anionic coat believed to be important in the maintenance of permselectivity. Cell electrophoretic studies were carried out on cloned, cultured GEC in order to understand the physico-chemical nature of the surface polyanion. Similar studies were carried out on NIH 3T3 fibroblasts for comparison. The GEC present a negatively charged surface which imparts a zeta potential of 29.0 ± 1.5 millivolts. Charge density on the cell was calculated to be 8.2×10^4 negative charge sites/sq micron. Fibroblasts showed a 20% lower charge density. Assuming uniform charge distribution, the average interchange distance for GEC was computed to be 35 Å. The pH at which the GEC charge became neutral (pI) was determined to be 1.98. A variety of polycations of various sizes were tested for their capacity to neutralize the GEC charge. All the polycations except cytochrome C (CC) and lysozyme (LZ) completely neutralized the cell. The dimensions of the latter approximate the interchange distance of 35 Å. Charge separation of 40 Å on fibroblasts allowed for greater neutralization by CC and LZ. CC and LZ could completely neutralize the GEC after chemical destruction of intra chain bonding which rendered them "flexible". Larger sized polylysines were more effective on molar basis in neutralizing the GEC than smaller sized polylysines presumably because of "flexibility" of polylysine molecules. We conclude that GEC presents a highly negatively charged surface *in vitro* and the negative charges are spatially fixed in a uniform arrangement with a specific inter-charge distance of 35 Å.

EXTRACELLULAR ATP ACTIVATES MULTIPLE ION TRANSPORT SYSTEMS IN THE RAT PAROTID CELL BY A NOVEL MECHANISM. S. Soltoff*, M. McMillian*, L. Cantley*, B. Talamo*, Tufts Univ. Dept. of Physiol., Boston, MA (intro. by L.J. Mandel)

Using a suspension of rat parotid acinar cells, we examined various aspects of stimulus-secretion coupling, especially the possibility that ATP may act as a neurotransmitter. Parotid acinar cells secrete fluids and electrolytes upon neural stimulation, and muscarinic, -adrenergic, and substance P receptors are linked to phospholipase C and stimulate IP₃ production. In normal medium, 1 mM ATP increased free Ca_i (Quin2) from 150 nM to over 1 μM, a larger increase than that produced by carbachol (to 680 μM). In Ca-free medium, ATP and carb each raised Ca_i, and thus released Ca from an intracellular pool(s); but since ATP did not stimulate IP₃ production, the effect of ATP was via a separate 2nd messenger system. ATP, carb, and ionomycin stimulated a Ca-activated K channel, which resulted in the rapid loss of 50% of the cell K; and all three stimuli were equally effective in activating Na/K/Cl cotransport (150 nmol Na/mg/min) and Na/H exchange (60). The stimulation of these Na uptake systems may involve cell shrinkage (25% within 10 sec), which accompanies the loss of K. The effect of ATP on Ca_i and ²²Na uptake has an app. K_{0.5} of 500 μM ATP, but was only 30 μM in the absence of divalent cations. This suggests that ATP⁴⁻ (not Mg-ATP) is the active moiety. The effects of ATP are reversible (by hexokinase) and blockable (by DIDS). Since ATP may be packaged with neurotransmitters, these studies suggest that a purinergic receptor provides an additional mechanism by which fluid and electrolyte secretion is regulated in these cells.

EPIDERMAL GROWTH FACTOR (EGF) AND PLATELET-DERIVED GROWTH FACTOR (PDGF) RAPIDLY ACTIVATE GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) IN RAT RENAL CORTICAL CELLS (RCC). RC Stanton*, DC Boxer*, E Zimmerman*, L Cantley* and JL Seifter. Harvard Med. Sch. and Tufts Univ., Boston, MA

Glucose metabolism via the hexose monophosphate (HMP) shunt leads to the production of ribose 5-phosphate, a necessary component of nucleic acids. We recently reported (Kidney Int. 31:182A) that EGF is mitogenic for cultured proximal tubule cells, and EGF in a Na⁺-free buffer causes a 0.2 to 0.3 pH unit decrease in cytoplasmic pH, the latter due to acid production from activation of the HMP shunt. We therefore evaluated the effect of EGF on HMP shunt enzymes G6PD and 6-phosphogluconate dehydrogenase (6PG). Aliquots of RCC were incubated for 15 min before addition of EGF (50nM), phorbol myristate acetate (PMA, 100nM), insulin (1μM), or PDGF (10nM). At 1 min, incubations were stopped and the cells lysed by adding Triton X-100. Activities of G6PD and 6PG were determined by monitoring the conversion of NADP⁺ to NADPH. EGF increased G6PD by 25±6% vs control. PDGF (which, like EGF, binds to a tyrosine kinase) increased G6PD by 27±3%. 6PG was not altered by EGF or PDGF. EGF addition to control whole cell lysates did not increase G6PD activity, suggesting that intact receptor coupling is necessary for EGF activation of G6PD. Neither PMA, an activator of protein kinase C (PKC) nor insulin (which also binds to a tyrosine kinase) altered activities of G6PD or 6PG. We conclude that EGF and PDGF augment the HMP shunt by activation of G6PD. This effect is independent of PKC, requires an intact cell and is not seen with insulin.

OXYTOCIN RECEPTORS MEDIATE A TRANSIENT INCREASE IN CYTOSOLIC FREE [Ca⁺⁺]_i IN LLC-PK1 CELLS. F.L. Stassen*, G. Heckman*, D. Schmidt*, M.T. Papadopoulos*, M. Gellai*, and L.B. Kinter, Smith Kline & French Labs., Depts. of Pharmacology & Molecular Pharmacology, Swedeland, PA.

We examined the effects of oxytocin (OT) on renal tubular epithelial LLC-PK1 cells. In cells loaded with Fura 2, 1 μM oxytocin induced a rapid increase in cytosolic free [Ca⁺⁺]_i from 120 nM to 250 nM within 12 sec. [Ca⁺⁺]_i then decreased and leveled at 148 nM. Calcium was mobilized from intra- and extracellular sources. Oxytocin also increased phosphatidylinositol (PI) turnover. Oxytocin-induced calcium mobilization was dose-dependent (EC₅₀ between 5 and 30 nM). Calcium transients were also induced by the oxytocin selective agonist Thr⁴Gly⁷OT, and blocked by the oxytocin selective antagonist desGlyd(CH₂)₅Tyr (Me)²Thr⁴AVT. dDAVP, a selective vasopressin V₂ agonist, and Phe²Ile³Orn⁸V⁹, a V₁ selective agonist, were only partial agonists and at least 10 to 100-fold less potent than oxytocin. The correlation between antagonism of oxytocin-induced calcium transients and pig kidney V₂ and rat liver V₁ receptor affinity was poor. We have also identified specific, saturable, high affinity oxytocin binding sites of low density on intact LLC-PK1 cells (K_D = 1.9 nM; B_{max} = 3.2 fmoles/10⁶ cells). We conclude that in LLC-PK1 cells, oxytocin stimulates a transient rise in cytosolic free [Ca⁺⁺]_i and PI turnover. These effects are not likely mediated by V₁ or V₂ vasopressin receptors, but by putative oxytocin receptors.

POSSIBLE ROLE OF 12-LIPOXYGENASE (L) PRODUCTS IN ANGIOTENSIN II (AII) EFFECT ON INTRACELLULAR CALCIUM (Cai) IN GLOMERULOSA CELLS (GC). N. Stern*, N. Yanagawa, J. Nadler and M. Tuck. Sepulveda VAMC, UCLA Sch. of Med., and LACMC, USC Sch. of Med., Los Angeles, CA.

AII stimulates GC aldosterone secretion via phosphoinositide-Ca messenger system. We have previously reported that in GC, (i) AII increases 12-L product, 12-HETE, (ii) AII effect on aldosterone secretion was selectively blocked by L inhibitors, and (iii) 12-HETE restored AII effect on aldosterone secretion in the presence of L inhibitors. We now report that 12-L pathway may be involved in AII effect on Cai. GC Cai was measured using Fura 2/AM (2 μM) with a microscope spectrofluorometer (Leitz, MPVSP). Effects of AII (10 nM), L inhibitor (BW 755C, 0.1 mM) and 12-HETE (10 nM) on Cai were studied. Addition of AII induced a transient and a sustained rise in Cai. Addition of BW 755C abolished the sustained rise of Cai by AII. Addition of 12-HETE, alone induced an immediate but sustained rise of Cai, and restored AII effect on Cai in the presence of BW 755C. Similar results were observed with different L inhibitor (Baicelain, 1 μM), and with another L products, 12-HPETE (10 nM). Combined with our previous studies, these results indicate that 12-L pathway's involvement in AII effect on steroidogenesis may be via changes in Cai.

PERMEABILITY OF THE EXTRAGLOMERULAR MESANGIAL CELL REGION AND MACULA Densa TO HORSE RADISH PEROXIDASE. Patricia L. St. John*, Dale R. Abrahamson and P. Darwin Bell. University of Al. at B'ham, Birmingham, Alabama.

Little is known regarding the permeability properties of the extraglomerular mesangial cell region and whether or not macromolecules may be restricted from entering or leaving the area directly beneath the macula densa cells. In order to investigate this issue, thick ascending limbs containing the macula densa segment and with attached glomeruli were dissected from rabbit kidney. Tubules were cannulated and perfused at 27 C for 30 min with an isotonic solution containing 1 mg/ml horseradish peroxidase (HRP) (mw=40,000) followed by fixation for light and electron microscopy (EM). There was no evidence that HRP crossed the tight junctions and entered the spaces between the macula densa cells. However, there was clear evidence for some uptake of HRP within apical endocytic vesicles located in cells of the macula densa. In other studies, HRP was added to the isotonic bathing solution and incubated at 27 C for 30 mins. Under these conditions, HRP penetrated the spaces between the extraglomerular mesangial cells and also fully penetrated the basement membrane area beneath the macula densa plaque. In addition, HRP was located in the intercellular spaces between macula densa cells. These results indicate that, except for the luminal tight junctions, there are no anatomical barriers restricting the movement of macromolecules into or out of the area beneath the macula densa. Thus it is possible that intercellular signaling between components of the macula densa-JGA complex may involve, or be influenced by, the movement of substances through these matrices.

THE 17 kDa SUBUNIT OF THE CLATHRIN-COATED VESICLE (CCV) PROTON PUMP IS A TRANSMEMBRANOUS PROTON CHANNEL. DK Stone, S-Z Sun*, and X-S Xie*, UTHSCD, Dallas, TX.

The 200-fold purified CCV proton pump is composed of a maximum of 8 subunits of MW 116,70, 58,40,38,34,33 and 17kDa. To date there has been no functional definition of subunit activity for this, or any other, vacuolar H⁺ ATPase. These studies were undertaken to define subunit composition and function.

ATPase and proton pumping activities of the purified, reconstituted CCV ATPase are inhibited 50% by dicyclohexylcarbodiimide (DCCD) at a ratio of 0.69 μmol DCCD/mg protein. At this DCCD/protein ratio, the 17 kDa subunit is exclusively labeled with [¹⁴C]DCCD. The 17 kDa subunit was isolated from 20 mg of purified enzyme by toluene extraction and was shown to label with [¹⁴C]DCCD at a ratio of 0.69 μmol DCCD/mg protein. To assess function of the isolated subunit, the toluene extract was evaporated and coreconstituted with the light-driven proton pump, bacteriorhodopsin. Bacteriorhodopsin-mediated proteoliposome acidification (measured as light-energized medium alkalinization) was ablated by coreconstitution of the 17 kDa subunit. Proteoliposome acidification was restored by treatment with DCCD at a ratio of 0.66 μmol DCCD/mg 17kDa protein. Moreover, the uncoupling effect of the 17 kDa subunit was trypsin sensitive.

It is concluded that the 17 kDa polypeptide present in the CCV proton translocating complex is a DCCD-inhibitable proton channel which facilitates transmembranous proton movement energized by the catalytic sector of the CCV H⁺ ATPase.

BASEMENT MEMBRANE HEPARAN SULFATE PROTEOGLYCAN (BM HSPG) SYNTHESIZED BY CULTURED GLOMERULAR EPITHELIAL CELLS (GEC). J.L. Stow^A, K. MacKay, L. Striker, G. Striker, and M.G. Farquhar^A. Department of Cell Biology, Yale University School of Medicine, New Haven, CT^A and NIDDK, NIH, Bethesda, MD.

We investigated the production and distribution of BM HSPG in a GEC line generated from outgrowths of normal mouse glomeruli. Confluent cell monolayers, grown on human fibronectin, were labeled for 1-24 h with [³⁵S]sulfate. Proteoglycans (PGs) were isolated from medium and cell layers by ion exchange chromatography, digested with chondroitinase ABC, and HSPGs were similarly purified from the digest. 80% of the total PGs in the medium and 87% in the cell layer were HSPG. The remainder were dermatan sulfate PGs. This high proportion of HSPGs is similar to that of other kidney epithelial cell lines (NRK and MDCK cells) studied previously (Stow and Farquhar, JCB 105:529-540, 1987). A distinct population of BM HSPG was identified in GEC using a specific antibody which recognizes the core protein of BM HSPG in GBM and other basement membranes. Most of the BM HSPG was found in the cell layer where 51% of the labeled HSPG was immunoprecipitated by anti-BM HSPG serum; < 10% of the HSPG in the medium was BM HSPG. When BM HSPG was localized in GEC cultures by EM immunocytochemistry it was found in large subcellular deposits of loosely-organized matrix which also contained laminin. BM HSPG was also localized intracellularly in rough ER and Golgi of GEC where it was seen more frequently than in other cultured epithelial cells. We conclude that BM HSPG are the predominant type of PG made by GEC in these cultures which will be useful for further studies on the biosynthesis and secretion of BM HSPG.

EFFECT OF OXALATE ON Ca-DEPENDENT FUNCTIONS OF RAT KIDNEY MITOCHONDRIA IN VITRO. T.Strzelecki* B.McGraw*, M.Menon* (intr.by D.Clive) Div.Urology Univ.Massachusetts Medical School, Worcester, MA

We investigated the effect of oxalate on mitochondrial functions to test whether oxalate may impair handling of calcium leading to renal cell injury. Exposure of isolated mitochondria to 3 mM oxalate had no effect on ADP-stimulated respiration with succinate or glutamate plus malate. Ca⁺⁺ influx into mitochondria was not altered by 3 mM oxalate:

Ca ⁺⁺ medium, μM	6	11	17
Ca ⁺⁺ influx		ngatom	Ca ⁺⁺ /mg/min
control	7.0±0.6	20.5±2.1	63.8±3.5
oxalate	5.8±0.4	23.2±2.3	58.7±1.6

Ruthenium red-insensitive efflux of calcium was unchanged by oxalate after accumulation of 20 ngatom Ca⁺⁺/mg protein (2.3 vs 2.3 ngatom/mg/min). Respiration of mitochondria stimulated by calcium cycling in the presence of ionophore A23187 was inhibited by 3 mM oxalate. In medium without Mg⁺⁺ the respiration rates were 211±6 vs 85±17 and with 1 mM Mg⁺⁺ 291±15 vs 202±9 ngatomO₂/mg/min, respectively. The apparent K_m of oxalate with respect to Mg⁺⁺ was 0.8 mM. Accumulation by mitochondria of 100 ngatom Ca⁺⁺/mg resulted in their spontaneous swelling, enhanced respiration and release of calcium, a phenomenon prevented by magnesium. Oxalate diminished the effect of Mg⁺⁺ on mitochondrial swelling. The app. K_m of Mg⁺⁺ was 0.2 mM without and 0.6 mM with oxalate.

In conclusion, these findings suggest that oxalate has no direct effect on mitochondrial Ca⁺⁺ handling. It affects Ca-dependent swelling and respiration of mitochondria indirectly via interaction with magnesium.

A NOVEL HORMONE RESPONSIVE GLYCAN-PHOSPHOLIPID IN CULTURED MESANGIAL CELLS. Dean A. Troyer and Jeffrey I. Kreisberg. Depts. of Pathology and Medicine, Univ. of Texas Health Science Center at San Antonio, San Antonio, Texas.

We have identified a glycan-phospholipid (X) in cultured glomerular mesangial (MS) cells. The lipid migrates between diphosphoinositide (PtdIns (4)P) and phosphatidylinositol (PtdIns) on HL TLC plates using CHCl₃:MeOH:20% methylamine (60:30:10) as the solvent system.

When cells are exposed to ³²P and vasopressin (VP) for 30 mins., label is incorporated into phospholipids as follows:

PTDINS(4)P	PTDINS	X	P-CHOLINE
+ VP:			
2557±133	14250±686	3165± 76	699±68
-VP:			
1246±256	2020±866	548±170	348± 61

Values show DPM±S.D.; thus, X contains phosphate and may participate in VP-stimulated phosphoinositide metabolism. Other labelled precursors were incorporated as follows:

	GLUCOSAMINE (12 HRS)	STEARIC ACID (48 HRS)	INOSITOL (48 HRS)
X/ PTDINS	1035/177	2221/19957	1215/15816

Values show DPM in X/DPM in PtdIns after the indicated time. Glucosamine is thus preferentially incorporated into X compared to PtdIns. Further studies are in progress to characterize the molecule and its function. A similar molecule has been linked to insulin action in studies of other cell types.

MOLECULAR CLONING OF A NOVEL EARLY GROWTH RESPONSE GENE RAPIDLY INDUCED BY KIDNEY EPITHELIAL CELL, FIBROBLAST AND LYMPHOCYTE MITOGENS. C. Tsai-Morris, S. Kartha, F. G. Toback, R. Taub, R. Hoover, and V. Sukhatme. Univ. of Chicago, Chicago, IL and Univ. of Pennsylvania, Philadelphia, PA.

Mitogens evoke many alterations in gene expression in eukaryotic cells. Genes activated rapidly and transiently, that are evolutionarily conserved and whose induction is shared by diverse cell types when exposed to different growth stimuli are likely to be of critical importance in transducing mitogenic signals and regulating cellular proliferation. C-myc and c-fos are the only known genes fulfilling these criteria. We report on the molecular cloning of a novel early growth response (egr) gene which also satisfies these conditions. In response to serum, its 3.7 kb mRNA is induced dramatically in mouse fibroblasts reaching a peak level at about 30 minutes that is ten times higher than the maximal value attained by c-fos mRNA. This transcript is induced by the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate and is "superinduced" by serum and cycloheximide together. Importantly, the gene is highly induced by different mitogens in a wide array of cell types: insulin stimulated rat hepatoma cells, adenosine diphosphate treated monkey kidney epithelial cells, and phytohemagglutinin stimulated human peripheral blood lymphocytes. An "organ blot" shows message present in adult brain, heart, lung and kidney. Partial sequence of the murine egr cDNA indicates that we have identified a novel transcript. Given the many properties that this gene shares with c-myc and c-fos, it may play a key role in the control of cell growth and differentiation in a wide variety of cell types including renal epithelial cells.

HORMONAL CONTROL OF DIVISION AND DIFFERENTIATION IN CULTURED PROXIMAL CELLS (PC). A. Vandewalle,* B. Baudouin,* M. Geniteau,* P. Verroust,* and P. Ronco,* INSERM U. 64 and U. 246, Paris, France (Intr. by C. Le Grimellec).

In initial experiments, PC isolated from young rabbit kidneys were reproducibly cultured in serum and EGF₂ free hormonally defined medium containing 5 x 10⁻⁸ M dexamethasone (Dex) and 5 µg/ml insulin (Ins), and studied at confluency (day 11). They exhibited high specific response to 5x10⁻⁷ M PTH (cAMP content x 21.9) and a rate of α -m.glucose uptake of 39.0 ± 17.0 pmol/min/mg. As compared to freshly isolated PC, l. amino-peptidase, endopeptidase, alkaline phosphatase and angiotensin converting enzyme were decreased by 2 to 5 fold. By contrast, γ glutamyl transferase (GT), dipeptidyl peptidase IV (DPP) and PEPCK, a neoglucogenic enzyme, were maintained (GT : 503.3, DPP : 171.6, PEPCK : 14.8 U/g, n = 6). We then tested the effects on cell division and differentiation of Ins, Dex and 5 x 10⁻⁷ M T3 added separately to serum free medium (SFM). Mitogenic effects were estimated by measuring ³H thymidine incorporation (Tdr) and cell density (CD) at days 7 and 11, respectively.

	SFM	+ T3	+ Dex	+ Ins	+ Dex, Ins
Tdr %	-	+24.5	-24.4	+16.1	- 4.2
CD %	-	+ 0.3	+ 8.9	+47.2*	+25.3*
GT U/g	375.7	374.0	769.0**	595.8**	624.5**
PEPCK U/g	5.3	6.4	9.6*	9.6*	11.0**

* p<0.05, ** p<0.01 vs SFM values.

In conclusion : 1. Both T3 and Ins stimulate DNA synthesis but only Ins induces cell division. 2. At variance with T3, Dex and Ins are promoters of PC differentiation.

IDENTIFICATION OF A VASOPRESSIN-INDUCIBLE WATER CHANNEL IN ENDOCYTIC VESICLES FROM RENAL PAPILLA. A.S. Verkman, W. Lencer*, D. Brown, D.A. Ausiello. CVRI, UCSF, CA & Renal Unit, MGH, Harvard Medical School, Boston, MA.

In the renal collecting duct, intramembrane particle aggregates believed to contain water channels are removed from plasma membrane by vasopressin-stimulated endocytosis. Osmotic water permeability of endocytic vesicles from renal cortex and papilla were measured by infusing vasopressin (VP) deficient Brattleboro rats with 6-carboxyfluorescein (6CF) ± VP for 15 min. 6CF was filtered by the glomerulus, concentrated in tubules and taken up into endocytic vesicles. Microsomes from renal cortex and papilla were prepared containing endocytic vesicles. On exposure to a 150 mM inward osmotic gradient, vesicle fluorescence decreased due to water efflux, vesicle shrinkage and quenching of entrapped 6CF. In cortical vesicles there were two time constants for vesicle shrinkage with different activation energies [SEM, 6 preps at 23°C]: 35±3ms, E_a=3.0 kcal/mol; and 0.67±0.03s, E_a=9.6 kcal/mol which did not change with VP infusion, suggesting presence of endosomes from proximal tubule known to contain water channels (JMB 96:107-119, 1987) and a second vesicle type with slower lipid-mediated water transport. In papillary vesicles -VP, >90% of the signal consisted of a slow exponential with time constant 0.78±0.06s and high E_a 13.0 kcal/mol; in papillary vesicles + VP there was in addition a fast process (30±4ms) with low E_a (3.8 kcal/mol) indicating presence of a water channel. These results define a VP-inducible population of endocytic vesicles from renal papilla which contain water channels, possibly the water channel responsible for regulation of collecting tubule water transport by VP.

INTESTINAL ALKALINE PHOSPHATASE IN HUMAN KIDNEY. Gonda F. Verpooten*, Patricia G. Hendrix*, Etienne J. Nouwen*, Marc F. Hoylaerts* and Marc E. De Broe* (intr. by George A. Porter). Univ. of Antwerp, Dept Nephrol.-Hypert., Antwerp, Belgium.

We have recently produced a specific monoclonal antibody (Mab) against intestinal alkaline phosphatase (IAP) which allowed biochemical and immunohistochemical identification of the intestinal isoenzyme in the human kidney. The Mab ("250") was produced by using purified human IAP. It showed specificity for adult and fetal IAP and a cross-reactivity of < .1% with all other AP isoenzymes. Biochemical measurements of IAP were performed using an enzyme immunoassay (EIA) which showed high sensitivity (detection limit .01 U/L). Using that EIA for extracts of different parts of kidney tissues indicated that IAP is localised mainly at the cortico-medullary junction. The Mab was also useful for immunohistochemical examination confirming the specific intrarenal localisation of IAP at the level of the brush border of proximal tubular cells of the S3 segment. Furthermore, specific measurement of IAP in urine using the same EIA showed that the IAP fraction in morning urine is $\pm 10\%$ (.2 U/L) of the total urine alkaline phosphatase content.

The specific localisation of IAP in the human kidney and the specific and sensitive detection of IAP in urine open new perspectives in the field of renal toxicology.

PRETREATMENT WITH COLCHICINE OR GENTAMICIN DECREASES RENAL CORTICAL UPTAKE OF GENTAMICIN IN RATS. Gert A. Verpooten*, Rubén A. Giuliano*, Falei Zheng*, Marc E. De Broe* (intr. by George A. Porter). University of Antwerp, Dept. of Nephrology-Hypertension, Antwerp, Belgium.

We investigated the effect of pretreatment with colchicine (COL) and gentamicin (GM) on further uptake of GM in the renal cortex of female Wistar rats. The animals were divided in the following groups: a) no pretreatment, b) COL 2 mg/kg IP 4 hr before the GM uptake kinetic study, c) GM 10 mg/kg IP, BID, during 4 days. Renal cortical uptake of GM was measured after a 4-hr continuous infusion which yielded constant serum concentrations ranging from 0.5 to 100 $\mu\text{g/ml}$. The cortical uptake rate of GM was nonlinearly related to serum levels and followed Michaelis-Menten kinetics.

	Km ($\mu\text{g/ml}$)	Vmax ($\mu\text{g/g/hr}$)
Controls	19.4 \pm 2.4	166.9 \pm 11.3
COL pretreatment	131.8 \pm 42.2	274.9 \pm 66.7
GM pretreatment	39.7 \pm 0.9	157.6 \pm 3.4

The increased Km after pretreatment with both drugs reflects decreased cortical uptake of GM at serum levels below 50 $\mu\text{g/ml}$. In the COL pretreated rats, this decrease may be secondary to the known effect of COL on microtubuli resulting in a decrease in the endocytic uptake of GM in the proximal tubular cell. In the case of GM pretreatment, these results suggest that GM accumulated in lysosomes interferes with some of the processes involved in the cellular uptake of the drug.

OXIDIZED ANF-ANALOG DISSOCIATES DIURESIS AND NATRIURESIS IN WISTAR RATS WITHOUT ELEVATING PLASMA OR URINARY cGMP LEVELS. Roland C. Willenbrock*, Johanne Tremblay*, Pavel Hamet*, (intr. by Vicentiu Beroniade). Clinical Research Institute of Montreal, Montreal, Que., Canada

Met (O)¹¹⁰-sulfoxide-ANF (Met-O-ANF) is formed by oxidation of human ANF (h-ANF) at the methionine in position 110. Compared to h-ANF, it is only slightly less vasorelaxant and hypotensive. It has less delayed and prolonged diuretic activity, but it does not enhance natriuresis. Moreover, it is able to reduce the diuretic and natriuretic activity of h-ANF, acting as an antagonist when injected prior to h-ANF. Contrary to h-ANF, Met-O-ANF causes no increase of plasma and urinary cGMP at concentrations which already have biological activity. At higher Met-O-ANF concentrations, a slight increase in cGMP levels is observed. Its effect in vascular smooth muscle and endothelium cells, and in isolated glomeruli and tubules is less than that of h-ANF but the degree of difference vary from tissue to tissue from less than one to several orders of magnitude. The specific biological effects of Met-O-ANF suggest heterogeneity of physiological function of ANF as well as of its molecular mechanism of action. Only the natriuretic effect but not the sodium independent diuresis of ANF is reflected by elevated extracellular cGMP levels, suggesting that either a part of the mechanism of action is cGMP-independent or that the cGMP-dependent diuretic effect is exerted at a site which does not lead to egression of the nucleotide.

"HUMAN RENAL MICROVASCULAR ENDOTHELIAL CELLS (RMEC) PRODUCE SINGLE CHAIN UROKINASE TYPE PLASMINOGEN ACTIVATOR (scu-PA)". J. Wojta*, R. Hoover*, and T. Daniel*, Departments of Pathology, Medicine, and Cell Biology, Vanderbilt University, Nashville, TN. (Intr by K. Badr)

Previous data have shown that tissue-type plasminogen activator (t-PA) is the major form produced by human vascular endothelial cells. We have compared the molecular form of PA produced by RMEC, derived from human renal cortex, with that of human omental microvascular endothelial cells (OMEC). Using combined immunoassays specific for u-PA and t-PA activity and antigen, we measured both in media conditioned for 24 h by 10⁵ cells.

	OMEC	RMEC
t-PA Activity (I.U.)	<0.15	0.19 \pm 0.01
Antigen (ng)	8.8 \pm 0.6	0.38 \pm 0.05
u-PA Activity (I.U.)	<0.15 / <0.15*	4.9 \pm 1.4 / 0.19 \pm 0.01*
Antigen (ng)	<0.9	55.4 \pm 13.1

(*Activity before plasmin activation)

Accumulation of activities and antigens were linear over a 48 h course and cell associated PA's accounted for <10% of total activity and antigen, demonstrating secretion. The requirement for u-PA activation by plasmin indicates the RMEC secreted product is scu-PA. Independent verification of the immunoassay data was obtained by SDS-PAGE followed by fibrin autography of conditioned medium depleted of either u-PA or t-PA by immunoabsorption using specific monoclonal antibodies. In contrast to OMEC, RMEC do not produce plasminogen activator inhibitor 1, as determined by reverse fibrin autography.

The alterations in renal microvascular capillary flow that attend the transcappillary fluid extraction accompanying glomerular filtration and medullary interstitial hypertonicity may necessitate active mechanisms for retarding coagulation. The synthesis and secretion of scu-PA appears to reflect a differentiated function of specifically renal microvascular endothelial cells that may serve this function.

STRUCTURAL ANALYSIS OF THE CATALYTIC CENTER OF THE CLATHRIN-COATED VESICLE PROTON PUMP (CCVPP). X-S Xie* and DK Stone, UTHSCD, Dallas, TX.

Proton pumping catalyzed by the CCVPP requires an ATP hydrolytic center and a coupling mechanism which links ATP hydrolysis to ventral proton movement through a transmembranous proton pore. These studies were undertaken to determine the structure of the ATP hydrolytic center.

ATPase activity and proton pumping catalyzed by the purified CCVPP is optimally supported by Mg ATP. However, Ca ATP can support ATPase activity ($7 \mu\text{mol Pi} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$) but not proton pumping of the CCVPP. This Ca ATPase activity shares with Mg ATPase activity the characteristics of NEM (1mM) and NO_3^- (5mM) inhibition and Cl stimulation (30mM). Ca ATPase activity was thus used for partial reaction analysis of the hydrolytic center. To determine which of 7 subunits (MW 116,70,58,40,38,34,33 kDa) participate in ATP hydrolysis, purified CCVPP was dissociated by urea treatment and glycerol gradient centrifugation, and the resultant components were analyzed alone, and after reconstitution, for Mg and Ca ATPase activities. No single fraction supported ATPase activities. Removal of the 116 kDa subunit resulted in a preparation which had Ca, but not Mg ATP hydrolytic activity. Reconstitution of Ca ATPase activity absolutely required the 70,40 and 33kDa subunits; contamination of subunit isolates with the 58kDa subunit precludes definition of its role at present. Thus, the catalytic center requirement for partial reaction function (Ca supported ATPase activity) is a minimum of 3 and a maximum of 4 subunits.

LOCALIZATION OF ACIDOSIS-INDUCED PHOSPHOENOL-PYRUVATE CARBOXYKINASE (PEPCK) mRNA IN RAT KIDNEY. S. Yamamoto*, E. Imai*, A. Yamauchi*, Y. Fukuhara*, Y. Orita*, T. Kamada*, P. K. Nakane*, and T. Koji*, Osaka Univ. Med. Sch., Osaka and #Tokai Univ. Med. Sch., Isehara. (intr. by J. S. Handler)

PEPCK is a key enzyme of gluconeogenesis which is an important pathway leading to a neutral endproduct of glutamine metabolism in metabolic acidosis. Localization of acidosis-induced expression of PEPCK mRNA along nephron segments should provide further understanding of renal gluconeogenesis and ammoniogenesis. We measured PEPCK mRNA level in whole kidney of control and acute acidotic rats, using RNA-cDNA dot blot hybridization (PEPCK cDNA, pPC116 provided by D. K. Granner, J. Biol. Chem. 260: 10748, 1985). PEPCK mRNA in acidotic rats administered NH_4Cl (serum HCO_3^- $9.2 \pm 2.0 \text{ mEq/l}$) increased from control values (serum HCO_3^- 22.4 ± 1.4) 354 to 802 cpm/ μg total RNA. Acidosis-induced PEPCK mRNA along the renal tubule was recognized by in situ hybridization with UV-irradiated thymine dimerized ($\text{T}^{\wedge}\text{T}$) cDNA probe. Hybridized DNA was detected immunohistochemically using rabbit anti- $\text{T}^{\wedge}\text{T}$ DNA antibody and peroxidase labeled goat anti-rabbit IgG (Acta Histochem. Cytochem. 20:229, 1987). Acute metabolic acidosis raised PEPCK mRNA levels mainly in the proximal tubules of inner cortex, while no PEPCK mRNA was detected in renal medulla. Our findings indicate that the increase in enzyme activity localized previously in microdissected tubules (Am. J. Physiol. 253: F246, 1978) is the result of increased mRNA accumulation.

BINDING TO APICAL AND BASOLATERAL (BL) INSULIN RECEPTORS OF CULTURED KIDNEY CELLS IS FOLLOWED BY INTERNALIZATION AND DEGRADATION. C. Yagil* and R. Rabkin, Stanford Univ. and VAMC, Palo Alto, CA.

In-vivo, filtered insulin (INS) is absorbed and degraded in proximal tubules after binding to the apical membrane. Peritubular removal also occurs and involves BL receptor binding and degradation. Whether BL internalization occurs is unknown. To answer this, a cultured opossum kidney cell line with proximal-like features and INS receptors were studied. Cells were grown in partitioned wells on filters. When confluent, apical and BL compartments are separated by the monolayer. Apical and BL binding, internalization and degradation were studied separately by incubating monolayers with ^{125}I -INS added to either the apical or BL side. INS associated with either pole in a specific manner. This interaction was competitively inhibited by cold INS, but not by unrelated peptides. Separation of surface-bound from internalized INS was achieved by lowering extracellular pH. At 4°C , 92% of the radioactivity added to either side of the monolayer was surface-bound, while at 37°C after 1 hr, 55% was surface-bound and 45% internalized. Affinity of apical and BL receptors were similar ($1-2 \text{ nM}$), but BL receptor number was greater, for at high INS concentrations (10^{-7} M) BL binding exceeded apical by 6 fold (685 ± 161 vs 101 ± 18 pm; $p < 0.01$).

Degradation followed exposure to either surface of the cell, but apical was greater (7 ± 0.2 vs 4.5 ± 0.5 /hr). Degradation products appeared after a delay of > 5 mins., suggesting that internalization is a prerequisite for degradation. Thus, cultured kidney cells possess INS receptors, with more located on BL than apical poles. Binding is followed by internalization and degradation. BL internalization with degradation is a novel finding, which if operative in vivo, could explain the large peritubular component of renal INS metabolism.

EFFECT OF L-TRIIODOTHYRONINE (T_3) ON Na^+ - H^+ EXCHANGE ACTIVITY IN CULTURED OPPOSUM KIDNEY (OK) CELLS. K Yonemura,* L. Cheng,* and B. Sacktor, Lab. Biol. Chem, NIA, NIH, Baltimore, MD.

Previous studies demonstrated that Na^+ - H^+ exchange activity in renal brush border (BB) membrane vesicles was increased in the hyperthyroid and decreased in the hypothyroid rat (PNAS 82:3606, 1985). To determine whether T_3 had a direct action on proximal tubular cells or if the alteration in BB activity was a secondary adaptive response, e.g. change in filtered Na^+ , we measured Na^+ - H^+ exchange activity in OK cells cultured for 4 days in DMEM + 10% FCS depleted of thyroid hormone and in cells cultured in the same medium supplemented with T_3 for 24 hrs prior to determining Na^+ (10 mM) uptake. T_3 did not significantly alter cell number, protein and DNA, thus ruling out cellular hyperplasia and hypertrophy. Immediately prior to measurement of uptake, cells were acid loaded (NH_4Cl). Initial rate (2 min) of amiloride-sensitive Na^+ uptake represented 80% of total uptake rate. T_3 had no effect on amiloride-insensitive Na^+ uptake. T_3 stimulated amiloride-sensitive uptake in a dose-dependent relationship, maximum increase of about 60% was obtained with 10^{-7} M and E_{50} with 10^{-9} M T_3 . T_4 was 30-times less effective. The increase in Na^+ uptake had a 12 hr lag period, suggesting a requirement for biosynthesis. The cells maintained an elevated rate of exchange for 48 hrs after removal of hormone, suggesting a slow turnover of the active state. These results indicate that thyroid hormone can stimulate Na^+ - H^+ exchange activity by direct action on tubular cells.

DIFFERENT MECHANISM OF INCREASE OF Na⁺/Pi CONTRANSPORT (Na⁺/Pi-COT) IN THE RENAL BRUSH BORDER MEMBRANE (BBM) ELICITED BY THYROID HORMONE (T₃) AND BY DIETARY PHOSPHATE DEPRIVATION (PD). Ahad N.K. Yusufi, Mirosława Szczepanska-Konkel, Hassan Moltaji* and Thomas P. Dousa, Neph. Res. Unit, Mayo Foundation and Clinic, Rochester, Minnesota.

We explored whether T₃ and PD increase the rate of Na⁺/Pi-COT across BBM in a similar manner with use of [¹⁴C]-phosphonoformate (PFA) as a probe for Pi-binding sites in BBM. The TPTX'd rats were either given T₃ (i.p.) or subjected to PD for 3 days, and then BBM vesicles (BBMV) were analyzed. Both PD and T₃ increased Na⁺/Pi-COT by BBMV, but the Na⁺-dependent [¹⁴C]-PFA binding increased only in response to T₃.

	Na ⁺ /Pi-COT (pmol/mg prot)	Na ⁺ -PFA (nmol/mg prot)	Alk. Pase (μmol/hr/mg)
Cont. (5)*	484 ± 57 (5)	2.8 ± 0.7 (8)	66 ± 3
T ₃ (6)	900 ± 58 ⁺ (6)	3.9 ± 0.8 (6)	56 ± 3 ⁺
LPD (5)	915 ± 23 ⁺ (5)	2.8 ± 0.7 (6)	81 ± 4 ⁺

* = No. of experiments; + = P < 0.05 from cont. The Na⁺/glucose uptake and [³H]-phlorizin binding did not differ between the 3 groups. The T₃-elicited increases of Na⁺/Pi-COT and [¹⁴C]-PFA binding were both due to increases in V_{max} and were correlated (r = 0.82, P < 0.01); in PD only V_{max} for Na⁺/Pi-COT increased. **Conclusion:** T₃ enhances the Pi transport by increase in number of Na⁺/Pi-COT units in BBM. In PD the number of Na⁺/Pi-COT units is not changed but transporters have faster rate of Pi translocation.

CLINICAL NEPHROLOGY

SHORT TERM EFFECT OF ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITOR, ENALAPRIL, IN INCIPIENT DIABETIC NEPHROPATHY. Salah H. Abu-Romeh* and Khalid Nawaz* (intr. by Richard L. Tannen). University of Kuwait, Faculty of Medicine, Depts. of Medicine and Nuclear Medicine, Kuwait.

Recently Enalapril, an ACE-inhibitor, was found very effective in preventing albuminuria and the development of glomerulosclerosis in experimental diabetic rat. We conducted a clinical study to investigate the effect of Enalapril on microalbuminuria and renal hemodynamics in normotensive patients (n:11) with incipient diabetic nephropathy (Type 1 diabetes). Microalbuminuria was assessed by 125I radioimmunoassay. Renal plasma flow (RPF) and glomerular filtration rate (GFR) were determined by hippuran and DTPA uptake utilizing a computerized program (Gates Method). After 2-week therapy with Enalapril (10 mg. once daily) patients (n:6) with an elevated baseline GFR and filtration fraction (FF) (154 ml/min and 0.28) responded with a significant decrease in both parameters (129 ml/min and 0.24 respectively), P value < 0.05. Concomitantly microalbuminuria was significantly ameliorated from baseline of 29 to 24 μg/min (P < 0.05). On the contrary, patients (n:5) with normal baseline GFR and FF (mean 116 ml/min and 0.24) responded in an opposite fashion (143 ml/min and 0.27 respectively), P < 0.05 only for GFR and there was no significant change in the degree of microalbuminuria.

It is concluded that Enalapril is effective in reducing GFR, FF and microalbuminuria in normotensive patients with incipient diabetic nephropathy and elevated baseline glomerular filtration.

USE OF ³¹P-NMR TO STUDY THE EFFECT OF HYPONATREMIA (HN) ON RAT BRAIN BUFFERING. S. Adler, V. Simplaceanu*, and C. Ho*. Montefiore Hosp., U. Pitt. Sch. Med. and Carnegie Mellon U., Depts. of Med. and Biol. Sci., Pittsburgh, PA

The presence of HN or its rapid correction appears to be associated with CNS lesions or dysfunction. As Na:H antiporters have been described in many tissues including brain synaptosomes we examined this problem in HN rats using ³¹P-NMR. In one experimental series infusion for two hours of a 500 mEq Na solution rapidly increased plasma sodium (PNa) from 123.6 to 147.0 mEq/l and raised brain pH (pHi) from 7.10 to 7.18. HN rats infused with a 150 mEq Na solution showed a small fall in pHi from 7.09 to 7.06 while PNa was unchanged (pHi and PNa p < .001 between groups). PCr and ATP levels in both groups were identical before and after infusion. In another experimental series CO₂ tension was raised then reduced acutely in HN and normonatremic rats (NN). Blood pCO₂ rose and blood pH fell identically in both groups (p > 0.5). After 15 minutes of hypercarbia calculated intrinsic buffering (Bi) was 45.5 and 49.1 in NN and HN respectively (p > 0.5). Bi progressively rose to 102.5 in NN but was only 48.5 after 55 minutes in HN (p < .001 between groups). CO₂ tension was then lowered acutely. Recovery of pHi was more rapid in NN (p < .01). At 15 and 35 minutes Bi was 61.0 and 85.6 in NN compared to 35.4 and 33.6 in HN (p < .001). The absence of late phase buffering in HN indicates interference with active H⁺ transport. The results suggest the presence of a physiologically important Na:H antiporter in rat brain which might play a role in the CNS dysfunction of HN.

EFFECTS OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS (NSAID) ON RENAL HEMODYNAMICS, SOLUTE AND H₂O HANDLING IN SICKLE CELL ANEMIA (SS). M. Alton, L. Lawson*, J.R. Eckman*, V. Delaney, E. Bourke. Dept. of Med., Emory Univ., Atlanta, GA.

Renal hemodynamics and solute and H₂O handling were evaluated in 19 SS patients and 8 normal subjects (N), during H₂O diuresis, before and after a NSAID. In contrast to N, indomethacin (IND) induced a 16% decrease in GFR, and dissociation in regulation of GFR and RPF in SS. Urinary prostaglandins (PG) decreased similarly in both groups. A fall in GFR (14%) following sulindac (SUL) in SS did not differ from that seen following IND. IND resulted in a slight increase in U_{osm} in N, but a significantly greater rise in SS (Δ, 89 vs 7 mOsm/kg, P < .02), although plasma vasopressin remained undetectable in both groups. Following IND, a significantly greater fall in FE_{Na} (P < .05), solute reabsorption in the diluting segment, C_{H₂O} (P < .01), and solute delivery to the diluting segment [C_{H₂O} + C_{Na+K}], (P < .01), was observed in SS vs N. Baseline fractional reabsorption of distally delivered solute, [C_{H₂O} / (C_{H₂O} + C_{Na+K})], was identical in SS and N. This remained unchanged following IND in N, but fell significantly from 0.86 to 0.69 (P < .01) in SS. Changes in solute and H₂O handling were of lesser magnitude following SUL, compared to IND. These results suggest that in SS: 1) supranormal renal hemodynamics are PG-mediated; 2) there is a PG-dependent decreased salt reabsorption in the medullary thick ascending limb of Henle, with enhanced proximal tubular reabsorption; 3) there is an IND-sensitive antinatriuretic effect in the diluting segment; and 4) SUL does not have a "renal-sparing" advantage.

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

To assess the efficacy and safety of IVPC in the treatment of severe LN, 20 Pts (x age 32 yrs; 15 female) have been treated with 5-6 monthly doses of IVPC (0.5-1g/M²) plus rapid oral prednisone taper. 11 Pts had lupus for >1 yr. Initial Bxs showed: 14 DPLN, 5 FPLN, 1 Memb LN. At Bx, 70% had extrarenal lupus activity, 65% were hypertensive, 90% had active urinary sediment. Pts have been followed up to 30 mo. 15/20 Pts completed the protocol, 4 are under therapy; 1 failed after 4 doses of cytoxin. Data at 6 and 12 mo include:

	N	Abnl P creat	Prot >1g/day	Prot >3g/day
Bx	20	11/20	16/20	10/20
6 mo	16	6/16	8/16	4/16
12mo	11	3/11	4/11	3/11

14/16 were serologically inactive by 6 mo. 1 Pt on dialysis failed to respond; one Pt had only a partial response to IVPC. Repeat Bx at 1 yr showed reduced activity index (AI) and increased chronicity index (CI) in 7 Pts. Third Bx in 3 showed further decrease in AI and stable or increased CI. Side effects have been mild with only 2 significant infections. In most Pts with severe LN, IVPC produced favorable early responses with correction of serology, proteinuria, and abnormal P creat, and reduced histologic activity on Bx. However, not all responded and IVPC did not prevent the development of chronicity on Bx suggesting the need for the additional treatment in some Pts.

VESICoureteric REFLUX (VUR) IN INFANTS AND YOUNG CHILDREN. REPORT OF THE SOUTHWEST PEDIATRIC NEPHROLOGY STUDY GROUP. Billy S. Arant, Jr. UT-Southwestern Medical School, Dallas, TX.

Reflux nephropathy is a major cause of hypertension and renal insufficiency in adolescents and young adults. VUR occurs in 30-50% of children with first UTI but is expected to resolve spontaneously with conservative therapy in most cases. However, the expected duration and relative risks of continued sterile VUR prior to resolution are unknown. 113 patients (92F, 21M) 1 mo-5 yrs old with grades I-III/V primary VUR after first UTI were entered for a prospective 5 yr follow-up study (closed 12-1-86). VUR was unilateral in 65 (58%; 27R, 38L) and bilateral in 48 (42%). Of the 226 renal units examined, VUR was grade IV in 4, III in 51, II in 81, I in 25 and 0 in 65. VCUG is done yearly until VUR resolves. Renal growth (planimetric surface area) and scarring are assessed by IVP done at 1, 3, 5 years. Renal function is evaluated yearly by GFR, maximal Uosm and FENa. Screening for hypertension is done yearly as blood pressure and PRA. To date, 87 patients have completed 1 year follow-up studies. Of 123 refluxing ureters at diagnosis, 43% had no VUR, 19% had lesser grade VUR, 27% had not changed and 11% had higher grade VUR at 1 year; none had increased to IV or V. Of 51 non-refluxing ureters at diagnosis, 7 (14%) had grade I-II VUR at 1 year. Unilateral VUR became bilateral in 5 patients. Renal scars were noted in 2 kidneys with grade II-III VUR. Therefore, clinical observations after 1 year of mild or moderate VUR in children with first UTI include: no VUR in 41%, improvement in 18%, no change in 25%, worsening in 16% and renal scarring in <2%.

CENTRAL DIABETES INSIPIDUS, CENTRAL DIABETES MELLITUS & HYPERNATREMIA COMPLICATING UNTREATED HYPONATREMIA IN WOMEN: A NEW SYNDROME. Allen L. Arieff & Cosmo L. Fraser. V.A. Med. Ctr. & Univ. of California, Dept. of Medicine, San Francisco, CA.

It is known that untreated symptomatic hyponatremia can cause seizures, respiratory arrest and permanent brain damage in healthy women. We now report the development of central diabetes insipidus (DI) and central diabetes mellitus (DM) as a result of brain damage from symptomatic hyponatremia. Ten healthy women with mean age of 34 years and no history of DI or DM were hospitalized for elective surgery (ENT, cosmetic dental, lumbosacral disc, breast reduction, hysterectomy). Admission serum Na & glucose (\pm SE) were 140 \pm 1mM & 96 \pm 4 mg/dl respectively. In all patients, serum Na fell to 115 \pm 2 mM in a mean of 27 hrs. Hyponatremia resulted from a combination of emesis, blood loss & IV administration of 285 mM glucose (5%D/W), or self-induced water intoxication in 1 patient. All patients had multiple non-osmotic stimuli for ADH secretion. After 27 \pm 3 hrs, all patients suddenly (10-30 min) went from alertness to seizures, respiratory arrest and coma. Within 24 hrs of coma, maximum urine volume spontaneously increased from 38 to 610 ml/hr. Mean serum Na rose from 115 to 164 \pm 1 mM and glucose from 96 to 513 mg/dl over 53 \pm 7 hrs with no therapy for the hyponatremia. All patients died without regaining consciousness. At autopsy, all had cerebral edema, brainstem herniation and ischemic damage, while 5 had pituitary infarction due to occlusion of the blood supply. Conclusions: Untreated symptomatic hyponatremia can lead to central diabetes insipidus and central diabetes mellitus with associated hypernatremia & hyperglycemia, due to pituitary damage from brain swelling and brainstem herniation. The devastating consequences of symptomatic hyponatremia in these patients are not related to therapy.

LIPID AND LIPOPROTEIN ALTERATIONS IN THE NEPHROTIC SYNDROME. Edwin K. Armitage, Domenic A. Sica, Judy Davis, Jane Baggett, and Mary E. King. Med. Coll. of Virginia, Depts. of Path. & Med., Richmond, Virginia.

We studied plasma lipid and lipoproteins from 33 nephrotic syndrome (NS) patients to compare changes seen in patients with NS patients with and without renal impairment, and in NS patients receiving and not receiving steroid therapy. Plasma lipoproteins were separated by discontinuous density gradient ultracentrifugation (d. 1.006-1.300 kg/L) in a Beckman Vti 80 vertical rotor. The gradient was collected in 20 fractions and the density of each was measured. Plasma and each fraction were assayed for cholesterol (TC), triglyceride (TG), and phospholipid (PL) by enzymatic procedures and apo AI, AII, and B by immunoturbidimetric methods.

The only significant differences found between the diabetic NS (n=14) and nondiabetic NS (n=19) groups were decreased plasma apo AI and decreased LDL, and VLDL TG concentrations in the diabetic group. The only significant differences between the renal compromised NS (n=14) and uncompromised NS (n=19) groups were decreased plasma PL and apo AI and decreased HDL TG in the compromised group. No significant differences were found between the steroid treated NS (n=9) group and untreated NS (n=24) group in any of plasma or lipoprotein lipids or apo. In conclusion, we found that additional changes in lipoprotein concentrations and composition due to diabetes, moderate renal failure, and steroid therapy are minor as compared to the marked alterations due to NS.

PLATELET ARGININE-VASOPRESSIN (PlatAVP): A MARKER OF THE LONG-TERM STIMULATION OF AVP; APPLICATION TO THE RAPID DIAGNOSIS OF CENTRAL VS NEPHROGENIC DIABETES INSIPIDUS. MF Arthus*, D Bichet, M Lonergan*, M. Razi*. Nephrol. Serv., Research Ctr., Hop. Sacré-Coeur, Univ. Montreal.

PlatAVP is AVP immunoreactivity in platelet-rich plasma. We recently demonstrated that PlatAVP is identical to plasma AVP (Pavp) and synthetic AVP on HPLC analysis and an estimate of AVP bound to specific platelet receptors (D Bichet et al., J Clin Invest, 79:881,1987). In the present report, we tested the hypothesis that a single PlatAVP measurement may be of diagnostic value in the evaluation of non-osmotic polyuric states. Twelve patients (pts) with central (neurogenic) diabetes insipidus (DI) and 5 male pts with congenital nephrogenic DI (CNDI) had measurements of Pavp and PlatAVP during dehydration and hypertonic saline infusion. All the pts were evaluated for their urinary responses to AVP and to dDAVP. These data were compared to Pavp and PlatAVP values obtained during dehydration and hypertonic saline infusion in 23 normal subjects and nomograms with 99% confidence limits of the relationship P_{Na}/P_{avp} were constructed. Pavp was low (<1.0 pg/mL) or undetectable in central DI and elevated for each level of plasma osmolality in CNDI. PlatAVP was less than 1.5 pg/mL in central DI, 5.7 ± 1.4 pg/mL (range 2.3 to 11.5) in normal subjects and 36 ± 16.8 pg/mL (range 9.7 to 102) in CNDI pts ($p < .01$ as compared to normal subjects). These results suggest that measurement of PlatAVP is a useful addition to standard dehydration tests in the evaluation of polyuro-polydipsic pts.

SEXUAL DIFFERENCE IN SURVIVAL WITH SEVERE SYMPTOMATIC HYPONATREMIA. J. C. Ayus, R. K. Krothapalli and A. I. Arieff. Baylor Coll. of Med. & U.C.S.F., Dept. of Medicine, Houston, TX & San Francisco, CA

It has been shown that symptomatic hyponatremia alone can result in death or permanent brain damage (New Eng J Med 1986;314:1529). Most such patients appear to have been women. Studies were designed to evaluate the role of gender in the morbidity from symptomatic hyponatremia. Studies were carried out in: a) 10 male (M); b) 34 female (F) patients; c) 4 groups of rabbits (n=80). Ten M subjects had prostate surgery with glycine/H₂O irrigation of the bladder: serum Na (sNa) fell from 139 ± 1 to 102 ± 2 mM in 4.5 hrs, 6 were lethargic & 4 had seizures, but none had a respiratory arrest. All were treated with 855 mM NaCl+furosemide, increasing sNa to 125 ± 1 mM at 4.6 mmol/hr. CT scans were normal and all recovered without sequelae. In 34 female subjects, sNa fell from 139 ± 1 to 115 ± 2 mM in 31±4 hrs after elective surgery. They all suffered respiratory arrest & seizures, and then received either no therapy, or NaCl to raise sNa at ≤ 0.6 mmol/hr. CT scans all showed cerebral edema, and all F patients either died or suffered permanent brain damage. The reasons for the different outcomes in M Vs F patients were investigated. In 53 rabbits, serum Na was lowered from 142 to 104 mM in 4.5 days. 86% of F died Vs 24% of M ($p < .01$). In 27 rabbits with serum Na = 106 ± 2 mM, brain (cerebral cortex) H₂O was less in M Vs F (4.1 ± 1 Vs 4.9 ± 3 ml/gm dry wt, $p < .01$), while both brain Na (2.82 Vs 3.32 mmol/gm dry wt) and K (3.74 Vs 4.85 mmol/gm dry wt) were significantly less ($p < .01$) in M Vs F.

Conclusions: a) Despite a greater fall in serum Na in less time, men had no morbidity Vs 100% in women; b) In hyponatremic rabbits, F mortality is 3.6 X M; c) F rabbits are less able to extrude K and Na from brain & prevent cerebral edema; d) differences in adaption of brain to hyponatremia in M Vs F may explain the differences in morbidity.

ASYMPTOMATIC POSTSTREPTOCOCCAL GLOMERULONEPHRITIS IN RELATIVES OF PATIENTS WITH SYMPTOMATIC GLOMERULONEPHRITIS; VALUE OF ENDOSTREPTOSIN ANTIBODY TESTING. Ali Azadegan*, Richard Bovie*, Hassan Majeed, Gene Seligson and Kurt Lange. Lenox Hill Hospital, Renal Immunology, Dept. of Med. New York, N.Y. and the Dept. of Pediatrics, Kuwait University, Kuwait.

Our previous studies have shown that Endostreptosin (ESS), a cytoplasmic protein of group A streptococci, is the causative agent of poststreptococcal glomerulonephritis (PSAGN). Antibody levels to ESS have been shown to be diagnostic of PSAGN and to correlate well with the pathologic disease process. Of 121 completely asymptomatic family members of 29 index cases of acute glomerulonephritis 26 (21.5%) had, however, one or more abnormalities including proteinuria, hematuria (18/26), low C3 (13/26 less than 100 mg/dl) and high ASO titers (15/26). Most of these family members had a significant elevation of ESS-Ab titers (24/26, 92.3%). The mean arithmetic titer for the CF test was 24 (normal mean 7.5) and the mean ELISA value 0.154 (mean normal value 0.075). With a certain percentage of PSAGN cases especially adults, progressing to chronicity, the ESS-Ab determination by complement fixation and/or ELISA may be simple methods to detect and to follow such asymptomatic patients at risk. The high incidence of asymptomatic PSAGN makes this data very significant for the question of the origin of chronic glomerulonephritis without a history of overt PSAGN. Since PSAGN is an infectious, often undiagnosed and asymptomatic disease it may contribute materially to the contingent of ESRD.

CONTRAST NEPHROPATHY IN SUBJECTS WITH PRE-EXISTING RENAL FAILURE: A PROSPECTIVE CONTROLLED STUDY. Barrett BJ, Parfrey PS, Griffiths SM, Withers J, Paul MD, McManamon PJ. Memorial University, Newfoundland Canada.

Renal failure (RF) may be a risk factor for contrast (C) nephropathy but no prospective controlled study has determined the extent of the risk. Therefore before and after intravascular C serial serum creatinines were performed on 94 nondiabetic subjects whose pre s. creat. was > 150 μ mol/l. 11% received nonionic C medium. A control group comprised 141 with s. creat. > 150 who had CT scanning without C or abdominal ultrasound. Mean s. creat rose by $\geq 25\%$ 2 days after the test in 12/94 of C and 11/141 of controls ($p = ns$, relative risk = 1.6). All patients were examined before imaging for predisposing factors to acute RF and patients who developed post-imaging RF were assessed by a nephrologist blind to whether patients received C. 6/12 of C and 9/11 of controls had a definite predisposing factor for acute RF other than C. 6.4% of C and 1.4% of controls had unexplained acute RF (relative risk = 4.6). There were no significant differences in demography, BP, initial s. creat, cardiovascular disease, or other potential risk factors for acute RF comparing C and controls who developed $\geq 25\%$ change in s. creat or those who did and did not develop acute RF in C. We conclude that the incidence of contrast nephropathy in patients with preexisting renal impairment is low.

RENAL DISEASE ASSOCIATED WITH ACUTE AND CHRONIC "CRACK" ABUSE. DT Barrido*, AJ Joseph*, TKS Rao, EA Friedman. SUNY, Health Science Center at Brooklyn, Brooklyn, New York.

"CRACK" is a purified and potent derivative of cocaine which is usually smoked and sometimes injected intravenously. From January through July 1987, of 361 renal admissions and consultations, 69 were for 54 abusers of heroin and cocaine. Of these 54 patients, 46 abused only heroin while 8 (5 women and 3 men), all black, used CRACK either solely or with heroin. CRACK abusers are young (mean age 33 years, range 25 to 44 years); 4 used only CRACK while 4 used CRACK and heroin.

In 2 patients (1 woman, 1 man), malignant hypertension (papilledema, BP 213/140, 180/140mmHg) and renal insufficiency necessitating dialysis followed CRACK intoxication. Within 48 hours, both patients became normotensive without medication but had persistent renal damage, 1 (also a heroin abuser) is on maintenance hemodialysis, and the other has a serum creatinine (Cr.) of 2.3 mg/dl. After acute CRACK use, 2 patients, (1 woman, 1 man) became unconscious with rhabdomyolysis and rapidly deteriorating renal and liver function (SGOT > 9000, LDH > 6000). 1 patient recovered renal function (Cr. 2.5 mg/dl) in 3 weeks, the other had severe stab wounds, a rising Cr. from 1.9 to 17.2 mg/dl, and refused dialysis but recovered some renal function (Cr. 5.8 mg/dl). Chronic renal failure was detected in 4 CRACK users: 1 with crescentic glomerulonephritis requires dialysis, 1 with SS disease has a serum Cr. of 6.5 mg/dl, 1 user for 1 year has a serum Cr. of 3.6 mg/dl, 1 with headache and hypertension (Cr. 13.3 mg/dl), became normotensive on dialysis.

We suggest that CRACK may induce malignant hypertension, rhabdomyolysis, renal failure, and liver failure. Elucidation of any chronic CRACK nephrotoxicity - distinct from that of heroin - requires further study.

ESTIMATION OF PREVIOUS SERUM CREATININE FROM FINGERNAIL CREATININE. RR Bergamo*, SA Laidlaw* & JD Koppke, Harbor-UCLA Med. Ctr., Torrance, CA.

When physicians treat azotemic patients who have no past history of renal failure (RF), it is often helpful to know whether the RF is of recent onset. We examined the findings of Levitt (Ann Int Med, 1966) that fingernail (FN) creatinine may indicate previous serum creatinine levels. Serum or plasma and FN creatinine were measured in normal adults and patients with acute RF, chronic RF, hemodialysis (HD, duration 55 ± 11 SEM months) and renal transplantation (TX) performed 1-6 weeks or 5-7 months previously. FN creatinine was measured in the distal 1-3 mm of the nails from 5 digits of both hands. The results indicate that FN creatinine was increased in chronic but not acute renal failure.

	N	Serum Creat (mg/dl)	Fingernail Creat (mg/100g)
Normal	15		19.0±1.4 ^{a,b}
Acute RF	6	8.8±1.1 (SEM)	16.1±2.1 ^{a,b}
Chronic RF	6	4.3±0.5	26.5±3.1 ^b
Hemodialysis	16	15.6±1.0	43.2±3.1
Renal TX			
0.7 months	4	2.7±0.6	50.3±3.8 ^{a,b}
6.2 months	4	1.7±0.3	20.7±3.4 ^{a,b}

Differs vs HD, ^ap<.003; TX at 0.7 mos, ^bp<.002. In the HD patients, the FN creatinine appeared to correlate best (r=0.608, p<.03) with the serum creatinine obtained about 7 months previously. In combined chronic RF and HD patients, FN creatinine also correlated with serum creatinine 7 months previously (r=0.757, p<.01). Thus, the FN creatinine may estimate the serum creatinine from about 7 months previously. FN creatinine may be a useful test for the chronicity of RF.

RENAL FUNCTIONAL AND SYMPTOMATIC RESPONSES TO REDUCING CYST VOLUME IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (APKD).

William Bennett, John Barry, Lawrence Elzinga, Thomas Golper and Vicente Torres. Oregon Hlth Sci Univ, Portland, OR and Mayo Clinic Rochester, Minnesota.

Cyst reduction surgery reduces refractory symptoms in APKD (Bennett et al., Journ Urol 137:620,1987). To prospectively examine effects of surgery on renal function, as well as symptoms, 8 additional patients had renal function testing, CAT scans and separated renal function assessment with ^{99m}Tc-dimercaptosuccinic acid (DMSA). Surgical indications were refractory pain alone (n=2) or symptoms plus progressive renal failure (n=6). There were 2 men and 6 women aged 47±10.

	Serum Cr (mg/dl)	GFR (ml/min/1.73m ²)	DMSA* (% uptake)
Baseline (n=8)	2.7±1.5	17.4±25	47.8±9
1-2 mos	2.6±1.4	18.7±26	*n=6

At last follow-up (3-12 mos), serum Cr was 3.0±1.4. Improvement in individual patients related to the degree of volume reduction. Symptoms disappeared in all patients. One female patient developed lower urinary tract infection at 2 months postoperatively. Mean hospital days were 6.1±1.6. Blood pressure control improved in 5 of 7 of patients who were hypertensive. **Conclusions:** Reduction of cyst volume regularly improves symptoms in APKD. Any long-term benefit on renal function or blood pressure derived presumably by reducing pressure on non-cystic parenchyma will await further experience and follow-up.

TREATMENT OF SEVERE IGA NEPHROPATHY IN CHILDREN. Jerry M. Bergstein, Sharon P. Andreoli, IUHC, Dept. of Peds, Indianapolis, Indiana

We treated 10 children with severe IgA nephropathy with prednisone (60 mg/M²/day for 8 weeks, then 60 mg/M² q.o.d.) and Imuran (2-3 mg/kg/day) for 1 year. Six were treated at diagnosis and 4 after repeat biopsy because of features suggestive of progressive disease (proteinuria [P]>1 gm/day, % crescent formation, segmental sclerosis, GBM deposition of IgA). Biopsies were scored for activity (% crescents, degree of mesangial proliferation, interstitial infiltrate, maximum score = 9) and chronicity (% fibrous crescents, % of glomeruli demonstrating segmental and global sclerosis, tubular atrophy and interstitial fibrosis, maximum score = 12). The serum creatinine after therapy (1.09 ± 0.47 mg/dl) was not significantly different from that prior to therapy; however P decreased markedly from 4052 ± 3190 mg/day to 1474.1 ± 1504 mg/day (P<.01). Among 7 children who underwent a biopsy after therapy, the chronicity scores before (5.4 ± 1.7) and after (5.8 ± 2.0) therapy were similar. However, the activity score decreased significantly (P<.01) from 4.35 ± .94 prior to therapy to 2.28 ± 0.75 after therapy. Mesangial deposition of IgA remained but GBM deposition of IgA was less prominent after therapy. At a mean follow-up of 2.25 years (range 1 month to 6.5 years), 8 children have stable renal function and protein excretion while 2 have developed mild renal insufficiency. Repeat biopsy in the latter two demonstrate progressive scarring with minimal inflammation. We suggest that prednisone and Imuran therapy is beneficial in children with severe IgA nephropathy.

RENAL HANDLING OF α -GALACTOSIDASE (aG) BY A RENAL GRAFT IN FABRY'S DISEASE (FD). R. Berty*, N. Basu*, R. Glew*, and S. Adler. Montefiore Hosp. and U. Pitt Sch. Med., Depts Med. and Biochem., Pittsburgh, PA

aG deficiency in FD leads to early death by renal failure, stroke or cardiac infarction. Unfortunately, it appears that renal grafts do not reliably supply the enzyme. We studied a 43 y.o. patient with FD post renal transplant. An initial study performed when the serum aG level had risen to half normal showed that water loading, water deprivation, and NaHCO₃ loading had no significant effect on fractional excretion (FE) of aG but I.V. acetazolamide (AZ) reduced FE from 15.6 to 3.5%. A repeat study in this patient and 4 age sex matched normals was performed 3 months later to examine renal handling of aG and 2 other lysosomal hydrolases, B hexosaminidase (BH) and B glucuronidase (BG). Using fractional changes in urinary enzyme/creatinine concentration as a clearance marker we found that in all 5 subjects AZ reduced the aG creatinine ratio but the decrease was non-significant, generally increased this ratio for BH, and had no effect on this ratio for BG. In this second study the patient's serum aG was 5% of normal and his FE aG was 2115%, 278% and 211% in 3 separate periods. These values significantly exceed 100% and significantly differ from the earlier study when his serum levels were tenfold greater. The results indicate that (1) aG is secreted by the renal graft, (2) renal handling of lysosomal hydrolases is selective and (3) reducing renal aG secretion could alter serum enzyme levels possibly improving prognosis in FD post renal transplant.

FACTOR VIIIc (F VIIIc), VON WILLEBRAND FACTOR (vWF), MEAN ARTERIAL BLOOD PRESSURE (MAP), PULSE, AND RENIN (PRA) RESPONSES TO dDAVP INFUSION IN PATIENTS (pts) WITH CONGENITAL NEPHROGENIC DIABETES INSIPIDUS (CNDI). D Bichet, M Razi*, M Loneragan*, MF Arthus*. Nephrol. Serv., Research Ctr., Hop. Sacré-Coeur, Univ. Montreal.

F VIIIc and vWF responses to 1-deamino-8D-arginine-vasopressin (dDAVP) have been reported to be absent in 2 pts with CNDI suggesting an extrarenal vasopressin receptor defect (Kobrinisky, Lancet i:1293,1985). We measured the coagulation and hemodynamic responses to a 0.3 ug/kg BW, 20 min dDAVP infusion in 7 male pts with CNDI, 6 obligatory transmitter female pts, 5 pts with central (neurogenic) diabetes insipidus (DI) and 4 normal subjects (N). Blood samples were obtained before and every 30 min for 120 min after the infusion. MAP and pulse were recorded every 5 min. Maximal responses are given in % as compared to baseline values. In central DI pts, dDAVP infusion decreased MAP (-13+2%) and increased pulse (+17+2%), PRA (+66+14%), F VIIIc (+181+33%) and vWF (+89+16%); all p<.01 (Dunnnett). Similar responses were obtained in N subjects. In CNDI pts, MAP (-4+2.5%), pulse (+5+2%), PRA (+6+4%), F VIIIc (35+11%) and vWF (25+9%) did not change significantly. Minimal responses were obtained in obligatory transmitters. Responses were significantly different (Anova) between CNDI and transmitters and the two control groups (central DI and N). These results suggest the existence of extrarenal V2 receptors possibly defective in CNDI pts. In these pts, all the above measurements after dDAVP infusion could be useful diagnostically.

SELECTIVE β -1 ADRENERGIC ANTAGONISTS ARE SAFER THAN NONSELECTIVE AGENTS IN DIALYSIS PATIENTS. M Bia, R A DeFronzo, P Castellino*. Yale Univ. School of Medicine, New Haven, Ct.

We have previously shown that adrenergic control of extrarenal potassium (K) balance is mediated via the β -2 receptor. To determine the clinical significance of these results under physiologic conditions, exercise induced hyperkalemia was evaluated in dialysis patients after nonselective (β -1 and β -2) versus selective (β -1) blockade. Seven nondiabetic dialysis patients (3 hemo; 4 CAPD) exercised for 40 mins on a bicycle ergometer at 40% V_{O2} Max after 3 days of (a) placebo (Ex + placebo); (b) 40 mg b.i.d. propranolol (prop) and (c) 50 mg b.i.d. metoprolol (metop). Each exercise was separated by 2 weeks. Results:

	Basal Plasma K	Δ Plasma K (mEq/L)
Ex + placebo	4.82 \pm 0.27	0.26 \pm 0.12
Ex + metop	4.99 \pm 0.27	0.29 \pm 0.08
Ex + prop	4.89 \pm 0.29	0.44 \pm 0.06 *

* p < 0.05 vs Ex + placebo and vs Ex + metop

The rise in plasma K was similar at all time intervals following placebo and metop. In contrast, plasma K rose significantly higher during exercise with prop. Plasma insulin, glucagon, aldosterone, epinephrine, norepinephrine, glucose and bicarbonate levels were similar during all 3 exercise sessions. The exercise induced elevations in blood pressure and heart rate were effectively and similarly decreased by both metop and prop. Thus, the higher rise in plasma K with prop could only be explained by inhibition of the β -2 adrenergic receptor. Conclusion: Selective β -1 blockade does not impair potassium homeostasis during exercise in dialysis patients and may offer a greater degree of clinical safety.

DISTURBANCES IN BRAIN MATURATION AND NEURODEVELOPMENT DURING CHRONIC RENAL FAILURE (CRF) IN INFANCY. G.H. Bock, C.K. Conners*, J. Ruley, C. Samango*, J. Conry*, C. David*. Nephrol., Beh. Med., Neurol & Genetics, Child. Hosp. Nat'l. Med. Ctr., Washington, D.C.

Fifteen infants with CRF since birth underwent serial developmental, neurophysiologic and clinical assessment. Age at study entry 3-16 mo. (median=5); study period 2-19 mo. (median=11); studies/patient 2-7 (\bar{x} =3.4); creatinine (Cr) clearance 5-37 ml/min/1.73m² (\bar{x} =19.7). Of the 15 patients, 6 had mental development scores (MDS) \leq 16 centile (-1SD) at entry (\bar{x} MDS=100.3) and remained stable or improved, 3 were less than 16 centile (\bar{x} MDS=50) and remained so and 6 were \geq 16 centile at entry (\bar{x} MDS=91.8) but had subsequent net decreases of MDS centiles of 27-99%. Patients were classified as GpI: MDS \leq or falling to -1 SD (n=7) and GpII: MDS maintained above -1SD (n=8). Outcome MDS for GpI and GpII = 65.3 and 107.2 (p < .01). Mean Cr for GpI=3.8; GpII = 1.3 (p < .01). Head circumferences (HC) were lower in GpI at entry (-1.8 SD vs -.2; p < .01 for Gp classification) and no significant changes in Gp HC SD occurred during the study period. Gross motor developmental quotients at entry were lower in Gp I (58.1% vs 83.9%, p < .05). These differences became insignificant over the study period (60.3% vs 82%). Serial analyses of spectral electroencephalograms (EEG) demonstrated slower disappearance of dominant lower frequencies (p < .01) and the appearance of significant left/right hemispheric asymmetries (p < .02) in GpI vs. GpII. Delayed cognitive development is significantly related to degree of CRF and the presence of impaired head growth. The progressive EEG asymmetry and delayed decrease in slow activity suggest the a progressive encephalopathy of undetermined origin and long-term consequence.

PROTEIN INTAKE: A MAJOR DETERMINANT OF GLOMERULAR FILTRATION RATE (GFR) AND CREATININE GENERATION IN NORMAL MAN. JP Bosch and S. Lew. George Washington University Medical Center, Department of Medicine, Renal Division, Washington, D.C.

To determine the effect of protein intake on GFR we studied 15 normal subjects with no evidence of renal disease who had different protein intakes. The subjects were in steady state at the time of measurements. A 24h urine was collected for creatinine clearance (CrCl) and urea excretion. Protein intake was calculated from urea nitrogen appearance rate. Result (N=15):

Protein Intake (g/kg/d)	Mean±SD	MIN	MAX
Protein Intake (g/kg/d)	0.6±0.2	0.3	1.2
Plasma Creatinine (mg/dl)	0.9±0.1	0.6	1.2
Creatinine Excretion (mg/d)	1088±465	391	1741
Weight (kg)	68.1±15.0	46.3	95.0
Creatinine Excretion/Body Weight (mg/d/kg)	15.6±4.8	6.5	20.4
Urea Excretion (mg/d)	5927±2646	1900	9611
Creatinine Clearance (ml/min)	84±34	29	130

CrCl was highly correlated with urea excretion ($r=0.92, p<0.001$). Creatinine excretion adjusted for body weight also correlated significantly with urea excretion ($r=0.85, p<0.001$).

These observations suggest that in normal man GFR is markedly influenced by diet. Creatinine generation is also affected by diet so the low GFR does not result in an elevated plasma creatinine.

PREDICTORS OF PROGRESSION TO CHRONIC RENAL FAILURE (CRF) IN IDIOPATHIC MEMBRANOUS GLOMERULONEPHRITIS (MG). G. Braden, A. Rastegar, J. Hession*, J. Garb*, N. Siegel, J. Fitzgibbons, M. Kashgarian, Baystate Med. Ctr., Springfield, MA, & Yale Med. Sch., New Haven, CT.

The long-term natural history of untreated MG in the USA has not been adequately documented. To identify predictors of progression to CRF in MG we evaluated initial clinical, laboratory and renal biopsy data in 55 consecutive untreated patients (pts) followed for a mean of 6.5 years. 19 pts progressed to CRF (end stage renal failure in 14, or creatinine >2.0 mg/dl in 5). 36 pts remained stable (creatinine <2.0 mg/dl). By univariate analysis 3 factors correlated with progression to CRF ($p<.05$): initial serum creatinine level, interstitial fibrosis, or interstitial infiltration. Age, sex, edema, 24 h urinary protein, serum albumin and mesangial indices were not useful predictors. By multiple logistic regression analysis (MLRA) 6 factors predicted progression to CRF (factor, p value): edema, .024; initial serum creatinine level, .012; age, .034; interstitial fibrosis, .044; tubular atrophy, .029, and interstitial infiltration, .076.

By probability analysis a 6 variable function was developed which classified pts with a low ($<10\%$) probability of progression to CRF; 30% of the pts fell into this group and none progressed to CRF.

We conclude: MLRA identifies pts with MG who have an excellent prognosis and who do not require immunosuppressive therapy.

DIAGNOSIS OF ACUTE PANCREATITIS (AP) IN END STAGE RENAL DISEASE (ESRD) PATIENTS (PTS). G. Braden, J. Rosenbaum*, A. McGuire*, M. Germain, J. Fitzgibbons, Baystate Med., Ctr., Springfield, MA., Tufts Med. School, Boston, MA.

To evaluate the utility of serum lipase activity (LA) in diagnosing AP we measured LA by a new sensitive Kodak colipase method (KC) and compared it to LA measured by the Dupont ACA method (DU) and amylase activity (AA) measured by the Kodak method. We studied 10 normals, 21 CAPD pts, 20 hemodialysis (HD) pts, 6 CAPD pts with peritonitis (PER), and 2 CAPD pts with AP diagnosed at laparotomy. Results are expressed as the number of pts in each group with normal (nl) or elevated LA or AA.

		HD	CAPD	PER	AP
LA by KC	nl	9	12	4	0
	up to 3X nl	10	8	2	0
	3X - 5X nl	1	1	0	0
	>5X nl	0	0	0	2
LA by DU	nl	13	14	5	0
	up to 3X nl	7	7	1	0
	>3X nl	0	0	0	2
AA	nl	10	13	5	0
	up to 3X nl	10	8	1	2
	>3X nl	0	0	0	0

Baseline LA&AA were elevated in ~50% of HD&CAPD pts. AP pts but no HD, CAPD, or PER pts had $>5X$ increase in KC LA or $>3X$ increase in DU LA. The AA levels in AP were elevated but in the range found in stable HD or CAPD pts. Peritoneal fluid (pf) from AP pts showed significant AA and LA by KC or DU not found in stable CAPD or PER pts.

We conclude: LA by DU $>3X$ elevated or LA by KC $>5X$ elevated in ESRD pts suggests AP; both are more sensitive than changes in AA. AA or LA in pf also suggests AP.

IMPAIRED URINARY AMMONIUM EXCRETION IN PATIENTS WITH PROXIMAL RENAL TUBULAR ACIDOSIS (PRTA). L.G. Brenes, M.I. Sanchez,* and J.N. Brenes.* Hospital San Juan de Dios, Univ. of Costa Rica Med. School, San Jose, Costa Rica.

Patients with isolated PRTA produce net renal acid excretion in appropriate quantities to balance their daily dietary input of hydrogen ion (Kidney Int. 10:561a). In spite of a chronic state of metabolic acidosis and normal glomerular filtration rate, when challenged with an acute acid load, their urinary ammonium excretion (NH_4^+) is inappropriately low (Am. J. Med 63:244). To test if this observation indicates a limitation in their maximum NH_4^+ , 8 patients with PRTA and 10 normal controls (C) were given daily NH_4Cl at 100mg/kg for 3 days. 24-hour urines were collected before (B) and during the third day of the test (3d) and titratable acidity (T.A.) and NH_4^+ were measured in mEq/24h/1.73m². (Mean ± SEM, *p .005):

	C		PRTA	
	B	3d	B	3d
U _{pH}	5.96	5.00	5.53	4.66*
	+0.15	+0.34	+0.19	+0.28
T.A.	23.4	45.9	29.1	48.5
	+2.5	+2.3	+4.3	+3.3
NH_4^+	32.7	76.2	27.0	47.7*
	+4.3	+5.7	+2.8	+4.4

These results show that a large and sustained acid load in PRTA leads to the expected increase in T.A. by comparison with C, but to a significantly decreased NH_4^+ which produces a lower urinary pH. We conclude that this impaired ammonium excretion is due to inadequate cellular production or to a decreased acidification in the proximal tubule.

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. Clinical Molecular Biology Unit, Toronto General Hospital and Div. of Nephrology, The Wellesley Hospital, Toronto, Ontario.

Adult polycystic kidney disease (APCKD) is a common autosomal dominant disorder which contributes 9% of individuals to chronic dialysis. A recently described DNA probe, 3'HVR has been closely linked to APCKD in European families (Readers et al. 1986 Nature 317:542). The purpose of the present study was to determine the accuracy and diagnostic reliability of 3'HVR for North American (NA) populations. This was done in collaboration with Readers who generously provided the 3'HVR probe. Buffy coat DNA was extracted by standard methods from 12 members of 2 unrelated families, one English and one French Canadian in origin. Pvu II digested DNA was electrophoretically separated in 1% agarose gels, transferred onto nylon membranes, hybridized with 3'HVR and autoradiographed. Polymorphic DNA fragments of the previously reported size range (1-8kb) correlated with the clinical picture of APCKD. The recombination frequency between APCKD and 3'HVR was 25% (2/8), slightly higher in our 2 families than previously reported for European populations (2-11%). There is no evidence of genetic heterogeneity. Further members of these families and additional families are now being studied to assess the reliability of these findings. If these results are substantiated the 3'HVR probe may be useful in preclinical diagnosis of APCKD in N.A.

FUNCTIONAL RENAL RESERVE INCREASES AFTER PROTEIN RESTRICTION IN DIABETIC NEPHROPATHY.

Ben H. Brouhard. Univ. of Texas Med. Br., Dept. of Pediatrics, Galveston, Texas.

Dietary protein restriction has been used to slow the progression of chronic renal disease. The rise in GFR after a protein meal defines functional renal reserve (FRR). To determine the effect of decreasing dietary protein on GFR (creatinine clearance) (CCr), albuminuria (alb) and FRR, we studied 11 diabetics with alb with or without decreased GFR. Five consumed their usual protein diet; 6 reduced intake to 0.6 g/kg/day. Data obtained before and 3 months after diet change included: hemoglobin Alc (HA_{1c}%), alb (µg/min), base Ccr (ml/min/sq m), peak rise in GFR (ml/min/sq m) and percent rise (%) after an 80 g protein (red meat) meal and blood pressure.

USUAL DIET

Time	Alb	Peak Rise in Ccr	Percent Rise in Ccr	Baseline Ccr
0	548±223	14±4	49±10	31±7.7
3m	435±252 (x±SEM)	7±2*	24±6*	38±10.0

LO-PROTEIN DIET

0	457±155	20±7.7	34±13	54±6.0
3m	86±17*	30±10.5*	49±20	56±4.3

* p<0.05

There were no differences in HA_{1c} or blood pressure (7.5±6 vs 8.0±.9%) (130±10/75±2 vs 137±5/82±5 torr) between the groups or time periods. Dietary protein restriction decreases alb and increases FRR in early diabetic nephropathy.

RAISED HEMATOCRIT (HCT) PERSISTS SIX-WEEKS AFTER STOPPING TREATMENT WITH HUMAN RECOMBINANT ERYTHROPOIETIN (r-HuEPO) IN AZOTEMIC, ANEMIC PATIENTS. Clinton D. Brown, Michael Kieran, Benzina V. Dossunmu, Zhong-Hua Zhao, Ralph H. Larson, Eli A. Friedman. SUNY, Health Science Center at Brooklyn, Dept. of Medicine, Brooklyn, New York.

13 azotemic (mean serum creatinine 5.6±2.9 mg/dl) adults (5 women and 8 men) with anemia (mean HCT 28.3±11.7%) secondary to renal disease in a double blind placebo controlled study received r-HuEPO 50 to 150 U/Kg or placebo intravenously (IV) thrice weekly for 54 days. Subsequently, all subjects then were given r-HuEPO (open label basis), individualized doses of 50 to 200 U/Kg, IV thrice weekly. Supplemental iron was administered to all subjects (daily dose 650 to 915 mg FeSO₄). Three subjects withdrew from study due to infection (osteomyelitis, decubitus ulcer, pneumonia).

Mean HCT in the 10 remaining patients (5 men, 5 women) rose from 29.7±2.8% to 41.2±1.2% over a mean of 3.3±1.3 months (range of 1 to 5 months). r-HuEPO therapy was discontinued once the HCT rose to 40% (men) and 37% (women). Subsequently, HCTs continued to rise in all patients, (to a mean of 41.2±1.2%). One subject developed a HCT of 49% 3 weeks after her last dose of r-HuEPO. However, increases in HCTs post-r-HuEPO were associated with marked reduction in RBC production (mean absolute reticulocyte count of 0.38 ± 0.14% compared to an r-HuEPO induced rate of 1.21±0.59%). Despite reduced erythropoiesis, r-HuEPO-induced HCT effect persisted for 1.49±0.5 months (range 0.7 to 2.2 months).

We conclude that in the dose range studied: (1) r-HuEPO is a safe and effective treatment for the anemia of renal insufficiency. (2) Post-r-HuEPO elevation in HCTs are not due to increased erythropoiesis but probably to longevity of r-HuEPO generated RBCs.

TREATMENT OF AZOTEMIC, ANEMIC PATIENTS WITH HUMAN RECOMBINANT ERYTHROPOIETIN (r-HuEPO) RAISES WHOLE BLOOD VISCOSITY (WBV) PROPORTIONAL TO HEMATOCRIT (HCT). Clinton D. Brown, Michael Kieran, Zhong-Hua Zhao, Ralph H. Larson, Eli A. Friedman. SUNY, Health Science Center at Brooklyn, Department of Medicine, Brooklyn, New York.

WBV and plasma viscosity (PV) were measured in 10 adult patients (5 men, 5 women) with anemia (mean of HCT 29.3±3.4%) and azotemia (mean serum creatinine of 5.2±3.2 mg/dl) before and after a 54 day placebo controlled study and 1 month of treatment with r-HuEPO (50 to 200 U/Kg given IV thrice weekly). Baseline and post-treatment HCT, WBV and PV were compared to 52 normal adult subjects (33 women, 19 men), (mean serum creatinine of 0.9±0.1 mg/dl, mean HCT of 42.4±3.7%).

To compare rheologic factors at subnormal HCTs, normal blood was diluted with autologous plasma to achieve a range of lower HCTs. Because the HCT of men is higher than in women, we compared each study patient with a normal group of the same sex. After 3 months of study participation: (1) Mean HCT rose 38% from 29.2 to 40.0% in men and from 29.1 to 41.2% in women; (2) WBV increased appropriately with respect to HCT (r=0.64); (3) WBV measured at high (230-1s) and low (23.0-1s) rates of shear in men and women was not significantly different from normals (p = >.5) for all values of HCT; (4) PV (1.67±0.15 for men, 1.65±0.08 for women) did not change in either sex and was not significantly different from PV of normals (1.63±0.14 for men, 1.57±0.08 for women).

We conclude that in the dose range studied: (1) r-HuEPO therapy restores HCT to normal in anemic non-dialysis patients with renal insufficiency, (2) WBV increases appropriately in both sexes with respect to the r-HuEPO-induced rise in HCT, and (3) PV is unaltered by r-HuEPO therapy.

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

As the course of MGN is variable, treatment efficacy is difficult to assess. To deal with this problem 5 consecutive patients with at least 6 months of documented progressive renal functional decline were treated with CY (100 mg qd x 12 mo). Four also received P (1.5 mg/kg originally then tapered). Each patient served as his own control. At start of treatment plasma creatinine (PCr) ranged from 2.1 to 3.0 mg/dl. Pre-treatment I/PCr slopes, negative in each patient, became positive in each case by 6 months post treatment. The mean \pm SEM pre Rx slope obtained at 3 month intervals was minus 0.07 ± 0.01 and post Rx was plus 0.1 ± 0.02 ($p < .01$). Mean values for the 5 patients were:

	-6 mo	at Rx	+6 mo	+12 mo
PCr mg/dl	1.8**	2.4	1.7**	1.8*
Palbumin g/dl	2.7	2.1	3.4**	3.7*
Pcholest mg/dl	501	532	374	348*
Uprotein g/240	7.5	11.0	5.4*	2.4*

**($p < .01$ vs at Rx)
* ($p < .05$ vs at Rx)

The 4 patients with edema pre Rx were edema free post Rx. Three patients were re-biopsied post Rx. Despite clinical improvement they showed progression from stage I to III, II to III-IV and III-IV to IV. By using the patient as his own control these results demonstrate a striking improvement in the clinical course of MGN with CY-P despite no improvement in renal histology.

CYCLOSPORIN (CY-A) TREATMENT OF NEPHROTIC SYNDROME (NS) IN CHILDREN. R. Burghard, W. Klein, J.U. Leititis, L.B. Zimmerhackl and M. Brandis (intr. by R.B. Sterzel). Dept of Pediatrics, Univ. Marburg, FRG.

In a prospective clinical trial 9 children with NS aged 2.7-15.9 y were treated with CY-A for 6 months. On renal biopsy 7 had signs of focal segmental glomerular sclerosis (FSGS), 2 minimal changes (MC). In 7 steroid resistant NS (6 FSGS, 1 MC) a 6 month treatment with CY-A with blood through levels between 100 and 400 ng/ml succeeded in complete remission in 5 patients. Concentration of proteins (PR), albumin (AL), cholesterol (CH) and urinary excretion of proteins (U-PR) before and after CY-A treatment were:

	PR (g/l)	AL (g/l)	CH (mmol/l)	U-PR (g/d)
BEFORE	46.0	14.9	15.7	10.0
AFTER	66.3	38.8	5.4	0.3
P<	0.05	N.S.	0.01	0.005

After discontinuation of CY-A, remission lasted for 1-16 months. In 2 pts CY-A had no influence on proteinuria, in 1 pat renal function deteriorated. In 2 pts with steroid dependency complete remission was maintained by CY-A; 1 pat relapsed immediately after discontinuation, 1 pat 4 months later.

SUMMARY: CY-A was partially successful for treatment of steroid resistant NS. In steroid dependant NS long lasting remission could not be obtained.

CONCLUSION: CY-A can be justified as an experimental treatment for steroid resistant nephrotic syndrome. A controlled multicenter study has been started.

B CELL FUNCTION IN UREMIC PATIENTS.

Carl J. Cardella, Camellia Mavandadi,* Joshua Weissgarten,* Eva Friedman,* Chang-Ming Ng.* Department of Medicine, Toronto Western Hospital, University of Toronto.

B cell function in uremic pts. may be different than normal controls. Cells from controls (n=10), pts. with chronic renal failure, (C.R.F.), (n=8) or on dialysis (D) (n=19), were stimulated with either PWM, PHA or SAC. IgG, IL-2, soluble IL-2 receptor (IL-2 R-S) and cell bound IL-2 receptor (IL-2 R-C.B.) were measured.

After PWM the IgG was 7747 ± 1831 ng/ml in controls and 2633 ± 510 ng/ml in uremic pts. ($p = 0.002$). The IgG in CRF pts. was 1153 ± 510 ng/ml and 3282 ± 673 ng/ml in D pts. ($p = 0.01$). After PHA T cells of controls produced 1.16 ± 0.24 units / ml of IL-2 as compared to 0.51 ± 0.1 units /ml of IL-2 from uremic pts., ($p = 0.003$). IL-2 R-S from T cells of controls was 3111 ± 529 units/ml and 2041 ± 221 units/ml in uremic pts., ($p = 0.03$). IL-2 R-C.B. was 1357 ± 209 units / 10^6 cells in controls and 809 ± 94 units / 10^6 cells in uremic pts., ($p = 0.007$). After SAC, the B cells of controls produced 471 ± 60 units/ml of IL-2 R-S as compared to 294 ± 42 units /ml for uremic pts., ($p = 0.02$). IL-2 R-S from B cells of CRF pts., was 104 ± 44 units /ml as compared to 396 ± 32 units /ml from D pts., ($p = 0.001$). IL-2 R-S in the serum of controls was 231 ± 16 units /ml as compared to 895 ± 52 units /ml in uremic pts. ($p = 0.0001$).

Ig production is impaired in all uremic pts. especially those with CRF. This may be due to inadequate amounts of IL-2, IL-2 R-C.B. and IL-2 R-S from cells which are exposed to high serum levels of IL-2 R-S.

ACID-BASE ABNORMALITIES IN CARDIOPULMONARY ARREST: VARYING PATTERNS IN DIFFERENT LOCATIONS WITHIN THE HOSPITAL. J.A. Chazan and D. McKay*, Division of Renal Diseases, Rhode Island Hospital, Brown University, Providence, RI

The records of all patients (67) who suffered cardiopulmonary arrest (CPA) over a 4-month period at RI Hospital were evaluated. The acid-base abnormalities in the first arterial blood sample obtained after resuscitation was instituted were analyzed. The patients were divided according to the location within the hospital where the arrest occurred: emergency department (ED) 23, general hospital bed (HB) 22, or intensive care units (ICU) 22. Acidemia was most severe in patients who presented either in the ED ($[H^+] = 71 \pm 31$ nEq/L) or in a HB ($[H^+] = 78 \pm 35$ nEq/L). This was the result of both metabolic ($[HCO_3^-] = 20 \pm 16$ or 15 ± 10 mEq/L) and respiratory acidosis ($paCO_2 = 59 \pm 30$ and 50 ± 24 mmHg). In contrast, patients on the ICUs were only mildly acidemic or even alkalemic ($[H^+] = 53 \pm 29$ nEq/L); hypercarbia was uncommon ($paCO_2 = 36 \pm 18$ mmHg) with a similar degree of metabolic acidosis ($[HCO_3^-] = 18 \pm 8$ mEq/L). Patients who were successfully resuscitated (20) did not differ from those in whom resuscitation failed in the degree of acidemia or the location of the arrest.

Conclusions: 1) Patients who have CPA on ICUs usually do not have severe acidemia because most have endotracheal tubes in place and are receiving mechanical ventilation prior to cardiopulmonary arrest. 2) The acidemia observed in patients in ED or HB is primarily due to hypercarbia; therefore, therapy should be directed toward adequate ventilation with few patients requiring $NaHCO_3$ administration.

CORRECTION OF SYMPTOMATIC HYPONATREMIA.

Jen-Chieh Cheng, Demetrios Zikos, Hal A. Skopicki,* Darryl R. Peterson,* and Kenneth A. Fisher. Dept. of Medicine and Physiology, Univ. of Health Sciences/The Chicago Medical School, and VA Medical Center, North Chicago, Ill.

There is considerable dispute between fast and slow correction for treatment of symptomatic hyponatremia (SH). A serum sodium level (SNa) of 110 mEq/L has been proposed to separate rapid correction (RC) from slow correction (SC). We studied, retrospectively, the outcome of 28 episodes of SH (≤ 120 mEq/L) in 8 psychogenic polydipsic patients. 10 of 12 episodes with SNa ≤ 110 mEq/L were treated with hypertonic saline (RC). 12 of 16 episodes with SNa > 110 mEq/L were treated with water restriction (SC).

Episode/Pt Number	Serum Sodium (mEq/L) (Mean \pm S.E.)	Correction Rate (mEq Na/L/hr.) (Mean \pm S.E.)
RC 10/6	106.1 \pm 1.2	2.52 \pm 0.38
SC 12/6	118.2 \pm 0.5	0.86 \pm 0.16

The difference in correction rate is significant ($p < 0.01$). 5 patients had episodes with SNa both above and below 110 mEq/L and were treated with SC and RC, respectively. In no instance did therapy induce hypernatremia. All patients have been followed for a minimum of 5 years and none have developed alteration in their baseline neurological examination.

We conclude that in SH associated with psychogenic polydipsia, it is safe to treat patients with SNa ≤ 110 mEq/L by RC into the 120-130 mEq/L range and equally safe to treat patients with SNa > 110 mEq/L by SC.

THE RELATIONSHIP OF SEROLOGICAL CHANGES TO EXACERBATIONS (EX) IN SLE. J. Clough*, M. Pohl, L. Hebert, R. Rohde*, and the Lupus Nephritis Collaborative Study Group (LNCSSG). Cleveland Clinic, Cleveland, Ohio, Ohio State University, Columbus, Ohio, and Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill.

In the Lupus Nephritis Collaborative Study, a randomized, controlled trial of the effects of plasmapheresis therapy on the clinical course of severe lupus nephritis, we investigated the ability of commonly measured serological parameters to predict SLE EX. EX were defined prospectively as clinical events requiring changes in treatment to one of five categories of major and minor, renal and nonrenal protocols. For the 86 patients (pts) studied, data were available for 55 EX (30 major and 25 minor) preceded by at least 16 weeks of uninterrupted steroid taper in 41 pts. 50 control periods were identified in 25 pts who had no EX. The probability of an EX within any 8-week period was 13.9%. Positive and negative predictive values (PPV and NPV) for EX within 8 weeks were calculated for anti-DNA, C1q-binding activity, cryoglobulin levels, C3 and C4 levels. PPV $> 50\%$ were found for anti-DNA > 100 units (sensitivity = 27%) (Farr assay, Amersham), C3 < 55 mg/dl (sensitivity = 36%), and C4 < 15 mg/dl (sensitivity = 44%). None of the tests alone had satisfactory NPV, but negativity of all 5 tests yielded NPV = 95.6%; however, only 38% of 8-week periods without EX met this criterion. None of the assays distinguished mild vs severe EX or renal vs nonrenal EX. We conclude that serological changes often associated with EX of SLE are not dependable predictors of increased disease activity.

URINARY ALBUMIN EXCRETION IS FIXED IN OLDER TYPE I DIABETIC PATIENTS: COMPARISONS OF DAY, NIGHT, AND TIMED SPECIMENS. A. Chonko, W. Moore*, J. Kyner*, M. Bernard*, and T. Wiegmann*. University of Kansas Med. Ctr., Kansas City, KS, and Veterans Administration Med. Ctr., Kansas City, MO.

Urinary albumin excretion rate (AER, ug/min) maybe an important early indicator of renal dysfunction and a predictor of diabetic nephropathy. A variety of techniques have been used for the determination of AER with some resultant confusion about definitions for abnormal AER from daytime (D) and nighttime (N) and timed morning specimens (S) in 135 patients with type I diabetes mellitus, grouped by age (I: < 18 years; II: > 18 years) and AER/24 hours (a: < 20 ; b: > 20 ug/min) Mean \pm SEM:

	N	D	N	S
Ia	60	6.39 \pm 0.9	3.09 \pm 0.4	9.49 \pm 1.5
Ib	12	91.52 \pm 25.2	66.66 \pm 29.5	225.98 \pm 166.4
IIa	45	7.86 \pm 0.9	5.41 \pm 1.0	11.15 \pm 2.6
IIb	18	156.93 \pm 37.9	164.78 \pm 45.36	133.27 \pm 38.8

AER from 24-hour values correlated best ($r=0.9$; $p < 0.01$) with D in all groups. AER was highest in S, and always lower in N than D in groups Ia, Ib, and IIa. This is consistent with observations on exercise induced proteinuria and reflects adaptive hemodynamic changes. The effect was lost in group IIb (older patients with an abnormal AER). We propose that these findings represent a loss of vascular/renal response in older patients.

THE ELECTROLYTES IN HYPONATREMIA (HN) D. Corish* and ML Graber. VAMC, Northport, and SUNY at Stony Brook, New York.

Retention of water is the dominant factor producing HN, and serum sodium concentration falls by this dilutional effect. To see if other electrolytes or cells are also diluted, we identified 51 patients with HN and compared their lab values when serum Na was normal to values at a time of HN (nml = 138 \pm 1 mEq/L, HN = 121 \pm 1 mEq/L). The median interval was 12 days.

Regardless of ECF volume status, serum Cl fell by the same % as Na, but there was no appreciable change in HCO₃ (26.1 \pm 0.6 vs 25.2 \pm 0.8), K (4.31 \pm 0.10 vs 4.33 \pm 0.15), RBC mean cell volume (89.9 \pm 1.5 vs 88.6 \pm 1.1), albumin, PO₄, or creatinine. Complete urine lytes were available in 31 patients: All 16 pts with clinically normal ECF volume had a UNa > 10 mEq/L, but 10 of the 16 had a FENa $< 1\%$. 11 of 15 patients with ECF depletion or excess had a FENa $< 1\%$, 4 had a UNa < 10 mEq/L, and 11 had a UNa < 30 mEq/L.

The data indicate 1) that UNa and FENa may not be reliable indicators of ECF volume status during HN, and 2) that unlike Na and Cl, most plasma electrolytes do not show effects of dilution. We propose that the stability of K and HCO₃ reflects the ionic changes which take place as cells attempt to regulate their internal volume in response to the hyposmolar state.

COST EFFECTIVENESS OF EVALUATION FOR NEOPLASIA IN PATIENTS WITH ASYMPTOMATIC MICROSCOPIC HEMATURIA. Howard L. Corwin and Marc D. Silverstein.* Dartmouth Medical School and University of Chicago, Hanover, NH and Chicago, IL.

Asymptomatic microscopic hematuria is a common clinical problem which may be a presenting sign of neoplasia. The cost effectiveness of evaluation these patients for neoplasia has not been analyzed. We used decision analysis to compare patient outcome with no evaluation. Outcome utilities assessed were quality adjusted life years (QALY) and diagnostic cost. We assumed that evaluation would result in a stage I diagnosis in 90% of patients with neoplasia. Estimates of test characteristics, disease prevalence, survival, disease staging, and test morbidity were taken from the literature. Analysis was performed for age ranges 30-75. In all age groups we found evaluation resulted in an increase in QALY for the 2% of patients with neoplasia (5-18 yrs at age 30 to 2-4 yrs at age 75). However, the average gain in QALY per patient evaluated was small (2-8 months at age 30 to 1-4 months at age 75). The marginal cost effectiveness of evaluation vs no evaluation ranged from \$4000 per QALY gained at age 30 to \$11,000 per QALY at age 75. The analysis was not sensitive to variations in short term treatment morbidity, disease prevalence, and test morbidity. We conclude that marginal cost effectiveness (cost per QALY gain) is high for all ages. However, it is lowest for younger patients. Evaluation results in a marked increase in QALY for patients with neoplasia, although the average gain in QALY in the total hematuria population evaluated is small.

RACIAL DISPARITY IN DIABETIC END-STAGE RENAL DISEASE (DM-ESRD) SURVIVAL AFTER CONSIDERING TREATMENT, DM TYPE AND HEALTH STATUS. CC Cowie,* FK Port, RA Wolfe,* PP Moll,* PJ Savage,* VM Hawthorne.* Univer. of Michigan, Ann Arbor, MI.

Cox survival analysis of Michigan (MI) Kidney Registry data for all DM-ESRD patients with incidence in 1974-1983 followed through 1985 revealed markedly greater survival in blacks (n=470 B) vs. whites (n=861 W). The opposite is expected if the higher DM-ESRD incidence reported in Bs is due to greater hypertension prevalence or poorer medical care. Greater survival is expected however if a higher percent of B relative to W DM-ESRD patients have less severe non-insulin-dependent DM. Further Cox analysis of the southeastern MI subpopulation (n=310 W, 284 B) age <65 at DM-ESRD with data by medical chart review on DM type and health status at DM-ESRD found 45% greater survival in Bs while assessing these other variable effects. Relative to those always on center dialysis, survival was greater by 45% in those ever on home dialysis and by 70% in those ever transplanted. Stroke and congestive heart failure before or at DM-ESRD decreased survival 36% and 31% respectively. Survival was decreased in patients with lower systolic blood pressure (BP) at DM-ESRD, extremes of diastolic BP, higher random blood glucose before DM-ESRD and earlier year of DM-ESRD. B relative to W survival was greater by 52% in those always on center dialysis, by 37% in those ever on home dialysis, and by 18% in those ever transplanted (p=.05 overall). DM type, age at DM-ESRD, and other cardiovascular indicators did not affect survival. These results suggest greater survival in blacks even after considering treatment, DM type and health status.

Tc-99m DTPA AND I-125 IOTHALAMATE (Io) PLASMA (P) CLEARANCES IN DIABETIC (DM) PATIENTS. B. Croft*, F. Pauley*, L. Noland*, and K. Bolton, Univ. of Va. Sch. of Med., Charlottesville, Va.

The renal clearance of Io is a recognized method for estimating GFR. In DM pts the urinary (U) bladder may not be emptied when U sampling is performed. If retained U remains constant, GFR is minimally affected. If not, estimation of U volume (V) and flow rate in an interval may be incorrect. The purpose of this study was to compare DTPA and Io clearances in DM pts, and assess the effect of retained U on GFR. Tc-99m DTPA was infused with Io for simultaneous GFR measurements and estimation of retained UV for 4 intervals each in 11 pts. Retained UV was estimated by imaging with Tc-99m with an Anger camera and computer before and after voiding. Corrections were made for the time elapsed between counting and voiding. A corrected Io GFR using voided plus retained U was then calculated. The average of 44 intervals of retained U measurements was 75±72 ml, (0 to 225 ml), while the average U voided was 254±147 ml (0 to 640 ml). Pts with ≥ 50 ml retained U had 20±27% variation in U flow vs 6±4% for those with < 50 ml; variation in GFR was 68±30% for retainers vs 25±12% for non-retainers, p<0.05. Steady state GFR in retainers was 73±14 (Io) vs 82±17 ml/min (corrected Io). The U/P values for Tc-99m correlated with Io: Tc-99m DTPA-U/P = -0.130 + 0.954 (Io-U/P), r = 0.987.

Thus, in these DM pts DTPA is equivalent to Io for P clearances; U retention influenced U flow and GFR; U retention/flow corrections can be made by DTPA but not Io. Corrected DTPA alone, or DTPA corrected Io may be preferable to Io for GFR estimates in DM pts.

OSTEOMALACIA (OM) IN MODERATE RENAL FAILURE (RF) DUE TO INTERSTITIAL NEPHRITIS. A. Czerwinski, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, Okla.

Moderate RF is associated with hyperparathyroidism, osteitis fibrosa, and minimally decreased levels of 1,25(OH)₂D. Skeletal symptoms are usually not observed. During the past 2 years, 4 patients (pts) with early or moderate RF (serum creatinine 2.8±1.4 mg/dl) presented with diffuse musculoskeletal pain and/or non-traumatic fractures. Three pts ingested large quantities of analgesics and all 4 pts had hyperchloremic acidosis. Laboratory findings (mean ± SD) including serum calcium (Ca), phosphorus (P), alkaline phosphatase (AP), PTH, [upper limits of normal (ULN) - 340], 25OHD (N 10-55), and 1,25(OH)₂D (N 20-76) were:

Ca	P	AP	PTH	25OHD	1,25(OH) ₂ D
(mg/dl)	(mg/dl)	(IU)	(pg/ml)	(ng/ml)	(pg/ml)
8.1±1.2	3.4±.8	253±117	5396±7585	16±6	19±9

Iliac biopsies revealed OM and were characterized by increased osteoid surface (77±24%, ULN 25%), relative osteoid volume (43±29%, ULN 5%), decreased mineralization, and no stainable aluminum. The decreased mineralization was characterized by a decreased number and broad tetracycline labels, and did not resemble aluminum associated OM. Treatment with 1,25(OH)₂D resulted in marked clinical improvement in all pts.

In summary, 4 pts with moderate RF due to interstitial nephritis presented with diffuse musculoskeletal pain and bone biopsies revealed OM. All had a marked clinical improvement with 1,25(OH)₂D. In conclusion, in moderate RF, OM can be induced by interstitial nephritis and may be secondary to impaired synthesis of 1,25(OH)₂D.

THE RENAL FUNCTIONAL RESERVE (RFR) IS NOT ALTERED IN CHILDREN WITH CHRONIC RENAL FAILURE (CRF). NG DeSanto, CG Capasso, P Anastasio, S Coppola, P Castellino, G Lama, C Giordano. Intr. by RA DeFronzo. I Facolta' di Medicina, Napoli, Italy.

There is a renewed interest in RFR which is reduced in adult patients with CRF. The goal of this work is to study RFR in normal children with CRF by means of a protein load (PL) of 2 gr/Kg/min of red meat. Clearance of Inulin (GFR) and para-amino-hyppurate (RPF) were evaluated before (3 thirty-minute baseline clearances) and after the protein load (6 thirty-minute clearances) in 12 normal (N) children (age 8-18 yrs) and 10 CRF children (age 10-18 yrs) with plasma creatinine above 3 mg/dl. Filtration fraction and percent increase over basal were also calculated for GFR and RPF. In N, basal GFR averaged 127 ± 26 ml/1.73 m² min and rose to a mean value of 154 ± 25 ml/1.73 m² min. In CRF basal GFR averaged 46 ± 15 ml/1.73 m² min (both $P < 0.05$). In N basal RPF averaged 560 ± 100 and rose to 670 ± 142 ml/1.73 m² min ($P < 0.05$) after the meal. In CRF basal RPF was 270 ± 154 ml/1.73 m² min and rose to 362 ± 201 ml/1.73 m² min ($P < 0.05$). No change was observed in the filtration fraction after the protein meal in both groups. The percent increase above basal for GFR and RPF in N was not different than in CRF. The data indicate that for this age group and this stage of renal failure: 1) RFR is normal, and 2) there is a lack of any role for the protein load test in assessing renal parenchymal damage.

COMPARISON OF DIETARY PROTEIN RESTRICTION TO ANGIOTENSIN CONVERTING ENZYME INHIBITION IN NEPHROSIS: A RANDOMIZED STUDY. B. Don,* F. Hutchison, G. Kayser, and M. Schambelan. Martinez VA and San Francisco General Hospitals, Univ. of Calif., Davis and San Francisco, CA

Both dietary protein restriction and angiotensin converting enzyme inhibition have been reported to reduce proteinuria in patients with renal disease. In the present study we compared these two treatments in 9 nephrotic patients ingesting a constant metabolic diet containing 1.6 g/kg protein. After baseline data were obtained, subjects were randomly assigned to either a low protein diet (LOPRO) or enalapril (ENAL). LOPRO (n=4) was effected by changing to an isocaloric 0.8 g/kg protein diet: proteinuria decreased significantly in only 2/4 (mean -0.8 ± 1.1 g/d, NS) whereas albumin synthesis decreased in all (-4.0 ± 1.3 g/d, $p < 0.05$). ENAL (n=5) was titrated to reduce mean arterial pressure (MAP) by 10 mmHg: in 3 with normal PRA (0.6-2.6 ng/ml/h), ENAL (20-80 mg/d) reduced MAP (-11 ± 1 mmHg, $p < 0.002$) and proteinuria (-2.9 ± 0.4 g/d, $p < 0.02$) without reducing albumin synthesis (-0.3 ± 2.6 g/d, NS); in a subject with low PRA (0.2) MAP and proteinuria were unchanged despite 80 mg/d; in a subject found to have hyperreninemia (PRA 13.3) and bilateral renal artery stenosis, transient hypotension and azotemia followed one 10 mg dose. Proteinuria remained reduced in 2/3 patients on long-term ENAL therapy. Thus, ENAL diminishes proteinuria in nephrotic patients with normal PRA without reducing the rate of albumin synthesis, an effect that may confer an advantage for inhibitors of angiotensin converting enzyme in the management of this disorder.

SURVIVAL ANALYSIS OF PATIENTS ON DIALYSIS. Louis H. Diamond, Mark V. Pauly, Philip Held. Georgetown Department of Medicine, D.C. General Hospital, Washington, D.C.

Health Care Financing Administration patient specific medical and financial files merged on 4,661 patients admitted dialysis 1977 and followed 4-5 years. Relative risk of death compared with an arbitrarily designated comparison group with all other covariates held constant:

Patient Covariates	Relative Risk of Death
male vs female	1.15
black vs. white	0.84
20-39 yrs. vs 40-59 yrs.	0.76
60-69 yrs vs 40-59 yrs.	1.67
70-79 yrs vs 40-59 yrs.	2.44
80+	3.10
open vs closed staffing	1.20
Reuse vs no reuse	0.88
Large units vs small	0.89
For profit freestanding vs hospital	0.88
High risk case mix vs low risk	2.61
Median family income of \$22,000 vs \$21,000	1.01
Low market concentration vs high concentration	1.03

The data demonstrate that various dialysis unit characteristics (type, profit status and market concentration and size) do influence patient mortality, as do certain patient characteristics (sex, race, age, income) and that the unit characteristics impact on patient mortality with patient covariates held constant.

CHARACTERISTICS OF MANNITOL-INDUCED (MI) ACUTE RENAL FAILURE. H. Dorman*, J. Sondheimer and P. Cadnapaphornchai. Wayne State University, School of Medicine, Detroit, MI.

The protective effect of (M) on renal ischemia is well known. However MI acute renal failure (MI-ARF) has not been well appreciated. We report herein seven cases of MI-ARF. All patients received M to reduce brain edema. There were 3 males and 4 females. Their ages ranged from 34-82 years (mean 61). ARF developed within 2-4 days after initiation of M in the absence of any other identifiable causes of ARF. The total doses of M were 628 ± 312 gm (mean \pm SD) given over 3 ± 1 days. Four patients developed oliguria and 3 non-oliguria. Characteristic renal tubular epithelial (RTE) cells containing large vacuoles were seen in the urinary sediments of patients whose urines were available, a finding not reported previously. Peak levels of BUN, creatinine and K⁺ were 56 ± 21 mg/dl, 5.4 ± 2.7 mg/dl and 5.7 ± 5 mEq/L respectively. Hyponatremia (serum Na 120 ± 8) and acidosis (CO₂ content 15 ± 3) developed in all patients. Peak osmolal gaps were 69 ± 41 . Changes in serum creatinines correlated significantly with osmolal gaps. ($p < 0.01$), an indication of M retention during reduced GFR. The daily doses of M also correlated significantly with serum creatinines suggesting a causal relationship ($P < 0.01$). When M was stopped, the serum creatinines returned to baseline within 7 days. We conclude that MI-ARF is not uncommon and is easily recognized. RTE containing vacuoles are characteristic. Recovery is rapid.

ENALAPRIL (E) IN NORMOTENSIVE TYPE 1 DIABETES (DM-1): A DOUBLE BLIND CROSS-OVER STUDY IN PATIENTS WITH ELEVATED GLOMERULAR FILTRATION RATE (GFR) AND RENAL PLASMA FLOW (RPF). K. Drummond, C. Levy-Marchal,* K. Laborde,* C. Kindermans,* C. Wright,* M. Dechaux,* and P. Czernichow*. Hôpital Necker Enfants Malades, Paris.

Since E reduces the transcapillary pressure gradient in experimental diabetes and since elevated glomerular pressure could be a factor in the glomerular hyperfiltration seen in DM-1 patients, we studied the effect of E on GFR, RPF, and mean arterial pressure (MAP) in 18 DM-1 patients with GRF over 155 ml/min/1.73m². GFR and RPF were measured by inulin and para-amino-hippurate clearance respectively. Mean age was 14 yr (SD 2.5, range 9-20) and duration of DM-1 6.2 yr (SD 4.2, range 1-15). Patients were randomly assigned to a course of E (0.5 mg/kg/day; maximum 20 mg) in divided doses followed by placebo (P), each for 4 weeks or vice versa, and studied after each 4 week period. Sequence of medication had no effect, and side effects were negligible.

	E		P		p
	mean	SD	mean	SD	
GRF (ml/min/1.73m ²)	170	29	162	31	N.S.
RPF (ml/min/1.73m ²)	790	131	743	151	N.S.
Filtration Fraction (%)	21	1.4	21	3.4	N.S.
MAP resting (mmHg)	77	8.0	85	8.3	.001
MAP upright (mmHg)	85	13.4	91	7.7	.05
Plasma renin (ng/ml.h)	35	27.6	2.6	1.7	.001
HbA _{1c} (%)	7.8	1.7	7.8	1.7	N.S.

Our study shows that although E altered the renin angiotensin system and reduced MAP it had no effect on GFR or RPF. This is compatible with the importance of factors other than glomerular hypertension in the pathogenesis of raised GFR in DM-1.

RELATION OF MICROALBUMINURIA AND GFR IN PATIENTS WITH NON-INSULIN DEPENDENT DIABETES MELLITUS.

Promod Duggal,* W. Gordon Walker, Judith Hermann,* George Tyler,* and Luis Gimenez. Johns Hopkins Hospital, Baltimore, Maryland.

Microalbuminuria (μ Alb) is reported to predict progressive nephropathy in IDDM implying that increasing μ Alb should regularly precede changes in GFR. μ Alb and GFR (Tc99 clearance) were measured in 36 NIDDM patients with hypertension presenting with normal serum creatinine (SCR). Demographic data plus fasting blood sugar (FBS), systolic blood pressure (SBP), spot μ Alb and GFR were obtained. Mean cohort age was 62 yrs and mean SCR was 1.04 mg/dl. The cohort was divided into two groups for analysis based on median GFR and the lower and higher GFR groups compared. No difference in age, sex, race or FBS was found. Significant difference was present in μ Alb, SCR and SBP of two groups. Group with low GFR (< the median of 77) had higher values for SCR (p=0.01), SBP (p=0.007) and μ Alb (p=0.02). Though mean SCR (low GFR group) > SCR (high GFR group) (1.13 vs 0.93), it was not high enough to provide clinically useful information for recognition of early renal involvement, thus SCR does not accurately reflect renal functional status in these patients. The data support previous findings that hypertension may adversely affect renal function in NIDDM and shows association of μ Alb with low GFR. However 1/3 of patients (6/18) with low GFR had no or low levels of μ Alb (<15 μ gm/ml) suggesting that other factor(s) may influence GFR in these patients or that renal impairment in NIDDM patients may not always be preceded by μ Alb.

PROGRESSION OF CHRONIC RENAL FAILURE (CRF) IN MAN DECREASED BY LONG-TERM THERAPY WITH THE CALCIUM BLOCKER NISOLDIPINE (NIS). HE Eliahou, D Cohen, D Herzog, B Hellberg, I Serban, S Gavendo, S Kapuler (Intr. by L Fine). Nephrology Dept. Chaim Sheba Med Ctr. Tel-Hashomer, Israel.

CRF patients with stable course were randomized A. Standard antihypertensive therapy+Placebo and B. Nis therapy. Patients were protein-restricted and on anti-hypertensive therapy. Slope of 1/serum creatinine versus months before and after intervention, was individually calculated. Protein intake assessed from urine N₂. Figures are Means SD.

	n	Slope X (10 ⁻³)		Protein Intake g/Kg/d	BP mmHg	
		Before	After			
A	14	4.67±3.3	7.9±6.6	0.85±0.16	146/93	137/92
		p(2s)=0.09			p(2s)<0.05	
B	16	7.58±4.8	4.87±4.6	0.85±0.2	151/91	141/86
		p(2s)=0.015			p(2s)<0.05	

A decrease in the rate of progression occurred in only 5/14 in A, but 12/16 in B. The % change in slope and % change in BP did not correlate. Dose of Nis group B averaged 20mg 17.7/d (range 10-60). Slope decreased significantly in Nis-treated and tended to increase in the placebo group (not significantly).

The calcium-blocker Nis reduced the rate of progression of CRF significantly in protein-restricted patients already treated for hypertension, with no correlation to BP when it is initially close to normal. This may be due to the prevention of calcium deposition in renal tissue, which occurs in CRF.

CORRECTION OF THE ANEMIA OF HEMODIALYSIS (HD) PATIENTS WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo): RESULTS OF A MULTICENTER STUDY. J.W. Eschbach*, and J.W. Adamson, Univ. of Washington, Seattle, WA., for the Cooperative Multicenter Epo Clinical Trial Group.*

We have treated 247 anemic HD patients with rHuEpo (AMGen, Inc.), 150-300 U/kg IV, 3 times/week for up to 4 months. All but 6 patients responded and the mean hematocrit increased from 22.9 to 33.5. Transfusions were required by 127 patients (average of 1.0 unit/month) prior to therapy but only 3 continued to require transfusions after rHuEpo therapy. Five non-responding patients had microcytosis (MCV <80). With rHuEpo therapy, the serum ferritin decreased by 45% after 4 weeks and 55 patients required IV iron to maintain iron stores and an erythropoietic response. Prior to rHuEpo therapy, 125 patients required antihypertensive medication. Of these, 21% had an increase in mean diastolic blood pressure (BP) of >10 mm Hg and another 26% required more medication. Of the originally normotensive HD patients, 17% had an increase in diastolic BP of >10 mm Hg. Thus, 32% of all rHuEpo-treated patients either increased diastolic BP >10 mm Hg or required more medication. Two patients experienced seizures associated with an increased BP. Four clotted their vascular access, and predialysis BUN, creatinine, and potassium did not increase. Antibodies to rHuEpo have been undetectable in 216 HD patients evaluated after 3-12 months of rHuEpo therapy. Our findings indicate that rHuEpo is effective in correcting the anemia of virtually all anemic HD patients. Higher blood pressure is the main adverse effect associated with correction of the anemia.

PREDICTING LEAD WORKERS AT RISK FOR IMPAIRED RENAL FUNCTION. Julian Espiritu,* John Vena,* and Rocco Venuto. SUNY at Buffalo, Depts. of Med. & Social and Preventive Medicine, Buffalo, New York.

We tested the hypothesis that duration of employment and blood lead levels (BPb) obtained from lead exposed factory employees while they were working may be used to predict late toxicity. The employees' health records were reviewed retrospectively in 1986, 4 yrs after the battery factory closed in 1982. Periodic BPb testing of these workers is mandated by Federal regulation. Workers with peak BPb on at least two determinations during employment of >75 mcg/ml and length of service (LOS) <3 yrs, and those with LOS >3 yrs with BPb of >55 mcg/ml were assigned to the High Risk Group (HRG) (n=63). The Low Risk Group (LRG) consisted of workers with employment BPb <55 mcg/ml (n=61). The age, blood pressure, BUN, serum creatinine (sCr), uric acid, and BPb were determined in 31 HRG and 23 LRG workers who agreed to participate in this study. High blood pressure (systolic >160 or diastolic <95 or on antihypertensive therapy was more common among HRG members but this difference failed to achieve substantial significance (p=0.08). Creatinine clearance (CrCl) was measured in 19 HRG and 23 LRG workers. The HRG had higher mean sCr and uric acid levels (p=.01) with 10 members having an abnormal CrCl (<90ml/min/1.73 m²) compared to 2 of 23 in the LRG (p=.05). These findings are independent of age. These data suggest that: 1) the HRG is associated with a higher rate of abnormal renal function and 2) that available BPb levels and LOS can be coupled with simple blood and urine tests to rapidly and inexpensively identify industrial lead workers at high risk for nephrotoxicity.

THE EFFECT OF BLOOD PRESSURE AND DIETARY PROTEIN INTAKE ON RENAL FUNCTION IN DIABETIC NEPHROPATHY. G.V. Evanoff, and E.J. Weinman. Univ. of Texas Medical School, Div. of Nephrology, Houston, Texas.

The rate of change (ROC) in renal function, 1/creatinine x 10⁻² per month (mean ± SEM) was investigated in 21 subjects with diabetic nephropathy.

The ROC was -1.36 with normal protein intake (NPD) and was -.35±.34 with restricted protein diet (RPD) (P<.05). The ROC was then correlated with the systolic (SBP) and mean arterial (MAP) pressures at each visit.

	RPD	NPD
SBP <140	.78±.59*	-1.11±.60
SBP 140-159	-.37±.59 ^o	-1.44±.66
SBP ≥ 160	-1.89±.31	-1.63±.36
MAP < 105	.23±.47**	-1.06±.39
MAP 106-115	-.39±.80	-1.78±.58
MAP > 115	-1.53±.54	-1.72±.77

*p<.01 vs. SBP>160 @p<.05 vs. SBP≥160
**p<.05 vs. MAP>115

During all intervals of RPD, the ROC was significantly lower with reduced SBP and MAP while no difference was evident during NPD. These findings suggest that reduction in systolic blood pressure slows the progressive renal dysfunction in individuals with diabetic nephropathy ingesting RPD.

ACCELERATED GROWTH FOLLOWING RECOMBINANT HUMAN GROWTH HORMONE (rhGH) THERAPY (R_x). Richard N. Fine, Vera H.K. Koch*, Barry M. Sherman*, Barbara H. Lippe*. UCLA Med Ctr., Dept. of Peds., Los Angeles, and San Francisco, CA.

The etiology of growth retardation in children with chronic renal failure (CRF) is multifactorial. To date no uniform approach has been effective in either reversing or preventing the phenomenon. Studies in uremic rats (Mehls and Ritz, Kid Int 24:S53, 1983) demonstrated significant improvement in short-term length gain following R_x with porcine growth hormone. We treated 5 males age 55.8±21.4 (r35 to 91) mos with CRF (Ccr 18.2±6.3; r14.3 to 29.5 ml/min/1.73m²) secondary to congenital diseases with rhGH (Protropin) 0.125 mg/kg thrice weekly subcutaneously for 6 mos. The mean Standard Deviation Score for height was -3.03±1.00 (r-1.8 to -3.9) at initiation of rhGH R_x and the pts had a mean annual growth velocity of 4.9±1.4 (r3.0 to 6.3) cm/yr prior to R_x. The bone age (BA) was 3.16±1.16 (r2.8 to 4.5) yrs at initiation of rhGH R_x. Following 6 mos of R_x the mean annualized growth velocity was 10.1±1.9 (r7.6 to 12.8) cm/yr (P<0.01), and Ccr was 16.6±3.9 (r13.0 to 22.8) ml/min/1.73m². Mean glucose tolerance data did not change after rhGH. BA advancement was consistent with mos of Rx. These preliminary data indicate that growth retarded children with CRF can respond to exogenous rhGH R_x with a marked acceleration in growth velocity.

SERIAL MEASUREMENTS OF 125 I-IOTHALAMATE CLEARANCE (IO), CREATININE CLEARANCE (CrCl) AND 1/CREATININE (1/Cr) IN PROGRESSIVE RENAL FAILURE. J. Fitzgibbons, G. Braden, M. Germain, Renal Division, Baystate Med. Ctr., Springfield, MA.

The decline in 1/Cr (Δ1/Cr) is frequently used clinically as a marker to estimate deterioration of renal function. However, it has not been validated by serial measurements of GFR using more direct methods. Furthermore, the rate of decline of GFR, and the constancy of this rate as renal function declines has not been reported.

IO has been used as a standard test for the evaluation of kidney disease in our laboratory for the past 10 years. We reviewed our records to identify patients who had serial measurements of IO demonstrating deterioration of renal function. 39 nondiabetics (ND) and 13 diabetics (D) were studied with a mean observation time of 20 ± 2.9 months.

Rate of Decline of GFR (Δ) (ml/min/month)

	ND	D
IO	.72 ± .09	1.25* ± .20
CrCl	1.05** ± .20	1.66** ± .27

(*p<.05 compared with IO, ND)

(**p<.05 compared with IO)

The Δ for IO was constant over the whole range of GFR's, but for CrCl the rate decreased as GFR decreased (r = .28 p<.05). Δ 1/Cr correlated with the rate of decrease for both IO (r = .43) and CrCl (r = .80) (p<.001 for both correlations.)

Conclusions: 1) The Δ IO is less in ND than in D. 2) The rate of decline of CrCl is greater than IO in both groups. 3) The Δ IO is constant but the Δ CrCl decreases as CrCl falls. 4) Δ 1/Cr correlates with Δ IO and Δ CrCl.

MALE-FEMALE DIFFERENCES IN RAT BRAIN SYNAPTOSOMAL SODIUM TRANSPORT. Cosmo L. Fraser, Philip Sarnacki*, and Allen I. Arieff. VA Med. Ctr. and Univ. of California San Francisco, Dept. of Med., Divisions of Nephrology and Geriatrics, San Francisco, California.

It has been shown that acute hyponatremia produces detrimental consequences in female patients, while complications are less severe in males with the same degree and duration of hyponatremia. To determine if the ability of the brain to transport sodium was influenced by gender, we first performed sodium transport studies in synaptosomes (membrane vesicles from brain) that were isolated from normonatremic male and female rats ($\text{Na}=138\pm 2$ meq/l). In the basal (unstimulated) state, sodium uptake in the male and female groups were similar to each other (0.58 ± 0.1 vs 0.47 ± 0.2 nmol/mg prot., respectively). With 50 μM veratridine, uptake was stimulated to 1.04 ± 0.2 and 1.48 ± 0.2 nmol/mg prot. in the male and female groups, respectively. This resulted in increased sodium uptake of 79% in male and 214% in the female group. We then made rats acutely hyponatremic ($\text{Na}=110\pm 4$ meq/l at 72 hours), by intraperitoneal administration of 285 mM dextrose and subcutaneous vasopressin. At 5 seconds, sodium uptake in the basal state in males was twice as large as in females (0.62 ± 0.1 vs 0.26 ± 0.01 nmol/mg prot.), however, with veratridine stimulation uptake in males was increased by 61% while in female it was increased by 353%.

These data show that veratridine stimulated sodium transport in synaptosomes is significantly greater in female than in male rats, and that the difference is magnified by hyponatremia. This difference may be due to either increased synaptosomal membrane permeability to sodium, and/or to decreased ability of the Na-K ATPase pump to extrude sodium from synaptosomes in female rats.

URINARY RED BLOOD CELL (RBC) VOLUME DIFFERENTIATES GLOMERULAR (G) AND NONGLOMERULAR (NG) HEMATURIA (HMT) P. Goldwasser*, A. Antignani*, A. Norbergs*, N. Mittman, F. DiPillo*, and M.M. Avram, The Long Island College Hospital, Brooklyn, NY & Metropolitan Hospital Center, New York, NY

It has been proposed that urinary RBC size distribution and morphology can differentiate G from NG HMT. We studied RBC size and morphology in 16 pts with trace or more HMT with prior classification as G (n=8) based on renal biopsy or clinical criteria, NG (n=6) based on urological evaluation, or probable G, in 2 subjects with low complement, +ANA and trace albuminuria. Sediment was prepared from 10-40cc urine within 1 hr of collection and was divided into 3 aliquots for analysis of (a) presence of at least 50% dysmorphic RBC by phase contrast microscopy (PCM), (b) RBC size distribution and mean cell volume (MCV) by hematology analyzer (HA) and (c) effect on RBC distribution of incubation of sediment in distilled H₂O to cause hemolysis (H).

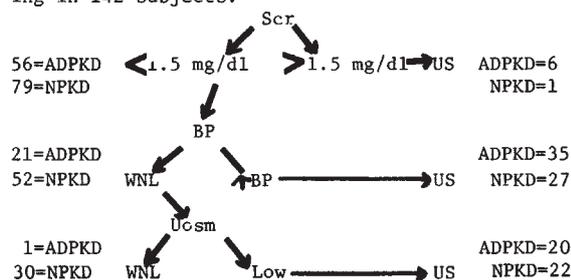
	N	MCV(range)	SEM	P(vsNG)	Dysmorphia
G	8	56fl (44-62)	2.5	<0.001	5/5
prob G	2	52 (43-61)	9.0	<0.01	2/2
NG	6	87 (81-93)	2.3		2/6

An MCV <72 was 100% sensitive and specific in identifying G HMT in this series. RBC size distributions were unskewed; they were abolished after H, confirming that the peaks represented cells rather than debris. After H, NG specimens manifested a new "RBC" distribution in the 10-50fl range strongly skewed to 10fl; this corresponded to RBC fragments under PCM.

We conclude (1) RBC volume is reduced in G HMT (2) RBC size analysis by HA can differentiate G and NG hematuria.

A PROPOSED SCREENING PROTOCOL FOR AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD). PA Gabow, AM Johnson*, IT Duley*, D Lezotte*, WD Kaehny. Univ of Co Hlth Sci Ctr, Denver, CO.

The diagnosis of ADPKD currently utilizes imaging studies, particularly ultrasonography (US) which is accurate, but moderately expensive. Therefore, we derived a potential screening protocol for ADPKD utilizing serum creatinine (Scr), blood pressure (BP) and renal concentrating ability [overnight dehydration, 5 units of aqueous vasopressin (SQ), measurement of urinary osmolality (Uosm)]. Subjects 18 to 69 years of age not receiving diuretics were included. Abnormal Uosm was defined with logistic regression techniques intended to maximize sensitivity of the screening protocol. The protocol demonstrated the following in 142 subjects.



Thus only 1 of 62 ADPKD subjects (1.6%) would have been missed and 30 of 80 NPKD subjects (38%) would have been spared US. Given that 600,000 Americans have ADPKD, this screening protocol could produce substantial economic benefits.

ESTABLISHMENT OF A CONTINUOUS TISSUE CULTURE SYSTEM FROM HUMAN POLYCYSTIC KIDNEY (HPKD) CELLS. Y. Granot, S.N. Summer,* V.J. Van Putten,* H. Granot,* P. Gabow, and R.W. Schrier. Univ. Colorado Sch. Med., Denver, CO.

The availability of a continuous cell culture of HPKD could provide an important methodological advance in studying these cellular and molecular abnormalities in this disease. We are reporting the first development of such a culture system. Primary cell cultures were obtained using the epithelial lining from HPKD kidney cysts which were allowed to reach 80-90% confluency. These cells were then successfully passed up to 10 times by trypsinization to 100mm plastic petri dishes (10^6 cells/dish) and were grown to 80-90% confluency. Subcultured cells were grown in RPMI-1640 media with 4 ng/ml dexamethasone, 50 ng/ml insulin, 20 ng/ml epidermal growth factor and 5% fetal calf serum. For future revitalization, HPKD cells were removed by trypsinization and resuspended 2×10^6 cell/ml in freezing media (growth media containing 8% glycerol) and stored submerged in liquid N₂. Subcultured and revitalized cells retained their characteristic epithelial polygonal and elongate shape and immunofluorescent staining for cytokeratin (>90%) throughout the passages. Cell numbers double every 3.5 days and reach 80-90% confluency after 10 days (5×10^6 cells/dish). The protein profiles of the subcultures, as determined by separation on polyacrylamide gels, are identical to primary HPKD cultures. This continuous tissue culture system will facilitate the further study of biochemical and physiological pathways involved in the development of HPKD.

THE PATHOLOGY OF THE LIVER LESION ASSOCIATED WITH AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE (ARPKD). P.C. Grimm*, D.A. Malatjalian*, M.R. Ogborn*, J.F.S. Crocker, Dalhousie Univ., Depts. of Peds. & Path., Halifax, Nova Scotia

The pathology of the liver in ARPKD is controversial. Different authors suggest very different anatomy. These include: dilation of the Canals of Herring, persistence of embryonic ductal plates and disorder of malformation and dilatation of the distal ducts in the portal tract. Techniques that have been used in studying these livers include stereologic, microdissection and serial sections with reconstruction. We studied the liver of a 2 day old infant. Kidneys were grossly enlarged and typical of ARPKD. The liver showed severe but typical histologic changes of the associated hepatic fibrosis at autopsy. Specimens were taken for histology, then Microfil® (Canton Biomedical) was injected into the common bile duct under 30 cm water pressure until the plastic was seen under the hepatic surface. The liver was removed and stored overnight at 4°C to harden the plastic, then dehydrated in graded alcohols, cleared in methyl salicylate and examined under the stereomicroscope. The distal intrahepatic bile ducts were dilated at multiple sites, occasionally flattened, and irregular compared to control human liver. In areas, a meshwork was formed with multiple anastomosis between ducts. No evidence of abnormal Ducts of Herring or a ductal plate abnormality was seen. Contrary to previous belief, the anatomy of the liver lesion in ARPKD is similar to that of the kidney with multiple areas of dilation of small ducts.

ATRIAL NATRIURETIC PEPTIDE (ANP) AND ENDOGENOUS DIGITALIS-LIKE NATRIURETIC FACTOR (EDNF) IN SIADH. P. Gross*, R. Lang**, M. Ketteler*, C. Hausmann*, E. Ritz*, H. Favre***. Univ. of Heidelberg, Depts. of Medicine and Pharmacology(+), Heidelberg, FRG, and Hospital Cantonal Universitaire de Geneve, Geneva, Switzerland(++). (Introd. by R.J. Anderson)

The potential roles of ANP and EDNF as indicators of ECF-volume status are incompletely understood. We therefore evaluated 22 patients with SIADH, measuring ANP (sensitive RIA) and EDNF (receptor assay) in their plasma, obtained under standard conditions at rest. Comparable measurements were also performed in additional hyponatremic patients suffering heart failure, n=25; cirrhosis, n=18; primary volume contraction, n=27. SIADH in hyponatremia was diagnosed a) after exclusion of the latter 3 conditions; in patients excreting >30 mM/l of urinary Na⁺ without being on diuretics; c) in the presence of a low PRA < 2 ng AI/ml·h without hyperkalemia or renal insufficiency.

We observed: ANP in chronic SIADH was 58.5±8.1 pg/ml, not different from control (54 pg/ml; range 12-85), as was ANP in cirrhosis (81.2±12.2) and volume contraction (81.5±18.6). ANP tended to be higher than control in hyponatremic heart failure (105.7±18.2; p N.S.). - EDNF was 130.6±13.8 nM/l in 8 water diuresing volunteers. EDNF was elevated above this value in hyponatremic patients: SIADH, 192.1±16.7 (p<.05); cardiac failure, 223±15.5 (p<.05); cirrhosis, 220.4±20.7 (p<.05); volume contraction, 247±25.3 (p<.05). Comparable findings were also obtained when EDNF was measured in 24-h urine of these patients.

In summary: In chronic SIADH, ANP failed to be stimulated above control. EDNF correlated more closely with hyponatremia than with ECF volume status.

SAFE AND EFFECTIVE TREATMENT OF THE HYPERLIPIDEMIA OF NEPHROTIC SYNDROME (NS) WITH GEMFIBROZIL. G.C. Groggel, A.K. Cheung, K. Ellis*, D.E. Wilson*. University of Utah School of Medicine and VA Medical Center, Salt Lake City, Utah.

Hyperlipoproteinemia, a known complication of the NS, has been associated with premature atherosclerosis in patients (pts) with persistent proteinuria. Until recently there has been no non-toxic, effective therapy for this disorder. We report the efficacy and safety of the use of gemfibrozil, an aryloxyalkanoic acid similar to clofibrate, in the treatment of nephrotic hyperlipidemia. 10 adults (2 diabetic, 2 females) with the NS were treated in a placebo-controlled, double blind, crossover study consisting of 6 weeks of gemfibrozil (1200 mg/day) and 6 weeks of placebo.

	Placebo	Gemfibrozil	P value
Cholesterol (mg%)	377±22	315±16	0.012
Triglyceride (mg%)	313±48	145±21	0.011
HDL Chol (mg%)	44±4	52±5	0.041
LDL Chol (mg%)	275±26	238±18	0.122
LDL/HDL	6.7±0.6	4.9±0.4	0.033
Apoprotein A-1 (mg%)	184±13	145±19	0.229
Apoprotein B (mg%)	167±10	110±16	0.011
Creatinine (mg%)	1.5±0.1	1.6±0.2	0.159
Albumin (gm%)	2.9±0.2	3.1±0.3	0.068
Proteinuria (mg prot./mg creat.)	3.5±1.7	4.9±3.6	0.291

mean ± SEM
Blood pressure did not change. There were no significant side-effects except for one pt who had a transient rise in CPK after vigorous exercise.

Gemfibrozil is well-tolerated and safe in nephrotic pts. Gemfibrozil appears to be effective in improving the hyperlipidemia of pts with the NS and should lower their risk for premature atherosclerosis.

DIURETICS ACCELERATE MICROALBUMIN EXCRETION RATES IN DIABETICS AND HYPERTENSIVES. W. Gordon Walker, Judith Hermann,* and Paul K. Whelton. Johns Hopkins Hospital, Baltimore, Maryland.

Our report (ISN 7-30-87) that hypertension accelerates albumin excretion rate (μAlb) in diabetics (NIDDM) has been pursued further in the longitudinal study of 86 non-insulin dependent diabetic subjects plus a cross sectional study of 432 elderly subjects (ES). Follow-up ΔμAlb is increased by maintenance thiazide diuretics (D) in persistently hypertensive (↑BP) and adequately controlled (norBP) NIDDM: norBP no D=22 ± 6; norBP (Rx) D=217 ± 100; ↑BP no D=118 ± 107; ↑BP D=1292 ± 440 (p<.0001). Further exploration in the 432 apparently healthy ES classified by BP, fasting blood glucose, presence, or absence of maintenance D Rx for ↑BP (171 norBP, 216 ↑BP, 44 NIDDM) revealed μAlb was significantly greater in ES receiving D than not (ND) (D=41.0 ± 11; ND=13.7 ± 2; p<.0001). This difference persisted after excluding hyperglycemics (D=31.2 ± 7.4; ND=13.0 ± 1.9 p<.0001) and remained when norBP and ↑BP were separated (norBP D=19.3 ± 5; ND=10.1 ± 2; p<.02; ↑BP D=40.5 ± 11.4; ND=16.2 ± 3.3; p<.002). These results were not explicable by differences in race, sex, age, serum creatinine or severity of hypertension. Most interesting was a correlation (r=+.44; p<.001) between μAlb and parathormone levels confined only to that subset of the cross sectional cohort receiving diuretics. All these findings support the hypothesis that diuretics may exert a deleterious effect on renal function in both NIDDM and hypertension.

RENAL HEMODYNAMICS (RH) IN RECENT ONSET ADULT INSULIN-DEPENDENT DIABETES (IDD): EFFECTS OF CAPTOPRIL (Ca) AND CYCLOSPORINE A (CyA).

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RH was studied before and after a single dose of 75 mg of Ca in 8 normal controls (N) and in 13 IDD, before (M0) and 3 months (M3) after chronic CyA administration (5 mg/kg.d). Glomerular filtration rate (GFR) and renal plasma flow (RPF) (ml/min. 1.73 m²) were measured by inulin and PAH clearances respectively. Fractional and absolute proximal fluid reabsorption rates (FPR % and ARP ml/min. 1.73 m²) were calculated from inulin and lithium clearances. In IDD, hemoglobin A1C decreased from 13.2±2.7 (M0) to 6.5±0.8 (M3) (p < 0.001). Results (mean ± SD) are :

	GFR	RPF	MAP	FPR	APR
N					
Before	123±17	649±92	89.9±10.7	0.71±0.04	88±15
After Ca	109±15**	670±93	85.3±6.2	0.67±0.04*	74±14**
IDD M0					
Before	143±21*	639±134	84.7±10.5	0.79±0.10*	114±25*
After Ca	135±23	678±126*	83.2±11.6	0.77±0.12***	104±23**
IDD M3					
Before	114±23§§	658±141	91.8±13.9§§	0.80±0.04	93±22§
After Ca	109±22	745±191*	90.6±13.9	0.76±0.06***	84±22*

MAP = mean arterial pressure (mmHg)

* p < .05 ; ** p < .01 ; *** p < .001 after Ca vs before Ca.

+ p < .05 M0 before Ca vs N before Ca.

§ p < .05 ; §§ p < .01 M3 before Ca vs M0 before Ca.

In conclusion : 1) Baseline GFR, FPR and APR were increased in IDD and did not return to normal values after Ca. The increase in GFR may be related to increased FPR, decreased distal delivery and depressed tubuloglomerular feedback. 2) CyA and control of diabetes decreased GFR without effect on RPF ; MAP increased slightly. Renal vasodilatory responses to Ca were unaffected after 3 months of treatment with CyA.

SIMILAR RISKS OF NEPHROPATHY IN TYPE I AND TYPE II DIABETES. Ch. Hasslacher, P. Wahl, E. Ritz (intr. by B. Brenner) Dept. Int. Med., Heidelberg, Germany.

It is commonly assumed that the risk of nephropathy in uremia is marked in type I and modest in type II diabetes. Since type II diabetes mostly concerns elderly individuals with limited life expectancy and high cardiovascular mortality, the true risk may have been underestimated. To further assess renal risk, we retrospectively analyzed all diabetics without severe secondary disease and with follow-up > 1 years who were seen in the outpatient clinic 1966-1983. 315 type I and 450 type II diabetics had no proteinuria and normal S-crea when first seen. 69/315 type I diabetics developed persisting proteinuria presumably from diabetic nephropathy (DN) and 7 from other causes (excluded from analysis); 64/415 type II diabetics developed pers. proteinuria from DN and 35 from other causes. Cumulative risk of proteinuria after 20 years (known) duration of type I or type II diab. was 32% and 28% respectively. The risk after 25 years was 58 and 73% respectively. Median (known) duration of diab. until onset of proteinuria in type I diabetics was 15 y. in males (8-31) and 21 y. (7-32) in females. Respective figures for type II diabetics 16 y. (9-32) in males and 15 y. (5-38) in females. Of the 69 type I diabetics with pers. proteinuria, elevation of S-crea (> 1.4 mg/dl) developed in 40% after 2.5 and 78% after 5 years. Respective figures in the 63 type II diab. 30% and 62%. Similar evolution of DN with time is surprising in view of relatively uncertain time of onset of type II diabetes. We conclude that similar risk of proteinuria and renal failure exists in type I and type II diabetics.

NUTRITIONAL MANAGEMENT OF INFANTS WITH CHRONIC RENAL FAILURE (CRF) WITHOUT DIALYSIS. W.E. Harmon, W.E. Grupe, and N.S. Spinozzi.* The Children's Hospital, Boston, MA and S.U.N.Y. Health Science Center, Syracuse, NY.

Previous data have shown that efficient nitrogen retention in infants with CRF is attained at energy intakes between 6 & 11.4 kcal/cm of height (\bar{x} =8.4 kcal/cm), providing protein intake does not exceed 0.15 gm/cm. The effect of this nutrient intake on growth and renal function using commercially available infant formula was studied prospectively in 7 infants 1-6 months of age who had congenital uropathies and CRF. Calculated urea nitrogen appearance (UGR) was used to monitor the efficiency of nitrogen retention. Under this protocol, all infants grew at a normal or accelerated rate as evaluated by Z scores. Three of 7 remained above the 5 %ile for length for the first 4 months of age; and two others accelerated growth to achieve at least the 5 %ile by 3 months of age. Neither the absolute UGR nor the BUN appeared to effect the rate of growth. Three infants with advanced renal failure had GFR's of 3.9-7.3% of normal for age (Scr levels of 6.0-10.1 mg/dl). With the production of an anabolic state and growth, GFR's improved 50-223% along with improvement in BUN, Cr, P & tCO₂. Dialysis was not necessary in any case. Growth rate declined after 6 months of age in 5 of 7 infants; the three with most profound CRF went on to transplantation while two others are currently being evaluated for transplant. Thus, attainment of energy intakes between 6 & 11.4 kcal/cm and protein intake that does not exceed 0.15 gm/cm has resulted in growth sufficient for transplantation without requiring dialysis.

COCCIDIOIDOMYCOSIS, AMPHOTERICIN B, AND THE KIDNEY. David Heaney, Visalia, CA.

Coccidioidomycosis usually affects the kidney on a microscopic level and not functionally. Amphotericin B has both reversible and irreversible effects on the kidney. Reversible effects include reduced creatinine clearance, distal RTA, renal potassium and magnesium wasting. Irreversible effects include acute renal failure without recovery, CRF accompanied by hypertension leading eventually to ESRD (end stage renal disease).

Five cases are presented. All had ESRD, 2 were transplanted, 2 died, 1 remains chronic hemodialysis. Two patients had IV Amphotericin B prior to starting chronic hemodialysis and developed ESRD. One had a bilateral nephrectomy 3 years post starting chronic hemodialysis which showed acquired cystic renal disease. Two patients had both tuberculosis and coccidioidomycosis. Both transplants are doing well. Mechanisms of renal damage by Amphotericin B included vasoconstriction, damage to distal tubular cells and indirectly by electrolyte depletive effects. Factors that decrease nephrotoxicity have not withstood controlled clinical trials in humans. Calcium channel blockers and angiotensin converting enzyme inhibitors have not been tried. IV Amphotericin B should be stopped at a serum creatinine of 2.5mg. These patients should have careful follow-up of both kidney function and blood pressure, as some of these patients go on to develop ESRD.

ACCUMULATION OF TRIMETHYLAMINES (TA) IN RENAL INNER MEDULLA AND BRAIN IN HYPERNATREMIA (HNa). CW Heilig*, and SR Gullans. Brigham and Women's Hospital, and Harvard Med. Sch. Boston, MA.

Osmolytes, including trimethylamines have been indentified in the renal inner medulla (IM). Since HNa increases idiogenic osmoles in the brain, we studied trimethylamines in the brains and IM of HNa rats. Perchloric acid extracts of brains and IM were prepared from control rats and from rats made chronically HNa (5-7 days) with daily 3cc gavages of 20% NaCl plus 0.32 M NaCl drinking water. HNa rats had significantly elevated plasma sodium levels (162 ± 7.5 SE vs 135 ± 1.8 meq/L). ^1H NMR spectra of brains revealed an increase in total TA from 2.9 ± 0.2 (6) to 4.7 ± 0.7 (6) umoles/gm wet wt ($p < 0.05$) with HNa. Glycerophosphorylcholine (GPC) and betaine, two specific TA solutes, were clearly identified but their overlapping peaks precluded quantitation. In the renal IM (Table) HNa caused a 2.5 fold increase in betaine although GPC and total TA did not significantly change. (mean \pm SEM, umoles/gm wet wt; * = $p < 0.01$):

	GPC	Betaine	Total TA
Control (5)	38.8 ± 4.7	13.2 ± 2.7	51.9 ± 6.2
HNa (6)	27.0 ± 2.9	$33.6 \pm 5.0^*$	60.6 ± 5.7

In conclusion, we have observed both GPC and betaine in normal and HNa rat brain and renal IM. Total TA was 10-20 times greater in renal IM than in brain. With HNa, there was an increase in total TA in the brain, whereas, in renal IM total TA remained nearly constant due to inverse changes in betaine and GPC.

EVALUATION OF Na-DEPENDENT DISTAL ACIDIFICATION (DA) IN PATIENTS WITH HYPERKALEMIC RENAL TUBULAR ACIDOSIS (RTA) USING BUMETANIDE (B) AND AMILORIDE (A). M Hizon* and DC Battle, Northw. Univ. and VA Lakeside Medical Center, Chicago, IL.

Na-dependent DA was studied in 13 patients with hyperkalemic RTA associated with selective aldosterone deficiency (SAD) and renal insufficiency (GFR 37 ± 8.9 ml/min, Aldo 14 ± 2.3 ng/dl). They were divided into two groups based on whether they could lower urine (U) pH below 5.5 during acidosis (Group I, n=7, pH 5.3 ± 0.09 ; group II, n=6, pH 6.4 ± 0.2). The inability to lower U pH of group II could be due to a defect in Na transport in the cortical collecting tubule (CCT) which resulted in reduced H⁺ and K⁺ secretion (i.e., a voltage defect) or due to a proton pump secretory defect coexisting with SAD. To distinguish between these two possibilities we used a single dose of B (2 mg po) to stimulate Na-dependent DA and A (20 mg po) to inhibit it. In controls with a GFR similar to that of the patients, B decreased U pH (from 5.4 ± 0.2 to 4.9 ± 0.08 , $p < 0.01$) and increased K excretion while A increased U pH from 5.3 ± 0.09 to 6.7 ± 0.1 , $p < 0.01$) and decreased K excretion. The U pH response was similar to that of controls in group I but not in group II patients whose U pH was only slightly influenced by B and A. In contrast, the kaliuretic response to both B and A was directionally similar to that of controls and not different between the two groups. We conclude that B and A are useful tools to assess Na-dependent DA. The ability to modulate K excretion in response to these agents argues against the presence of a voltage-dependent defect. A proton secretory defect best explains the inability to lower U pH seen in some patients with hyperkalemic RTA.

BODY FLUID VOLUMES AND TRANSCAPILLARY COLLOID OSMOTIC GRADIENT IN DIABETIC NEPHROPATHY. E Hommel*, K Aukland*, ER Mathiesen*, and H-H Parving* (intr. by S Anderson). Hvidøre Hospital, Klampenborg, Denmark.

We examined transcapillary fluid balance in Type I diabetic nephropathy. Plasma (P) and subcutaneous interstitial fluid (IF) (suction blister technique), colloid osmotic pressure and albumin (Alb) concentration, plasma volume (^{125}I -Alb), glomerular filtration rate (GFR) and extracellular fluid volume (^{51}Cr -EDTA) were measured in 9 normal subjects (Group I), 9 normoalbuminuric Type I patients (Group II), 16 Type I diabetics with nephropathy (III), and 14 Type I diabetics with slight peripheral edema due to nephropathy (Group IV). Results:

Group	GFR ml/min/1.73 m ²	PV/IFV	IF/P Alb
I	103 ± 13	0.30 ± 0.03	0.43
II	120 ± 19	0.27 ± 0.04	0.44
III	111 ± 19	0.25 ± 0.03	0.42
IV	$94 \pm 25^*$	$0.23 \pm 0.04^{**}$	0.30**

(Means \pm SD; * $p < 0.05$, ** $p < 0.01$ vs. other groups). Values for GFR, plasma volume/IF volume (PV/IFV) ratio, IF/P Alb ratio, and the transcapillary colloid osmotic gradient were reduced in Group IV compared to the other groups. The decreased interstitial fluid albumin concentration and colloid osmotic pressure can be partly explained by simple dilution, and partly by a reduction in interstitial protein mass (enhanced lymph flow). Thus, the wash-down of subcutaneous interstitial protein reduces the tendency to edema formation in diabetic nephropathy.

LIMITED UTILITY OF ROUTINE SEROLOGIC TESTS IN THE DIFFERENTIAL DIAGNOSIS OF THE ADULT IDIOPATHIC NEPHROTIC SYNDROME (INS). A Howard, S Gouge, J Lockard, K Melton, W Paulson*, D Tietjen*, J Moore. Nephrology Svc, Walter Reed Army Medical Center, Washington, DC.

From 1980-85 we biopsied 87 adults with INS. We tested whether serologic studies obtained routinely at time of biopsy improved clinical diagnostic accuracy. Each patient's record was edited to remove serologic data. History, physical exam, CBC, chemistry panel, urinalysis, and urine creatinine and protein were evaluated in a blind fashion by 3 nephrologists (N). Based only on this information, each N predicted whether the patient had INS or secondary nephrotic syndrome (SNS), and the most likely histopathologic entity. Six months later, each N re-evaluated the record, to which the results of serologic tests (VDRL, RF, FANA, complements, cryo's HbsAg, and sed rate) had been added. Each N again predicted whether each patient had INS or SNS, and the most likely histopathologic entity. Histopathology was established by renal biopsy. We analyzed the concordance between N¹ choices and biopsy results both before and after serologic tests were available with a kappa statistic. Pre-serology concordance was moderate ($k=0.52$), and identical to post-serology concordance ($k=0.51$) for both INS vs SNS and actual histopathology. Serologies were rarely abnormal without clinical suspicion, and added \$350 to the cost of each evaluation. These results suggest routine serologic testing does not improve diagnostic accuracy in adult INS.

MESANGIALPROLIFERATIVE GLOMERULONEPHRITIS (mes GN) IN SOUTHWESTERN (SW) AMERICAN INDIANS. WE Hoy, SM Smith,* DM Megill,* MD Hughson.* Lovelace Medical Foundation and Univ. of New Mexico, Albuquerque, NM, Phoenix AZ and Providence Hosp. El Paso, TX.

SW Indians have high rates of renal disease and renal failure. Over half is nondiabetic, poorly described and of unknown etiology. To better understand renal disease in these groups we reviewed all 143 Indian renal biopsies processed since 1971 at our centers: these probably comprise more than 90% of Indian biopsies from New Mexico and northeast Arizona. We compared them with an Anglo and Hispanic control series chosen at random from over 1200 biopsies over the same period. Rejecting transplants (TP) and inadequate tissue were excluded from all series.

Mes GN with immunoglobulin (Ig) deposition and mes electron dense deposits comprised 70% of Indian biopsies: 31/42 in the Zunis, 48/75 in the Navajo and 10/14 in the Rio Grande Pueblo Indians, Apache and Ute Indians. In comparison, 22.6% of 235 controls had Ig positive mes GN ($p < 0.001$).

60% of Indians with mes GN had nephrotic proteinuria, and 60% had renal insufficiency at biopsy. In 10% the disease was preceded by an HSP-like illness, and at least 11% were alcoholic. Male:female ratio was 1:1 in Indians and 2:1 in controls. IgA was the sole Ig in 5% of Indian biopsies, IgM alone in 7%, and both were present in 66%. Ig deposition changed in sequential biopsies in one individual. Mes IgA was found in a donor kidney biopsied at time of living related TP. The disease has recurred in transplants.

Mes GN is a major cause of ESRD in these tribes: the potential roles of infectious agents, alcohol use and an hereditary predisposition need study.

PROGRESSION IN CHRONIC RENAL FAILURE AND THYROID HORMONE. Yasuhiko Iino, Ken Tachibana*, Shouichi Ootsuka*, Kiyohide Fushimi*. Kimio Tomita, Naoki Yoshiyama*, Kouichi Taniguchi*. Tokyo Medical and Dental University, Renal Center, Tokyo, Japan

To elucidate the pathophysiology of the progression in chronic renal failure, thyroid function (free T₄, total T₄, total T₃, T₃ RSU, TBG) were evaluated in patients with chronic renal failure for more than 2 years, whose serum creatinine varied from 2 to 9 mg/dl (n=37) and blood pressure were well controlled. The progression of chronic renal failure was estimated by the changes in the reciprocal of serum creatinine. Free T₄ level was decreased in patients with chronic renal failure, although total T₄, total T₃, T₃ RSU, TBG were within normal limits. Renal function in patients with low free T₄ level (lower than 0.9 ng/dl) deteriorated faster than that with slightly low to normal free T₄ level (higher than 1.0 ng/dl). In addition, deterioration of renal function was correlated negatively with free T₄ level. The progression of the renal failure is thought to be deeply related to protein metabolism. Therefore, these decrease in free T₄ might be secondary effects related to protein metabolism. In other possibility, low free T₄ level actually indicates tissue hypothyroidism which affects cellular function and deteriorates renal function. In conclusion, free T₄ level in patients with chronic renal failure was low, and these changes might be related to protein metabolism which affected progression of renal failure, or might indicate tissue hypothyroidism which deteriorated the renal failure.

THE SINGLE KIDNEY - AGENESIS VS NEPHRECTOMY (Nx) B Ihle, M El-Khatib*, G Becker*, P Kincaid-Smith. Dept. of Nephrology, Royal Melbourne Hospital, Melbourne, Australia.

Focal glomerular sclerosis (FGS) is regarded as a consequence of loss of renal mass. We postulated that the greater capacity of the foetal kidney for hypertrophy should make FGS more likely in the remnant kidney secondary to unilateral renal agenesis (URA) rather than following Nx.

Biopsies from 17 patients (F=7, M=10) with single kidneys, proteinuria and/or impaired function were reviewed (\bar{x} age 44 ± 15 yrs). Biopsies were either done as an open procedure or under CAT control. Seven patients had had a Nx. The \bar{x} time from Nx to biopsy was 23 ± 8 yrs. At the time of biopsy, 4 were normotensive. The \bar{x} [creat] was 0.3 ± 0.2 mmol/l (range 0.13 - 0.85). Thirteen patients had protein excretion >1.0 gm/24 hrs (\bar{x} 2.6 ± 2.0 gm). Renal function in those with URA was worse: 0.34±0.13 vs 0.25±0.1 ($p < 0.05$).

Histomorphometrics was performed on all biopsies. Total glomerular sclerosis was \bar{x} 35±18% (range 11-78%). There was a significant correlation between [creat] and % of total sclerosis ($r=0.87$) $p < 0.001$, but insignificant ($r=17$) between sclerosis and proteinuria. Glomerular surface area (GSA):-

	Nx	URA	CONTROL
\bar{x}	7.5±2	10±2	3±0.5) x10 ⁻² mm ²
Range	5.1-10.8	7.8-12.3	2-3.9

$p < 0.01$ Nx vs URA

$p < 0.001$ C vs (Nx+URA)

Patients with URA develop larger glomeruli and worse function. This may reflect greater hemodynamic adaptation of the young kidney with a greater propensity to develop FGS.

HYPERVOLEMIA, RENAL HORMONES AND HEMODYNAMICS IN BLOOD PRESSURE CONTROL IN PATIENTS WITH PYELONEPHRITIC RENAL SCARRING. Stefan H Jacobson*, Carl M Kjellstrand, Lars-Eric Lins*. Dept. Med., Karolinska Hospital, Stockholm, Sweden.

Patients with pyelonephritic renal scarring are at risk of developing renal failure and hypertension.

We studied the mechanism of blood pressure elevation by following glomerular filtration rate (GFR), renal plasma flow (RPF), filtration fraction (FF), systolic (SBP) and diastolic (DBP) blood pressure, fractional sodium, potassium and phosphate excretion, peripheral renin activity (PRA), plasma aldosterone (p-Aldo), urinary albumin excretion (U-Alb) and urinary β_2 -microglobulin excretion (β_2 -M) in hydropenia (HP) and during transition to 3 % volume expansion (VE) with isotonic saline infusion in 22 female patients with renal scarring and 9 healthy controls. We also studied how fast patients and control defended their volume against VE.

The patients had significantly lower GFR (81 ± 24 vs 106 ± 14 ml/min, $p < 0.01$), higher SBP (127 ± 15 vs 113 ± 12 mm Hg, $p < 0.05$) and higher PRA (1.41 ± 1.22 vs 0.79 ± 0.30 ng/ml x h, $p < 0.05$) in HP, but there was no significant difference in RPF, FF, DBP or p-Aldo. After VE; SBP (135 ± 17 vs 118 ± 11, $p < 0.05$), DBP (89 ± 12 vs 74 ± 8, $p < 0.01$), PRA (0.54 ± 0.46 vs 0.21 ± 0.11, $p < 0.01$) and p-Aldo (242 ± 77 vs 107 ± 84 pmol/l, $p < 0.001$) were significantly higher in patients than in controls. Transition to 3 % VE was associated with similar increase in SBP (8 vs 5, NS) in both patients and controls whereas DBP increased significantly more in the patients (6 vs 0, $p < 0.01$). The patients with renal scarring had the same capacity to excrete sodium (18 % vs 18 %) and water (15 % vs 19 %) during transition to VE as the healthy controls (pNS).

These data suggest that the development of hypertension in this group of patients is due, to an abnormally activated renin-angiotensin system and not episodic hypervolemia.

RELATIONSHIP BETWEEN AGE AND DISEASE PROGNOSIS IN IgA NEPHROPATHY (IgAN). B.A. Julian, F.B. Waldo, R. Wyatt, J. Galla and E.C. Kohaut. Univ of Ala at Birmingham, Depts of Peds and Med, Birmingham, AL and Univ of Tenn, Dept of Peds, Memphis, Tenn.

Increased age at diagnosis is reported as a risk factor for disease progression in IgAN. We have studied this relationship in 35 children and 67 adults with IgAN. Age at onset was defined by the first clinical or historic evidence of nephritis. Follow up averaged 8.5 yrs. Rate of progression was calculated from the slope of 1/Cr vs time.

Age onset	n	Abnl Cr		ESRD		HTN %	init-ial Cr	median slope 1/Cr
		n	%	n	%			
<11	19	2	11	2	11	21	1.0	-10.5
11-17	16	3	19	3	19	31	1.0	-9.0
18-29	34	3	9	8*	23	55	1.6	-7.0
>30	33	3	9	15*	45	69	3.1	-7.0

*Three males in each group presented with ESRD and 28/33 patients with progression were male (p<.01). Patients >30 were more likely to develop ESRD (p<.01), had more hypertension (p<.05), and higher initial Cr (p<.05) than younger patients. Children <11 were less likely to progress but had lower initial Cr, and less hypertension than older patients (p<.05). The slope of progression varied within each age group but the median slopes were not significantly different among the groups. The rate of progression did tend to decrease as age increased. We conclude that 20% or more of prepubertal children with IgAN may progress to ESRD at a rate similar to that in adults. The increased incidence of ESRD in adults may be related to delay in diagnosis (elevated Cr at onset) and an increase incidence of hypertension which promotes glomerulosclerosis.

INFLUENCE OF SEX ON LIVER MANIFESTATIONS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD). WD Kaehny, M Manco-Johnson*, AM Johnson*, DJ Tangel*, PA Gabow. University of Colorado Health Sciences Center, Denver, CO.

Sex hormones are known to affect growth characteristics of a variety of cells including liver cells. Grunfeld et al reported that women with ADPKD have a higher prevalence of liver cysts (75%) than do men (44%). Therefore, we postulated that estrogens may influence the development of liver cysts in ADPKD. In order to test this we compared liver cyst occurrence in males (M) and females (F) with and without ADPKD. We found the following: 1). Liver length (cm) per unit height (m) was greater in control F (9.6) vs control M (8.6) and in ADPKD F without cysts (9.7) vs ADPKD M without cysts (8.6). 2). The frequency of liver cysts in non-endstage ADPKD F (68%) did not differ from that in ADPKD M (58%). In order to look at the effect of estrogens in the premenopausal female without serious renal failure we compared only subjects under 50 years with serum creatinine less than 1.6 mg/dl. Women had a higher prevalence of cysts, 60% vs 38% (p=0.041). 3). Women had more cysts regardless of age or renal function (41% F vs 20% M with greater than 6 cysts). 4). Women with liver cysts had more pregnancies than women without cysts and the number of cysts correlated directly with the number of pregnancies. These findings suggest that estrogens influence growth of both normal and cystic liver tissue.

FRACTIONAL EXCRETION OF UREA, AN INDICATOR OF RENAL EFFECTS OF ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITION. Andre A. Kaplan & Orly F. Kohn*, Univ of Conn Hlth Ctr, Farmington CT

The effects of ACE inhibition on renal function have been shown to vary greatly in relation to the preexisting state of renal hemodynamics. When efferent arteriolar resistance is elevated, filtration fraction rises and post glomerular capillary oncotic pressure increases. Increased proximal reabsorption of urea decreases its fractional excretion (FEUr). We have identified two patients with severe CHF, on furosemide, in whom a preexisting low value for FEUr (10-15%) responded to ACE inhibition with resulting increases in FEUr and net urea clearance. In one patient the response was found with both captopril and enalapril. Renal function before and after ACE inhibition is listed below.

Pt.	CrCl ml/min	UrCl ml/min	FEUr %	FENa %	[Na] mEq/l	urine ml/day
1. pre	17.2	2.6	15	1.5	135	1800
capto	18.2	7.4	46	0.6	135	1200
2. pre	9.1	0.9	10	2.4	139	600
capto	16.8	2.9	17	3.8	142	1500
2. pre	2.9	0.4	12	1.3	135	200
enala	15.4	3.5	23	4.3	141	1400

[Na]=serum, CrCl=creatinine clearance, UrCl=urea clearance, FENa=fractional excretion of Na

Prior to ACE inhibition, the FEUr remained low despite diuretic induced increases in FENa and urine volume. We conclude that, in the context of CHF, a low FEUr may serve as a useful indicator of patients likely to respond favorably to ACE inhibition.

NO CHANGE NEPHROTIC SYNDROME: H. Keyvan, S.A. Sadjadi, V.A. Medical Center, Wilkes-Barre, PA

A 52 year old white man with history of COPD and on respirator who had no history of Diabetes Mellitus or arterial hypertension or previous kidney or heart or liver disease was found to have proteinuria of 4700 to 10500 mg per day on several occasions, Creatinine clearance 65/ml/min, serum Albumin 3, total protein 8 grams/dl., normal C3, C4, CH 50, Negative ANA, Rheumatoid factor, Cryoglobulins, VDRL and hepatitis screen and normal kidney size on ultrasound. At necropsy, light and immunofluorescence were normal. Electron microscopy did not reveal flattening or fusion of pedicells or immune deposits. Conclusion: Nephrotic syndrome could be associated with normal ultrastructural glomerular histology. Proteinuria in this case could result from alteration of charge or selectivity of the glomerular basement membrane. This patient exemplifies a pristine example of minimal change or nil change nephrotic syndrome.

EFFECT OF PARATHYROID HORMONE (PTH) ON LYMPHOCYTE IN NORMAL AND UREMIC SUBJECTS Marian Klinger*, Thomas O. Pitts and Shaul G. Massry, Div. Nephrol. Univ. So. Calif. Los Angeles, CA

Data suggest that PTH may affect lymphocyte blastic transformation (LBT). Also LBT is impaired in uremia. It is possible that excess PTH in uremia underlies the impaired LBT. We examined the effects of 1-84 PTH and 1-34 PTH on spontaneous and phytohemagglutinin (PHA) induced LBT in 5 day cultures of lymphocytes from normal and uremic subjects. Both peptides augmented ($p < 0.01$) spontaneous and 1/7 optimal PHA induced LBT of cells from normal subjects, and there was a dose-response relationship. Inactivation of PTH abolished this effect. LBT in response to optimal doses of PHA was inhibited ($p < 0.01$) by both moieties of PTH. These effects were greater with 1-84 than 1-34 PTH. Both intact and 1-34 PTH had no effect on LBT of cells from 7 dialysis patients. The data show that the normal lymphocyte is a target organ for PTH and the hormone affects its immune response. It is possible that enhanced entry of Ca by PTH into lymphocytes stimulates interleukin 2 production and the latter stimulates LBT. Chronic exposure to PTH, as in uremia, may cause a chronic loading of cells with Ca and this underlies the lack of action of PTH on the lymphocytes from patients.

UNIQUE INFILTRATIVE CHANGES IN THE MYOCARDIUM OF PATIENTS WITH END STAGE RENAL DISEASE (ESRD)-THE GLISTENING SPECKLED APPEARANCE (GSA). Krane, N.K., Martinez, J.A.,* Cain, C.,* Bleich, S.* & Phillips, J.H.* Sections of Nephrology & Cardiology, Tulane Univ. School of Medicine, New Orleans, Louisiana.

The echocardiograms (echo) of 55 ESRD patients (those on maintenance dialysis therapy or renal transplant recipients) at Tulane Medical Center who underwent evaluation from 1982 to 1986 were reviewed. In 44 (80%) of the patients, a previously undescribed GSA was noted in the left ventricle; the right ventricle was involved in 8 (14.5%) patients. In keeping with previous descriptions of the echo in ESRD, left ventricular hypertrophy was found in 39 (70%) of our patients, pericardial effusion without tamponade in 32 (58%), mitral annulus calcification in 10 (18.1%); thickened or calcified mitral valve in 18 (32.7%), and aortic sclerosis in 27 (49%) of the patients. The GSA was limited only to the echo of patients with ESRD and to a lesser degree in some patients with chronic renal failure and was not seen in over 200 echos of other patients with normal renal function.

This previously undescribed phenomenon of unknown significance resembles an infiltrative process similar to amyloid or pre-amyloid deposition but differs in that myocardial contractility and movement are not as severely affected as other infiltrative processes. Because of some similarity of the GSA on echo to amyloidosis, and the suggestion by previous investigators that β -2 microglobulin deposition may be increased in hemodialysis patients, current studies are evaluating this possibility.

TWENTY-FOUR HOUR INTEGRATED GROWTH HORMONE (GH) DETERMINATIONS IN CHILDREN WITH CHRONIC RENAL FAILURE (CRF). Vera H. Koch*, Barbara H. Lippe*, Barry M. Sherman*, Richard N. Fine. Dept. of Peds., Div. of Neph. and Endo., UCLA Med. Ctr., Los Angeles and San Francisco, CA.

15 pts aged 2 11/12 to 17 8/12 yrs who were <Tanner Stage III puberty underwent every 30 minute GH determinations over a 24 hr period. 5 pts had CRF with a Ccr of 14.3 to 29.5 ml/min/1.73m²; 5 pts were undergoing continuous cycling ambulatory dialysis (CCPD) and 5 pts were post-transplant with a Ccr of 16 to 76 ml/min/1.73m². All pts were growth retarded with a height standard deviation score (SDS) of >-2.0. Three patterns were obtained: 1) insufficient amplitude and frequency of GH peaks (3 pts); 2) failure of GH levels to return to baseline (2 pts); and 3) normal GH peaks (10 pts). Mean 24 hr GH levels did not correlate with either the pts SDS ($r = -0.28$) or growth velocity during the year prior to the study ($r = 0.19$). 11 pts were treated with recombinant human growth hormone (rhGH), 0.125 mg/kg subcutaneously thrice weekly for 4 to 6 mos. There was no relationship between the increment in growth velocity following rhGH treatment and the results of the 24 integrated GH determinations ($r = 0.5$). Therefore, 24 integrated GH determinations do not predict the potential response to exogenous rhGH therapy.

PROTEINURIA DETERMINES PROGNOSIS IN TYPE 2 (NON-INSULIN-DEPENDENT) DIABETES. C.L. Kunzelman*, R.G. Nelson*, W.C. Knowler*, D.J. Pettitt*. (intr. by J.C. Stivelman). NIH, NIDDK, Phoenix, Az

It is generally believed that proteinuria is of less clinical significance in type 2 (non-insulin-dependent) diabetes than in type 1 (insulin-dependent) diabetes. The effect of proteinuria on progressive renal disease and death was determined in Pima Indians, a population with a high prevalence of type 2 diabetes. Proteinuria, the ratio (P/C) of urine protein (gm/L) to urine creatinine (gm/L) was defined by a $P/C \geq 1.0$, which approximates a urine protein excretion rate of 1.0gm/day. The cumulative incidence of proteinuria in persons with diabetes was 2%, 6%, 21%, and 50% after 5, 10, 15, and 20 years duration of diabetes respectively. Proteinuria was strongly predictive of renal insufficiency (RI) [serum creatinine ≥ 2.0 mg/dl]. The incidence rate of RI increased from 5 cases/1000 person-years at risk to 105 cases/1000 person-years after 5 and >15 years of diabetes respectively in those with proteinuria, whereas in those without proteinuria, the rates were 0 cases/1000 person-years and 5 cases/1000 person-years after 5 and >15 years of diabetes respectively. Ten years after the onset of proteinuria, the cumulative incidence of RI was 65% in diabetic persons with duration of diabetes >10 years. Most of the excess deaths associated with diabetes occurred in persons with proteinuria. Fewer than 50% survived 5 years after the onset of proteinuria. The incidence rates of proteinuria in type 2 diabetic Pimas are as high as those reported in type 1 diabetes in other populations, and proteinuria heralds progressive renal disease and excess mortality.

NEPHROTOXICITY AND HYPERKALEMIA IN AIDS PATIENTS RECEIVING PENTAMIDINE (PEN). Mohsen Lachaal,* and Rocco Venuto, SUNY at Buffalo, Buffalo, N.Y.

Pen is used for the treatment of Pneumocystic carinii pneumonia and certain protozoal diseases. Impaired renal function has been reported to occur in approximately 25% of patients (Pts) given Pen, (Pearson, R.D. Ann Int Med 1985;103:782-786). Hyperkalemia, however, has been reported only in 0.5% of the Pts. We, retrospectively, analyzed the charts of 22 AIDS Pts given Pen when admitted to the county hospital over a 2 year period. Collectively, these Pts were admitted 28 times during this period and received a total of 23 courses of Pen. During 5 of these admissions, Pen was not given. Therapy ranged from 5-33 days (mean:13.4 days). Three admissions were excluded because of insufficient laboratory data or concomitant use of therapies which could effect the parameters being studied. In 19 of the remaining 20 admissions, the Pts treated with Pen were observed to have elevations of: potassium (5.1-8.7 mEq/L), creatinine (1.5-11.8 mg/dl, BUN (27-183 mg/dl) and a decrease in serum HCO₃⁻ (14-21 mEq/L). Of the 19 Pts exhibiting these abnormalities, most required Kayexalate and 2 required dialysis. During the admissions when Pen was not given, hyperkalemia was not observed. After discontinuation of Pen therapy these metabolic derangements normalized in all Pts except one who expired while still in acute renal failure. Four Pts received more than one course of therapy and upon reinstatement of Pen treatment, the same metabolic abnormalities recurred. In conclusion, Pen is more nephrotoxic than previously reported and can cause life threatening hyperkalemia in AIDS Pts.

THE BUBBLE CELL R Lamia,*, E Pastoriza,* B Lane,* and M Gräber. VAMC, Northport, and SUNY at Stony Brook, NY.

Examination of the urinary sediment often provides valuable information in assessing the etiology of renal syndromes. We have recently seen 3 patients with a distinct urinary sediment and a uniform clinical picture: The urinalyses were characterized by >20/HPF large cells filled with clear vesicular structures resembling bubbles. Cells resembling oval fat bodies were also present, as were red and white blood cells, and muddy brown casts. Clinically all three patients had acute renal failure due to hypotension or toxins with creatinines peaking over 7 mg/dL. Urinary sodium concentrations were > 50 mEq/L, and the patients were all nonoliguric and recovered function. By electron microscopy the bubble cells were epithelial cells with grossly distended endoplasmic reticular spaces. Intact nuclei and compact mitochondria suggested the cells were viable. Epithelial cells filled with fat droplets were also identified by EM.

We conclude that the bubble cell and cells resembling oval fat bodies can be seen by light microscopy of the urinary sediment in acute renal failure, and may be indicative of severe but reversible types of injury. We hypothesize that these are proximal tubular cells which have been acutely but not lethally damaged.

INCREASED OXALATE APPEARANCE (OXA) IN HEMODIALYSIS (HD) PATIENTS. D.M. Landwehr, J. Brothi*, G. Landwehr*, S. Halbedl* and J. Costello* Allegheny General Hospital and Allegheny-Singer Research Institute, Pittsburgh, PA.

Accumulation of oxalate (OX) in renal failure (RF) may be due to increased OXA and relate to greater conversion from ascorbate (A) or B₆ deficiency. To estimate minimal OXA and evaluate the role of A and B₆, OX removed by HD (DOX) and urinary excretion (UOX), plasma A (PA), dialysate ascorbate and adequacy of B₆ stores, measured as ratio of stimulated to basal erythrocyte glutamate pyruvate transaminase activity (EGPT), were measured in 26 pts receiving maintenance HD. UOX, DOX, measured from total dialysate collected in 3.5 N HCl and OX in plasma ultrafiltrate (POX), were determined by a double enzyme method in which recoveries were monitored with ¹⁴C OX. Mean ± SE pre-dialysis POX, 491±61µg%, was markedly greater than control (C), 27±7µg%, and was decreased significantly by HD, 186±25µg% (p<.001). Mean UOX for 24h prior to HD was 15±4mg and mean DOX was 98±8mg. When corrected for number of dialysis treatments per week, minimum OXA was 49±4mg/24h, compared to 26±7mg/24h in C, as measured from UOX excretion (p<.001). Although 8 pts had increased PA (>1.0mg%) and 7 had B₆ deficiency (EGPT ratio > 1.25), PA and EGPT ratio were not correlated with POX or OXA in these pts or the group as a whole (all r values between 0 and .52, all p values > 0.05). We conclude that OXA is increased in HD pts and that the increased total body OX burden in RF is not solely due to decreased excretion. Although this increase in OXA may result from increased synthesis or GI absorption, B₆ deficiency or increased PA do not play a role.

RAPID RENAL FAILURE IN AIDS NEPHROPATHY. C Langs, GR Gallo, RG Schacht, DS Baldwin. NYU Sch Med, NY, NY

We report the clinical and morphologic features of AIDS nephropathy in 18 patients observed between the years 1984-1987. Fourteen were black, all but 2 were male; mean age 39 years. Risk factors for AIDS were homosexuality in 9, IV drug abuse in 7, both in one, and unknown in one. Renal disease was already present at the time of AIDS or ARC diagnosis in 17. At presentation, 2 were in renal failure, mean serum creatinine was 2.1 mg/dl in the others; urinary protein averaged 5.6 g/24 hr. Only one developed hypertension. Histopathology showed focal glomerulosclerosis (FGS) in 14, mesangial proliferation in 3, and membranous nephropathy in one. End stage renal failure developed within 3 mos of presentation in 5, after 4 to 10 mos in 4, and at 27 mos in one. Histology in the FGS patients who progressed to uremia revealed segmental and global collapse of glomerular capillary walls with wrinkling of the GBM and luminal narrowing, often without advanced end-stage glomerular sclerosis. Interstitial mononuclear cell infiltration was a common feature. At latest follow-up, 2 had expired with renal failure, 5 died after a mean of 7.2 mos on dialysis, 5 are on dialysis for a mean of 5.2 mos, and 6 are alive for a mean of 12 mos with creatinines between 1.8 and 7.8 mg/dl.

In summary, many patients with AIDS-associated FGS pursue a fulminant course to uremia within a year and lack histologic evidence of advanced glomerular obliteration by sclerosis. We suggest that irreversible renal hemodynamic mechanisms play a major role in the pathogenesis of rapidly evolving renal failure in AIDS nephropathy.

PERCUTANEOUS ABSORPTION OF LINDANE IN UREMIC SUBJECTS. Kenneth D. Lempert, Marc J. Thibonnier. Medical College of Ohio, Department of Medicine, Toledo, Ohio.

Topical application of 1% lindane (L) lotion (Kwell) is a common treatment for scabies. The percutaneous absorption of L has not previously been studied in patients (pts) with renal insufficiency. L pharmacokinetics was studied in 33 subjects who were divided into 3 groups: normal renal function (N), moderate renal failure (RF), and hemodialysis (HD). Subjects were further classified into excoriated (EX) and non-excoriated (N-EX) categories. L lotion (30 ml) was applied to the skin surface from the neck down and removed by washing 8-12 h later. Blood samples were obtained prior to L application, and at 6, 12, 24, and up to 120 h later. HD pts had levels determined before and after their following HD treatment. Measurements were in whole blood using a gas chromatographic technique. L peak level at 6 h was greater in EX than in N-EX subjects (30.9 ± 8.0 ng/ml vs. 11.3 ± 1.6 ng/ml, $p < 0.05$, mean \pm SEM). Noncompartmental analysis revealed that L mean residence time was significantly longer in HD pts (233 ± 67 h) than in N subjects (107 ± 27 h) and RF pts (102 ± 16 h), $p < 0.001$. HD had little effect on L clearance. We conclude skin excoriation and renal failure are potential risk factors for L intoxication after percutaneous application and that HD is unlikely to be useful for the treatment of L intoxication.

ACUTE ABDOMEN AND PERINEPHRIC EFFUSIONS MAY BE DUE TO SPONTANEOUS RENAL DECAPSULATION. C.M. Link and L. Agodoa, Dept. Med., Walter Reed Army Medical Center, Washington, DC

Spontaneous decapsulation of the grafted kidney has been reported in a few patients after transplantation (Koene et al, NEJM 300:1030, 1979). Large volumes of transudate are formed, thought to originate in the interstitium and leak from the kidney surface. We report a case of spontaneous decapsulation of the native kidneys.

A 27-year old white male, previously in good health, except for gross hematuria following a skiing accident at age 16, presented with symptoms of acute abdomen. Ascites was found at exploratory laparotomy, and both kidneys were enlarged on palpation. CT showed bilateral perirenal fluid collection, with extrinsic compression on the left and a subcapsular fluid collection on the right. VCUG, IVP, RPR, RF, ANA, HIV antibody titre, and cultures were all negative. Percutaneous drainage on the left yielded a transudative fluid with an electrolyte profile identical to serum. Reaccumulation was rapid, and percutaneous drainage resulted in 100-200 cc's per day. Exploration of the left kidney revealed a thick, fibrous perirenal cyst, and the renal capsule was partially detached from the parenchyma. A small fistula was found connecting the cyst to the subcapsular cavity in the parahilar area. The cyst wall and the left capsule were both excised. Pathology showed a non-epithelialized cyst wall with chronic inflammation.

Follow-up evaluation showed reaccumulation of the fluid around the left kidney and a 5% decrement in function on the right.

CLINICAL APPLICATION OF A SPECIFIC IMMUNOADSORPTION COLUMN FOR THE REMOVAL OF ANTI-NATIVE DNA UTILIZING MONOCLONAL ANTI-IDIOTYPE ANTIBODIES. E.J. Lewis, B. McLeod*, R. Katz*, S. Korbet, T. Schnitzer*, W. Wong*, A. Neighbour*. Rush Medical College, Department of Medicine, Chicago, IL and E.I. du Pont de Nemours and Co., Glenolden, PA.

Anti-native DNA antibodies which express a common idiotype have been demonstrated in the serum and in immune deposits in the glomerulus of patients with SLE nephritis. In an initial feasibility study, plasma immunoadsorption was carried out on 3 active SLE nephritis patients using a device which employed mouse monoclonal anti-idiotypic antibodies (designated anti-3I). Immunoadsorption was accomplished on plasma separated using a standard plasmapheresis apparatus, with post-immunoadsorption plasma being returned to the patient. A single immunoadsorption was associated with a mean reduction of anti-native DNA = 34.8 ± 5.7 (SD) (range 28-40%) per treatment. The maximum decrease in serum anti-native DNA antibodies recorded was 90 units/ml/hr (Farr RIA). Column eluate contained only IgG. Total IgG adsorbed ranged from 45 to 130 mg. The adsorbed IgG was highly enriched for anti-native DNA, mean = 86 ± 17 (range 64-100%). No significant alteration was noted in plasma C3a desArg, C5a desArg, serum Clq, C3, C4, or Clq binding activity. Hematologic and coagulation status were unchanged by the immunoadsorption. We conclude that employment of monoclonal anti-3I idiotype antibody as an immunoadsorptive agent is a safe and efficient way to selectively effect an acute removal of anti-native DNA in active SLE.

PROSTAGLANDIN SYNTHESIS INHIBITION DOES NOT NORMALIZE GFR OR DECREASE ALBUMIN EXCRETION IN MODERATELY HYPERFILTRATING DIABETES MELLITUS. Tommy Linné*, Anna Körner*, Susanne Rudberg*, and Bengt Persson.* Department of Pediatrics, Karolinska Institute, St. Göran's Hospital, Stockholm, Sweden.

The short-term effects of prostaglandin synthesis inhibition (PGSI) (single dose 500 mg of naproxen) on renal function were studied in six women (age: 22.3 ± 1.66 yrs) with diabetes mellitus (DM) of 14.3 ± 2.8 yrs' duration, and in nine age- and sex-matched controls. The diabetics had no overt signs of nephropathy (Albustix^R neg, normal serum creatinine and blood pressure). The clearance of inulin (C_{in}) and PAH, the filtration fraction (FF), and the excretion of Na, albumin and PGE₂ were studied under water diuresis on two separate mornings, without and with PGSI. All individuals decreased their PGE₂ excretion. The C_{in} and FF were significantly ($p < 0.05$) higher in the diabetics than in the controls both without (129.4 ± 23.9 ml/min/1.73m² and 23.4 ± 2.82 %, vs. 107.6 ± 10.3 and 19.7 ± 1.62) and with (133.7 ± 29.4 and 22.6 ± 2.06 , vs. 106.8 ± 10.3 and 20.1 ± 1.47) PGSI. The diuresis and Na excretion decreased significantly in both groups. The albumin excretion was significantly higher in the diabetics under both conditions (29.9 ± 16.6 and 34.2 ± 19.9 µg/min/100 ml GFR, vs. 14.5 ± 10.6 and 12.9 ± 8.3).

Thus, the increased GFR in moderately hyperfiltrating DM does not seem to be PG dependent, and PGSI gives no immediate effects on the albumin excretion.

METABOLIC ALKALOSIS IMPAIRS THE RESPONSE TO BUMETANIDE. N.R. Loon,* C.S. Wilcox, R. Nelson,* and M. Mounts,* Div. of Nephrology and Htn., Univ. of Florida, and VAMC, Gainesville, FL.

We showed that daily doses of a loop diuretic lead to tolerance (natriuretic and chloruretic actions reduced by 20%) and to generation of a metabolic alkalosis (ALK). To explore the potential role of ALK in diuretic tolerance, 8 normal subjects were studied while they received a regulated daily dietary intake of Na⁺ (10 mmol), K⁺ (70 mmol) and acid equivalent (70 mmol). This was supplemented for 3 days on 3 occasions with 110 mmol/d of either NaCl (normal acid-base status, N), NaHCO₃ (ALK) or NH₄Cl (acidosis, ACID). Thereafter, they received 1 mg of IV bumetanide (B). Plasma aldosterone (ng/dl) was increased (p < 0.01) during ACID (107±24) compared to N (31±4) or alkalosis (ALK) (34±2) whereas PRA, GFR, ERPF, FF and BP were similar. ALK decreased the natriuretic (ΔFE_{Na} ; p < 0.01) and chloruretic (ΔFE_{Cl} ; p < 0.05) responses to B by 17-20%:

	N	ALK	ACID
ΔFE_{Na} (%)	+5.9±0.2	+4.7±0.3	+5.6±0.3
ΔFE_{Cl} (%)	+9.1±0.4	+7.5±0.5	+8.8±0.4

B excretion was unchanged by ALK or ACID; thus, the ratio of Na⁺ to B excreted was decreased (p < 0.01) by 24% by ALK (462±64 mmol/ μ g) compared to N (606±53) or ACID (617±80). Conclusions: ALK impairs the natriuretic and chloruretic responses to B independent of changes in renal hemodynamics, BP, PRA, aldosterone or delivery of B to its active site on the tubular lumen. Therefore, development of ALK may contribute to tolerance and/or resistance to loop diuretics in man.

RECOMBINANT HUMAN ERYTHROPOIETIN (r-HuEPO) TREATMENT ENHANCES EXERCISE TOLERANCE IN HEMODIALYSIS PATIENTS (HD). AP Lundin, BG Delano, R Stein, RM Quinn, EA Friedman SUNY, Health Sci. Cntr. at Bklyn, Bklyn, New York.

Treatment with r-HuEPO (150 IU/kg, IV, thrice weekly) increases the hematocrit (HCT) in HD patients. In 35 of 37 patients (18 men and 19 women), of mean age of 48.3±14.1 years, the HCT rose from a mean of 20.4±3.5% (range 13.5-26.5%) to an initial peak of 34.3±5.1% (range 24-43.4%) (p<.001) in a mean of 65±25 days. This increase in HCT occurred without rise in blood pressure (141.1±21.7/78.8±12.0mmHg at the start vs. 137.3±25.5/82.5±25.5mmHg at maximal HCT) (p=NS). No study patient had a seizure associated with hypertension or increase in HCT. One patient, suffered seizures and death due to cocaine toxicity.

Subjectively, 32 of 35 reported improved well being and exercise capacity. In 6 patients, a multistage, graded treadmill exercise test protocol using 1 minute stages with workload increased by elevations of 2.5% while speed was held constant was employed. Measured $\dot{V}O_{2max}$ rose from 12.4±4.5 ml/kg/min (range 7.5 to 20.5 ml/kg/min) at a mean HCT of 20.5±3.8% (range 14.1 to 25.7%) (Low fitness) to 20.7±4.4ml/kg/min (range 12.9 to 27.6, p<.02) (Fair Fitness) at a mean HCT of 30.4±2.9% (range 26.9 to 35.9 ml/kg/min). At a HCT below 20% (18.1±2.5%) mean $\dot{V}O_{2max}$ was 11.7± ml/kg/min. When the HCT exceeded 35% (37.2±1.9%) mean $\dot{V}O_{2max}$ was 24.9±2.5ml/kg/min (p<.02 compared with HCT <20%).

We conclude that r-HuEPO can be used to raise the HCT in HD patients to low normal ranges with few complications - over the short-term - resulting in an objective improvement in exercise tolerance.

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PROTEIN PERMEABILITY IN GLOMERULOPATHIES.

Robert L. Luke, Maine Medical Center, Dept. of Pathology, Portland, Maine.

Selectivity of proteinuria has been defined as the slope of the regression line of the logarithm of the clearance (dependent variable) and the logarithm of molecular weight (independent variable) of proteins. If the square of the radius of a hydrated sphere is substituted for the independent variable, then the relation is consistent with membrane models that have been derived using the technics of statistical mechanics. This method was used to analyze the clearances of twelve serum proteins (molecular weight 44 to 900 kilo-daltons) in 64 patients with proteinuria; results were compared with glomerular histopathology. The intercept is the theoretical maximum clearance of these proteins and was greatest in those diseases associated with separation of visceral epithelial cells; the slope (inverse square root of) is the average half distance between fibers in the membrane and was greatest in those diseases associated with extensive abnormalities in the lamina densa. In follow-up studies, changes in protein excretion correlated directly with changes in the intercept (integrity of slit diaphragm); changes in creatinine clearance correlated inversely with changes in average half distance between fibers (radius of pore).

PREDICTING RENAL FAILURE IN MEMBRANOUS NEPHROPATHY. Jeanne Macrae, Monica M. Beyer, Anthony D. Nicastrì, C.K. Chen, and Eli A. Friedman. SUNY at Brooklyn, N.Y.

Sixty-four cases of idiopathic membranous nephropathy were reviewed retrospectively to determine if factors found at presentation might be useful in predicting outcome. Patients had been followed for a mean of 5 years. Sixteen patients developed end-stage renal failure (Group I). Twenty-two patients had an apparently benign course with no detectable decline in creatinine clearance (Group II). Patients who died of non-renal causes had incomplete data or whose outcome was equivocal at the end of the study were not considered. Group I and Group II were compared using 9 clinical and 5 pathologic variables. Patients who developed renal failure were found to be older (mean 50.2 vs 37.5) and to present with a significantly higher serum creatinine (1.98 mg/dl) and lower serum albumin (2.14 gm/dl) than patients in Group II (1.06 mg/dl, 2.91 gm/dl respectively). Biopsy specimens from Group I exhibited tubular atrophy, interstitial fibrosis and obsolescent glomeruli more frequently than specimens from Group II. These 3 pathologic findings were considered to be risk factors for renal failure as were the following clinical variables: age greater than 50 serum creatinine greater than 2 mg/dl and serum albumin less than 2 gm/dl. Using the presence of 2 or more risk factors as our criterion, the outcome could have been predicted in 33 of 38 patients (89.5%). We conclude that the 5 year outcome in membranous nephropathy can be predicted with a high degree of success using a combination of clinical and pathologic variables.

EFFECT OF ACIDOSIS ON RENAL GLUCOSE REABSORPTION DURING SEVERE HYPERGLYCEMIA. P.O. Magner and M.L. Halperin. Renal Division. St. Michael's Hospital, Toronto, Ontario, Canada.

Glucose is reabsorbed in the proximal tubule by a Na-linked process, the rate of which is dependent on the driving force for Na reabsorption. When the blood glucose is 55 mM (1000 mg/dl), daily glucose reabsorption is 3-fold larger than the glucose pool and is a major determinant of hyperglycemia. Our purpose was to determine if metabolic acidosis might influence renal glucose reabsorption. The hypothesis to be tested was that lowering Na/H⁺ antiporter flux could augment Na/glucose co-transport. Rats were infused with 10% glucose to induce hyperglycemia and glucosuria; GFR was monitored by exogenous creatinine clearance. Glucose reabsorption (filtered minus excreted) was measured in 3 control periods (30 min) and expressed per ml GFR. In 9 rats, acetazolamide (10 mg/kg bolus and 10 mg/kg/hr) was given to diminish Na⁺/H⁺ antiporter flux; an additional 7 rats received HCl to lower the plasma HCO₃ to 15.1 ± 0.8 mM. (*p .05 vs control)

	Blood [Glucose] mM	Gluc. Reabs. Umol/ml GFR
Control	27.6 ± 1.7	13.9 ± 1.0
Acetazolamide	30.5 ± 2.2*	17.9 ± 1.5*
Control	29.9 ± 3.4	18.7 ± 1.0
HCl	42.4 ± 6.0*	22.2 ± 1.6*

Conclusion: Reducing HCO₃ reabsorption increased glucose reabsorption by up to 30%. This could have a major impact on the degree of hyperglycemia in diabetic ketoacidosis.

CONTRAST NEPHROPATHY IN AZOTEMIC DIABETICS UNDERGOING CORONARY ANGIOGRAPHY. C. Manske, J. Strony, J. Wang. University of Minnesota, Minneapolis, MN. (Intr. by T.H. Hostetter).

The high incidence of contrast-induced acute renal failure in diabetics with advanced renal disease has been considered a contraindication to cardiac catheterization in this group. We studied 15 insulin-dependent diabetics with impaired renal function (mean CrCl=13 ml/min; range=9-18) who underwent coronary angiography. Nonionic contrast was used and dye load was minimized through use of biplane filming (mean volume=37.5 cc, range=10-175cc). All patients received 1000 cc D₅NS with 25 g mannitol at 150 cc/hr beginning 2 hr pre-procedure. Five patients had no acute change in renal function measured as serial inverse serum creatinine ratios. Nine had decrements within the first 3 days post-procedure (mean=16.7%, range=7-27%) and one became transiently anuric. These acute decrements were directly correlated with amount of contrast given (r=.95). Persistent diminution in function measured as change in 1/Cr 4-6 weeks post-procedure, occurred in only 3 patients. However, only one patient (who received 175 cc dye and became anuric) required dialysis; 2 patients lost 32% and 19% of function respectively but did not require initiation of permanent dialysis. We conclude that the risk of contrast-induced renal failure in azotemic diabetics who require cardiac catheterization can be minimized by use of small quantities of contrast. Forced diuresis may also afford protection. Advanced renal failure is not necessarily a contraindication to cardiac catheterization in patients with severe diabetic nephropathy.

MOLECULAR FORMS OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN THE PLASMA AND URINE OF RENAL DISEASE PATIENTS. F. Marumo, K. Fujii*, N. Umetani*, A. Kurokawa*, T. Kurosawa* and K. Ando*# Dept. Med., Kitasato Univ. and #Bristol-Myers, Japan

A comparison was made of the molecular forms of ANP in the plasma and urine of patients with nephrotic syndrome and chronic renal failure (CRF), and healthy subjects, using reverse phase HPLC and GPC. 20 µl of a concentrated sample from 1 ml of plasma obtained with a Sep-Pak C18 were applied onto HPLC. 0.5-2.0 ml of plasma or urine were applied directly onto GPC. The elution positions of α, β and γ-ANP were identified by authentic α and β-ANP in the HPLC and by ¹²⁵I-α-ANP, authentic α and β-ANP and RNase (1.34kdalton) in GPC.

The ANP molecular forms in the plasma of 5 out of 15 healthy subjects showed both α and γ-ANP peaks, the others showing only α-ANP peaks. In the urine, a single peak of α-ANP was seen in all subjects. (FE_{ANP} <1%). In nephrotic patients, both α and γ-ANP peaks in the plasma and urine were found in 4, and a single α-ANP peaks in 3 patients. (FE_{ANP}=2.42±1.02%). In 9 CRF patients, not only α but β and γ-ANP peaks could be seen in the plasma and urine (FE_{ANP}=5.26±2.10%). 2 HD patients showed essentially the same tendency as that noted in non-HD patients. The ANP molecular forms in the plasma obtained from the coronary sinus and vena cava by heart catheterization in cardiac disease patients were essentially the same. In conclusion, various ANP molecular forms in the plasma of kidney disease patients appeared possibly as a result of change in ANP secretion from the atrium but not owing to processing of ANP during circulation. β and γ-ANP in the urine were observed in patients with high FE_{ANP}.

REDUCED URINARY EXCRETION OF EPIDERMAL GROWTH FACTOR IN INCIPIENT AND OVERT DIABETIC NEPHROPATHY. E. Mathiesen*, E. Nexo*, E. Hommel*, and H-H Parving* (intr. by S. Anderson). Hvidovre Hospital, Klampenborg, Denmark.

To study the relationship between glomerular and tubular function in diabetes, we investigated urinary albumin excretion rate (AER), glomerular filtration rate (GFR), and urinary excretion of epidermal growth factor (EGF), a mitogenic peptide synthesized in renal tubular cells, in normal subjects (Group I, n=7) and in insulin-dependent diabetics with and without microalbuminuria. The following diabetic groups were studied: diabetics with normal AER (Group II, n=11); with incipient nephropathy (Group III, n=11); with overt nephropathy and normal GFR (Group IV, n=12); and with reduced GFR (Group V, n=10). Results:

Group	GFR ml/min/1.73m ²	EGF nmol/24 hr
I	100 (87-116)	7.9 (4.1-10.5)
II	129 (111-150)	6.7 (1.3-9.2)
III	131 (95-136)	5.0 (3.6-7.4)*
IV	116 (90-132)	4.1 (2.5-15.1)
V	39 (13-50)	1.4 (0.8-5.3)**

(Means and ranges; *p<.02, **p<.01 vs Group I). There was a significant correlation between the excretion of EGF and GFR (p<0.001), and a negative correlation between the excretion of EGF and AER (p<0.01). Thus, urinary excretion of EGF diminishes with increasing diabetic glomerular involvement, and renal tubular function is reduced early in the development of diabetic renal disease.

AIDS-ASSOCIATED NEPHROPATHY IS NOT SEEN AT SAN FRANCISCO GENERAL HOSPITAL. Sami Mazbar* and Michael H. Humphreys, Division of Nephrology, San Francisco General Hospital, San Francisco, CA.

From 1982 to 1987 we received 49 requests for inpatient consultation for renal-electrolyte abnormalities in patients with AIDS or AIDS-related complex. Of these, 20 had chronic renal insufficiency (Scr > 2 mg/dl), proteinuria > 1 gm/24^o, or both; 11 had nephrotic range proteinuria. Twelve were black, 17 gay or bisexual, and 9 had intravenous drug abuse (IVDA) as risk factors. Five pts, all black, developed ESRD; two died before leaving the hospital and three are on chronic dialysis (2 hemo, 1 peritoneal) for 4 to 13 months. Renal tissue was available in 8 patients and showed variable pathology: 2 had focal glomerulosclerosis (FGS), 3 proliferative glomerulonephritis, 1 interstitial nephritis, 1 IgA nephropathy, and 1 with normal glomeruli. Clinical course in the other patients was not one of rapidly advancing renal failure. Review of 90 autopsies done at SFGH from 1981-1986 on patients with AIDS revealed only one case with significant proteinuria not seen antemortem; glomeruli in this case were normal by light microscopy. No additional case of glomerular disease was observed. These data do not confirm the high incidence of malignant nephrosis and renal failure in AIDS patients reported from New York and Miami, nor do they support the existence of a specific glomerular lesion of FGS. These east coast findings may rather reflect the greater predilection for blacks to suffer from diverse renal diseases including FGS, and for patients with IVDA to develop FGS.

RENAL FAILURE IN ADULT LIVER TRANSPLANT PATIENTS. J. McCauley*, D.H. Van Thiel*, T.E. Starzl*, and J.B. Puschett. University of Pittsburgh, Pittsburgh, PA. Departments of Medicine and Surgery.

We reviewed the charts of 105 adult patients (pts) receiving orthotopic liver transplants (tx) to determine the incidence, etiology, and outcomes of pts with acute renal failure (ARF) in the perioperative period and the incidence of chronic renal failure (CRF) after 35 months (mo) of follow up. Ninety-nine (94.3%) of 105 pts developed ARF defined as serum creatinine (Scr) increase > 50% of pre-tx values. Mean pre-tx Scr was .92 ± .62 mg/dl and peak Scr was 2.72 ± 1.4 mg/dl (p < .0005). Etiologic factors included: a) Acute tubular necrosis (ATN), 46; 34 ischemic, 10 aminoglycosides, 2 volume depletion b) Cyclosporine toxicity, 17 c) Pre-renal azotemia, 7 d) Hepatorenal syndrome, 4 e) Interstitial nephritis, 1 f) Unknown, 23. Graft rejection was not a significant cause of ARF and no correlation existed for peak or mean cyclosporine levels, bilirubin, or SGOT. Ischemic ATN resulted in the greatest mortality (41%) which was usually due to sepsis. Pre-tx renal failure (Scr > 2.5 mg/dl) occurred in 5 pts, but did not increase mortality. Only 1/10 pts requiring dialysis survived. Eighty-five pts (81%) survived and data is available for 79 pts up to 35 mo. Discharge Scr was 1.39 ± .6 mg/dl. Mean Scr after 35 mo was 1.93 ± .9 mg/dl and in 69/79 has reached 1.7 mg/dl or greater. Cyclosporine toxicity is the probable cause of progressive CRF. In summary, both ARF and CRF are common complications of liver tx. Pts developing ischemic ATN or requiring dialysis are at significant risk, although pre-existing renal failure is not a negative prognostic factor. Progressive CRF may become a major factor in morbidity after liver tx.

ANEMIA IS A RISK FACTOR FOR BLEEDING COMPLICATIONS AFTER PERCUTANEOUS KIDNEY BIOPSIES (KBx). DF Middendorf, FG Cosio, WH Bay, NS Nahman Jr*, AA Imm* and T Gelety*. Ohio State University, Department of Medicine, Columbus, OH.

Anemia results in prolonged bleeding times in patients with renal diseases. Thus, anemia itself may be a risk factor for bleeding in renal patients. We tested this hypothesis by examining the relationship between hemoglobin (Hb) level and bleeding complications after percutaneous KBx. Hospital records were reviewed in 213 patients who had percutaneous KBx performed between 1983 and 1986. By ANOVA, we analyzed the correlation between the occurrence of bleeding complications and the following factors: Age, sex, weight, hypertension, medications, tissue specimen adequacy and prebiopsy serum creatinine and Hb levels. Of all factors analyzed only the preBx Hb level was significantly correlated with the occurrence of bleeding complications. Thus, for patients with preBx Hb ≤ 10 (N=60), 10 to 12.5 (N=78) and > 12.5 g/dl (N=75) the incidence of complications was 35, 46 and 26% respectively (Pearson's chi=6.34, p=0.04). Specific complications occurred as follows:

Complication/Prebiopsy Hb	≤10	10-12.5	>12.5
Gross hematuria	20%	24%	13%
Flank hematoma	7%	4%	0%
Obstruction (clot)	2%	6%	0%
Transfusions	17%	13%	4%

Severe bleeding complications occurred in 11 patients, all of whom had preBx Hb < 12.5. Conclusion: Anemia is a major risk factor for bleeding following percutaneous KBx. PreBx blood transfusion should be considered in patients with low Hb undergoing percutaneous KBx.

SURGICAL ANATOMY OF THE LIVER IN ADULT POLYCYSTIC KIDNEY DISEASE (APKD). D. M. Nagorney*, V. E. Torres, J. Rakela, T. J. Welch. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Despite their frequency, liver cysts usually cause little morbidity in APKD. Some patients, however, may become incapacitated by mechanical problems produced by massively enlarged polycystic livers. To provide information on anatomic distribution of these cysts and treatment of symptomatic pts, we reviewed 147 consecutive CTs of the abdomen in 57 men and 62 women with APKD. Seventy-two percent of men and 84% of women had liver cysts. The cysts were randomly distributed in all hepatic segments. Six men and 18 women had moderate to severe hepatomegaly with cysts involving more than 30% of the liver. In 21 of these 24 pts, the cystic disease was focal, with some liver segments being relatively spared. Spared segments were in the right lobe (segments V-VIII) in 11 pts, left lobe (segments I-IV) in 3 pts and both lobes in 7 pts. The segmental distribution of cystic liver disease allowed segmental hepatectomies for pts with massively enlarged livers and incapacitating symptoms. We performed segmental hepatectomies in 4 women with incapacitating symptoms. Segments VII-VIII were resected in 3 and segments II-VI resected in one pt. Hospital stay was 10 ± 2 days. Postoperative morbidity was limited to reactive right pleural effusions in 2 pts. Marked symptomatic improvement occurred in 3 pts. In 3 additional pts, large, complicated cysts had percutaneous drainage without complications. These preliminary observations suggest that surgery and interventional radiology may benefit a small subset of pts with severe polycystic liver disease.

EXCRETION OF TAMM-HORSFALL GLYCOPROTEIN (THP) DURING PREGNANCY: A DEFENSE AGAINST NEPHROLITHIASIS. Y. Nakagawa*, D. Sirivongs*, P. Maikranz, M. D. Lindheimer, and F. L. Coe. Nephrology Program, Univ. of Chicago, Chicago, IL

Although pregnant women are hypercalciuric (243 ± 23 mg/day vs 121 ± 61 mg/day), they do not develop kidney stones. Investigations in our laboratory indicate that THP prevents the aggregation of calcium oxalate crystals. Aggregation of small crystal nuclei is one important mechanism by which hypercalciuria could cause calcium oxalate nephrolithiasis. To determine if THP excretion increases in pregnancy, THP levels in urine of pregnant women were analyzed quantitatively using a modified ELISA sandwich method (Hunt, J. et al., *J. Immunol. Methods*, 91, 35-43 (1986)). THP levels were 200.51 ± 24.15 ug/ml during the first trimester, and 301.82 ± 26.16 and 249.11 ± 21.57 ug/ml by the second and the third trimesters, respectively. In the two to three months period after delivery, levels of THP remained 281.66 ± 23.00 ug/ml. All THP level exceed 90.69 ± 6.37 ug/ml in 6 normal women not recently pregnant. High level of THP during pregnancy may prevent nephrolithiasis that hypercalciuria could initiate.

PROTEINURIA AND PROGNOSIS IN IgA NEPHROPATHY. K Neelakantappa, GR Gallo, DS Baldwin. NYU Sch Med NY, NY.

Clinicopathological data in 74 patients with IgA nephropathy were analysed to identify clinical markers of poor prognosis. A serum creatinine concentration (S Cr) in excess of 2 mg/dl (renal insufficiency = RI) was used as the end-point for life table analysis of "renal survival" according to the method of Cutler and Ederer. Relative risk of developing RI was 12.9 times greater in patients who were 30 years of age or older at presentation compared to those under 30 ($p < 0.001$). Proteinuria of 3 gm/day or more (heavy), 1-2.9 gm/day (moderate) and under 1 gm/day (mild) each occurred in approximately equal numbers of patients. One-sixth of those with more than mild proteinuria developed end-stage renal failure, while S Cr never exceeded 2 mg/dl in any with mild proteinuria. Renal survival at 5 years after presentation was 100% in patients with persistent mild proteinuria, 87% in those whose proteinuria reached the moderate range and 69% when heavy or nephrotic range proteinuria developed. The relative risk of RI in patients with moderate proteinuria at presentation was 2.5 times and with heavy proteinuria 6.3 times greater than those with mild proteinuria ($p < 0.05$). Only rarely (in 4 of 33 patients), did mild proteinuria increase to higher levels. Moderate or heavy proteinuria typically preceded the onset of hypertension (in 20 of 22 patients) and invariably occurred prior to development of RI. A correlation existed between the magnitude of proteinuria at the time of renal biopsy and severity of morphological changes. Our results underscore level of proteinuria as an early marker of poor prognosis in IgA nephropathy.

PERCUTANEOUS RENAL ANGIOPLASTY (PTRA) FOR ACUTE RENAL FAILURE (ARF) IN HIGH-RISK ELDERLY PATIENTS. RM O'Donovan*, OH Gutierrez*, JL Izzo, Jr, Depts. of Medicine and Radiology, Univ. of Rochester, Rochester, New York, USA.

Elderly patients (pts) with ARF have poor survival despite hemodialysis. In 17 high-risk pts (8M, 9F, 73 + 7 yrs old, serum creatinine (SCr) 3.8-17 mg/dL), 19 PTRA's were attempted to reverse ARF. Aortography followed clinical suspicion, renogram and ultrasound; high grade stenosis or occlusion was present in all cases. Complicating problems included: hypertension (17), severe arteriosclerosis (16), ischemic heart disease (15), diabetes mellitus (8), congestive heart failure (8), hemodialysis dependency (7), acute myocardial infarction (5).

Seventeen of 19 PTRA's were technically successful. Seven pts had reversal of ARF, with mean SCr falling from 6.4 to 2.1 mg% ($p < 0.01$); 2 discontinued dialysis. 3 of 4 episodes of acute oliguria were reversed ($p < 0.05$). Four pts had no further deterioration in SCr. Five pts died; 4 from cardiac causes unrelated to PTRA, 1 from aortic thrombosis after PTRA. Other complications were femoral hematoma (4), minor peripheral embolism (3), and renal artery thrombosis (2) or dissection (1). All 11 pts with improvement or stabilization of renal function survived hospitalization; mean length of stay was 17 days. In contrast, 5/6 PTRA failures requiring maintenance hemodialysis died ($p < 0.05$); mean length of stay was 38 days ($p < 0.1$). BP med requirements were reduced in 8/12 survivors.

Conclusions: PTRA for renovascular disease in high-risk elderly pts can reverse ARF, improve survival, and lower hemodialysis needs and cost.

FAVOURABLE OUTCOME OF CHILDHOOD HEMOLYTIC UREMIC SYNDROME (HUS) WITH STEPPED AGGRESSIVE THERAPY. M.R. Ogborn*, J.F.S. Crocker, D.R. Barnard*, P.C. Grimm*. Dalhousie University, Dept. of Pediatrics, Nova Scotia, Canada.

HUS remains a common cause of acute renal failure in young children. It is reported to have a significant risk of mortality and chronic renal and non-renal morbidity. Since 1979, 55 children consecutively admitted to the IWK hospital for children with HUS have been treated with a stepped care protocol. Mild cases (20/55) receive fresh frozen plasma and are maintained in good fluid balance. The more common patients (35/55) with uncompensated hemolysis, extra-renal complications or rapidly evolving renal insufficiency receive early hemodialysis and exchange transfusion in addition to the baseline therapy. There was no mortality and no patients have progressed to chronic renal insufficiency. Mild neurologic deficit occurred in one patient. One patient developed a late colonic stricture requiring resection. Hypertension, usually requiring a single pharmacologic agent, occurred in approximately one-quarter of patients and was the only common long term sequelae. Infection and minor hemorrhage from arterio-venous shunt sites was the most common treatment related morbidity. One major transfusion reaction occurred. No cases of transfusion acquired viral infection have been detected. Non-familial childhood HUS is a disease that has an excellent prognosis and justifies early and intensive medical intervention.

PREVENTION OF CENTRAL PONTINE MYELINOLYSIS (CPM) BY DEXAMETHASONE (D) OR COLCHICINE (C) IN RATS. Man S. Oh, Kyu C. Choi*, Jaime Uribarri, Joanna Sher*, Chandrakant Rao*, and Hugh J. Carroll. State Univ. of New York, Health Science Center at Brooklyn, Dept. of Medicine and Pathology, Brooklyn, New York.

CPM is a well known complication of rapid correction of hyponatremia (HNa), but the mechanism is unclear and no means of prevention or treatment exists. The investigation was carried out to determine whether D and C prevent CPM by preserving the blood-brain barrier and inhibiting mobilization of macrophages respectively. HNa was induced in 63 rats in 3 groups, with pitressin and 2.5% dextrose. On day 4, HNa was corrected with 5% NaCl. Group 1 received 4 mg of D twice on day 4 and once on day 5. Group 2 received 150 ug of C once each on days 4 and 5. Group 3 received neither. On day 8 rats were sacrificed, and the brains were examined. The results are analyzed on 30 rats with HNa below 120 mEq/L followed by a rise of 20 mEq/l or more with 5% NaCl. Demyelinating lesions were seen in the pons, cerebellum, thalamus, subcortical white matter, anterior commissure, corpus callosum, and mid brain, always accompanied by infiltration of macrophages. Degree of HNa and extent of correction were the same among 3 groups. All rats on no drug (9 of 9) had the lesions, whereas 13 of 21 (62%) that received either D (3 of 6) or C (10 of 15) had the lesion; D and C may prevent CPM.

A FAMILIAL RISK OF CHRONIC RENAL FAILURE AMONG BLACKS ON DIALYSIS? Terry J. Opgenorth, Rollington Ferguson*, and Clarence E. Grim. C. R. Drew University, Los Angeles, CA.

We recently noted that several hypertensive blacks entering dialysis gave a history that other family members had also been on dialysis and/or died of kidney disease. Because hypertension (HT) is the leading cause of end stage renal disease (ESRD) in blacks and is known to be familial, we hypothesized that ESRD may also be familial. This was tested by the case-control method in 114 black patients from 3 area dialysis centers and 99 black neighborhood controls. Among cases, 26% of patients reported a relative as having had chronic kidney failure or dying of kidney disease as compared to 11% among the controls. The Mantel-Haenszel odds ratio (ORMh) was calculated and demonstrated that family history of HT (ORMh=1.92, 95% CI=0.96-3.68), individual history of HT (ORMh=5.14, 95% CI=2.29-11.56) and history of ESRD in a first or second degree relative (ORMh=2.30, 95% CI=1.09-4.83) were all "risk" factors for being dialysed for ESRD. Stratification analysis demonstrated the risk of chronic renal failure due to HT was independent of family history of diabetes.

In conclusion, individual history of HT, family history of HT, and family history of ESRD are all significant risk factors for the development of ESRD in the population of interest. Because HT and decreases in renal function may develop long before ESRD, aggressive detection and treatment of hypertension in the relatives of black patients with ESRD may prevent ESRD in this group and merits further investigation.

EFFECTS OF TWO DIFFERENT PROTEIN DIETS ON PROGRESSION OF EARLY CHRONIC RENAL FAILURE (ECRF): A SELF-CONTROLLED STUDY. L. Oldrizzi*, C. Rugiu*, G. Maschio. Division of Nephrology. Verona. Italy.

A self-controlled study to compare the progression of renal failure on 2 different protein and phosphate intakes was planned. Ten patients (p) with ECRF on long-term protein restriction were studied. We decided for each p 2 study-periods: period A (low protein diet: 0.6 g/kg bw, 600 mg of PO₄) averaged 30 months; period B (normal protein diet: 0.9 g/kg bw, 900 mg of PO₄) had a mean duration of 9 months. GFR (125 I-iothalamate clearance) was evaluated twice in each period (start/end). Serum and urine biochemistries were checked every 2 months. Protein and PO₄ intakes were extrapolated from UNA and U-PO₄. No changes in drug therapy had been made throughout the study. The results are shown in the table.

	PERIOD A		PERIOD B	
	Start	End	Start	End
GFR (ml/min)	43.6±14.6	45.3±13.6	51.4±14.8	42.7±15.0
SCr (mg/100 ml)	1.89±0.49	1.63±0.49	1.72±0.56	2.15±0.75
Slope 1/SCr	0.0022±0.0032		-0.0193±0.013*	
U-Prot. (g/day)	0.68±0.71		1.34±0.82*	
Prot. Intake (g/day)	43.7		60.3	
PO ₄ Intake (mg/day)	486		742	
MAP (mm Hg)	114.7±7.8		115.4±10.8	

* p < 0.01. MAP: mean arterial pressure

The results show a worsening of renal function in p with ECRF (GFR between 25 and 60 ml/min) when dietary protein and phosphate intakes exceed 0.6 g/kg and 600 mg/day, respectively.

THE CORRECTION OF ANEMIA IN HEMODIALYSIS PATIENTS USING RECOMBINANT HUMAN ERYTHROPOIETIN (r-HuEPO): HEMODYNAMIC EFFECTS. Emil Paganini, Theodore Thomas*, Fetnat Fouad*, Jose Garcia, and Emmanuel Bravo. Cleveland Clinic Foundation, Dept. of Hypertension/Nephrology, Cleveland, Ohio.

The correction of renal failure induced anemia with the use of r-HuEPO has been associated with the production of hypertension in some chronic hemodialysis patients. To assess this, we studied ten hemodialysis patients prior to and after attaining a target hematocrit, nine of whom were on antihypertensive therapy which remained unchanged during the study. Hemodynamic evaluation was obtained by radionuclide angiography, blood pressures measured by the cuff method, and heart rate by EKG monitoring. The levels of catecholamines, aldosterone, renin, and ADH were determined. Baseline mean arterial pressures varied from 75-132 mmHg (median 99 mmHg). After correction of the anemia, hematocrit increased (24 to 40% p < 0.05), mean arterial pressure remained unchanged (5.96-7.17 L/M, p < 0.05) and total peripheral resistance index (TPRI) decreased (33-28 units/m², p < 0.05). CO increased due to increases in cardiopulmonary volume (CPV), (737-913 ml p < 0.05), the ratio of CPV to total blood volume, and ejection fraction (EF) (48-54% p < 0.05). In conclusion, correction of anemia with r-HuEPO has hemodynamic effects different from those seen with anemia correction by transfusions, did not worsen hypertension in our population, and improves cardiac performance as measured by CO and EF. The improved cardiac performance may be due to improved myocardial oxygenation and redistribution of blood volume.

THE IMPACT OF END STAGE RENAL FAILURE TO CANADIAN PUBLIC HEALTH. G.A. Posen, Ottawa Civic Hospital, University of Ottawa, J. Silins,* Statistics Canada, A.M. Ugnat,* Health & Welfare, Canada.

The purposes of this study are to use the Canadian Renal Failure Register data to estimate the probability of dying and life expectancy of renal failure (RF) patients; to determine the number of lives gained and the potential years of life that may be saved; and to project the number of new cases and the total cases in the future.

The probability of dying for the first 5 years is estimated from the registered data. A linear model is used to project the probability of dying up to 10 years. This data is used in a life table to compute the life expectancy of RF patients. The number of lives gained and the potential years of lives saved are estimated for each treatment group, by comparing non treatment to reference treatment. The number of new cases is projected by using a linear model for the age groups 0-14 and 75 and over, and an exponential model is used for age groups 15-74. The probability of dying is applied to the registered data of every year to determine the number of prevalence cases.

The probability of dying is "U" shaped curve according to ages. The low peak of the curve is ages 15-34. Females and diabetic patients have a higher probability of dying. Transplant patients have a lower probability. Younger aged patients lose more life expectancy than older patients. If the treatment of renal disease remains the same there will be a continued exponential increase in the number of new patients and total patients for the foreseeable future.

TEMPORAL RELATIONSHIPS BETWEEN PLASMA GROWTH HORMONE (GH), PLASMA IGF-1 AND RENAL FUNCTION AFTER GH INJECTION. H. Rabb†, R. Hirschberg*, R. R. Bergamo* and J. D. Kopple. Harbor-UCLA Medical Center and UCLA, Torrance and L.A., CA.

GH does not acutely alter renal hemodynamics, but repeated GH injections increase renal plasma flow (RPF) and GFR. Since plasma IGF-I rises slowly after GH injection, we examined whether the changes in RPF and GFR correlate temporally with changes in plasma IGF-I. PAH and inulin clearances were measured in 7 normal adults on 3 consecutive days. On Day 1, after baseline clearances, a single i.m. dose of GH, 0.15 mg/kg, was given. RPF, GFR, MABP and plasma GH and IGF-I were measured over 5.5 additional hrs on Day 1 and for 2 hrs on Days 2 and 3. 2 to 5 hrs after injection, plasma GH rose markedly and then fell to near baseline on Day 2 and to baseline by Day 3. Plasma IGF-I did not increase on Day 1, but was elevated on Days 2 and 3. Changes in RPF, GFR and TRVR (total renal vascular resistance) (table) paralleled the rise in IGF-I but not the

	Baseline	Day 1	Day 2	Day 3
RPF ¹	546±19 ³	567±46	715±21 ^C	662±25 ^b
GFR ¹	100±3	106±5	130±5 ^b	113±5 ^a
TRVR ² x 100	9.3±1.7	8.6±1.4	6.8±1.5 ^b	7.6±1.4 ^a

¹ml/min/1.73m². ²mmHg.min/ml. ³SEM. Differs from baseline: ^ap<.05, ^bp<.01, ^cp<.001.

changes in GH. Thus, one GH dose causes a delayed increase in RPF, GFR and plasma IGF-I. These findings support the possibility that IGF-I affects renal hemodynamics and mediates the changes in renal function induced by GH. Since plasma IGF-I also is influenced by nutrient intake, the chronic effects of diet on RPF and GFR may be partly regulated by IGF-I.

NEPHROPATHY AS THE INITIAL MANIFESTATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) DISEASE. TKS Rao, LR Mallis, EA Friedman. SUNY, Health Science Center at Brooklyn, Brooklyn, New York.

AIDS-associated nephropathy (AAN) defines a nephrotic syndrome (NS) in an AIDS patient who has enlarged kidneys, showing focal and segmental glomerulosclerosis (FSGS), with progression to uremia in weeks to months. To meet the CDC definition of AIDS, at least one opportunistic infection is required. During the first six months of 1987, however, we encountered 6 patients (aged 18 to 40), 2 black women and 4 men (1 white, 3 black) who were HIV antibody positive and manifested a syndrome similar to AAN but lacked clinical evidence of AIDS. All 6 patients presented in a nephrotic syndrome.

In 2 patients (the wife of an intravenous drug user and a white homosexual man), HIV antibody had been detected 4 and 3 months, respectively, prior to the onset of massive proteinuria. This woman had enlarged kidneys, showing FSGS, and required maintenance hemodialysis for uremia within 2 months. The homosexual man, declined biopsy and had a normal creatinine clearance with proteinuria persisting at 5 g/day. In the other 4 patients (1 Haitian, 1 homosexual man, 1 homosexual male sex partner, and 1 woman drug abuser), HIV antibody was detected at initial evaluation for the nephrotic syndrome. In 2 of these 4 - with large kidneys and FSGS - uremia developed in 4 and 12 months. Each of the other 2 patients declined renal biopsy and have present serum creatinine levels of 3.0 and 3.1 mg/dl. Only 1 of 6 patients manifested an opportunistic infection (cryptococcal pneumonia) while the other 5 have no signs of AIDS 3-18 months after detection of HIV antibody.

We suggest that the term HIV-Associated Nephropathy replace AAN to encompass a rapidly progressive renal syndrome which may be manifested prior to clinical AIDS.

PARADOXICAL NATRIURESIS IN ACUTE MYOCARDIAL INFARCTION IN THE DOG. M. M. Redfield*, J. A. Shirger*, D. M. Heublein*, and J. C. Burnett, Jr., Mayo Medical School, Rochester, MN

Preliminary studies have suggested a maintenance of renal hemodynamic and excretory function during acute myocardial infarction (AMI) despite a decrease in arterial pressure. The present studies were designed to document renal hemodynamic and excretory responses as well as endocrine function during uncomplicated AMI. Studies were performed in 6 open-chested anesthetized dogs. Systemic hemodynamics, renal hemodynamic and excretory function, and hormones affecting renal function were measured. Data were collected before and during the five hours following occlusion of the left anterior descending coronary artery.

	Control	Peak
GFR, ml/min	28±4	27±4
RBF, ml/min	162±20	179±20
FE _{Na} , %	.71±.22	2.08±.59†
CO, L/min	3.3±0.2	2.6±0.2
MAP, mmHg	103±5	98±4†

†p<.05, peak vs control

All animals experienced a transient natriuresis despite a significant decrease in arterial pressure. This response was variable in onset but averaged 123±55 min (range 10-300 min) after occlusion. No significant changes were observed in atrial pressures, atrial natriuretic factor, renin, vasopressin, or aldosterone.

We conclude that a natriuresis may occur in uncomplicated AMI which is independent of systemic and renal hemodynamic or hormonal mechanisms. This paradoxical natriuresis may serve to prevent intravascular volume overload during acute myocardial infarction.

PLASMA INFUSION (PI) FOR HEMOLYTIC UREMIC SYNDROME (HUS) IN CHILDREN: RESULTS OF A MULTICENTER CONTROLLED TRIAL (MCT). G. Rizzoni*, A. Claris-Appiani*, A. Edefonti*, P. Facchin*, F. Franchini*, R. Gusmano*, E. Imbasciati*, L. Pavanello*, F. Perfumo*, G. Remuzzi* (intr. by M.J. Dunn). Nephrology Unit of Padua, Milan, Firenze, Genoa, Sondrio, Bergamo - Italy.

A MCT on PI for the treatment of children HUS has been performed between February '81 and February 85. Admission criteria: a) observation within 8d from first symptoms, b) dialysis treatment required, c) no special treatments and no more than 25ml blood/kg previously received. Children were divided according to age (under and over 3yrs), and randomly assigned to treatment with PI or symptomatic therapy. 32 children (16M, 16F), age 4mos to 6yrs entered the study: 17 received PI (P+group) and 15 symptomatic therapy (P-group). Mean follow-up was 16mos. Surgical renal biopsy was performed 29-49d after onset in 11 P+ and 11 P- children and 33 histological parameters were semiquantitatively evaluated. Plasmatic activity stimulating prostacyclin was normal in all patients at admission. No death occurred in either groups. No difference was found in blood pressure, proteinuria at the end of follow-up; renal biopsies showed predominant glomerular involvement in all patients. On electron microscopy (EM) no vascular changes were observed in 7 children of P+group, while in 5 of 7 in P-group thickening of lamina rara interna and arteriolar damage were present. It is concluded that in children with predominant glomerular involvement and treated in a very early phase of HUS, PI did not influence significantly the short and medium term clinical outcome of the disease. A longer follow-up is needed to ascertain whether the higher degree of endothelial damage by EM in children who were not given PI is of clinical relevance.

INCREASE IN MAXIMAL EXERCISE CAPACITY IN HEMODIALYSIS (HD) PATIENTS FOLLOWING CORRECTION OF THE ANEMIA WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo). H.T. Robertson, N.R. Haley, J.W. Adamson, and J.W. Eschbach*, Univ. of Washington, Seattle, WA.

Thirteen anemic HD patients underwent exercise testing before and after correction of their hematocrit with rHuEpo (AMGen, Inc.) in order to quantify the improvement in exercise capacity. Subjects underwent two baseline progressive work studies, followed by a third study after normalization of the hematocrit. All studies were performed on non-dialysis days, when antihypertensive drugs were withheld. The study protocol used a cycle ergometer with 15 watt power increments each minute. Testing consisted of measuring blood pressure (BP) and subjective fatigue index each minute and heart rate, minute ventilation and oxygen consumption continuously. Maximal oxygen consumption (VO2 max) was predicted from age, height and sex. Improvement in VO2 was significant

	Hct (%)	VO2 max (ml/Kg/min)	VO2 max (% predicted)
Pre-rHuEpo	22.5±4.1	14.5±7.2	43.0±15.6
Post-rHuEpo	35.5±2.1	16.3±6.5	48.5±14.2

($p < .05$). Although the maximal increase in heart rate decreased with correction of the anemia from 80% to 76.6% of the age-adjusted predicted value, the difference did not reach statistical significance. We conclude that, acutely, rHuEpo therapy results in modest increases in maximal exercise tolerance in unconditioned HD patients. These findings suggest factors other than anemia contribute to the exercise impairment of chronic HD patients.

RENAL FUNCTIONAL RESERVE (RFR) IN PREGNANCY
Claudio Ronco*, Alessandra Brendolan*, G. La Greca*, P. Mentasti* (intr. by Paul Kimmel), Depts. of Nephrology and obstetrics, City Hospital, Vicenza-Italy.

Creatinine Clearance was evaluated in 5 normal and 29 pregnant women under baseline conditions (R-GFR) and after acute protein loading (T-GFR). The test was carried out at different stages of pregnancy as suggested by Bosch et Al. to measure the filtration capacity under stimuli. R-GFR increased from the 1st month of gestation (99.8ml/m') to the 9th month (149.6ml/m'). All subjects increased significantly their GFR after protein loading (163.8ml/m') independently on the stage of gestation (SD=6.5) suggesting that filtration capacity remains unchanged during pregnancy being related to the functioning renal mass. On the contrary R-GFR increases during pregnancy utilizing progressively the renal functional reserve which is the difference between T-GFR and R-GFR. Thus the possibility to increase GFR during pregnancy depends on an intact RFR and filtration capacity. Subjects with early renal disease not yet clinically evident with normal R-GFR but reduced filtration capacity and RFR may encounter clinical problems during pregnancy being unable to increase GFR in response to hormonal stimuli.

The test is simple and permits for a low cost to predict the behavior of the kidneys during future pregnancies in normal women without evidence of renal disease and might prevent some risks in pregnant women with early renal disease not yet clear.

PROGRESSION OF CHRONIC RENAL FAILURE (CRF) IN PATIENTS (p) WITH SOLITARY KIDNEYS (SK). C. Ruggiu*, L. Oldrizzi*, G. Maschio. Div. Nephrology, Verona, Italy.

Very little is known on the rate of progression of CRF in p with SK as compared to that of p with bilateral primary chronic renal disease. We prospectively followed for 36 to 48 months 4 groups of p on protein-restricted diet (0.6 g/kg bw). Group 1 had 90 p with Chronic Glomerulonephritis (CG); Group 2 had 44 p with Polycystic Kidney Disease (PKD); Group 3 had 93 p with Chronic Pyelonephritis (CP); Group 4 had 30 p with congenital (9) or acquired (21) SK. All p in Group 4 had normal renal function and BP after nephrectomy. The 4 groups were matched for age, sex, initial SCR, known duration of hypertension and follow-up. The frequency of hypertension was not statistically different in the 4 groups and all hypertensive p received the same drugs.

Follow-up (months)	35.8±28.8	35.8±25.5	41.4±28.0	48.1±30.0
SCR (mg/dL) start	2.34±1.42	2.66±1.42	2.54±1.15	2.12±1.84
end	4.34±3.12	4.84±3.18	4.37±3.58	4.64±4.26
M.I.SCR (mg/dL/mo)	.056±.15	.060±.10	.044±.14	.031±.13
A.S.P. (per cent)	48	45	70	60*

M.I.SCR: monthly increase in Serum creatinine

A.S.P.: Actuarial Survival Probability (assuming as renal death a serum creatinine > 10 mg/dL) at 72 months of follow-up.

* $p < 0.05$ vs both CG and PKD

The prognosis of CRF in p with SK seems better than that in p with CG or PKD when early dietary protein restriction is prescribed.

LARGE VOLUME ABDOMINAL PARACENTESIS (P) IN THE TREATMENT OF RESISTANT ASCITIS: S.A. Sadjadi, R.M. Shah, V.A. Medical Center, Wilkes-Barre, PA

Three patients with advanced liver cirrhosis and resistant ascitis were treated with repeated (58, 12, 16) abdominal paracenteses over an 18 month period. Each time 4 to 6 liters of ascitic fluid was removed. There was no significant change in serum urea nitrogen, creatinine, sodium, potassium, renin, aldosterone, antidiuretic hormone level and serum osmolality. Arterial blood pressure change before and after P was significant ($p < 0.05$) but was not associated with any symptom. Plasma albumin rose significantly ($p < 0.001$). Renal and hepatic function remained stable throughout.

Acute renal and electrolyte effects of large volume paracentesis:

Before (B) and After (A)

	Patient 1		Patient 2		Patient 3	
	B	A	B	A	B	A
SUN mmol/l	1.8	2.3	2.3	2.3	3.5	3.5
S _{cr} μmol/l	79.2	88	70.4	79.2	167.2	167.2
SN _a mmol/l	138	139	130	128	143	142
S _k mmol/l	4.7	4.9	3.0	2.9	3.7	4.9

S: serum

SN_a: serum sodium

UN: urea nitrogen

S_k: serum potassium

cr: creatinine

Conclusion: Repeated large volume abdominal paracentesis is a reasonable and safe alternative in some patients with cirrhotic ascitis.

NEPHROTIC SYNDROME IN CHRONIC LYMPHOCYTIC LEUKEMIA: RESPONSE TO THERAPY. M. Schiff, P. Phatak, J. Olson, M. Owens and M. Lichtman, D. Kamm. Dept. of Med., U. of Rochester Sch. of Med., Rochester, NY.

The association of nephrotic syndrome with chronic lymphocytic leukemia (CLL) is well documented; the most frequent glomerular lesion appears to be membranoproliferative glomerulonephritis. A review of the literature shows that of twenty reported cases, eight were treated with cytoreductive or immunosuppressive therapy. A partial response occurred in three patients and complete response in one patient on two separate occasions. We report five cases of nephrotic syndrome in CLL. In all five patients, treatment with cytotoxic agents resulted in improvement or complete remission of their nephrotic syndrome. Protein excretion (G/day), serum creatinine (mg/dl) and WBC were 10.2 ± 2.7 , 1.42 ± 0.21 and $54,600 \pm 13,200$ before and $1.0 \pm 0.43^*$, 0.98 ± 0.07 and $15,900 \pm 8,700^*$ after therapy. None of the patients had either amyloidosis or increased excretion of light chains. One patient had four separate relapses of over a fifteen year period, each associated with an increase in her lymphocytosis and anemia followed by worsening proteinuria and renal function. Each of these episodes responded to chemotherapy. These results indicate that a trial of cytoreductive or immunosuppressive therapy is warranted in the management of the nephrotic syndrome in patients with CLL. A reduction of the tumor burden will often result in a remission of the nephrotic syndrome.

*Different from before, $p < 0.05$

HAS RECOMBINANT ERYTHROPOIETIN (r-EPO) AN HYPERTENSIVE EFFECT?

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The blood pressure response of 75 originally severely anemic dialysis patients treated with r-EPO (Genetic Inst./Boehringer Mannheim) was studied in a randomized multicenter study (r-EPO 40, 80, or 120 U/kg BW). When the target hematocrit of 30-35% had been reached, the predialysis systolic blood pressure (SBP) was compared to that before r-EPO treatment. The patients were categorized as hypotensive (SBP <100 mmHg; group I), normotensive (SBP 100-160 mmHg) without (group II) or with medication (group III), and hypertensive (SBP >160 mmHg; group IV). No significant change in mean SBP was observed in any of the four groups. However, in 12 patients of group III the antihypertensive medication had to be increased; 9 remain normotensive, while 3 have mean SBP of >160 mmHg. In 4 patients the r-EPO therapy had to be discontinued because of severe hypertension. No correlation could be found between the SBP increase and the weekly rise in hematocrit or the r-EPO dose administered. We conclude that patients of group III and IV are most prone to a further increase of SBP and require special attention during r-EPO therapy.

PREDICTIVE VALUE OF STEROID PATTERN TO BIOPSY (Bx) AND DIFFERENCES IN THE THERAPEUTIC RESPONSE IN Bxs VS STEROID PATTERN IN CHILDREN (Ch) WITH NEPHROTIC SYNDROME (NS). Seth L. Schulman, *Bruce A. Kaiser, * and H. Jorge Baluarte, Dept. of Pediatrics, Temple Univ. School of Medicine, Philadelphia, PA.

We reviewed 91 Ch mean age 5.0 ± 3.9 yrs biopsied for NS prior to therapy with cytotoxic agents. Bxs were defined as focal segmental glomerulosclerosis (FSGS) in 39 Ch when there was evidence of focal sclerosis, lipoid nephrosis (LN) in 27 Ch with normal glomeruli or with mesangial prominence (MP) and questionable (Q) in 25 Ch with evidence of focal global sclerosis, IgM deposits on immunofluorescence or MP and tubular changes. Steroid patterns were defined as steroid resistant (SR) in 40 Ch with NS after 8 wks of steroids, steroid dependant (SD) in 32 Ch who relapse on QOD therapy or within 2 wks of therapy and frequent relapse (FR) in 19 Ch who have at least 4 relapses/yr.

	FSGS	Q	LN	
SR	23	12	5	$p < .01$ agreement between two methods
SD	14	9	9	
FR	2	4	13	

Cyclophosphamide (126 ± 37 mg/kg) or Chlorambucil (12.6 ± 5.8 mg/kg) was given to 83 Ch. Using actuarial survival techniques NS relapse curves were generated. Comparing Bx results, statistical significance between curves was only found for FSGS vs LN ($p = .05$) and not LN vs Q or FSGS vs Q ($p = ns$). Curves generated comparing steroid patterns revealed greater significance; between SR vs FR and SR vs SD ($p < .005$) though SD vs FR was not significant. R_{50s} (the time in mos when 50% of Ch relapsed) were extrapolated from the curves. R_{50s} were 4, 14, 25 mos for FSGS, LN and Q respectively and 0, 13 and 44 mos for SR, SD and FR respectively. Our data demonstrate the ability to predict the Bx based on steroid patterns and suggest that steroid patterns correlate better with response to cytotoxic therapy than Bx results.

CONTRAST NEPHROTOXICITY: A PROSPECTIVE RANDOMIZED TRIAL OF IONIC VERSUS NON-IONIC RADIOGRAPHIC CONTRAST. SJ Schwab, M Hlatky,* K Morris,* D Mark,* C Davidson,* T Skelton,* T Bashore.* Duke University Medical Center, Durham, NC.

One hundred forty-six patients to date have been enrolled in a prospective trial to assess renal dysfunction following intravenous radiographic contrast used in cardiac angiography. High risk patients in this study (49) were determined by serum creatinine (Cr) >1.5 mg%, congestive heart failure, or diabetes mellitus. Seventy-eight patients (mean Cr 1.1 mg%) were randomized to ionic contrast (diatrizoate), while 67 patients (mean Cr 1.1 mg%) received non-ionic contrast (iopamidol). The average dose of contrast was 150 ml in both groups. All patients were hydrated with 1-1.5 L of 0.45% saline pre-study. Serum Cr increased significantly ($P < .001$) in both groups (ionic 0.13 mg% (non-ionic 0.10 mg%) following angiography. Six of 78 (8%) who received diatrizoate increased serum Cr >0.5 mg% compared with only 2 of 67 (3%) who received iopamidol ($P = .22$). There were no episodes of acute renal failure. Neither urinalysis, urinary Na, nor uric acid excretion were useful indicators of nephrotoxicity. Data from this ongoing trial demonstrate that both contrast agents have potential nephrotoxicity. Preliminary data suggest non-ionic contrast may be less nephrotoxic than ionic contrast.

HEREDITARY XANTHINE OXIDASE DEFICIENCY: SEVERE LEUKOPENIA FOLLOWING AZATHIOPRINE ADMINISTRATION. TR Schwab, JL Johnson*, Dept. of Medicine, Mayo Clinic, Rochester, MN, Dept. of Biochemistry, Duke University Medical Center, Durham, NC

The initial serum uric acid was decreased (0.8 mg/dl) in a 25 year-old male taking no medications who developed renal failure due to acute glomerulonephritis. Chronic hemodialysis was required and repeated serum uric acid concentrations were 0.8 mg/dl or less. Following renal transplantation prednisone (0.5 mg/kg/day) and azathioprine (1.5 mg/kg/day) were administered for immunosuppression. Within two weeks of starting therapy severe leukopenia ($<10^3/\mu\text{L}$) developed. Several weeks after azathioprine discontinuation the leukopenia resolved. Our evaluations one year later disclosed a GFR of 29 mL/min/1.73m², a serum uric acid concentration of 0.8 mg/dl, increased serum hypoxanthine (0.07 mg/dl) and xanthine concentrations (0.9 mg/dl); increased urinary excretion of hypoxanthine (46 mg/d) and xanthine (127 mg/d) with decreased urinary uric acid excretion (5 mg/d). There was no evidence of xanthine urinary calculi in the native or allograft kidneys. Bioassay of xanthine oxidase content of jejunal mucosa demonstrated no detectable activity (0%) compared to a normal control subject (65 nmoles uric acid produced/min/ μg protein).

While not reported previously, we postulate that azathioprine treated subjects with hereditary xanthine oxidase deficiency may develop severe leukopenia from accumulation of 6-mercaptopurine, an active metabolite of azathioprine which requires xanthine oxidase for its degradation. As expected renal transplantation does not correct hereditary xanthine oxidase deficiency.

AUGMENTATION OF ANTI-INFLUENZA ANTIBODY(AIAb) RESPONSE IN HEMODIALYSIS(HD) PATIENTS(Pts) BY THYMOSIN ALPHA-1(Tal).

S. Shen, J. Josselson, Q. Cortez*, S. Gravenstein*, W. Ershler*, J. Sadler, P. Chretien*. Division of Neph., Univ. of MD, Baltimore, MD; Div of Geri., Univ. of WI, Madison, WI.

Antibody response to influenza(V) vaccination(V) is poor in HD pts. In this double-blind placebo controlled study we evaluated the effect of Tal on AIAb production after I V in 97 HD Pts. Each patient was matched for age and randomized into one of two groups, A and B. All Pts received an injection of 1986 monovalent I vaccine (Taiwan Virus A). Group A(48 Pts) also received Tal injections (900 ug/m² of body surface area) twice weekly for 4 weeks(wks) after V; group B(49 Pts) received placebo at the same schedule. Peripheral T-cell subset counts (PTSC), and sera for specific AIAb determinations by enzyme-linked immunosorbent assay were collected from all Pts before V, and at 4 and 8 wks after V. Response to V was defined as a 4-fold rise in AIAb from the pre-V level at 4 wks and thereafter post-V. Response rates at 4 wks after V were 71% in group A and 43% in group B ($p < 0.002$, Chi-Square), and at 8 wks, 65% VS 24% ($p < 0.001$). 17 Pts in group A VS 2 Pts in group B were able to sustain an 8-fold rise in AIAb 8 wks following V. Response rates decreased with age in group B, but did not in group A. PTSC increased after 4 wks of Tal injections. Pts who responded to V had significant increases in PTSC, especially in group A Pts. These results suggest (1) that AIAb production after I V is age and T-cell dependent, and (2) that Tal augments AIAb response in HD Pts by increasing PTSC and possibly rectifying the immune impairment associated with aging.

DIABETIC HYPOURICEMIA AS A PREDICTOR OF CLINICAL NEPHROPATHY. M. Shichiri*, S. Sasaki, K. Tomita, Y. Iino, N. Yoshiyama*, H. Iwamoto*, T. Shiiigai*. Tokyo Medical and Dental University and Toride Kyodo Hospital, Tokyo, Japan.

We studied whether low serum uric acid level would be associated with glomerular hyperfiltration and would predict the development of clinical nephropathy in maturity-onset diabetes mellitus.

Of 201 maturity-onset diabetic patients without diminished glomerular filtration rate, 66 patients (32.8%) showed moderate hypouricemia of less than mean-1SD of 201 non-diabetic control. 13 (6.5%) showed marked hypouricemia of less than mean-2SD. Hypouricemic patients showed normal daily urinary urate excretion with a markedly elevated urate clearance/creatinine clearance ratio. Most of them were under poor glycaemic control and presented either negative or intermittent clinical proteinuria. However, neither poor glycaemic control, nor presence of proteinuria or retinopathy alone significantly affected the serum uric acid level of the whole diabetic population. The hypouricemic group showed significantly higher endogenous creatinine clearance and a lower serum β -2 microglobulin level than the non-hypouricemic group, suggesting that the hypouricemic group had a higher glomerular filtration rate. Long-term observation of up to 12 years on the above patients revealed that in most patients persistent hypouricemia was observed prior to an initial appearance of intermittent proteinuria.

These findings suggest that diabetic hypouricemia develops during glomerular hyperfiltration in early diabetic nephropathy. It may also predict near-future progression of incipient diabetic nephropathy.

INCREASE OF NEPHROCALCIN (NC) IN URINE OF RENAL CELL CARCINOMA (RCC) PATIENTS: A POSSIBLE TUMOR MARKER FOR RENAL CELL CARCINOMA. D. Sirivongs*, Y. Nakagawa*, N. Vogelzang* and F. L. Coe. Nephrology Program and Hamatology/Oncology, Univ. of Chicago, Chicago, IL

RCC comprises over 80% of all primary malignant renal tumors. Its site of origin has been believed to be proximal tubular epithelial cells. Previously we have reported that mouse proximal tubule cell cultures produce NC, a calcium oxalate crystal growth inhibitor. To determine if urine from RCC patient contains high level of NC, 24-hr urine collections from 16 RCC patients were obtained and analyzed. These 16 patients all had pathological diagnosis of RCC from tissue obtained by nephrectomy. A one ml aliquot of each urine sample was dialyzed against a 0.7 M NaCl buffer solution (pH 7.0) and NC was quantitated by a competitive ELISA method. NC levels of 15 patients were at least 2- to 5-fold higher than the normal value (2.23 ± 0.36 ug/ml), and as the metastated carcinoma progressed the level of NC also increased. One patient who has no evidence of metastases showed a normal level of NC in urine (3.22 ± 0.67 ug/ml). The urine of two breast cancer patients contained NC in normal amount. Tumors from 2 of 16 patients were cultured in standard media, both patients excrete high amount of NC. NC was purified from the cultured media and characterized by biochemical and immunological methods. The level of NC in urine could be usable as a marker for RCC.

ENALAPRIL(E) REDUCES ALBUMIN EXCRETION IN DIABETIC PATIENTS WITH MICROALBUMINURIA(MA). L. Sliemowitz*, R Bergamo*, R Hirschberg, & J D Kopple. Harbor UCLA Med Ctr, Torrance, CA.

Persistent MA (>15 ug/min or 22 mg/day) precedes the onset of frank diabetic nephropathy. Some workers suggest that if the MA is reversed, diabetic nephropathy may not develop. Since E impedes the development of diabetic nephropathy in rats, we examined whether E could reverse MA in 5 diabetic men (age, $58 \pm SE$ 3 yrs) who received insulin for 15 ± 1 yrs. Serum creatinine was 1.0 ± 0.1 mg/dl. Subjects lived in a CRC for 8-10 wks and ingested a similar diet each day that provided 28.1 ± 1.9 kcal/kg/day and 1.3 ± 0.1 g protein/kg/day. After a 14 day baseline, patients were given E (5-15 mg/day) for 28 days. They were then monitored for 14 days without E. Urine albumin was measured by radioimmunoassay.

	Pre	Enalapril	Post
(N = 5)			
Albumin, mg/day ¹	Wks 1&2	Wks 5&6	Wks 7&8
24 hr alb/creat ¹	33.2± 4.6*	22.8± 4.7	32.3±2.3*
Spot alb/creat ²	.026±.004*	.018±.003	.023±.002*
MAP, mmHg	.016±.003*	.012±.002	.016±.001
	91 ± 2*	84 ± 4	91 ± 2*

Mean of the means ± SE. ¹Mean of two 48 hr collections obtained at the end of each week.

²Mean of 14 first morning voided urine specimens collected during each period. *p<0.05 compared to the E treated period.

The data suggest that in normotensive diabetics with persistent MA, E reduces and sometimes abolishes MA. E may be of benefit to diabetics with MA. Studies are needed to assess whether longterm E therapy in diabetics can prevent MA and stop or retard progressive renal failure.

ACETAZOLAMIDE (A) REDUCES PROXIMAL TUBULAR REABSORPTION AND GLOMERULAR FILTRATION RATE (GFR) IN DIABETIC NEPHROPATHY. P Skótt*, E Hommel*, S Arnold-Larsen*, NE Bruun*, and H-H Parving* (intr. by S Anderson). Hvidøre Hospital, Klampenborg, Denmark.

A double-blind, placebo (P) controlled, crossover study of a 3-day course of A (250 mg TID) was performed in 7 normals (Group I) and in 8 insulin-dependent diabetics with nephropathy (Group II). GFR was measured with the single bolus ⁵¹Cr-EDTA technique in all subjects, and fluid flow rate from the proximal tubule into the thin descending loop of Henle was assessed by measurement of the renal lithium clearance (C_{Li}) in all subjects except one patient in Group II. Results: (Mean±SD, *p<.02 vs. placebo):

Group	GFR	C _{Li}		APR	FPR
		ml/min			
I P	108±11	22±6	85±11	79±5	
A	82± 9*	27±8	56± 7*	67±8*	
II P	71±19	14±5	55±17	79±5	
A	54±14*	15±4	37±6*	72±6*	

In both groups a 24% decline in GFR occurred during A treatment, while the renal lithium clearance was unchanged. Absolute proximal tubular reabsorption (APR) of water was reduced by about a third, and fractional proximal reabsorption (FPR) of water and sodium declined. Renal sodium clearance and distal fractional reabsorption of sodium were unchanged, while a slight increase in distal fractional water reabsorption occurred. Thus, A reduces GFR and proximal tubule water reabsorption in parallel, while output of fluid from the proximal tubule remains nearly constant.

BIOCHEMICAL CORRECTION OF TYPE I PRIMARY HYPEROXALURIA WITH COMBINED LIVER-KIDNEY TRANSPLANT. L. Smith, J. Perkins,* D. Wilson, J. McCarthy, and R. Wiesner.* Mayo Clinic, Rochester, Minnesota.

We report a 36 y.o. male with pyridoxine resistant Type I Primary Hyperoxaluria (PHI) who received a combined liver-kidney transplant (LKTx) on 2/11/87. Despite conventional therapy (Vit B₆ and oral phosphate) progressive renal failure occurred and led to kidney transplant (KTx) 7/86. Rejection and cyclosporin toxicity caused KTx failure. Oxalate removal from KTx to LKTx by native kidney function (3.84 ± 0.14 mM/24 h, n=42) and hemodialysis (2.89 ± 0.32 mM/dialysis, n=20) led to no oxalosis on bone marrow/muscle biopsies at LKTx. The effect of LKTx on oxalate metabolism is shown in table (representative data).

	Serum C ₂ O ₄	Urine C ₂ O ₄	GFR	C ₂ O ₄	FE _{C₂O₄}
	μM/L	mM/24 hr	ml/min	ml/min	%
Pre Tx	94.6	3.84	16	31.7	2.02
2/14	26.3	2.88			
3/12	4.3	1.59	77	387	433
4/13	3.0	1.26	73	359	422
6/23	1.3	0.66	70	266	326
7/16		0.43			

Enzymological studies done in England showed a marked peroxisomal alanine:glyoxylate aminotransferase deficiency (patient 0.54 μmol/h/mg protein; 11% of control). Post LKTx the urinary excretion of glycolic acid returned to normal by day 7 but it took over 5 months for urine oxalate to normalize suggesting a large oxalate store even in the absence of microscopic oxalosis. LKTx in this patient has corrected the biochemical abnormalities present in PHI. Its use in selected patients with PHI who fail conventional therapy offers potential cure for an otherwise fatal disease.

CLOTTING INHIBITORS IN NEPHROSIS: CHANGES IN PROTEIN C AND S. Q. Soffer, M. Allon and B.L. Evatt. Dep. of Med. Div. of Neph., V.A. Med. Center, Emory Univ. Sch. of Med. and C.D.C., C.I.D., Div. of Host Defence, Atlanta Ga.

Preliminary studies in nephrosis revealed changes in plasma proteins C and S, natural inhibitors of coagulation, presumably related to nephrosis. We measured protein C and free protein S (by electrophoresis) in 42 patients (21 nephrotic) with Diabetes Mellitus (DM), Focal Glomerulosclerosis (FGS), Membranous Glomerulonephritis (MGN) and Chronic Renal Failure (CRF), in order to investigate the relationship between these changes and potential contributing factors.

Abnormalities in C were noted in 38% and in S in 29% of the patients. High C was noted in 36% (primarily in nephrotics). Low S was found in 56% of DM (3/7 nephrotic and 2/2 non nephrotic) and in 1/9 with FGS. Multivariate regression analysis between plasma C or S and type of renal disease, age, sex, serum albumin and degree of proteinuria, revealed that C or S were not associated with degree of proteinuria or serum albumin, either for the entire group or for nephrotic and non nephrotic patients. There was a significant association between the type of renal disease and C and S. Elevated C was present in FGS and MGN, and low S in DM. However, the mean levels of C and S in nephrotic and non nephrotic were not different. Although C was significantly higher in nephrotic FGS vs non nephrotic FGS.

Thus, these results suggest that in different types of nephrosis protein C and S are abnormal, but this is most strongly related to the type of the underlying disorder (DM, FGS) rather than to heavy proteinuria. In evaluating changes in clotting inhibitors in nephrosis, the type of renal disease must be considered.

HYPERCALCIURIA IN CHILDHOOD HEMATURIA: A RISK FACTOR FOR FUTURE UROLITHIASIS. A PROSPECTIVE STUDY OF THE SOUTHWEST PEDIATRIC NEPHROLOGY STUDY GROUP (SPNSG). E. Bruder Stapleton, Memphis, TN.

A prospective multicenter study was designed to determine the frequency and prognostic importance of hypercalciuria (HCU) in children with hematuria. Urinary calcium (UCa) excretion was examined in 215 pts with unexplained isolated hematuria (no proteinuria, urolithiasis, infection or systemic disorder). HCU (UCa >4 mg/kg/d) was identified in 78 pts (36%). Among SPNSG centers, the incidence of HCU ranged from 21-41%. Compared to pts with normal UCa, HCU was characterized by male preponderance (51/78, P<0.04), white race (70/71, P<0.04), family history of urolithiasis (33/78, P<0.003), gross hematuria (30/70, P<0.03) and Ca oxalate crystals (15/78, P<0.0001). Renal biopsies were performed in 9 pts with UCa 0.4-2.5 mg/kg/d; 3 had IgA glomerulonephritis, 2 had glomerular basement membrane thinning, 1 with proliferative glomerulonephritis and 3 were normal. Renal biopsies in 2 pts with HCU (UCa 4.6 and 4.1 mg/kg/d) showed focal global glomerulosclerosis or no abnormalities. UCa after one week of dietary Ca restriction was higher (5.8 mg/kg/d) in renal HCU than in other HCU pts (3.4 mg/kg/d), P<0.01. Oral Ca loading tests showed renal HCU in 27 pts, absorptive HCU in 16 pts and were not diagnostic in 35 pts. Serum parathyroid hormone, bicarbonate and phosphorus and U cAMP concentrations were similar in the 3 groups of HCU pts. 1-4 yr follow-up was available for 184 pts. 8 of 60 HCU pts developed urolithiasis or renal colic compared to 2 of 124 pts with normal UCa (P<0.001). HCU is a common etiology of isolated hematuria and represents a risk factor for future urolithiasis in children with hematuria. Oral Ca loading tests offer little diagnostic benefit over 24 hr UCa following dietary Ca restriction.

RAPID CORRECTION OF HYPONATREMIA: RISK WITHOUT BENEFIT. R. Sterns, and D. Thomas*. University of Rochester School of Medicine, Rochester, NY.

Although widely advocated, rapid correction of severe hyponatremia has not been proven beneficial in animal models or in patients. We made rats hyponatremic (95 to 98 mEq/L) over 72 hours and then corrected the disturbance either rapidly (3 mEq/L/hr, n=12) or slowly (0.3 mEq/L/hr, n=12). After rapid correction to 129 mEq/L, animals became more alert and appeared normal, but one to two days later, 83% deteriorated neurologically and 58% died; all slowly corrected animals steadily improved and survived, neurologically intact. Results were similar in a retrospective study of 20 consecutive patients with serum sodiums of 105 mEq/L or less. After slow correction to 120 mEq/L (0.3 ± .04 mEq/L/hr, n=9), all patients recovered uneventfully. Symptoms initially improved after rapid correction (1.1 ± 0.2 mEq/L/hr, n=11), but in four cases, additional neurologic findings--more severe than the original hyponatremic symptoms--appeared two to ten days after treatment; one of these patients died and one was permanently disabled. Delayed neurologic deterioration was also observed after rapid correction in six additional patients with serum sodiums of 115 mEq/L or less who were treated at two Rochester hospitals in the past eight years. Only one of the ten patients with complications had a major anoxic event before correction of hyponatremia. We conclude: the prognosis of severe hyponatremia is good when corrected slowly; attempts to rapidly increase the serum sodium concentration to "safe" levels may risk potentially fatal delayed neurologic complications.

A SPECTRUM OF HISTOLOGICAL CHANGES IN CHILDREN WITH ARC/AIDS AND NEPHROTIC SYNDROME (NS). J. Strauss*, V. Pardo*, G. Scott*, G. Zilleruelo*, C. Abitbol, A. Paredes*, W. Parks*, and C. Mitchell*. Univ. of Miami School of Medicine, Depts. of Pedi., Pathol., & Micro., Miami, FL.

Adult patients with ARC/AIDS develop NS and chronic renal failure (CRF), with focal segmental sclerosis (FSS) or mesangial hyperplasia (MH). In ARC/AIDS pediatric patients with NS/CRF it is not known whether FSS/MH are the only lesions found. An assessment of renal disease was undertaken in ARC/AIDS children admitted between January 1981 and June 1987. Of 155 children with perinatally acquired HIV infection, 12 had NS [3 FSS, 3 MH, 2 "lupus like changes" (SLE) and 1 minimal changes (MC)]; 2 had no renal histology (UH). Focal tubulo-interstitial nephropathy (TIN) was observed in 4 patients (2 FSS, 1 MH, 1 MC). Five patients (2 SLE, 1 FSS, 1 MH, 1 UH) were treated with prednisone and evidenced no response. Two patients (1MH, 1 MC) had spontaneous remissions. Six patients (4 FSS, 1 SLE, 1 UH) rapidly progressed to CRF; 1 presented with an apparent acute renal failure. Nine patients (4 FSS, 2 MH, 2 UH, 1 SLE) have died, 3 without CRF; 3 patients (2 MH, 1 MC) are alive and relatively well, up to 17 months after proteinuria was diagnosed. Prednisone, immune suppressors, or plasmapheresis seem to have little effect on NS children with ARC/AIDS and FSS or SLE. It is concluded that in infants and children, ARC/AIDS and its associated nephropathy can occur independently of drug addiction, that they may develop NS with a variety of lesions, that NS may be present without CRF for many months, and that death may occur with or without CRF.

HYPONATREMIA (HN) IN PATIENTS WITH ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) and the AIDS RELATED COMPLEX (ARC). Winson W. Tang,* Eben I. Feinstein, Shaul G. Massry, Div. Nephrol, Univ. So. Calif, Los Angeles, CA.

Fluid and electrolyte disturbances are common in patients (pts) with AIDS or ARC. We prospectively evaluated HN in 210 pts with AIDS (165) or ARC (45) during a 3 months period. 99 episodes of HN occurred during 259 admissions, for a prevalence of 34%. In 58% of pts, HN was present on admission. These pts were hypovolemic and had gastrointestinal losses associated with mycobacterial infection. Two hypovolemic pts had adrenal insufficiency. In 47 episodes of HN, the pts were euvolemic and HN developed in most of them in the hospital. These pts usually had pneumocystis carinii infection and the etiology of HN was consistent with nonosmotic stimulation of vasopressin secretion. Hypervolemia was detected in 9 episodes of HN; 8 of these patients had renal insufficiency and 2 of them had also the nephrotic syndrome. Pts with HN stayed in the hospital longer than normonatremic ones (16.8 ± 1.3 vs 9.1 ± 0.7 days $p > 0.001$) and the mortality rate was also greater in HN pts with AIDS or ARC (30 vs 15.6%, $p = 0.05$). The data show that HN is common in patients with AIDS or ARC, has multiple etiologies, occurs in eu-, hypo-, or hypervolemic states, and is associated with a poor prognosis.

HYPERURICEMIA IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD). DJ Tangel*, WD Kaehny, IT Duley*, AM Johnson*, PA Gabow. University of Colorado Health Sciences Center, Denver, CO

Several reports suggest a positive relationship among hyperuricemia, gout and ADPKD. This suggestion is based on studies with few subjects and were not controlled for other factors known to affect uric acid (UA) handling. To evaluate this relationship we studied 79 subjects with ADPKD and 97 unaffected relatives as controls (C). All subjects were at least 18 years old, had serum creatinine levels less than 1.6 mg/dl and were not taking diuretics or allopurinol. The ADPKD and C had similar mean serum uric acid (SUA) levels (4.9 ± 0.1 vs 5.2 ± 0.1 mg/dl), mean 24 hour UA excretions (583 ± 15 vs 553 ± 19 mg/24), mean fractional excretion (FE) of UA ($8.7 \pm .3\%$ vs $7.9 \pm .4\%$) and similar frequency of hyperuricemia (3.8% vs 2.1%) and hyperuricosuria (7.6% vs 8.2%). The ADPKD and C groups had similar prevalence of gout (5% vs 6%). To analyze factors known to affect UA handling, covariance analysis was utilized with sex, age, presence of ADPKD, hypertension (HTN) and creatinine clearance (CCr) as covariates. SUA level was significantly related to HTN, CCr and sex. FE was significantly related to CCr and sex. Neither SUA or FE was related to ADPKD. No relationship was found between the severity index (based on cyst number, cyst size and % normal renal parenchyma) and SUA. We conclude that UA handling is normal in ADPKD with normal renal function, and factors affecting UA handling are the same in ADPKD as in the general population and include HTN, CCr and sex.

NATRIURETIC RESPONSE TO VOLUME EXPANSION (NRVE) IN ADULT POLYCYSTIC KIDNEY DISEASE (APKD) WITH NORMAL RENAL FUNCTION. V. E. Torres, D. M. Wilson, K. P. Offord,* J. C. Burnett, Jr., J. C. Romero. Mayo Clinic and Mayo Foundation, Rochester, MN.

Whether APKD pts with normal renal function have blunted NRVE that contributes to hypertension (HT) is controversial. Nine APKD pts (5 with HT, 4 normotensive) and 9 age- and sex-matched normotensive controls (NTC) received 6.5 ml of 0.9% NaCl/kg/hr for 4 hrs. They were on no medications and had similar baseline Na⁺ excretion (147 ± 33 vs. 164 ± 33 mEq/24 hrs). FE_{Na⁺} was measured during and for 3 hrs following NaCl infusion.

	APKD		NTC	
	Pre	4th hr	Pre	4th hr
MABP(mmHg)	105±15	107±15	88±7	90±7
C _{in} (ml/min/SA)	105±26	106±23	101±12	106±14
C _{pAH} (ml/min/SA)	454±109	468±116	522±103	513±119
FF(%)	23.5±4.6°	23.1±3.9	20.1±2.6°	21.7±4.0
FE Na ⁺ (%)	1.7±0.7§	3.8±1.3§	1.6±0.3†	2.8±1.4†
Mean±SD.	§,†: p<0.01, °: p=0.064.			

During NaCl infusion, FE_{Na⁺} increased at a faster rate in APKD (36±7% of the Na⁺ load excreted vs. 27±9% in NTC $p = 0.03$). For the 3 hrs following NaCl infusion, APKD and NTC excreted 11±5% and 15±8% of the Na⁺ load. At the peak of the NRVE, the pressure-natriuresis regression line was shifted to the right by 21 mmHg ($p = 0.056$) in APKD pts with HT. Pre-expansion p. aldosterone and FE_{K⁺} were lower in APKD. No other differences in p. aldosterone, PRA or ANF were detected. These observations suggest that (1) in APKD NRVE is not blunted as compared to NTC, (2) in APKD pts with HT there is a resetting of the pressure-natriuresis that could contribute to development and/or maintenance of HT.

PLATELET-VASOPRESSIN (AVP)-VOLUME RELATIONSHIPS IN IDIOPATHIC NEPHROTIC SYNDROME (INS). H. Trachtman* & B. Gauthier, Dept. of Peds., SUNY-Stony Brook, Schneider Child. Hosp. of LIJMC, New Hyde Park, NY.

The pathogenesis of edema accumulation in INS and the role of primary intravascular volume depletion in the syndrome remain controversial. The plasma renin activity (PRA) has been the standard measure of the volume status of these patients. The plasma AVP could serve as an index of the effective circulating blood volume in nephrotic children, but has previously shown poor correlation with PRA levels. Since 90% of circulating AVP is bound to platelets, we reinvestigated the AVP-PRA relationship in INS by measuring AVP in platelet-rich (PRP) and platelet-poor (PPP) plasma and correlated these values with PRA.

Blood was collected from 16 children with INS during disease relapse. One sample was spun at 130 g x 20 min to yield PRP and the second was spun at 950g x 20 min to yield PPP. AVP levels in both fractions and PRA were determined using standard RIAs. The mean levels (mean±SEM) of PRA (ng/ml/hr), PPP-AVP and PRP-AVP (pg/ml) were 13.9 ± 2.9 , 3.5 ± 0.7 and 11.9 ± 4.2 , respectively. The platelet-bound AVP levels, obtained by subtracting PPP-AVP from PRP-AVP levels were 8.4 ± 4.0 and the % AVP binding to platelets were lower (55%) in nephrotic patients than previously reported in normal controls. The PPP-AVP level was significantly correlated with PRA ($r = 0.83$, $p < 0.001$), while no such relationship was observed with PRP-AVP or platelet-bound AVP levels.

These results indicate that plasma AVP levels may be a useful index of effective intravascular volume in INS if they are measured in PPP.

ENDOTOXEMIA, BACTEREMIA AND UROSEPSIS.

S.J.H. van Deventer*, I. de Vries*, L.W. Stadius van Eps, W. Pauw*, H.R. Buller*, A. Sturk* and J.W. ten Cate*. Depts. of Internal Medicine and Bacteriology, Slotervaart Hospital and Dept. Hemostasis and Thrombosis, Academic Medical Center, Amsterdam, The Netherlands.

Endotoxins are the most important bacterial factor in the development of Gram-negative septicemia. In this study we assessed the predictive value of endotoxemia and bacteremia for septicemia in consecutive febrile patients with Gram-negative urinary tract infections. At entry in the study blood was obtained for bacterial culture and endotoxin tests (using the chromogenic Limulustest, detection limit 5 pg/ml). In a subsequent period of 72 hours the development of septicemia was determined, defined as the presence of one or more of the following symptoms: shock (systolic blood pressure < 90 mmHg), oliguria, metabolic acidosis or thrombocytopenia. Of 76 consecutive febrile patients 14 (18%) developed septicemia. Gram-negative bacteremia was present in 38 patients (11 septic), sensitivity and specificity being 79% and 56% respectively. The positive predictive value was 29% (neg. 92%). In contrast, the presence of endotoxemia was a reliable harbinger of septicemia as 11 of 15 patients developed septicemia, sens. and spec. were 79% and 95%, whereas the positive predictive value was 73%, neg. pred. value 95%. We conclude that the chromogenic Limulus assay has high predictive values for septicemia in febrile patients with symptomatic urinary tract infections.

DISSOCIATION OF HYPERPERFUSION AND HYPERFILTRATION FROM PROTEINURIA IN NEPHROTIC RATS TREATED WITH FISH OIL DIET (FOD). Z. Varghese*, A. Ando*, J. Senior*, J. Persuad*, P. Sweeney*, J.F. Moorhead. Royal Free Hospital, Pond St., London, UK.

The effect of diets with high fat content and omega-3 fatty acids (fish and oil diet) on the development of proteinuria and renal function were examined in two models of nephrotic syndrome.

5/6th Nephrectomised Sprague-Dawley Rats (SD): 3 groups of 5/6th nephrectomised SD rats were studied. 1-Normal diet (ND), 2-high fat diet (HFD), and 3-FOD. The HFD group had more severe hyperlipidaemia, earlier and heavier proteinuria. The FOD group had higher GFR and EPRF than those on ND. FOD also reduced plasma lipids and proteinuria.

Uninephrectomised Obese Zucker Rats (OZR): 3 groups of uninephrectomised OZR were studied. 1-ND, 2-restricted diet [as for normal lean Zucker rats (LZR)] and 3-FOD. Controls were uninephrectomised LZR on ND. OZR developed proteinuria earlier than LZR. Proteinuria was higher and the hyperlipidaemia was more extreme in the ND OZR than the other groups. The FOD group had higher GFR and EPRF at 24 weeks yet significantly lower proteinuria. Hyperlipidaemia can significantly accelerate renal damage. FOD reduced hyperlipidaemia and proteinuria but increased GFR and EPRF. Therefore, FOD dissociated hyperperfusion and hyperfiltration from proteinuria.

CYCLOSPORINE (CSA) INDUCED RENAL FUNCTION (RF) IMPAIRMENT IN PATIENTS (Pt) WITH PRIMARY BILIARY CIRRHOSIS (PBC). J. A. Velosa, R. H. Wiesner*, R. Jorgensen*, E. R. Dickson*. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

We studied the effects of CSA on RF in Pt with normal RF and no other confounding variables known to affect renal performance. We measured RF in Pt with PBC (an autoimmune cholestatic liver disease) enrolled in a double blind randomized controlled trial using CSA. By design, CSA trough levels were maintained between 80-140 ng/ml (HPLC on whole blood), a level lower than generally utilized in organ transplantation. Thirty-four PBC Pt were randomized to CSA 19, and placebo 15. Iothalamate clearances (CIOT, ml/min) were performed at baseline and one year and serum creatinine (SCr, mg/dl) at baseline and monthly intervals. The mean CSA dose was 4.7 mg/kg/day/yr (range 2.7-15), and mean CSA trough levels were 132 μ g/L/yr (range 50-330). In the treatment group, the mean CIOT declined by 24 ml (p=.001) while mean SCr increased by .13 mg/dl (p<.05) from baseline. CSA levels did not correlate with changes in RF.

Renal Function	Treatment	Placebo
CIOT baseline	n=19 93.7 \pm 12.5*	n=11 96.4 \pm 15.3
1 year	n=15 71.2 \pm 14.8	n=6 101.3 \pm 19.2
SCr baseline	n=19 0.78 \pm 0.2†	n=12 0.82 \pm 0.1
1 year	n=15 0.91 \pm 0.3	n=6 0.75 \pm 0.1

* p=.001 † p<.05
In CSA treated vs. controls there was a 25% loss of RF. Renal dysfunction was unrelated to dose or trough CSA levels. Whether these changes are stable, reversible, or progressive remains to be determined.

SURVIVAL PATTERNS IN LUPUS NEPHRITIS WITH END-STAGE RENAL DISEASE. N.K. Wadhwa*, K.S. Kant, D.H. Clyne, M.R. First, V.E. Pollak, Dept. of Medicine, University of Cincinnati and V. A. Medical Center, Cincinnati, Ohio.

The prognostic implication of systemic lupus erythematosus (SLE) in patients with lupus nephritis (LN) on end-stage renal disease (ESRD) therapy is controversial. To evaluate SLE as a risk factor, we studied 20 consecutive patients with ESRD secondary to LN out of 554 patients (3.6%) who started treatment at this center from 1973-86. The follow-up period ranged from 1.5 to 12 years after the development of ESRD. Twelve patients started hemodialysis while 8 started continuous ambulatory peritoneal dialysis (CAPD) as the initial treatment modality. Four patients died at 3, 7, 9, 10 months following the initiation of dialysis treatment. Nine patients subsequently received renal allografts (5 cadaver, 4 living related). No patient died after transplantation. However, 4 renal allografts were lost at 9 days, 15 days, 13 months and 25 months post-transplantation. When SLE was analyzed as one of 22 independent risk factors in 554 patients starting ESRD therapy at this institution over the same period by the Cox Multivariate Hazard analysis, it did not significantly influence the patient survival. A similar analysis in 230 of the 554 patients who received transplants showed that the presence of SLE did not affect the graft survival. We conclude that SLE does not adversely affect the survival in patients treated either by dialysis or transplantation.

LEUKOCYTOSIS, AGE, AND RENAL FAILURE ARE THE MOST IMPORTANT RISK FACTORS IN IDIOPATHIC RENAL VASCULITIS. R. D. Wagoner, J. A. Velosa, M. J. Wilkowski,* K. E. Hötley, V. E. Torres, J. V. Donadio, K. P. Offord,* C. Chu*. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

We studied 142 patients (Pt) with idiopathic renal vasculitis; focal segmental necrotizing glomerulonephritis (FSNGN) alone (80 Pt), or combined with small (33 Pt) or medium sized (29 Pt) artery vasculitis. Observed Pt survival was not different among the 3 groups, therefore, analysis of risk factors was based on all Pt. Univariate analysis of clinical, pathologic, and laboratory variables determined at initial evaluation defined the following risk factors.

	Pt n	%Pt Survival		
		1 yr	3 yr	5 yr
Age <40	28	92(.92)*	87(.87)	87(.87)
(Yrs) 41-70	86	78(.80)	68(.72)	53(.59)
71+	28	77(.83)	63(.80)	63(.93)
WBC <12x10 ³	104	87	77	65
(cells/μl) >12x10 ³	36	65	56	49
Serum Cr <4.0	105	83	74	67
(mg/dl) >4.0	37	72	62	42

(*) relative survival (observed/expected in general population)

Leukocytosis, age, and serum creatinine were each associated with Pt survival ($p < .007$, $p < .006$, $p < .001$). Observed and relative Pt survival was significantly decreased for 41-70 year old Pt compared to younger age. Severe but not lesser degrees of renal insufficiency indicated higher mortality. These findings underscore leukocytosis as a marker of disease severity and may be of help in patient management and the design of prospective treatment protocols.

DIETARY PROTEIN RESTRICTION MODIFIES LIPOPROTEIN ABNORMALITIES IN INSULIN-DEPENDENT DIABETICS (IDDs) WITH NEPHROPATHY. J Walker, M Mattock, R Dodds, P Kontessis, J Bending, G C Viberti. Intr. by F Pugliese. Unit for Metabolic Medicine, UMDS, Guys Campus, London, UK.

Abnormal lipoproteins in IDDs with persistent proteinuria (P) may contribute to their increased risk of cardiovascular death. We compared fasting lipoprotein fractions in 77 IDDs without proteinuria (NP) (41M aged 48±6y; 36F aged 52±6y) with 34 IDDs with P (24M aged 43±13y; 10F aged 38±13y, mean (SE) GFR 69 (8)ml/min/1.73m²) and investigated the effect of a low protein diet (LPD) in 16 IDDs with P. Mean Body Mass Index, tobacco and alcohol consumption were not different between NP and P. In female P total cholesterol (TC) was significantly higher (6.96±1.83) than in NP (6.0±0.98mmol/l, $p < .05$) with an increased LDL-Cholesterol (LDL-C) (4.47±1.43 vs 3.68±0.92mmol/l, $p < .05$). HDL cholesterol was not different between the two groups. Male P showed no difference from NP in lipoprotein fractions. LPD reduced protein intake from 84±19g/24h to 46±5g/24h, cholesterol from 403±184 to 141±53mg/24h, fat from 97±38 to 70±19g/24h and increased P/S ratio from 0.27±0.12 to 0.56±0.33. Although TC did not fall significantly on LPD, LDL-C fell from 3.5±1.43 to 2.96±0.3mmol/l ($p < .05$). On usual protein diet there was a negative correlation between triglyceride and GFR ($r = -0.4$, $p < .05$) which disappeared on LPD. TC and LDL-C are elevated in females with diabetic nephropathy. LPD favourably modifies lipoprotein abnormalities in diabetic nephropathy.

STONE DISEASE IN SAUDI ARABIA: AN INITIAL REPORT. V.R. Walker,* W.G. Robertson,* R.A.L. Sutton, N. Bissada,* H. Hughes,* S. Barkworth,* and R.G.G. Russell.* King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia and Vancouver General Hospital, Vancouver, Canada.

Urolithiasis in Saudi Arabia (S.A.) accounts for 1/3 of all patients treated by the urologists. It is a major disorder in this hot and often dry region. Our purpose was to study this problem by a number of approaches. The present report presents some of our preliminary findings from screening studies in 230 S.A. subjects, 142 classified as male idiopathic stoneformers (SF). In 2-hr fasting urine collections differences in Ca, P, Mg, Na, K, Cl, and urate were not found between this group of SF and matched normal controls (n=29). However, when comparing these parameters with Vancouver studies performed using an identical protocol, differences were observed in the two populations of SF, particularly in regard to urate:

	S.A. (142)	Vancouver (42)	
Serum urate (mg/dl)	6.9 ± 0.16	6.0 ± 0.1	$p < .001$
Urine urate/creat (mg/mg)	0.42 ± 0.01	0.35 ± 0.02	$p < .05$
FE urate (%)	7.2 ± 0.3	6.0 ± 0.3	$p < .01$

Values shown are Mean ± SEM, n in parentheses.

These initial observations indicate that uric acid may play a greater role in the genesis of renal calculi in S.A. than in Western countries. High purine intake, low urine volume and a more acid urine may contribute to the higher number of uric acid containing stones in S.A. However, we have also found that the fasting excretions of Ca, Na and Mg in normal S.A. subjects are greater ($p < .01$) than in normal Western subjects. Thus several factors have been identified which may predispose this population to renal stone disease.

SERUM CARNITINE LEVELS AND CARNITINE ESTERS OF PATIENTS WITH CHRONIC RENAL INSUFFICIENCY. C. Wanner,* P. Schollmeyer,* W.H. Horl,* Univ. of Freiburg, Dept. of Medicine, Freiburg, FRG (intr. by T.F. Luscher).

It has been shown that all forms of renal insufficiency show a disturbed pattern of acyl- to free carnitine.

Therefore, we investigated serum levels and urinary excretion of carnitine and carnitine esters in 7 patients (mean creatinine 2.8 ± 0.4 mg/dl) and 7 healthy control subjects (CO) before and 2, 4, 11 and 24 hours after a single intravenous dose of L-carnitine (10 mg/kg body weight). Free carnitine (FC) short chain- (SCC) and long chain acylcarnitine (LCC) were measured with an improved radiochemical enzymatic assay (Rössle et al. Clin. Chim. Acta 149: 263-268, 1985).

Azotemic patients (AP) showed a significantly higher ratio of acyl/free carnitine as compared to healthy controls (0.32 ± 0.04 vs 0.23 ± 0.02; $p < .01$). 24 h urinary excretion of SCC was lower in azotemic patients. Two hours after L-carnitine administration a significantly higher increase of acylcarnitine formation was found in AP (SCC: 86 ± 7.6 % vs 114 ± 15.4 %; $p < .05$. LCC: 54 ± 5.1 % vs 84 ± 7.9 % $p < .01$). This effect was also present during 4 and 11 hours. 24 hours after L-carnitine administration serum carnitine esters returned to baseline values in CO (SCC-0h vs 24h: 7.2 ± 0.6 vs 8.3 ± 1.3 μmol/L; LCC-0h vs 24h: 2.6 ± 0.2 vs 2.6 ± 0.1 μmol/L) but were still higher in AP (SCC-0h vs 24h: 19 ± 2.7 vs 29.3 ± 6 μmol/L; LCC-0h vs 24h: 3.8 ± 0.3 vs 4.5 ± 0.4 μmol/L). However, 24 h urinary excretion of SCC was comparable in AP and CO (SCC: 510 ± 51 vs 582 ± 40 μmol/L).

Our results document, that carnitine acylation is not impaired in chronic azotemic patients. Therefore, increased serum values of short chain- and long chain acylcarnitine in patients with chronic renal insufficiency seem to depend on the excretion capacity of the diseased kidneys.

POTASSIUM EXCRETION IN SEVERELY HYPERGLYCEMIC PATIENTS: A REVERSIBLE DIMINISHED RESPONSE TO ALDOSTERONE. M.L. West, P.O. Magner and M.L. Halperin. Renal Div., St. Michael's Hosp., Univ. of Toronto, Toronto, Canada.

In patients presenting with the hyperglycemic hyperosmolar syndrome (HHS) or diabetic ketoacidosis (DKA), the plasma [K] is usually in the high normal range despite severe K depletion. Normokalemia is said to reflect K redistribution from the ICF to the ECF secondary to insulin deficiency. HHS and DKA should be associated with very marked renal K loss because both the prerequisites required for K excretion should be present - a large daily urine volume (osmotic diuresis) and aldosterone action (response to the ECF volume contraction). 3 patients with HHS and 5 with DKA had typical findings on presentations; the mean plasma [K] was 5.3 ± 0.2 mM in HHS and 5.0 ± 0.2 mM in DKA. Plasma aldosterone levels on presentation were above the upper limit of normal in 7/8. However, the urine [Na] and [K] were 35 ± 3 and 28 ± 2 mM and the distal nephron transtubular [K] gradient (TTKG) was only 2.4 ± 0.3 (expected value 7). 8 hours after therapy was begun, the TTKG rose to 7.8 ± 0.3 despite a fall in aldosterone level.*

Conclusion: HHS and DKA are associated with a reversible aldosterone resistance on admission and this response was normal after 8 hr of therapy. This resistance prevents the development of much more severe K depletion.

* Min Elect Metab 12:234, 1986.

OPTIMAL DETERMINATION OF URINARY ALBUMIN EXCRETION AND RENAL FUNCTION IN TYPE I DIABETES. I. Wiegmann*, W. Moore*, and A. Chonko. Veterans Adm. Medical Center, Kansas City, MO, and University of Kansas Medical Center, Kansas City, KS.

Urinary albumin excretion rate (AER, ug/min) may be an important early indicator of renal dysfunction and a predictor of diabetic nephropathy. There is disagreement regarding best technique for determination of renal function and AER. We examined 15 Type I diabetic patients (age <18 yrs) with normal 24 hr AER (<20 ug/min). Creatinine clearance (CL cr) and AER were determined after waterloading during supervised 30 minute collections (Mean \pm SEM):

Min	Clcr ml/min	% Diff	AER ug/min	% Diff
0-30	79.9 ± 11.4	from the	11.38 ± 3.42	from the
30-60	83.8 ± 9.9	mean of	4.84 ± 1.18	mean of
60-90	82.7 ± 9.8	120-240	3.38 ± 0.56	120-240
90-120	82.8 ± 9.2	minutes	3.74 ± 0.95	minutes
120-150	85.1 ± 9.5	5.1 ± 2.5	4.10 ± 1.15	5.1 ± 6.9
150-180	88.2 ± 9.7	1.2 ± 2.7	4.75 ± 1.29	6.0 ± 4.5
180-210	90.2 ± 9.5	1.9 ± 2.3	4.58 ± 1.11	5.8 ± 3.9
210-240	91.9 ± 9.6	4.2 ± 2.5	4.13 ± 0.75	6.8 ± 6.9

Serial AER was characterized by an early peak AER, consistent with washout, while later collections were similar to daytime (5.03 ± 0.89) but not to night (2.10 ± 0.71) collections. We suggest that AER and renal function are conveniently and reliably measured 3-4 hours after waterloading. Early collections may reflect lack of steady state; variability is small under supervised conditions.

THE SPECTRUM OF IDIOPATHIC RENAL VASCULITIS. LONGTERM OUTCOME OF 142 PATIENTS (Pt). M. J. Wilkowski*, J. A. Velosa, K. E. Holley, K. P. Offord*, C. Chu*, V. E. Torres, J. T. McCarthy, J. V. Donadio, R. D. Wagoner. Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

The outcome of Pt with focal segmental necrotizing glomerulonephritis (FSNGN) alone, or combined with small (micro-PAN) or medium size artery vasculitis (PAN) is not well established. All Pt with idiopathic FSNGN evaluated over a 15 year period were classified into these 3 groups according to size of renal or extrarenal vessel involved on tissue biopsy. Differences in serum creatinine at initial evaluation were not significant: FSNGN, $2.3(.9-29.0)$ median(range); Micro-PAN, $2.2(.9-17.1)$; PAN, $1.6(0.8-8.8)$ mg/dl. Treatment was prednisone (P) alone (74%) or combined P plus cytotoxic (CTX) drugs (26%). More Pt with micro-PAN (36%) or PAN (41%) received P plus CTX compared to FSNGN alone (16%) (p=.002). Patient and renal survival (renal survival = Pt survival without renal failure) were analyzed using actuarial survival techniques.

Group	Pt n	% Pt Survival			% Renal Survival		
		1yr	3yr	5yr	1yr	3yr	5yr
FSNGN only	80	84	72	61	81	67	54
Micro-PAN	33	83	73	68	83	69	65
PAN	29	70	65	55	70	65	55
Total	142	NS (log-rank)			NS (log-rank)		

Differences from expected survival were significant for each group. Observed Pt survival was not different among groups. These results show progressive and comparable mortality among these 3 groups of Pt with renal vasculitis and underscore the need for better treatment modalities.

CYCLOSPORIN-TRIAL (CY-A) IN CHILDREN WITH NEPHROTIC SYNDROME (NS): EVALUATION OF NEPHROTOXICITY. L.B. Zimmerhackl, W. Klein, R. Burghard, U. Helmchen*, J.U. Leitis and M. Brandis (intr. by R.B. Sterzel). Dept of Pediatrics, Univ. Marburg and *Dept of Pathology, Univ. Göttingen, FRG.

To evaluate the risk of CY-A induced nephrotoxic effects in NS we studied 9 children aged 2.7-15.9 y in a prospective trial treated with CY-A for 6 month. 7 children were steroid resistant, 2 steroid dependent. On renal biopsy 7 showed focal segmental sclerosis (FSGS), 2 minimal changes. CY-A blood trough levels were maintained between 100 and 400 ng/ml as measured by RIA. Children were investigated prior to CY-A and after treatment for: creatinine (S-CR), urea-N (S-U); clearance of creatinine (C-CR), inulin (C-IN), PAH (C-PAH), phosphate (C-P); fractional phosphate reabsorption (TRP), fractional excretion of α 1-microprotein (A1), B2-microprotein (B2); and morphology by kidney biopsy.

RESULTS: Mean renal function in 8 patients was:

	S-CR	S-U	C-CR	C-IN	C-PAH	C-P	TRP	A1	B2
(μ mol/l)	6	140	96	557	12	85	0*	0.02	
(mmol/l)	6	120	113	626	9	88	0*	0.09	

* = below limit of detection

One child with FSGS had reduced renal function (C-IN 22) prior to therapy and deteriorated under CY-A requiring dialysis 6 month later. After CY-A renal biopsy revealed microcalcification in 1 case, and mild tubular atrophy in another case. CONCLUSION: Short term CY-A treatment of children with NS and normal renal function did not demonstrate nephrotoxic signs. Decreased renal function may be severed by CY-A, however.

DIALYSIS: HEMODIALYSIS

MECHANISMS OF IMPROVEMENT IN ALUMINUM (Al) INDUCED MICROCYTIC ANEMIA (MA) FOLLOWING LONG-TERM DEFEROXAMINE (DFO) TREATMENT IN HEMODIALYSIS (HD) PATIENTS. Kenneth Abreo, Stephen T. Brown, M'Liss Sella*. LSU Medical Center, Shreveport, LA.

Deferoxamine (2mg/wk) was administered to 14 HD patients with Al induced MA. Baseline and monthly plasma Al (P-Al, $\mu\text{g/L}$), red blood Al (RBC-Al, $\mu\text{g}/10^6$ cells), RBC indices, reticulocyte count (R-C) and serum ferritin (S-F) were obtained. Eight patient normalized RBC-MCV ($80-100\mu\text{m}^3$) after 6.4 ± 2.1 months of treatment. In 2 patients MA with high RBC-Al recurred after discontinuing DFO. There were no major side effects. Results were as follows:

	PreDFO	PostDFO	P
RBC-Al	35.6 ± 2.5	6.4 ± 1.4	<.001
P-Al	200 ± 28	281 ± 24	<.05
RBC-MCV	65.9 ± 1.1	82.9 ± 2.3	<.001
Hematocrit	27.2 ± 1.4	33 ± 1.7	<.001
Hemoglobin	8.4 ± 0.4	10.2 ± 0.5	<.001
RBC count	4.1 ± 0.2	4.2 ± 0.5	NS
R-C	1.2 ± 0.6	1.1 ± 0.6	NS
S-F	1437 ± 590	376 ± 160	<.02

In vitro studies: RBC-Al and P-Al were measured before and 24 hrs after incubation of RBC's in plasma (P) containing 0.2 mg/dl of DFO. There was no change in RBC-Al or P-Al.

These data suggest that (1) Decrease in RBC-Al is associated with improvement in MA (2) Correction of MA is a result of increase in RBC-MCV and hemoglobin rather than RBC count and R-C (3) Decrease in RBC-Al does not indicate completion of DFO therapy as MA can recur if tissue Al is not adequately depleted (4) P-Al increases with DFO therapy as a result of mobilization of tissue Al (5) DFO does not transfer Al from RBC's to P.

APPLICATION OF UREA KINETICS (UK) TO NUTRITIONAL THERAPY. Sergio Acchiardo, Linda W. Moore,* Debbie Bannister.* Univ. of TN, Dept. of Med., Memphis, TN.

Frequently nephrologists are confronted with nutritional problems, the treatment of which requires dietary prescriptions, oral supplements, and/or i.v. administrations of nutrients. UK provides the tool needed for clinical nitrogen (N) balance studies. Using routine BUN determinations it is possible to calculate urea generation and protein catabolic rate (PCR) that in stable dialysis patients (pts) corresponds to dietary protein intake (DPI). Using this system, during the last 9 y. we have followed 233 pts on conventional or high flux hemodialysis and 32 pts on CAPD. We counseled HD pts with $\text{PCR} < .8$ (17.6%) and those with $\text{PCR} > 1.4$ gm/Kg/day (3.4%). A group of pts with low PCR, BUN and serum albumin were treated aggressively with intra-dialytic hyperalimentation. CAPD pts had a mean PCR 0.96 gm/Kg/day and an intake of 25.9 KCal/Kg/day. With this regimen most of the patients remained in positive N balance. For these calculations we considered stools and non-urea metabolic losses constant 1.5 gm/day. We also applied UK to pts with acute or chronic renal failure (CRF) receiving hyperalimentation (16 pts) and to a group of pts on continuous arterio-venous hemofiltration (8 pts). These pts were followed with daily measurements of BUN and urine urea excretion. Since we knew their protein intake and we measured their PCR it was possible to adjust their caloric intake to a level sufficient to prevent catabolism. Finally, we applied UK to the follow up of pts with CRF to determine compliance with their diet. In summary, UK is a rapid, simple and effective way to evaluate and modify the nutritional status of pts with renal disease.

CONTINUOUS TRANSCUTANEOUS GAS MONITORING IN ADULTS ON REGULAR DIALYTIC TREATMENT (RDT)

E. Agazia, L. Guarda, and E. Saporiti (intr. by Paul L. Kimmel) Nephrology Dept. Venice, Italy

We had tested a transcutaneous (tc) oxygen (O_2) and carbon dioxide (CO_2) sensor (Radiometer) both in 15 normal adults (NA) and in 20 patients on RDT (RDT-pts)

We had compared the tc gas values (PtcO_2 , PtcCO_2) with the arterial tension (PaO_2 , PaCO_2) measured with an ABL3 blood gas analyzer. This comparison was done once in NA and in RDT-pts at the end of a 30-minutes-test, and six times during 25 RDT (every 60')

During the 30-minutes-test: (* $p < .01$ ** $p < .05$)
 PtcO_2 vs PaO_2 $r = .72^*$ (NA) $r = .41^{**}$ (RDT-pts)
 $\text{PtcO}_2/\text{PaO}_2 = .84\pm .12$ (NA) $.68\pm .16$ (RDT-pts)
 PtcCO_2 vs PaCO_2 $r = .59^{**}$ (NA) $r = .76^*$ (RDT-pts)
 $\text{PtcCO}_2/\text{PaCO}_2 = 1.02\pm .12$ (NA) $1.09\pm .12$ (RDT-pts)

During 14 acetate dialysis changes as Δ % vs time 0':

Δ PaO_2 -12.7 ± 3.5 (at 60') $+4.8\pm 8.8$ (at 240')
 Δ PtcO_2 -6.0 ± 1.0 (at 60') $+9.7\pm 9.0$ (at 240')
 Δ PaCO_2 -17.7 ± 3.4 (at 240') -1.8 ± 2.7 (1 h later)
 Δ PtcCO_2 -15.8 ± 4.5 (at 240') -3.2 ± 2.4 (1 h later)

As in NA, in RDT-pts there is a good relationship between PtcCO_2 and PaCO_2 values. In NA the absolute PtcO_2 values are always lower than PaO_2 (-14.4 ± 10 mmHg); in RDT-pts this negative difference is more considerable (-27.6 ± 15.1). During RDT the trend was the same between the tc and a values. The tc-sensor seems to be useful in continuous monitoring of pts suspected to develop hypoxemia, especially during AD but it cannot substitute the blood acid-base values

ENHANCEMENT OF ERYTHROPOIESIS DURING DEFEROXAMINE (DFO) IN PATIENTS WITH LOW LEVELS OF ALUMINUM (Al) ACCUMULATION. Altmann P*, Plowman D*, Marsh F*, Cunningham J*, (intr. by E Slatopolsky). The London Hospital, London, UK.

Anemia in hemodialysis (HD) patients may be exacerbated by severe Al accumulation. Such patients (now rarely seen) usually have very high serum [Al] ($>400\mu\text{g/l}$), with microcytosis and hypochromia, despite normal iron stores. The effect of lesser degrees of Al accumulation (as may arise in large numbers of HD patients) is unknown. We have examined the effect of intensive Al chelation therapy using DFO on [Hb] in a group of 15 patients with no clinical evidence of Al toxicity, normal red cell indices and only modest Al accumulation (serum [Al] $55\pm 11\mu\text{g/l}$). After 1 month of DFO therapy, total serum [Al] (including chelated Al) had risen to $167\pm 28\mu\text{g/l}$ and these levels correlated well with the patients' bone Al content ($r=0.72$, $p=0.002$). [Hb] increased steadily during DFO therapy and by 3 months was 23% above baseline (8.5 ± 0.7 to 10.4 ± 0.8 g/dl, $p=0.002$). Both MCV (87 ± 2 to 91 ± 2 fl, $p<0.001$) and MCHC (33.1 ± 0.2 to 33.8 ± 0.1 g/dl, $p=0.001$) also increased. Following withdrawal of DFO therapy [Hb] decreased and after 2 months had returned to baseline. Neither serum ferritin, creatinine nor urea concentrations changed during the study. The maximum rise in [Hb] correlated well with the increment in serum [Al] after 1 month of DFO therapy ($r=0.85$, $p=0.002$). This relationship was curvilinear and indicated that the greatest benefit occurred in patients with moderate Al accumulation (baseline serum [Al] $15-75\mu\text{g/l}$). Above this level the effect was less marked, suggesting that more prolonged chelation therapy might be needed in the more Al loaded patients

The results indicate that exacerbation of anemia by Al is much more common than previously thought and that even very modest Al accumulation may have a pronounced effect on Hb synthesis in HD patients. Such patients may benefit from Al chelation. The implications of these findings for erythropoietin therapy remain to be defined.

LIPIDS IN DIABETIC AND NONDIABETIC ESRD PATIENTS
M.M. Avram, P. Goldwasser*, E. Quinones*,
A. Antignani*, and N. Mittman. The Long Island
College Hospital, Brooklyn, New York

Lipid and N-terminal PTH levels were compared in Type I (DM1)(n=10) and Type II (DM2)(n=20) diabetics, and nondiabetics (ND)(n=63) on chronic maintenance hemodialysis. The groups differed in age (44±2, 65±1.5, 55±2 mean ±SEM years)(p<.001); systolic and diastolic BP (152/84, 135/74, 126/72) (p=.005, p=.014) respectively, but not in race, sex, months on dialysis, fasting state, dialyzer membrane, bath or androgen use.

	DM1	DM2	ND
HDL-cholesterol (mg/dl)	46±7	41±3	38±1
cholesterol (mg/dl)	178±13	185±15	183±6
ratio*	3.3±0.47	3.8±0.42	4.2±0.26
fasting TG (mg/dl)	181±41	166±35	172±18
PTH (pg/ml)	664±224	225±54	473±89
alk.phos. (U/L)	148±15	148±22	235±29

*ratio=non HDL-cholesterol/HDL-cholesterol

HDL-cholesterol correlated with cholesterol (r=0.23 p<.02) and inversely with fasting TG (r=-0.54 p=.001). Longer duration of dialysis in nondiabetics was associated with lower cholesterol (r=-0.34 p=.004) but not HDL-cholesterol. This may reflect diminished cardiac risk and mortality in the long-term survivors. Diabetics, however, showed associated lower HDL-cholesterol (r=-0.4 p=.005), but not cholesterol, with longer duration. DM1 showed the highest HDL-cholesterol and lowest ratio, the highest PTH but not alk.phos and a strong inverse correlation between PTH and HDL-cholesterol, even when controlling for duration (r=-0.99 p=.025).

These provocative findings merit further investigation.

INVESTIGATION OF BLOOD/MEMBRANE INTERACTION WITH ELECTRONMICROSCOPY IN HEMODIALYSIS: MEMBRANE MORPHOLOGY AFTER CLINICAL USE AS A BIOCOMPATIBILITY MEASURE. Hans v. Baeyer*, Hannelore Hampl*, Andreas Debrandt*, (Intr. by Friedrich K. Port). Dept. of Internal Medicine, Klinikum Charlottenburg, Berlin-West, F.R.G.

Transmission and scanning electronmicroscopy was performed on 7 types of dialysis membranes in native state and after 4-5 hours dialysis in ≥5 pts for each membrane: Cuprophane (CU), Haemophane (HA), Celluloseacetate (CA), Polysulfone (PS), Polyacrylonitrile (PA), Ethylvinylalcohol (EV) and Polymethylmethacrylate (PM). The findings are membrane specific and suggest a classification in three types of morphological reaction to clinical blood exposure. Type 1: continuous layer of fibrin as identified by incubation with plasmin solution, and deposition of granulocytes (CU, HA, CA). Type 2: continuous layer of fibrin with very few granulocytes (PA, EV), unchanged membrane surface free from any deposits (PS). Type 3: formation of a mural white thrombus (PM). These morphological findings do not correlate with complement activation during dialysis. Granulocyte deposition is closely linked to generation of both elastase and β-2 microglobulin during dialysis. Formation of a mural thrombus or of a fibrin layer seems to have an inhibitory effect on the clotting potential. Degranulation of white blood cells initiated by adhesion as well as liberation of cytokines may be hazardous in long-term dialysis. Electronmicroscopy should routinely be used in the evaluation of biocompatibility.

A SIMPLIFIED APPROACH TO UREA KINETIC MODELLING. RH Barth, GM Berlyne. Brooklyn VA Medical Center, Brooklyn NY.

The quantity KT/V, where K=dialyzer urea clearance, T=dialysis time, and V=urea distribution volume, is widely used as a measure of adequacy of hemodialysis. Its calculation is hampered by inexact knowledge of K and the difficulty of measuring V.

K, however, may be defined as

$$\frac{\text{Total urea removed/time}}{\text{BUN}_{\text{Midpoint}}}$$

and V as

$$\frac{\text{Total urea removed}}{\text{BUN}_{\text{Pre}} - \text{BUN}_{\text{Post}}}$$

Substituting for K and V, the following relationship results:

$$\frac{KT}{V} = \frac{\text{BUN}_{\text{Pre}} - \text{BUN}_{\text{Post}}}{\text{BUN}_{\text{Midpoint}}}$$

KT/V was calculated for 9 dialysis treatments using K and V derived from direct measurement of dialysate urea. Calculation of KT/V for the same dialyses using the above formula closely matched the directly measured values (r=0.9979, mean difference 0.02), and were more accurate than values calculated from the single-compartment urea kinetic model (r=0.7625, mean difference 0.09).

We conclude that using the reported formula, KT/V may be accurately calculated without need for exact knowledge of K and V, affording a simple and reliable method for monitoring adequacy of hemodialysis.

ALTERED INTERLEUKIN-1 (IL-1) PRODUCTION IN PATIENTS UNDERGOING HEMODIALYSIS

M.Blumenstein*, B.Schmidt*, R.A.Ward, H.W.L.Ziegler-Heitbrock* and H.J.Gurland*. Nephrology Department and Institute for Immunology, University of Munich, F.R.G.

The capacity of peripheral blood mononuclear cells (PBM) to produce IL-1 spontaneously was examined in 19 patients undergoing intermittent hemodialysis for greater than 3 months with low-flux cuprophane (CU) and polymethylmethacrylate (PMMA) membranes and high-flux polysulfone (PS) and polyacrylonitrile (PAN) membranes. PBM were isolated from pre-dialysis blood samples and stored in liquid N₂. Cells were then incubated for 8 hrs at 37 C in a 5% CO₂ atmosphere. Extracellular and cytoplasmic IL-1 were assayed by biologic and radioimmunologic methods. Results from the patients were compared to those of 10 healthy controls. Spontaneous IL-1 activity in PBM supernatants was very low and there were no significant differences between patients using various membranes and the controls. However, intracellular IL-1 concentrations were greatly increased in the 3 patients treated with PAN (8x normal) and in the 10 patients treated with CU (5x normal). In contrast, they did not differ significantly from controls in 3 patients on PS and 3 patients on PMMA. We conclude that PBM from patients predialysis may show functional signs of preactivation. The underlying mechanism of this preactivation may be independent of direct cell-membrane interactions.

EFFECT OF ERYTHROPOIETIN (Ep) TREATMENT ON HEMOGLOBIN O₂-AFFINITY AND PHYSICAL PERFORMANCE IN PATIENTS WITH RENAL ANEMIA. A. Böcker, E. Reimers, B. Nonnast-Daniel, K. Kuehn, K.M. Koch, P. Chigalla, K.M. Braumann, R. Brunkhorst, D. Böning. Med. School, Hannover, FRG, (intr. by M. Lorenzen).

In 16 not transfused and not nephrectomized anemic patients on regular hemodialysis a) hemoglobin (Hb) b) standard-P₅₀ as measure of oxygen affinity c) red cell 2,3-diphosphoglycerate (DPG), and d) workload at a heart rate of 130 beats/min (PWC 130) were determined before and after 3 months (mo) treatment with human recombinant Ep (40-120 U/kg, 3 times/week). Prior to Ep therapy values for P₅₀ (28±SD 3.3 mm Hg) and DPG (15.2±5.2 μmol/g Hb) scattered considerably. The usually observed correlations between P₅₀ and Hb as well as P₅₀ and DPG were not significant before therapy and restored (p<0.01) after treatment. Probably red cell adaptation to anemia via DPG was disturbed in untreated patients. After Ep treatment there was a slight but not significant increase of the mean values of P₅₀ (+1.8 mm Hg) and DPG (+2.6 μmol/g Hb) possibly resulting from a high proportion of young erythrocytes and the remaining moderate anemia. Initially high P₅₀ decreased, initially low P₅₀ increased (r=-0.84, p<0.01). The observed changes in Hb and P₅₀ should improve oxygen transport from lung to tissue. This is in accordance with the observed increase (73.3±27.9 to 97.6±38.4 Watt, p<0.05) of PWC 130 which is a measure of aerobic performance.

LABELLED BETA-2-MICROGLOBULIN (B2m) REMOVAL USING HEMOPERFUSION, CONVENTIONAL AND HIGH FLUX DIALYSIS MEMBRANES. D.Branaccio*, A.Anelli*, S.Barbesti*, P.Padovese*, G.Colantonio*, G.L.Tarolo*, M.Gallieni*, L.Ubertalli*, E.Sabbioni*, R.Pietra*, A.Berlin* (intr. by N.Tessitore). Renal Units Osp. S.Paolo, Milano and Como, Italy; Ist. Med. Nucleare, Univ. Milano, Italy; JRC, Ispra, Italy; C.E.C., Luxembourg.

Mechanisms of B2m removal during dialysis have not been clearly elucidated by clinical studies. Therefore we evaluated B2m kinetics in an ex vivo dialysis circuit in which ¹²⁵I-labelled B2m was added to normal human blood; we also evaluated the removal efficiency of a combined hemodialysis-hemoperfusion (HD-HP) system (Cuprophane-Activated charcoal (AC)) in the same ex vivo circuit and in vivo as well. Our data show that 1) no B2m clearance is seen using cuprophane 2) Polyacrylonitrile (PAN), Polysulphone (PS) and Polymethylmetacrylate (PMMA-BK) can remove 80% of the labelled protein after 2 hours of dialysis; besides, B2m adhesion to PAN is an additional mechanism of removal 3) PAN, PS and PMMA-BK further increase B2m clearance when used in hemodiafiltration (+8±2.4%) 4) no variation of B2m plasma levels was observed when HD-HP was performed in vivo (+4%; n:10). The ex vivo experiments showed otherwise a decrease of 80% of the labelled protein and low levels of B2m in the dialysate at the end of dialysis; after rinsing, 63% of the labelled B2m was found in AC, suggesting a high affinity of AC for B2m.

THE EFFECT OF HEMODIALYSIS ON THE CONCENTRATION AND FUNCTION OF PLASMA FIBRONECTIN (FN).

ST Brown, R Hendrix*, FB Gelder*, CA Moore*, L Bairnsfather*, LSU Med. Ctr., Shreveport, LA.

Plasma FN is significantly (p<.005) reduced in hemodialysis patients (n=11) compared to continuous ambulatory peritoneal dialysis patients (n=14) and normals (n=83). The collagen binding function of FN (FN-CBF) is also reduced (p<.005). To determine if the changes in FN are a direct result of blood-dialyzer contact, FN and FN-CBF were measured at 0 (pre), 15, 30, 60, and 180 (post) min of dialysis using cuprophane 1st (Cu1) and 6th (Cu6) use and PMMA (PM) dialyzers.

Time	0	15	30	60	180	
Cu1	266±40	247±55	238±46	259±72	259±63	(FN)
(n=11)	35±14	31±11	33±10	38±12	40±15	(%)
Cu6	306±79	292±81	279±61	308±75	293±86	(FN)
(n=3)	34±17	31±22	34±15	33±16	34±15	(%)
PM	218±12	262±43	262±41	245±13	257±45	(FN)
(n=4)	21±4	34±17	34±16	35±15	26±11	(%)

Cu1 and Cu6 show a reduction in FN by 30 min with return to baseline at 180 min. PM shows the reverse trend with higher than baseline FN at 180 min. The reduction in FN-CBF worsens during dialysis but is less for PM at 180 min. Pre-dialyzer FN values were not significantly different from post values. FN-CBF significantly decreased (p=.03) pre to post the dialyzer with Cu1 but not Cu6 or PM. We conclude that blood-dialyzer contact does not directly reduce FN but may reduce FN-CBF. The results using dialyzers of differing biocompatibility suggest that the changes in FN and FN-CBF are the consequences of indirect as well as direct blood-dialyzer contact.

COMPARATIVE BIOCOMPATIBILITY ASSESSMENT OF HEMODIALYSIS MEMBRANES IN AWAKE SHEEP AND THE ROLE OF CYCLOOXYGENASE. K. Burhop*, J. Simpson*, D. Chenoweth* and J. Borgia* (intr. by S.K. Webster). Baxter Healthcare Corp., Round Lake, Illinois 60073.

Comparative hemodialyzer membrane biocompatibility assessment studies were performed on 40 awake sheep prepared with carotid-jugular and femoral arterio-venous shunts. Five groups of dialyzers were examined: Travenol CF-1511 (Cuprophane, 1.0M²); Travenol ST-25 (Cuprophane, 1.6M²); Travenol HT-15 (Hemophan, 0.9M²); Travenol CTA-110 (cellulose triacetate, 1.1M²); and Hospal AN-69HF (polyacrylonitrile, 1.15M²). Cuprophane membranes consistently produced transient 2-3 fold increases (p<0.01) in mean pulmonary artery pressure (MPAP) and vascular resistance (PVR) which were significantly greater (p<0.01) than those produced by the AN-69HF, HT-15, and CTA-110 dialyzers. The increases in MPAP and PVR produced by the Cuprophane dialyzers were correlated (p<0.01) with increases in the plasma concentration of TxB₂ (e.g., from 52±11 pg/ml initially to 16,100±6,900 pg/ml at 10 min post-dialysis with CF-1511). Compared to the AN-69HF, the Cuprophane dialyzers produced significant decreases (p<0.01) in the number of circulating PMN's and caused prolonged increases (p<0.01) in the plasma conc. of C3a. There were no significant differences detected between the AN-69HF and the CTA-110 in any measured parameter. Pretreatment (n=5) with sodium ibuprofen (30 mg/kg) prevented the CF-1511 induced rises in MPAP and PVR but did not prevent the generation of C3a or the decrease in PMN's. These results suggest that complement activation is the physiologic transducer of the Cuprophane response but that the final effector pathway is mediated by TxA₂.

A PROSPECTIVE RANDOMIZED DOUBLE-BLIND STUDY OF RECOMBINANT HUMAN ERYTHROPOIETIN (r-HuEPO) IN CHRONIC HEMODIALYSIS. Canadian Erythropoietin Study Group

The clinical efficacy and pharmacokinetics of r-HuEPO were examined in a prospective double-blind study. 24 patients on chronic centre hemodialysis were randomized into 4 groups (n=6) to receive treatment with placebo, or r-HuEPO at a dose of 50, 100, or 200U/kg 3 times weekly post-dialysis. All subjects received iron supplementation (mean: 300mg ironferon/wk), and clinical care and reporting of adverse effects was undertaken by a Nephrologist blinded to the treatment and hematologic response. There was a slight fall in Hg in all 4 groups during the first 14 days, being greatest in the placebo treatment group (mean: 6g/L). Hematologic response to r-HuEPO was first observed by 21 days of treatment, with an increase from mean baseline Hg in the 3 treatment groups of 44% (50U/kg/d), 59% (100U/kg/d), and 40% (200U/kg/d). The placebo group had a mean increase of Hb of 9% from baseline at this timepoint. Mean % change in reticulocyte count was greatest in the group receiving 200U/kg (540%) and not directly correlated with hematologic response suggesting that at this dose (200U/kg) overdrive of the marrow leads to ineffective erythropoiesis. Mean serum ferritin rose throughout the study period in both the placebo and 50U/kg treatment groups (increment: 122 and 71 ng/ml respectively), but fell despite continued iron supplementation in those receiving r-HuEPO 100 and 200U/kg (decrement: 57 and 73 ng/ml respectively) by the second month of treatment. Change in Hg occurred without a concomitant alteration in other formed elements. At stable state, Hg could be maintained within a 15g range by weekly dose titration. Frequency distribution analysis revealed the maintenance dose required to lie between 50 and 100 U/kg 3 times weekly. Withdrawal of r-HuEPO or iron supplementation resulted in a rapid cessation of erythropoiesis.

EXPERIENCE WITH 100 CONSECUTIVE INTERNAL JUGULAR CATHETERS FOR TEMPORARY ACCESS. George E. Cimochowski*, W. E. Rutherford, Joan Blondin, Herschel Harter, Edward Worley*, Jo Ann Sartain*. Monroe, Louisiana.

We have previously demonstrated superiority of the internal jugular route with respect to long-term venous strictures when compared to the subclavian vein. We have now prospectively analyzed 100 consecutive internal jugular catheters for temporary access in end stage renal disease and temporary renal failure. Ages ranged from 15 to 92. Reasons for insertion were: acute renal failure 11%, chronic renal failure 39%. All catheters were inserted in the OR under fluoroscopic control, 81% in the right internal jugular, 19% in the left internal jugular. Short term complications include: unable to insert 0%, pneumothorax 1%, hemothorax 0%, carotid artery puncture 1%. Longterm complications: infection 6%, thrombosis 6%, dislodgement 1%, unacceptable dialysis flows 0%, delayed perforation of atrium or great vessel 0%. Venograms in 20% (20/100) one mo. to 24 mos. after removal revealed no venous strictures in the venous access route of the upper extremity in any patient. The internal jugular route is safe both short and longterm, has comparable complications with respect to the subclavian route, but has 0% venous stricture formation, in marked contrast to a 50% subclavian stricture as reported in our previous experience.

The internal jugular vein is the ideal route for insertion of the temporary access catheter at this time.

BINDING OF COMPLEMENT (C) REGULATORY PROTEINS FACTORS B AND H TO CUPROPHAN (CuM) AND CELLULOSE ACETATE (CAM) MEMBRANES. Alfred K. Cheung, Charles J. Parker*, Jarmila Janatova* and Linda Wilcox.* V.A. Med. Ctr. and Univ. of Utah, Salt Lake City, Utah.

During hemodialysis, CuM activate more C than CAM. CAM are cellulosic membranes on which most of the surface hydroxyl groups are acetylated. We have previously shown that the decreased C activation by CAM is not due to blockage of covalent binding sites for nascent C3b by acetyl residues. To investigate further the molecular basis for the difference in C activating potentials of CuM and CAM, the binding of the C regulatory proteins, factors B and H to the two membranes was examined. Activated B is the catalytic subunit of the C3 convertase of the alternative pathway of C (APC), and its binding to C3b amplifies APC activity. In contrast, binding of H to C3b inhibits the APC.

We found that native CAM bound 40X more radiolabeled H compared to native CuM. When the membranes were incubated with normal human serum to allow activation and binding of C3, subsequent studies showed that the specific binding of radiolabeled B to CuM was 3X higher than that to CAM. This binding of B could be inhibited by H in a dose-dependent fashion, suggesting that these two regulatory factors compete for binding sites on membrane-associated activated C3. Scatchard analysis demonstrated that the affinity of B for binding to the two membranes were similar, but that the number of B binding sites was markedly lower on CAM. Further, we found that after exposure to serum, CuM bound 4X more B than H, whereas the B:H binding ratio approached unity for CAM.

These observations are consistent with the concept that CuM have higher C activating potentials than CAM because in the former, the binding of B to membrane-associated C3 is favored over the binding of H. The binding of these two regulatory proteins to activated C3 on cellulosic membranes appears to be modulated by the presence of acetyl residues.

VALUE OF SERUM ALUMINUM (Al_S)-MONITORING IN DIALYSIS. Jan P. Clement*, Patrick C. D'Haese*, Monique M. Elseviers*, Marc E. De Broe* (intr. by George A. Porter). Univ. of Antwerp, Dept. of Nephrology.-Hypertension, Antwerp, Belgium.

Data on Al_S-monitoring and its clinical implications are scarce. In a multi-center study (total N of participating units: 16), a retrospective analysis of a total N of 1,130 dialysis patients was performed. Semestrial Al_S-determinations were performed in our central lab, using thermal atomic absorption spectrometry. Precision and accuracy were assured by a monthly international quality control program. Throughout the whole study period '84-'87, an overall mean Al_S of 43 µg/L was found in a total of 3,441 tests performed. After a peak value of mean 53 µg/L begin '85, there was a generalized trend of declining Al_S towards a minimum of mean 37 µg/L begin '87. An individually assessed diminution of the cumulative Al-intake appeared as the sole important determinant of this evolution since water treatment was optimal (< 7 µg/L) in all centers from the start on. Conditions recently associated with higher risk for Al-accumulation gave following mean Al_S in comparison to the overall Al_S of 43 µg/L:

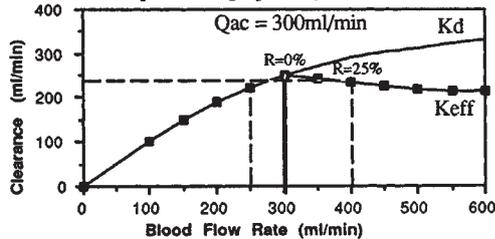
Patients at risk	N tests	Mean Al _S	p-value
overt liver disease (n=7)	39	101	< 0.001
osteomalacia (n=10)	75	71	< 0.001
encephalopathy (n=3)	10	213	< 0.001
diabetes I or II (n=15)	44	66	< 0.01
iron overload (n=40)	185	48	n.s.

Al_S-monitoring is clinically relevant if performed by a qualified lab.

RECIRCULATION AND EFFECTIVE CLEARANCES.

A. Collins, G. Hanson*, R. Berkseth and P. Keshaviah*. Regional Kidney Disease Program, Hennepin County Medical Center, Minneapolis, MN

Higher blood flow rates (Q_b) are being increasingly used to shorten treatment time. If Q_b exceeds the access flow (Q_{ac}), recirculation (R) will occur with the effective clearance (K_{eff}) being less than the dialyzer clearance (K_d). It is not intuitively obvious if increasing Q_b to maximize dialyzer performance will achieve a net gain in K_{eff} despite the increase in recirculation. In order to study the tradeoff between dialyzer performance and recirculation, the equations for dialyzer clearance and recirculation were mathematically manipulated. The results for small solutes are presented graphically below:



For $Q_b < Q_{ac}$, $K_{eff} = K_d$ and increasing Q_b increases K_{eff} . Maximum K_{eff} is achieved at $Q_b = Q_{ac}$, $R = 0$. When $Q_b > Q_{ac}$, $K_{eff} < K_d$, the decrease due to recirculation exceeding the increase due to improved dialyzer performance. With increasing Q_b , K_{eff} decreases to values achievable at a lower Q_b with no access recirculation. As shown above, at $Q_b = 400$ ml/min, $R = 25\%$ and $K_{eff} \sim 230$ ml/min, a value equal to that achievable at $Q_b \sim 250$ ml/min and $R = 0$. Solute removal is maximized when $Q_b = Q_{ac}$. Q_{ac} can be calculated from an appropriate reformulation of the recirculation equation for $Q_b > Q_{ac}$. In the absence of such corrections therapy adequacy may be significantly compromised.

DIALYZER UREA AND CREATININE CLEARANCES NOT SIGNIFICANTLY CHANGED IN r-HuEPO TREATED MAINTENANCE HEMODIALYSIS (MD) PATIENTS. RG Delano, AP Lundin, R Galonsky, RM Quinn*, TKS Rao, EA Friedman. SUNY, Health Sci. Cntr. Bklyn, Bklyn, New York.

Anemic MD patients treated with r-HuEPO respond by increasing both their hematocrits (HCTs) and whole blood viscosity (Brown, CD et al, ASN 1987). Whether altered blood rheology will adversely effect solute extraction and thus efficiency of routine hemodialysis treatments is a concern in use of r-HuEPO. Of 37 MD patients (18 men and 19 women) treated with r-HuEPO, 35 responded with an increase in HCT from a starting mean of $20.4 \pm 3.5\%$ to a peak of $34.3 \pm 3\%$ within a mean of 65 ± 25 days.

A subset of 8 stable MD patients (4 men and 4 women) of mean age 42.3 years (range 22-73 years) had dialyzer clearances measured before and after their HCTs were increased by r-HuEPO treatment. Each had the same brand and size dialyzer used for pre- and post-r-HuEPO clearance studies. Dialyzer creatinine (C_{cr}) and urea (C_u) clearances at a blood flow rate of 300 ml/min were measured 1 hr after initiation of hemodialysis. A dialysate sample was analyzed at the same time for mass balance. Pre-r-HuEPO C_u and C_{cr} were 190 ± 12 and 146 ± 20 ml/min respectively (ranges 170-211 and 109-172 ml/min). Mean HCT was $19 \pm 4\%$ when r-HuEPO was begun. Repeat dialyzer clearances when HCT increased to $34 \pm 6\%$ were: C_u 173 ± 47 and C_{cr} 137 ± 46 ml/min, representing insignificant decreases of 9% and 6% respectively. Although dialysis efficiency for the group was unaltered, significant decreases were observed in 2 patients who had reduction in C_u of 39 and 54%, and in C_{cr} of 50 and 46%. No patient had significant increases in serum creatinine, BUN or serum potassium.

We conclude that an r-HuEPO-induced HCT increase does not necessarily reduce hemodialysis efficiency.

ACETATE CAUSES ENDOTHELIUM-INDEPENDENT INCREASES IN CYCLIC AMP, BUT HAS NO EFFECT ON CYCLIC GMP, IN THE RAT CAUDAL ARTERY. J.T. Daugirdas, V. Swanson*, D. Kim*, X. Wang*, R. Fiscus*. Hines-Loyola Medical Center, Hines, IL.

The mechanism of the vasorelaxant action of acetate is unknown. We studied the effects of acetate on tissue cyclic AMP and GMP levels in rat caudal artery. All studies were done in the presence of 250 μ M IBMX.

Acetate (16mM) induced an increase in tissue cyclic AMP levels whether endothelium was present (E+: control: 15 ± 1.6 pmoles/mg protein; 16mM acetate: 26 ± 3.8 , $p < 0.05$), or absent (E-: control 14.5 ± 2.4 ; 16mM acetate: 29 ± 4.6 , $p < 0.05$). There was no difference in cAMP levels in E+ or E- arteries. The increase in cAMP was near maximal as early as 2 minutes after exposure to acetate, and was sustained for at least 30 minutes in the continued presence of acetate. The effect at 2 minutes of 4mM acetate on tissue cyclic AMP ($+60\% \pm 17$) was as great as that of 16mM ($+55\% \pm 8.4$) or 64mM acetate ($+70 \pm 17$), p NS. The rise in cyclic AMP levels appeared to be consistent with the time course of acetate-induced vasorelaxation. In contrast, acetate had no effect on tissue cyclic GMP levels in E+ (control: 6.6 ± 0.78 pmoles/mg protein; 16mM acetate: 6.7 ± 1.27 , p NS) or E- (control: 1.5 ± 0.42 , 16mM acetate 2.1 ± 0.61 , p NS) arteries. Forskolin, an agent that increases tissue cyclic AMP, also induced vasorelaxation in this model.

The results suggest that acetate causes an increase in tissue cyclic AMP levels that is not dependent upon presence of a functioning endothelium.

THE EFFECTS OF INTRAVENOUS 1,25(OH)₂D₃ ON HYPOCALCEMIC STIMULATION OF PARATHYROID HORMONE IN UREMIA. James Delmez*, Carol Tindira*, Patricia Grooms*, David Windus, Eduardo Slatopolsky. Dept. of Medicine, Washington Univ., St. Louis, Mo.

We have shown that intravenous 1,25(OH)₂D₃ (IV D) suppresses PTH secretion in uremic subjects before an increment in serum ionized calcium (I Ca) is detected. In addition, calcium infusion studies (Kidney Int 31:382A) suggested that the effect of IV D may be in part mediated by increasing the sensitivity of the gland to I Ca and correcting the abnormal set point for calcium present in uremia. To further elucidate the effect of IV D on PTH secretion, 8 patients underwent hemodialysis with a dialysate low in calcium (1 mEq/L) to stimulate PTH release. Blood was obtained at frequent intervals for I Ca and amino-terminal PTH. After control studies were performed, the patients then received 3 μ g of IV D following each dialysis for two weeks, during which time calcium supplements were discontinued to prevent hypercalcemia. Basal PTH levels were 180 ± 58 pg/ml before and 118 ± 42 pg/ml ($P < 0.01$) after 2 weeks of IV D despite no change in I Ca (4.47 ± 0.17 vs. 4.60 ± 0.16 mg/dl, $P = N.S.$). After the patients received IV D for two weeks, the release of PTH during acute hypocalcemia was blunted. The peak PTH response to hypocalcemia during control studies was 454 ± 158 pg/ml vs 263 ± 104 pg/ml, after administration of IV D. ($P < 0.01$). The degree of hypocalcemia was comparable in both studies. The maximal rise in PTH levels correlated with baseline values, both with and without IV D. Since it is known that 1,25(OH)₂D₃ suppresses the cellular pre-pro PTH mRNA content in vitro and gene transcription for PTH in vivo, our results suggest that the blunted response of PTH to hypocalcemic stimulation likely reflects a lower basal secretory rate in the presence of 1,25(OH)₂D₃.

EFFECTS OF HEMODIALYSIS (HD) AND DIALYZER MEMBRANE ON PULMONARY MECHANICS. George A. DeVault, Jr., Gary T. Kinasewitz, Lori Stevens*, Stephen T. Brown, LSU Medical Center, Shreveport, LA.

Peri-dialytic ventilatory function may be influenced by inter-dialytic weight gain (IDGAIN), total lung water, and biocompatibility of dialyzer membranes. To assess the effect of HD on pulmonary mechanics, we obtained serial pre- and post-dialysis spirometry on stable HD patients without known lung disease. Cuprophane (CU) dialyzers were used for 147 treatments in 16 patients whereas the more biocompatible PMMA membrane was used for 49 treatments in 7 patients. The increase in the ratio of post- to pre-HD FEV1 (FEVR) and FVC (FVCR) correlated positively with IDGAIN and IDGAIN/dry weight (WTR) ($p < 0.0001$). Improvement in pulmonary function was significantly greater in patients dialyzed with the PMMA membrane.

	n	FEVR	FVCR	WTR
CU	147	1.10±0.01*	1.08±0.01†	0.051±0.002*
PMMA	49	1.17±0.02*	1.14±0.01†	0.073±0.003*
	$\bar{x} \pm \text{SEM}$	* $p < 0.001$	† $p < 0.05$	

Even after multiple uses of CU (≥ 6) and PMMA (≥ 3) dialyzers, these differences in FEVR and FVCR persisted ($p < 0.01$). No significant difference in pulmonary mechanics was observed when "initial use" and "multiple use" data sets were compared within either CU or PMMA group. The slopes of the relationships between WTR and improvement in FEV1 or FVC were similar in both groups. While dialyzer membrane biocompatibility may affect pulmonary vascular hemodynamics and gas exchange, pulmonary mechanics remain unchanged. Since the improvements in FEV1 and FVC reflected the removal of excess body water, spirometry may prove helpful in the assessment of dry weight in HD patients.

ABSENCE OF IN VITRO GENERATION OF BETA-2-MICROGLOBULIN (β_2m) BY CUPROPHANE (CUP) DIALYZERS. M. Dratwa, L. Galand, P. Bergmann, P. Colle, C. Tielemans (Intr. by C.C. Tisher). Hôpital Universitaire Brugmann, Brussels, Belgium.

In the search for pathogenetic mechanisms of "dialysis β_2m amyloidosis", it has been suggested by some that hemodialysis with Cup leads to increased plasma levels of β_2m , possibly by acute shedding of β_2m from blood cells membranes.

To test this hypothesis, β_2m plasma levels were measured hourly during an in vitro recirculation dialysis using a .65 m2 Cup dialyzer (Travenol ST 12). The volumes of normal blood and bicarbonate dialysate compartments were 200 and 400 ml while blood and dialysate flows were set at 100 and 200 ml/min, respectively. Ultrafiltration was maintained below 5 ml/hr and β_2m values were corrected for hemoconcentration. The following results (means of 6 experiments \pm SEM) were obtained:

Time (min)	0	60	120	180
β_2m (mg/L)	1.7±.1	1.7±.1	1.8±.1	1.9±.2 (NS)

Experiments using reused dialyzers led to similar results. Total mass transfer of β_2m was near zero. Similarly, when Cup membrane fragments were incubated 1 hour with uremic blood, β_2m levels did not change (NS, n=6).

Time (min)	0	60
β_2m (mg/L) controls	37.8±10.9	31.6±10.6
Cup	33.9±10.7	33.4±10.6

Thus, it appears that the mere contact of blood with Cup is not sufficient to increase β_2m plasma levels as observed in patients during Cup dialysis. Other mechanisms operating in vivo such as liberation of β_2m from the pulmonary microcirculation induced by complement activation (as recently proposed by De Broe) must be searched for.

EFFECT OF CARTRIDGE pH ON SERUM SODIUM AND ACID BASE STATUS IN PATIENTS (PTS) DIALYZED WITH THE REDY SYSTEM. R. Dunlavy*, J. Pederson, C. Williams* and F. Lisch. Dept. of Med., Univ. of Okla. HSC and VAMC, Okla. City, OK.

The Redy hemodialysis system uses a sorbent cartridge to regenerate the bath. The cartridge, containing zirconium phosphate (ZP), removes urea from and releases bicarbonate (HCO_3^-) with Na to the bath. The ZP pH influences the magnitude of Na release and HCO_3^- generation. This study evaluates changes in acid base status and serum sodium (SNa) among 6 hemodialysis pts. These pts, ages 59±7 years, were treated thrice weekly in a crossover study, with low (L) pH (5.75) and high (H) pH (6.80) cartridge dialysis.

Following H there were significant ($p < 0.05$) increases in arterial pH, (7.42±.06 to 7.53±.04); pCO_2 , (37±4 to 42±3 mmHg); HCO_3^- (25±5 to 42±5 mEq/L); and SNa (141±3 to 148±3 mEq/L). No comparable changes were noted following L. Thirst was common to both H and L. Interdialytic weight changes and the $\% \Delta$ BUN during dialysis were similar for the two groups.

The $\% \Delta$ SNa = 4.37 ZP pH - 25.67±1.55 ($r=0.61$, $p < 0.001$) when 30 additional treatments (ZP pH from 5.75 through 6.25) were considered.

In summary: Dialysis with cartridges of higher pH resulted in greater alkalemia and increases in SNa. A linear correlation between the increment in SNa and ZP pH of the cartridge existed. In conclusion: The ZP pH is a direct and major determinant of the generation and release of HCO_3^- and Na from the sorbent cartridge. The possible benefit of utilizing a spectrum of selectable ZP pH cartridges remains to be assessed.

A STRUCTURED COMPUTERIZED DATABASE FOR TRACKING HEMODIALYSIS PATIENTS AND THEIR MEDICAL DATA. Irving Dunn, Peter K. Wayne*. Interfaith Medical Center, Division of Nephrology, Brooklyn, N.Y.

A simple, menu driven computer database program was designed to operate on the IBM compatible micro-computer. Its purpose is to organize, store and retrieve the medical information that accumulates on each hemodialysis patient. Separate but integrated files were developed in the following areas: patient demographics, medications, laboratory results, hospital admissions, medical summaries, access site history, dialysis parameters or "orders", EKG results, diagnostic procedures, and the long and short term care plans. A crucial decision was not to include data from each dialysis treatment thereby making the system compatible with monthly rather than daily data entry. The benefits of such a database program operating in an eighty patient Hemodialysis Unit for over a year and one half include: no misplaced data, certainly of rapid retrieval of data, legibility of data, self checking and organizational report functions that increase the efficiency of operation. The ability to query the database enables the health care professional to test out hypothetical relationships and has implications for quality assurance and clinical research.

EFFECTS OF NANDROLONE DECANOATE (ND) ON NUTRITIONAL PARAMETERS IN ELDERLY UREMICS
Arnold R. Eiser, Martin S. Neff, Robert F. Slifkin
 Dept. of Medicine, City Hospital Center at Elmhurst and the Mt. Sinai School of Medicine, New York, NY

Androgens are widely used to treat the anemia of uremia. Some evidence suggests they also exert a general anabolic effect. This study was designed to assess such an effect in elderly uremics.

Six male maintenance hemodialysis patients ≥ 65 years old were studied during placebo and again after 4 months of ND 2mg/Kg weekly. Lymphocytes (lym), weight, transferrin (TF), albumin (Ab), arm muscle circumference (AMC), handgrip strength (HGS) by dynamometry were measured. AMC = mid-arm circumference $-(0.314 \times \text{triceps skin fold in mm})$.

	Lym (per m ³)	TF (mg/dl)	AB (gm/dl)	MAC (cm)	HGS (lb)	WT (Kg)
Placebo	1180	263	3.72	23.9	49.3	72.1
ND	1677	270	3.39	24.3	43.6	72.3
Change	+497*	+007	-.33**	+0.4	-5.7**	+0.2

* P. < 02 **P. < 05 two-tailed t-test

Lymphocytes were increased significantly by ND while other parameters were not. Thus it appears that in geriatric uremic patients ND has a specific effect on lymphocytes without enhancing general nutritional parameters. Lack of effect on nutritional parameters may reflect aging effect or failure to increase caloric and protein intake. The lymphocytosis may represent a receptor specific growth or differentiation enhancement analogous to the effect of androgens on erythropoiesis.

Further studies are needed to assess whether immune competence is also increased.

BLOOD COOLING PREVENTS DEGRANULATION OF NEUTROPHILS (PMN) DURING HEMODIALYSIS (HD). G.Enia, Q.Maggiore, W.H.Hörl, C.Catalano (Intr. by G.Orlandini)
 Centro Fis.Clin.CNR Reggio Calabria Italy and University Medical Clinic Freiburg FRG.

Leukocyte activation during HD is associated with a marked leukopenia and the release of neutral proteinases (P) contained in the granules of PMN. Since it is known that temperature (T) changes can influence the function of PMN we investigated whether cooling of the extracorporeal blood during HD could prevent the release of P.

Plasma levels of granulocyte elastase in complex with alpha 1-proteinase inhibitor (E- α 1PI) were determined with a highly sensitive enzyme-linked immunoassay in 9 patients both during hypothermic and control HD. HD was carried out by manipulating blood and dialysate T in such a way that blood T within the dialyzer averaged $22.7 \pm \text{SEM } 0.04$ °C in hypothermic HD and 33.1 ± 0.02 °C in the control HD. Hollow fiber cuprophane dialyzers of 1.2 m² were employed for all the treatments.

Maximal fall in WBC count averaged $78 \pm \text{SEM } 1.4$ % during control HD and 19 ± 3.8 % during hypothermic HD (p < 0.001). Plasma E- α 1PI levels increased from $128 \pm \text{SEM } 7.6$ ng/ml to 368 ± 18 ng/ml during control HD but only from 112 ± 7.9 to 200 ± 15 during hypothermic HD (p < 0.001).

We conclude that PMN activation can be largely prevented by cooling of the extracorporeal blood during HD.

THE EFFECT OF DESFERRIOXAMINE (DFO) ON ALUMINUM BONE DISEASE AND PARATHYROID HORMONE (PTH). A. Felsenfeld, M. Rodriguez, M. Coleman, D. Ross, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, Okla.

Aluminum (AL) toxicity in dialysis patients (pts) is associated with decreased bone turnover and a relative PTH deficiency. Previous studies in AL associated osteomalacia (OM) have shown: 1) DFO to be an effective treatment and 2) decreased PTH secretion during hypocalcemia. In this study, 17 pts with AL associated OM or aplastic bone disease were evaluated with both bone biopsy and a calcium-free dialysis before and 1 year after DFO. AL(OH)₃ was continued during DFO therapy. Pre and post studies, including serum AL (sAL), stainable bone AL (bAL), basal PTH (bPTH), maximum increase in PTH during hypocalcemia (m Δ PTH), osteoblastic osteoid (OB), osteoclastic resorption (OR), relative osteoid volume (ROV), and bone volume (BV) were:

	Pre	Post	Pre	Post
sAL (μ g/L)	167 \pm 37	193 \pm 35	OB (%)	1.3 \pm 5
bAL (%)	44 \pm 4	14 \pm 4*	OR (%)	0.9 \pm 3
bPTH (pg/ml)	152 \pm 42	124 \pm 36	ROV (%)	8.8 \pm 2.3
m Δ PTH (pg/ml)	309 \pm 63	292 \pm 62	BV (%)	15.6 \pm 1.4
x \pm SE	*p < 0.05 pre vs. post			20.9 \pm 2.2*

In summary, in these 17 pts, 1 year therapy with DFO resulted in marked decrease in stainable bone AL and improvement in bone histology. Serum AL did not decrease and the PTH status (basal and stimulated) did not improve. In conclusion, 1) removal of AL from bone may result in increased bone activity; and 2) the lack of improvement in PTH secretion may be due to a defective parathyroid gland or high serum AL possibly due to continued AL(OH)₃ therapy.

β -ENDORPHIN LEVELS IN PATIENTS WITH REGULAR HEMODIALYSIS TREATMENT. R.D.Fitzgerald, G.Sunder-Plasman, F.Stockenhuber, P.Balcke. 1st Medical Clinic, University of Vienna, Austria.

β -endorphin is known to participate in several regulatory mechanisms including blood pressure immune system, gastrointestinal system as well as cerebral mechanisms and stress. β -endorphin is produced together with ACTH which is known to appear in elevated blood levels in patients with chronic renal failure and regular hemodialysis treatment.

We compared β -endorphin plasma levels of 33 patients with end stage renal failure and chronic hemodialysis treatment with plasma levels of 16 healthy volunteers. Levels were significantly (p < 0,0005) elevated in the hemodialysis group ($24,3 \pm 4,4$ pg/0,1 ml) compared with the volunteers. Blood samples taken at different times of day showed no diurnal rhythm. Concentrations at beginning and end of hemodialysis showed no conclusive changes. These findings may suggest that β -endorphin could be involved in various dysfunctions related to chronic renal failure so as blood pressure dysregulation, amenorrhea and dysfunctions of the immune system.

COLD HEMODIALYSIS MAINTAINS STABLE BLOOD PRESSURE IN DIABETIC PATIENTS. P.M. Fitzpatrick* and S.B. Kurtz, Dept. of Medicine, Mayo Medical School, Rochester, MN.

Cold hemodialysis has been shown to be useful in ameliorating dialysis-induced hypotension in nondiabetic dialysis patients. Hypotension is a frequent complication during hemodialysis in diabetic patients as well. This study was designed therefore to test the usefulness of cold hemodialysis in maintaining stable blood pressure in chronic diabetic dialysis patients. The diabetic patients studied (n=7) had a mean age of 48.7 years (range 24-81), were on hemodialysis a mean of 48.8 months (range 4-132), were taking no antihypertensive medications and had a history of frequent episodes of dialysis-induced hypotension. Subjects received 3 warm hemodialysis (WHD, dialysate 37°C) sessions and 3 cold hemodialysis (CHD, dialysate 34°C) sessions. The mean arterial pressure (MAP) and mean oral temperatures (Temp) prior to and at 60 minute intervals during CHD and WHD are depicted below:

(min)	MAP(mmHg)		Temp(°C)	
	CHD	WHD	CHD	WHD
0	90±3	93±3	35.6±.2	35.5±.2
60	96±3	88±3*	35.3±.2	35.9±.2*
120	94±3	84±3*	35.1±.2	36.3±.1*
180	89±3	79±3*	35.2±.2	36.2±.1*

*p .05 CHD vs WHD

Weight loss, heart rates, and hematocrits did not differ between groups. Results show that CHD improved blood pressure stability and was well tolerated in these diabetic subjects. We conclude that CHD is an effective means of ameliorating dialysis-induced hypotension in susceptible diabetic patients.

VASOPRESSIN IN SEVERE RECURRENT DIALYSIS HYPOTENSION. Ullrich Friess*, Peter Gross*, Albert Schömig*, Eberhard Ritz*, Wolfgang Rascher**. Univ. of Heidelberg, Depts. of Medicine and Pediatrics(+), Heidelberg, FRG. (Intro. by R.J. Anderson)

Hypotension is known to be a potent stimulus for vasopressin release. The role of vasopressin in dialysis hypotension has not been clarified. We therefore studied vasopressin in 27 patients (age: 56+ .3 years) on chronic hemodialysis (6.4+ .3 years; duration of single dialysis: 4.5 h; weight loss/dialysis 2.4+ .3 kg; dialysate: acetate 18, bicarbonate 9) who suffered from severe recurrent dialysis induced hypotension (H). A control blood sample (C) was obtained after the first hour of dialysis, when systolic blood pressure was unremarkable and 138+7 mm Hg; a blood sample was obtained again 3 min after the onset of any subsequent symptomatic drop of systolic blood pressure to a value <70 mm Hg (H: 66+2 mm Hg).

We observed: In 8 patients experiencing nausea during H vasopressin rose from 9.8 pg/ml (C) to 93.4 +33 (H; p<.05). In the remaining 19 patients vasopressin failed to change significantly (C: 8+1 pg/ml; H: 13+ 3; p N.S.), as did pulse rate (C: 81+ 2.5/min; H: 96+ 3.4; p N.S.), adrenaline (C: .2+ .04 nM/l; H: .2 + .04; p N.S.) and noradrenaline (C: 2.2+ .2 nM/l; H: 2.1+ .2; p N.S.). In 14 of these 19 patients vasopressin fell during H. We concluded: The vasopressin response to nausea appeared to indicate an intact secretory mechanism (efferent limb) of the hormone. The failure to respond adequately to severe symptomatic hypotension suggests an autonomic dysfunction localised in the afferent limb by the deficient rise of vasopressin in most patients. Our findings contribute data to the multifactorial pathogenesis of severe recurrent dialysis hypotension.

A UREMIC FACTOR INHIBITS HUMAN RBC Na-K-ATPase AND Ca-ATPase ACTIVITIES. U. Gafter*, T. Malachi* and J. Levi, Nephrology, Hasharon Hosp., Petah-Tikva; Tel-Aviv Univ. Med. Sch., Israel.

Na-K-ATPase and Ca-ATPase activities in uremic RBC are depressed. It has been shown that hemodialysis (HD) increases the activity of both enzymes in RBC from patients with end stage renal disease (ESRD). The hypothesis that there is an association between the inhibition of these enzymes was tested. First, Na-K-ATPase and Ca-ATPase activities were determined simultaneously in RBC ghosts from controls (C) (n=9) and patients (n=9) before and after HD. Second, RBC from healthy subjects (n=10) were incubated in vitro with normal plasma, uremic plasma and urea. Ghosts were prepared and Na-K-ATPase and Ca-ATPase activities were measured (nmol Pi/mg protein/h). Activities of both Na-K-ATPase and Ca-ATPase in patients were lower than C (p<.01). Following HD, activities of both enzymes increased significantly (p<.02).

	Control	Before HD	After HD
Na-K-ATPase	199.7±24.9	123.8±14.2	158.8±11.6
Ca-ATPase	846.5±55.5	452.8±108.0	798.7±120.3

Uremic plasma inhibited Na-K-ATPase activity 184.7 +18.9 vs. 221.2±2 with normal plasma (p<.01) and Ca-ATPase activity 776.5±75.1 vs. 999.7±64.6 (p<.001). The decrement in Ca-ATPase activity caused by uremic plasma correlated with that of Na-K-ATPase activity (r=0.749, p<.01). Urea had no effect on the ATPases.

In conclusion, RBC Na-K-ATPase and Ca-ATPase are inhibited in patients with ESRD. This inhibition is reversible, can be induced by uremic plasma but not urea, and may be caused by the same dialyzable uremic factor.

COMPLEMENT (C) RECEPTORS (R) ON NEUTROPHILS (PMN) AND MONOCYTES (MC) DURING HEMODIALYSIS (HD).

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During HD with cuprophane (CU) membranes, C is activated resulting in transient neutropenia, followed by rebound leukocytosis. This has previously been attributed to down-regulation of C receptors on PMN. Using an indirectly fluoresceinated recombinant human C_{5a} and flow cytometry, we investigated the relationship between C activation and C_{5a} receptors on PMN and MC during HD. These were determined in 6 chronic HD patients, using new CU membranes, at 0, 15, 30, 60 and 240 minutes following initiation of HD. C_{5a} was determined by standard RIA techniques. The cytometer was calibrated by fluorescent beads (100 fluorescent units = 57,000 receptors/cell). The results are tabulated below (mean ± SEM):

Minutes	Pre	15	30	60	240
C _{5a} (ng/ml)	3.1 ±0.27	47.1* ±11.9	28.7** ±8.6	16.7** ±2.1	12.1* ±1.2
% PMN	100	20** ±3.2	45** ±6.6	100 ±6.3	135* ±4.6
PMN C _{5a} R	332 ±43	282** ±34	302* ±34	306* ±36	340 ±43
MC C _{5a} R	206 ±29	152 ±14	145* ±11	158** ±23	209 ±20

*P<.05 **P<.01 (paired t-test)

Thus, although C_{5a} receptors remain down-regulated on both PMN and MC up to 60 minutes, neutrophil count returns to baseline values. Thus down-regulation of C_{5a} receptors is not sufficient to explain the reversal of granulocytopenia during hemodialysis.

AN AUTOMATED TECHNIQUE FOR CONTINUOUS ARTERIOVENOUS HEMODIALYSIS (CAVHD) FACILITATING THE MONITORING OF PERFORMANCE. M. Goldstein, J. McDougall,* A. Thomas,* S. Doubt,* and K. Jindal,* St. Michael's Hospital, Toronto, Ontario.

Continuous therapy has become the preferred mode of management for acute renal failure. We have devised an automated approach for CAVHD using conventional equipment and monitoring blood flow rate and the diffusive and convective performance of the dialyzer. Two automated IMED pumps (model #980) deliver dialysate to and from a high flux dialyzer providing excellent ultrafiltration capacity and volumetric control. The stable rate of ultrafiltration enables the calculation of blood flow rate by hematocrit difference. The constant dialysate flow rate facilitates determination of the clearance from blood and dialysate samples, simplifying antibiotic dosing. Monitoring the peak negative pressure in the dialysate compartment reflects the convective performance of the dialyzer. Using these three parameters of performance, one can make rational decisions concerning replacement of the dialyzer. Bookkeeping of the rate of ultrafiltration relative to dialysate flow is markedly facilitated by the automated recordings of the pumps which can be reset hourly. As all the equipment is conventional, the apparatus is easily operated by ICU nurses.

HIGH SENSITIVITY AND SPECIFICITY OF INDIUM-111-LEUCOCYTE SCAN IN INFECTIOUS COMPLICATION IN PATIENTS UNDERGOING REGULAR DIALYSIS TREATMENT. R. Götz, E. Heidebreder, W. Becker, A. Heidländ; Div. of Nephrol. and Dept. of Nuclearmed., Univ. of Würzburg, FRG (intr. by J.D. Kopple)

Serious problems in the diagnosis of infectious raise especially in patients suffering from adult polycystic kidney diseases (APKD). The high sensitivity and specificity of the In-111-leucocyte scans in infections were promising to study this technique also in these renal diseases. In 39 patients undergoing RDT 47 In-111-leucocyte scans were performed. The etiology of the renal insufficiency was: APKD n=15; chronic glomerulonephritis (GN) n=10; interstitial nephritis (IN) n=9 and diabetic nephropathy (DN) n=5. The indication for the leucocyte scan was fever and /or severe leucocytosis of unknown origin.

Eight of the 23 studies in patients suffering from APKD showed a positive leucocyte scan. Six of these were proven surgically. In 2 others a cyst infection only could be diagnosed clinically. Eight of 24 patients with DN, GN, or IN showed a positive leucocyte scan with different localization of infections: abdominal abscesses, osteomyelitis, pericarditis, infection of secondary acquired cysts. All infections could be proven surgically, radiologically or by biopsy. In-111-leucocyte scans successfully localize the origin of infections in patients undergoing RDT. In patients with APKD the leucocyte scan is of great importance, other imaging procedures are not very reliable methods.

SUCCESSFUL TREATMENT OF CYTOMEGALOVIRUS (CMV) PNEUMONIA WITH GANCICLOVIR (DHPG) IN A CHILD ON DIALYSIS. Carl M. Grushkin, Carl Lanarsky*, J.P. Sommadossi*, Ellin Lieberman. Childrens Hospital of L.A. and University of Southern California School of Medicine, Los Angeles, CA and the University of Alabama, Birmingham, Alabama.

A 9 year old boy treated with antithymocyte globulin and monoclonal antibody for transplant rejection became severely immunocompromised with no lymphocyte response to antigens including CMV. Fever, thrombocytopenia and leukopenia developed. He was successfully treated for candida sepsis and esophagitis following transplant nephrectomy. He continued to have fever and developed pulmonary infiltrates, became oxygen dependent and CMV pneumonia was diagnosed via positive culture from bronchial washings. He was treated with DHPG 2.5 mg/kg following each hemodialysis for 4 weeks. Fever decreased by 7 days and resolved after 14 days. Oxygen requirement resolved after 18 days. Chest x-ray improved slowly. Pre and post dialysis DHPG blood levels ranged from 2.8-4.6 mcg/ml to 1.5-2.1 mcg/ml respectively indicating that a significant amount is removed during a 2-3 hour dialysis. No side effects were observed from DHPG. The child is doing well on dialysis 4 months post therapy with no residual illness. DHPG was effective, safe and shown to be hemodialyzable. Additional study should be carried out to determine appropriate dosage for patients with renal failure on dialysis.

SURVEY OF WATER TREATMENT IN MARYLAND DIALYSIS FACILITIES. Donald R. Hamilton*, R. Todd Penry*, Kevin O'Brien* and John H. Sadler. Food and Drug Administration, Rockville, MD. and Univ. of Maryland, Baltimore, MD.

Water used for the production of dialysate solution must be treated to remove undesirable chemical and bacterial contaminants. Recommended guidelines for treating dialysis water have been published by the Association for the Advancement of Medical Instrumentation (AAMI). These guidelines provide limits for both chemical and bacterial contaminants.

Information available to the Food and Drug Administration (FDA) from problem reporting systems, literature searches and other sources indicates that inadequate water treatment can be a significant cause of adverse effects to patients during hemodialysis treatments. The inadequate treatment of water may be a result of poorly designed, controlled or monitored purification systems. The training of the health care professional team may be deficient causing the physicians, nurses, and technicians to be unaware of the potential problems.

With the support and assistance of hemodialysis personnel, the FDA recently conducted a pilot survey of water treatment in Maryland dialysis facilities. Samples of the feed and treated water were collected and analyzed against the AAMI guidelines for chemical and bacterial levels. A short questionnaire was also completed which provided information on the purification system, its operation and verification, and the monitoring system in place. Results of the survey findings and a look at future FDA plans in this area will be presented.

INFLUENCE OF PARATHYROID HORMONE (PTH) ON EXOGENOUS ERYTHROPOIETIN (EPO) STIMULATED ERYTHROPOIESIS IN HEMODIALYSIS (HD) PATIENTS. H. Hampl, E. Riedel, G. Wendel, U. Stabell and M. Kessel (intr. by W. Shapiro) Departments of Nephrology & Biochemistry, Free University of Berlin, FRG.

Erythropoiesis in HD patients is modulated by both EPO and PTH. We compared the effects of EPO on hematocrit (HCT) and the age distribution of erythrocytes (RBC) as determined by Percoll-density gradient and expressed as fraction of younger RBC's (FyRBC). Studies were performed in 6 anemic HD patients with normal levels of intact PTH (<10 pmol/l) and in 8 anemic HD patients with high PTH levels (>30 pmol/l). All patients received intravenous injections of EPO at a dose of 50 IU/kg after each HD treatment thrice weekly for 4 weeks. Results (mean±SD):

Before EPO			After EPO		
HCT (%)	FyRBC (%)	PTH (pmol/l)	HCT (%)	FyRBC (%)	PTH (pmol/l)
20±3	40±14	<10	28±6*	57±11*	<10
26±4	42±11	>30	35±7*	51±16*	>30

* p<0.01 compared with values before EPO.

EPO administration resulted in a significant increase in the HCT and FyRBC in both groups. The rise in HCT was similar between the two groups; however, in patients with normal PTH the rise in FyRBC was significantly greater (p<0.01) than in the group with elevated PTH. These data demonstrate that in HD patients with high PTH levels RBC production in response to EPO administration is not diminished but is modified so that the RBC population consists of more old cells than in patients with normal PTH.

SCREENING DIALYSIS PATIENTS FOR HYPERSENSITIVITY: SPECIFIC IgE-ANTIBODIES VERSUS C3a GENERATION A. Heidland¹, R.M. Schaefer¹, and H.D. Lemke² (introd. by S.G. Magsry); ¹Dept. of Int. Med., Univ. of Wuerzburg, FRG, ²Enka Research Inst., Obernburg, FRG.

Both generation of C3a and specific IgE-antibodies against ethylene oxide (IgE-AB) have been incriminated to contribute to hypersensitivity reactions during hemodialysis (HD). The present study was performed to investigate as to whether IgE-AB or C3a formation were correlated with the occurrence of hypersensitivity. Seven of 129 dialysis patients (pts) had a history of severe hypersensitivity at the onset of HD. All of these 129 pts were screened for elevated serum IgE-AB. Five of the 7 symptomatic pts displayed elevated IgE-AB, whereas only 2 of 116 non-symptomatic pts had elevated levels of IgE-AB. Thus, the sensitivity to detect a symptomatic pt was calculated to be 71% and the specificity was 98%. In addition, C3a generation both in vivo and in vitro using zymosan was followed in the group of symptomatic pts and adequate controls. For the in vivo studies symptomatic pts were dialyzed with gamma-radiated cuprophane membranes. Peak C3a levels were 9,276 ± 3,750 for symptomatic as compared to 9,638 ± 3,817 ng/ml for non-symptomatic pts. In vitro complement activation resulted in C3a values of 25,235 ± 10,119 for symptomatic pts comparing to 32,394 ± 9,657 ng/ml for non-symptomatic pts. Taken together, these data evidence that IgE-AB are a valuable tool to screen for dialysis pts prone to hypersensitivity, whereas the extent of C3a generation did not correlate with hypersensitivity during HD.

SUPEROXIDE (O₂⁻) GENERATION BY NEUTROPHILS (PMN), AND MONOCYTES (MC) BY CUPROPHANE (CU) MEMBRANE. J. Himmelfarb*, R. Hoffman*, J.M. Lazarus, R.M. Hakim. Brigham & Women's Hospital, Boston, MA, and Vanderbilt University Medical Center, Nashville, TN.

Superoxide generation by activated PMN is an integral part of host-defense mechanisms; however, its unregulated production has also been associated with endothelial cell lysis, myocardial tissue damage, and increased risk of neoplasms. We studied O₂⁻ production by PMN and MC during dialysis. Six patients on chronic HD were studied at 0, 15, 30, 60 and 240 minutes following initiation of HD with new CU membrane. PMN were harvested, loaded with dichlorofluorescein-diacetate which fluoresces when oxidized. The extent of fluorescence was measured using flow cytometry. The results are tabulated below (mean±SEM):

min	0	15	30	60	240
fluorescence	191	263**	218	217	180
channel	±30	±33	±28	±32	±26

**p<0.01 (paired t-test)

Thus, during dialysis with CU membrane PMN had significantly higher O₂⁻ generation at 15 min. We further studied the residual capacity of these PMN to respond to additional stimuli by incubating them in the presence of recombinant human C₅a (2x10⁸M). The % increase of O₂⁻ production from baseline is shown below:

min	0	15	30	60	240
% increase	22.8	7.2*	7.7*	9.8*	22.2
	±5.1	±2.2	±2.3	±2.5	±4.3

*p<0.05 (paired t-test)

Thus, the responsiveness of these PMN to C₅a was significantly reduced up to 60 minutes. Similar results were obtained with MC.

ATTITUDES REGARDING CARDIOPULMONARY RESUSCITATION (CPR) AND WITHDRAWAL OF DIALYSIS (WD) BY A POPULATION OF CHRONIC DIALYSIS PATIENTS. J. Holley, T. Finucane† A. Moss, WV Univ., Morgantown, WV.

Nephrologists must frequently discuss issues of life-support therapy with acutely ill dialysis patients (pts). The attitudes of this population toward life-support therapies are not well known. We collected the responses of 86 stable chronic dialysis pts (51 in-center hemodialysis and 35 CAPD pts, mean age 57 yrs in both groups) to questions concerning critical illness, withholding CPR (WCPR), and WD in hypothetical situations. In the event of acute illness, 93% of pts always want to be informed of conditions and 71% want to participate in medical decision-making. CPR if required at the time of the study was approved by 71%. With brain injury and coma, 68% request WCPR and 41% WD (X²p=.07). With permanent coma, pts are more accepting of withholding chronic ventilation than WD (X²p=.03). CAPD pts were more likely to accept WD than were hemodialysis pts (X², p=.02). More pts had previously considered CPR issues than WD. Ninety-four % of pts approved discussion of these topics. We conclude that most dialysis pts want to participate in decisions regarding life-supporting therapy, favor CPR, have seldom considered stopping dialysis, are more willing to refuse CPR than to discontinue dialysis in the event of brain injury, and support discussion of these issues.

PITFALLS IN INTERPRETATION OF CHANGES IN PLASMA BETA-2-MICROGLOBULIN (B2M) CONCENTRATIONS UNDER HEMODIALYSIS (HD) AND HEMOFILTRATION (HF)

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(introduced by KF Kopp)

Plasma and ultrafiltrate B2M concentrations were determined (RIA, Pharmacia) during HD (cuprophane, GF120H, Gambro) and HF (polysulfone, HF80, Fresenius) under various conditions in order to evaluate the elimination kinetics of B2M. Calculations were done on the basis of plasma-water-concentrations (PWC).

Elimination of B2M follows first-order kinetics ($r > 0.97$) and the volume of B2M distribution was calculated to be $17 \pm 2\%$ of body weight. This reflects the extracellular volume (ECV). Under provoked fluid shift from ECV to intracellular volume and vice versa by varying sodium (Na) concentration in substitution fluid or dialysate B2M was measured in HD and HF at identical weight loss. At comparable B2M removal individual intratreatment B2M concentration changes at low Na load were $>15\%$ smaller than those at high Na load. B2M mass balance in HF even allowed an estimate of ECV changes, induced by Na load.

Conclusion: Calculations of intratreatment changes of B2M concentration should base on PWC because of the varying degree of simultaneous plasma-volume changes. Interpretation of intratreatment B2M changes must take into account elimination, but also changes of distribution volume.

SAFETY AND EFFICACY OF DIALYSIS WITH A VERY LOW POTASSIUM BATH.

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Hemodialysis (HD) has been used for acute treatment of hyperkalemia in patients with renal failure but the safety and efficacy of HD with very low K baths has not been established. We dialyzed 11 chronic stable HD patients with dialysate containing 2 mEq/L (2 K bath), 1 mEq/L (1 K bath) and 0 mEq/L (0 K bath) of K to assess their safety and efficacy. Fluid removal, blood flow and dialysate Ca, glucose and acetate were standardized. Dialysate K concentration was measured prior to dialysis. Spent dialysate was collected. Its K concentration was measured and K removal was calculated. Serum K was measured hourly during HD and 1 hour after HD. Results were as follows:

4 hour K removal in mEq	Serum K at 4 hours mEq/L	K at 4 hrs. mEq/L
2 K 50.6 ± 6	$3.8 \pm .45$ N.S.	$1.8 \pm .5^*$
1 K 62.9 ± 5	$3.2 \pm .13$ N.S.	$1.7 \pm .2^*$
0 K 78.05 ± 2.6 $p < .01$	$3.1 \pm .33$ N.S.	$2.1 \pm .3^*$

Cardiac rhythm was monitored by holter monitor from the beginning of HD for 12 hrs., 8 hrs. after dialysis with on each bath. Isolated PAC's and PVC's were common and did not vary with the K concentration in the bath. Only one patient had potentially dangerous arrhythmias. She had frequent PVC's on all baths and runs of V tach on the 0 K bath. In her, serum K fell below 3 mEq/L on all baths. Frequency of arrhythmia decreased and occurrence of dangerous arrhythmias disappeared when she was dialyzed on 3 K bath. We conclude that 1) 0 K bath is significantly more effective in K removal than 1 K and 2 K baths. 2) K removal by standard dialysate accounts for less than half of daily intake. 3) Dialysis with 0 K bath is safe in most patients but because of arrhythmias in occasional patients, cardiac monitor should be used in the first HD on 0 K bath.

*Differences between baths not significant.

CORONARY ARTERY BYPASS GRAFTING (CABG) IN END-STAGE RENAL DISEASE (ESRD): LONG TERM SURVIVAL.
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CABG can be performed with acceptable risk and results in symptomatic improvement in patients with ESRD. However, the effect of CABG on long term survival in these patients is unknown.

We identified 39 patients who underwent CABG for intractable angina between Jan. 1975 and Feb. 1987. A control group of 39 dialysis patients was selected matching for age, sex, year of initiation, and presence of diabetes mellitus and atherosclerotic heart disease at initiation. Additionally, controls were selected only if they had survived on dialysis at least as long as their matched study patient. Using life-table analysis, survival probability (with 95% confidence intervals) was determined from the time of CABG for study patients or after an equivalent period of time on dialysis for controls. Study end points included death, transplantation, transfer to other dialysis facilities, reoperation, and alive on dialysis.

Operative mortality (30 days) was 2.6% and 2.56 ± 0.75 (mean \pm S.D.) vessels were bypassed. Mean followup post CABG was 34.9 ± 30.1 mos. Two yr. survival was 91.7% in the study patients and 51.4% in controls ($p < 0.05$). Survival at 4 yrs. was not significantly different between study patients and controls (51.3% vs 32.7%), but the number of entrants at 4 yrs. was small (9 vs 4, respectively).

These data demonstrate a remarkably low 2 yr. mortality rate post CABG and suggest that CABG has a positive impact on life expectancy in patients with ESRD.

COMPLEMENT ACTIVATION AND POLYMORPHONUCLEAR LEUKOCYTE FUNCTION DURING HEMODIALYSIS.

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Complement (C) activation during hemodialysis (HD) results in increased C receptor expression and leukopenia. To test the hypothesis that C activation during hemodialysis alters respiratory burst and phagocytosis (phag), we measured H202 production, phag, and C3bi receptor (CR3) expression by polymorphonuclear leukocytes (PMN) in whole blood by flow microfluorimetry expressed as relative fluorescence intensity (rfi), and C3a des Arg and C5a des Arg generation in serum from patients before, during and after HD. Comparisons were made to non-dialyzed normal control subjects. The data for HD are below:

	Before	15 min	After
H202 (rfi)	1341 ± 159	1219 ± 228	1045 ± 107
Phag (rfi)	130 ± 8	107 ± 9	132 ± 5
CR3 (rfi)	115 ± 8	$155 \pm 14^+$	$172 \pm 11^+$
C3a (ng/ml)	139 ± 10	$784 \pm 82^+$	333 ± 87
C5a (ng/ml)	1.9 ± 0.4	$4.4 \pm 0.3^+$	1.9 ± 0.2

+ $p < 0.05$ compared to before HD (mean \pm SEM)
Respiratory burst was greater in dialysis patients than in controls. HD did not increase respiratory burst despite C activation and increased CR3 expression. Phag decreases during peak C activation, however, this decrease was not statistically significant. We conclude that PMN from HD patients are primed for an enhanced respiratory burst. Phag appears to be impaired during HD, but respiratory burst is independent of C activation. The increased infection rate in HD patients cannot be attributed to impaired PMN respiratory burst.

PRURITUS IN DIALYSIS PATIENTS DUE TO IRON DEFICIENCY. Paul G. Jenkins. Univ. of Wisc. Med. School, Mt. Sinai Med. Ctr., Milwaukee, WI.

Pruritus (PR) occurs in a majority of dialysis patients at some time during their illness. As a rule, no specific cause of PR is identified and no specific or uniformly effective treatment exists.

We have evaluated 6 episodes of severe PR in 3 dialysis patients with each episode associated with a decreased serum ferritin level. The PR improved within several weeks and resolved completely within 1-2 months after iron (Fe) therapy. Two hemodialysis and 1 CAPD patient had 3, 2, and 1 episodes of PR and had been on dialysis 84, 54, and 11 months respectively before PR first developed. Iron dextran (1 gm) was given to the hemodialysis patients and oral iron to the CAPD patient. Pertinent hematologic data are summarized.

		Hct	MCV	Ferritin
Pre Fe	Mean	46%	84	34 ng/ml
	Range	27-55%	74-88	<5-76
Post Fe	Mean	44%	84	154
	Range	26-55%	75-90	93-106

We conclude: (1) Fe deficiency can cause PR in dialysis patients. (2) Neither anemia nor microcytosis need be present. (3) Response to Fe therapy is relatively rapid and complete. (4) Fe deficiency may be the most common specific cause of PR in dialysis patients.

THE IMPORTANCE OF DIALYZER AND MEMBRANE SPECIFIC FEATURES IN SEVERAL HIGH FLUX DIALYZERS (HFD). K. Jindal,* B. Woods,* L. Nowakowski,* and M. Goldstein. St. Michael's Hospital, Toronto, Ontario.

As there is increased use of HFD we questioned whether there were major differences between the various membrane types (MB) in the clearance of large versus small molecular species. The membranes studied were: 2 polyacrylonitrile (PA 1 and PA 2), 2 cellulose acetate (CA 1 and CA 2), 1 polymethylmethacrylate (PMMA) and 1 polysulphone (PS). Clearances based on timed dialysate collections were measured for urea (U), creatinine (C), and Beta 2 microglobulin (B2M). Sieving coefficient (SC) for B2M was measured.

MB	Clearances (ml/min)			SC
	U	C	B2M	B2M
PA 1	201	144	4.3	.05
PA 2	215	170	17	.21
CA 1	270	199	0	0
CA 2	212	152	11.4	.27
PMMA	265	223	0	0
PS	225	186	31.3	.47

There was a 32% fall in serum B2M from pre to post dialysis with PMMA despite 0 SC suggesting adsorption. The results indicate major differences in B2M clearances not predictable from the U clearance or the ultrafiltration index. Complete assessment of high flux dialytic therapy should include not only urea kinetics but also B2M kinetics.

TREATMENT OF ACUTE RENAL FAILURE AND HYPERKALEMIA IN A NEWBORN USING CONTINUOUS ARTERIOVENOUS HEMODIAFILTRATION (CAVHD).

Randall D. Jenkins,* and Elizabeth C. Jackson:* (intr. by Nancy H. Holland). Univ. of Kentucky Med. Ctr., Dept. of Pediatrics, Lexington, Kentucky.

We have found continuous arteriovenous hemofiltration (CAVH) to be a powerful option in the treatment of neonatal renal failure. CAVH has been inadequate in controlling hyperkalemia, or azotemia in some infants. We report the first use of CAVHD (adding a dialysate to CAVH) in a neonate. The increased ability of CAVHD to control hyperkalemia is demonstrated with clearance data and serial chemistries. A 3500 gm term infant at 29 hours of life developed a tension pneumothorax with severe acidosis and hypoxia followed by acute anuric renal failure and DIC. Despite initiation of dialysis with CAVH, potassium level increased from 7.2 to 8.1 meq/l. After brief ventricular tachycardia, CAVHD was begun with Travenol^R dialysate pumped through the hemofilter. Hyperkalemia resolved promptly. K⁺ clearance (0.54 ml/min) was 3 times that from CAVH alone (0.17 ml/min). Results of chemistries are as follows:

	PRE-Rx	POST-CAVH	POST-CAVHD
Creatinine	2.5 mg/dl	2.7	2.8
Sodium	142 meq/L	150	133
Potassium	7.2 meq/L	8.1	4.8

CAVHD was continued for 51 hours with no complications. Urine output and renal function returned to normal. CAVHD may be superior to CAVH for therapy of hyperkalemia with renal failure in the newborn.

ANAPHYLATOXINS C3a AND C5a ADSORPTION ON POLYACRYLONITRILE (PAN) MEMBRANE OF DIALYZER - IN VIVO STUDY. ¹Aljoša Kandus*, ¹Jože Drinovec*, ¹Silvester Kladnik*, and ²Peter Ivanovich. ¹University Medical Center, Ljubljana, Yugoslavia, ²V.A. Lakeside Medical Center, Chicago, Illinois, USA.

Recent in vitro studies confirmed significant adsorption of C3a and C5a on PAN membrane of dialyzer. The purpose of our prospective clinical study was to investigate adsorption of C3a and C5a on PAN membrane in 10 patients during regular hemodialysis (HD). Blood passed first through cuprophane (CU) dialyzer and then through PAN dialyzer, which was not in contact with dialysis fluid. Blood samples were drawn at the efferent line of both CU and PAN dialyzers at 15, 60 and 240 min of HD. C3a and C5a plasma concentrations were determined by radioimmunoassay. Results are presented in the table ($\bar{X} \pm SD$).

	min HD	CU line	PAN line	p
C3a	15	9472 [±] 2588	3165 [±] 1502	<0.001
	60	3979 [±] 1125	1475 [±] 329	<0.001
	240	2225 [±] 830	922 [±] 414	<0.01
C5a	15	53.9 [±] 23.6	18.8 [±] 7.9	<0.001
	60	29.5 [±] 5.3	12.8 [±] 4.0	<0.001
	240	12.1 [±] 4.1	8.3 [±] 3.2	<0.05

Results suggest that considerable adsorption of C3a and C5a on PAN membrane occurred throughout HD. It is possible that adsorption on PAN membrane prevents significant elevations of C3a and C5a plasma levels during HD with PAN dialyzer.

HERPES ZOSTER IN DIALYSIS PATIENTS. Lois A. Katz
NY VA Medical Center, NYU School of Medicine,
New York, NY

Herpes zoster (HZ) infections are frequent in immunocompromised individuals including renal transplant recipients, but have not been noted to occur commonly in dialysis pts. In a population of 22 men on home dialysis, 3 black pts, aged 40-61 yrs, on dialysis 3-6 yrs, had herpes zoster in the last year. One pt was on CAPD and 2 were on hemodialysis (HD). One had inactive systemic lupus erythematosus, one is a former IV drug abuser, and the third is being treated for tuberculosis which developed 6 months prior to the HZ. None of the pts was receiving immunosuppressive drugs or had evidence of AIDS.

Two pts presented with chest pain 1-2 wks prior to the vesicular eruption. Each had an extensive evaluation to determine the etiology of the pain. The other man had pain and a rash when he presented. All were treated with acyclovir. The recommended dose for patients whose $C_{CR} \leq 10$ ml/min is 200 mg every 12 hrs; acyclovir is dialysable. The CAPD pt received 200 mg daily for 2 wks; his pain lasted for 6 wks after beginning treatment. One HD pt received 400 mg after each dialysis for 3 wks; the other received 200 mg twice daily for 10 days. The different doses seemed to have similar therapeutic results; all infections remained localized. There were no adverse effects. Only one other pt in our unit has had HZ; he was a 70 yo diabetic who became ill one month after starting CAPD in 1984; he received prednisone.

The occurrence of HZ in dialysis pts is probably related to their impaired cellular immunity. Acyclovir therapy is safe in dialysis patients.

ERYTHROCYTE (RBC) CATION TRANSPORT IN HEMODIALYSIS (HD); ROLE OF ACUTE CHANGES IN MEMBRANE LIPIDS. Kelly RA*, Canessa ML*, Steinman TI, Smith TW, Mitch WE. Depts. Med., Harvard Med. Sch., Boston, MA and Emory Sch. Med., Atlanta, GA.

Abnormal active and facilitated cation transport occur in RBCs from some HD patients. HD can transiently improve this by unknown mechanisms. As we (J.B.C. 260:11396) and others find that nonesterified fatty acids (NEFA) in plasma can inhibit the Na pump, we measured plasma and RBC membrane NEFA and cation flux in RBCs of 34 chronic HD patients before and just after HD. The average maximal Na pump activity, [3H]-ouabain binding and Na/Li countertransport were unchanged by dialysis; Na/K cotransport flux rose slightly (0.56 ± 0.06 to 0.69 ± 0.07 mmol/L cell/hr; $\bar{X} \pm SE$, $p < 0.02$). Plasma NEFA rose 87% with HD but RBC membrane NEFA fell (72 ± 8 pre vs 56 ± 7 post, nmol/mg/protein; $p < 0.001$). In 24 patients whose RBC membrane NEFA decreased $>10\%$ after HD, Na pump activity exponentially increased ($r = 0.56$; $p < 0.01$). These changes in membrane NEFA and Na pump activity were mimicked by incubating predialysis RBCs with delipidated albumin. Thus, acute changes in membrane NEFA may modulate cation transport in uremic erythrocytes.

A RE-APPRAISAL OF THE NATIONAL COOPERATIVE DIALYSIS STUDY (NCDS). P. Keshaviah* and A. Collins, Regional Kidney Disease Program, Hennepin County Medical Center, Minneapolis, MN.

The NCDS is a widely published and publicized study that is being invoked as a gold standard for prescribing hemodialysis therapy. We recently received the final edited computer files of the NCDS courtesy of Dr. Lowrie, the principal investigator of the study. A re-analysis of this data raises some disturbing questions regarding the validity of conclusions drawn from other analyses of the NCDS:

1. The mean KT/V values for Groups I - IV were 1.16, 0.61, 0.95 & 0.55 respectively. Despite the study intent, Groups I & III, the low BUN groups, did not receive equal therapy prescriptions. In Group I, 73 % of the patients had a $KT/V > 1$ compared to only 34% in Group III. The slightly higher incidence of F2 failures in Group III may not, therefore, reflect the influence of treatment time or middle molecules but may be a consequence of the lower KT/V .

2. The relationship of % F2 failures to KT/V is not discontinuous but linear with a negative slope and correlation coefficient of 0.86. At a KT/V between 0.8 and 1 the % failure is ~20 % and regresses to zero at a $KT/V \sim 1.3$.

3. The mean PCR was identical in the successful and failed sub-populations of the study and was a poor predictor of failure when $PCR > 0.8$ gm/kg/day. At lower PCR, by virtue of the study design, the KT/V was also low and < 0.8 . The strong influence of the KT/V on the higher % failure in this sub-group cannot be ruled out.

The NCDS clearly demonstrates how not to dialyze patients. Connoting a $KT/V = 1$ with adequate dialysis may not be justified by virtue of the analysis above. Finally, the significance of PCR on therapy outcome appears exaggerated.

INTRADIALYTIC BICARBONATE INFUSION (BiIn) DURING ACETATE DIALYSIS (AcDi); EFFECTIVE ALTERNATIVE TO BICARBONATE DIALYSIS (BiDi) FOR ACETATE (AcDi) INTOLERANT PATIENTS WITH ESRD. S. Khan, A.B. Schwartz, A. Olshan, J. Brezin, L. Krevolin, M.B. Lim, J. Chinitz. St. Agnes Medical Center & Hahnemann University, Philadelphia, PA

Methods: BiIn 44 mEq onset and 22 mEq q 1/2 h during AcDi was compared to Std BiDi and Std AcDi with pts single blinded. Studies performed on mid Rx of Std 3X week schedule: pH, pCO_2 , pO_2 , HCO_3^- , tCO_2 , Na, K, Cl, CBC, BP, P, wt, symptoms, fluids.

Results: With BiIn post $HCO_3^- = 23.7$ mEq/L, equalled BiDi post $HCO_3^- = 23.7$ and $> AcDi$ post $HCO_3^- = 21.9$ ($p < .05$). Pre BiIn HCO_3^- following BiIn = 21.5 which was $> BiDi$ pre $HCO_3^- 19.2$ ($p < .002$) and $> AcDi$ pre $HCO_3^- 19.2$ ($p < .002$) and $> AcDi$ pre $HCO_3^- 19.3$ ($p < .10$).

	HCO_3^-		tCO_2		pH		pCO_2	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BiIn	21.5*	23.7	21.5*	7.36	7.42	37.9	36.5	
BiDi	19.2*	23.7	18.8	7.34	7.42	35.1	36.9	
AcDi	19.3	21.9*	19.0	7.36	7.42	33.4	33.4*	

There was N.S. difference in wt, or IV saline. Post Rx Mean BP was highest with BiIn = 87.3 mm Hg vs BiDi = 84.9 and AcDi = 84.6. Pre Rx mean BP was highest with BiIn = 93.7, BiDi = 96.5 vs AcDi = 87.3. Pre Rx Na were similar. Na concentration was unchanged throughout Rx in: BiIn 135-135; BiDi 137-137 vs; AcDi 136-134.

Conclusion: BiIn increases HCO_3^- and maintains pH without decreasing pCO_2 . BiIn maintains favorable BP, is an easy alternative method of delivering HCO_3^- to AcDi intolerant pts without untoward effects when BiDi machine is unavailable.

DIALYSIS OF DOGS USING ACETATE AND SUCCINATE: CARDIOVASCULAR AND METABOLIC EFFECTS. Paul L. Kirkendol, Ted A. Kasperek*, James E. Pearson, Norman W. Robie*, Efrain Reisin and Francisco M. Gonzalez. LSU Med. Ctr., Depts. of Pharmacology and Medicine, New Orleans, Louisiana.

Succinate (SC) appears useful as a substitute for acetate (AC) in dialysis due to cardiovascular stability. Thus, acutely nephrectomized dogs were dialyzed for four hours with AC, SC or 50:50 AC:SC and monitored for two hours post-dial. BP, CO and blood gases were determined at 0, 5, 30, 60, 120, 180, 245, 275, 305, and 365 min. All solutions produced similar decreases in BP (about 40%). During post-dial. only the AC animal's BP remained depressed. Similar decreases in CO were seen with all three solutions. This would suggest that the SC and 50:50 mixture had less effect on TPR than AC alone in the post-dial. period. AC produced least loss of HCO₃ (4 mEq/L) and SC the most (11 mEq/L). During post SC dial., plasma HCO₃ returned the slowest. During AC and 50:50 dial., pH did not change though HCO₃ decreased, thus, suggesting a loss of CO₂. During SC dialysis, pH decreased, reflecting the decrease in HCO₃. In the post-dial. period for all solutions, the pH and HCO₃ tended to return toward pre-dial. levels, but were still significantly decreased after 2 hr. Blood chemistries taken pre-, post- and 2 hr post-dial. showed values consistent with those expected for dial. therapy. Even though SC may offer greater CV stability for dial., its conversion to HCO₃ is too slow to be useful. The 50:50 mixture is more suitable, but its conversion to HCO₃ also appears to be too slow. The best mixture may be one with less than 50% SC.

POSTDILUTION (POD) IS AS EFFECTIVE AS PREDILUTION (PRD) IN CONTINUOUS ARTERIOVENOUS HEMOFILTRATION (CAVH). Sidney M. Kobrin*, Mark S. Kramer, Stephen J. Goldstein, Maria Mendez and Rasib M. Raja. Albert Einstein Medical Center, Kraftsow Division of Nephrology, Philadelphia, PA.

Intermittent hemofiltration is performed using the POD mode. Investigators have claimed administering replacement fluid via the PRD port during CAVH increases urea (U) clearance (C), possibly by increasing U transport out of red cells and/or decreasing oncotic pressure and enhancing ultrafiltration. The effect of PRD or POD modes of CAVH on the C of larger solutes has not been reported. We performed a total of 40 hrs of CAVH in 4 anesthetized dogs. A loading dose followed by a constant intravenous infusion of creatinine (Cr), U and inulin (I) was given to maintain plasma U at 200 mg/dl, Cr at 10 mg/dl and I at 25 mg/dl. Every ½ hr, replacement fluid at a rate of 400 ml/hr was alternated between POD and PRD sites, arterial blood and ultrafiltrate (UF) samples were collected, and blood pressure (BP) and UF volumes recorded. U, Cr and I concentrations were determined and clearances calculated. Results (mean ± SEM):

MODE	C _u ml/min	C _{Cr} ml/min	CI ml/min	BP mmHg	UF ml/min
PRD	6.1±0.6	5.9±0.6	4.7±0.5	134±2.4	6.86±0.6
POD	6.3±0.6	6.2±0.6	4.7±0.4	134±2.5	6.65±0.6

These data suggest there is no significant difference in the C of small and middle molecular weight solutes between PRD and POD modes of CAVH. Significant transfer of urea out of red cells is, therefore, unlikely during PRD mode of CAVH. Like intermittent hemofiltration, POD may be preferred during CAVH since less replacement fluid is required.

SYNTHESIS OF BETA 2-MICROGLOBULIN IN DIALYZED PATIENTS. Kazuo Kumano*, M.Nanbu*, S.Kusakari*, T.Sakai*, K.Sakurai.*(intr. by Dr.Y.Tsukamoto) Kitasato Univ., Kidney Ctr., Hashimoto Clinic. Sagami-hara, Kanagawa, Japan

Beta 2-microglobulin (B2M) has been identified as a major component of amyloid deposit. The serum concentration of B2M does not differ between patients on CAPD and those dialyzed with cuprophane dialyzer, although the former removes a significant amount of B2M and the latter does not. This may indicate some changes either in synthesis or in degradation of B2M among these patients. To elucidate this possibility, B2M synthesis was studied in dialyzed patients. Mononuclear cells (MNC) were isolated in peripheral blood from healthy volunteers, patients dialyzed with cuprophane dialyzer and patients on CAPD. MNC were cultured for 96 hours in medium of RPMI 1640 with or without presence of IL-1, IL-2 or interferon (INF-γ). To study the effect of dialyzer membrane on B2M synthesis, uremic blood was recirculated extracorporeally for 2 hours through cuprophane or synthetic membrane (PMMA, EVAL) dialyzer and isolated MNC were examined. B2M of cultured cell and its supernatant were detected by enzyme immunoassay. B2M synthesis was significantly decreased by 25% in patients on hemodialysis when IL-2 or INF-γ was present in medium, however, there were no significant difference between healthy volunteers and CAPD patients. B2M synthesis of MNC was increased 30-70% by extracorporeal blood circulation with all types of dialyzers and even a blood line itself. In conclusion, B2M synthesis of MNC was decreased in hemodialysis patients, but extracorporeal blood circulation per se increased B2M synthesis.

BODY COMPOSITION OF CHRONIC HEMODIALYSIS PATIENTS (HD) DETERMINED BY BIOELECTRIC IMPEDANCE (BI) PLETHYSMOGRAPHY. Paul S. Kurtin and Hiroko Tomita*, Depts. Ped. and Med., New Eng. Med. Ctr. Boston, MA.

BI is a widely used, non-invasive method of determining total body water (TBW) in healthy subjects. Routine dialysis acutely alters TBW in HD. We evaluated the utility and reliability of BI (RJL Systems Model BIA-101) in 10 stable, adult HD. TBW, fat-free mass (FFM), and body fat were assessed pre and post dialysis. An hydration factor of .72 was used to calculate FFM.

	WT(kg)	TBW(l)	FFM(%)		FAT(%)	
			M	F	M	F
PRE	62.9	37.1	81.8	72.0	18.2	28.0
	+10.8	+5.6	+6.6	+3.9	+6.6	+3.9
POST	60.6	34.5	78.5	69.7	21.5	30.3
	+10.6	+5.3	+6.7	+4.6	+6.7	+4.6

CHANGE p<.0001 p<.0001 p<.0001 p<.0001

Values represent means ± S.D. Difference pre vs. post determined by paired t-test. The FFM/HT ratio was .30±.02 in males and .22±.02 in females. In healthy controls the FFM/HT ratio is .38 in males and .29 in females. By linear regression there was no significant correlation (r=.43, p>.05) between change in weight and change in TBW. FFM determined by BI correlated highly (p<.01) with FFM determined by anthropometry. Conclusions: 1) The low FFM/HT ratio may represent chronic malnutrition in HD. 2) BI does not accurately reflect acute changes in TBW. 3) When TBW is expanded as in HD, FFM determined by BI is a maximal value and likely overestimates the true FFM. 4) BI may be more useful in following single patients longitudinally.

IN VITRO HEMODYNAMICS IN CONTINUOUS ARTERIOVENOUS HEMOFILTRATION (CAVH). S Lew and JP Bosch, George Washington University Medical Center, Department of Medicine, Renal Division, Washington, D.C.

The efficiency of CAVH depends on the hydrostatic pressure gradient and blood (QB) and ultrafiltration (UF) flow rates (ml/min). To determine the effect of circuit design on hemodynamic parameters, an in vitro CAVH circuit simulating the in vivo CAVH circuit was used to measure pressure (P) and flow. Whole blood was used to simulate in vivo blood composition. The in vitro CAVH circuit consisted of a blood pump, a 14 gauge angiocatheter, and an Amicon filter. Two filters with different lengths and fiber number were studied. P was measured in mmHg before and after the catheter, and before and after the filter. QB was varied by the blood pump and was measured at the venous end. Resistance (R) (dynes/cm²) was calculated. Results:

QB	D10-UF	P1	P2	P3	P4	D10+UF	P1	P2	P3	P4
100	5	8	10	5		7	6	9	4	
200	19	18	18	10		16	15	16	8	
300	38	28	26	12		35	25	23	14	
400	59	39	35	18		56	35	32	21	
100	D20-UF	9	7	10	5	D20+UF	12	6	6	3
200	26	16	19	9		23	15	18	8	
300	46	26	29	14		40	26	30	11	
400	69	38	42	20		58	40	42	16	

Pressure gradient in the CAVH circuit varies directly with filter resistance. Filter design and UF rate determines the filter resistance. A low resistance filter is indicated in the hypotensive patient.

LYSINE VASOPRESSIN (LV) IN THE TREATMENT OF REFRACTORY HEMODIALYSIS INDUCED HYPOTENSION (HIH). Jill S. Lindberg*, Kenneth Melton*, Charles Wade*, John B. Copley. Nephrology Svc, Brooke Army Med Ctr, Ft Sam Houston, TX.

The etiology of HIH is multifactorial and includes autonomic dysfunction. Because of recent data showing LV to be efficacious in the treatment of Shy Drager syndrome, we assessed the clinical response to LV of patients with HIH.

Six patients with refractory HIH, defined as three documented episodes of hypotension (H) with a mean arterial pressure (MAP) of <70 mm Hg or a fall in systolic blood pressure \geq 30 mm Hg on high sodium and bicarbonate dialysate, were enrolled. Prior to study entry all had pericardial effusion excluded by echocardiogram, a normal cardiac ejection fraction by MUGA, stable serum chemistries, no evidence of occult blood loss, and all drugs associated with H withdrawn. Formal autonomic testing was accomplished and found to be abnormal in all. Intranasal LV and placebo (2 sprays each nostril bid) were assessed in a double-blind crossover fashion. High sodium, bicarbonate dialysate was utilized throughout the study with fluid removed to an established dry weight. With LV the mean number of hypotensive episodes, defined as a MAP <70 occurring after dialysis initiation, was significantly less than the number which occurred with placebo (0.9 + 0.8 vs. 1.5 + 1; t = 3.95; p < .05). Additionally the total number of IV fluids administered for symptomatic H episodes was significantly less when LV was utilized (155 + 57 cc vs. 280 + 123 cc; t = 2.977; p < .05). This suggests that LV may be efficacious in alleviating refractory HIH.

INDUCTION AND REMOVAL OF TUMOR NECROSIS FACTOR (TNF) AND INTERLEUKIN-1 (IL-1) DURING IN VITRO HEMODIALYSIS (HD). G. Lonnemann, J.W.M. van der Meer*, S. Endres*, S. Shaldon**, K.M. Koch, C.A. Dinarello*. Med. School, Hannover, FRG; Tufts New England Med. Center, Boston*, MA; Univ. Hosp. Nimes**, France, (intr. by G.M. Eisenbach).

The monokines TNF and IL-1 share inducing mechanisms and biological activities. As HD was found to induce monocyte IL-1 production a simultaneous stimulation of TNF seemed likely. We studied induction and removal of both, TNF and IL-1, during in-vitro HD with cuprophan (Cu) and AN 69 (AN) membranes. Normal human donor (n=5) blood was circulated for 2 hrs in a closed-loop system against acetate dialysate (n=5 for each membrane). Mononuclear cells (MNC) were separated before and after circulation and incubated at 37°C for 18 hrs. MNC were lysed and total TNF, IL-1-alpha and -beta were measured by radioimmunoassay. In addition the removal of ¹²⁵I labelled TNF/IL-1-β from the blood compartment was studied (n=3). Results: (ng/ml, mean ± SEM).

	pre-HD (n=5)	2 h Cu-HD (n=5)	2 h AN-HD (n=5)
TNF	0.2 ± 0.05	0.5 ± 0.1	0.35 ± 0.1
IL-1-a	0.3 ± 0.2	1.3 ± 0.6	0.6 ± 0.2
IL-1-β	0.35 ± 0.1	1.5 ± 0.4	0.5 ± 0.1

Total mass of ¹²⁵I TNF or -IL-1-β in the blood compartment did not decrease during Cu-HD. In contrast during AN-HD there was a loss of approximately 75 % of both monokines. Only 25 % were recovered in the dialysate, the mass balance error possibly being due to sorption to the AN membrane. The results demonstrate that simultaneous induction of TNF and IL-1 is occurring during hemodialysis with both membranes, but effective sorption and removal of the monokines only with AN.

CONTRAST DYE AND THE NEED FOR SUBSEQUENT HEMODIALYSIS. T.W. Medcalf*, R.L. Fisher*, M. Rodriguez, and J.A. Pederson. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, OK.

Immediate hemodialysis after contrast dye is sometimes recommended. However, effects of contrast dye in hemodialysis patients are not well described. In 9 hemodialysis patients, mean arterial blood pressure (MAP), pulse, hematocrit (Hct), hemoglobin (Hgb), serum electrolytes, osmolality (Osm), and percent change in plasma volume (% ΔPV) were obtained before (pre) and 45 minutes after (post) contrast dye infusion. The following table summarizes the results [mean ± (S.E.); *p < .05]:

	Hct	Hgb	MAP	Osm	Osm Gap	K	%ΔPV
Pre	26.5	8.7	103	305	-10	4.6	0
	(1.2)	(.3)	(4.7)	(4.3)	(3)	(.3)	(0)
Post	24.6*	8.1*	102	308	3.4	5.0*	9.7*
	(1.2)	(.3)	(7.3)	(4)	(1.6)	(.2)	(2.5)

Changes in serum electrolytes and pulse were not significant. Changes in systolic blood pressure, MAP, and pulse correlated significantly with % ΔPV.

In conclusion, the results do not show any specific indication for immediate hemodialysis following contrast dye. The increase in potassium is unexplained but never reaches dangerous levels. The increase in plasma volume after contrast dye is likely the result of intracellular fluid shifts to the plasma space secondary to the osmotic activity of the contrast dye.

HEMODIALYSIS-ASSOCIATED SUBCLAVIAN VENOUS STENOSIS (SVS): PREVALENCE AND PREDISPOSING FACTORS; TREATMENT WITH TRANSILUMINAL ANGIOPLASTY (TLA). J.P. Middleton,* LD Quarles, M Saeed,* SJ Schwab. Duke University Medical Center, Durham, NC.

This prospective study was undertaken to define the factors predisposing to SVS and to clarify treatment of this condition. Thirty-six patients underwent upper arm venogram as a result of fistula dysfunction. SVS was documented in 12. All 12 had elevated venous dialysis pressure (196 ± 15 mmHg), and 6 had arm edema. All 12 previously had subclavian cannulation on the side of the fistula. Twenty-four patients showed no evidence of SVS. Eleven of these had previous subclavian cannulations on the side of the fistula. The mean age of the fistula at the time of venogram in patients who had subclavian catheters was 13.6 months (patients with SVS), vs. 6.1 months (patients without SVS). TLA was performed on all patients with SVS and lowered venous dialysis pressure and restored patency to fistulae in 100%. Based on 120 patients maintained on hemodialysis, the prevalence of SVS was 10% during this study. We conclude SVS is a common problem and elevated venous pressures are a first indicator of this condition. Predisposing factors include prior ipsilateral subclavian cannulation and the duration of high venous flows related to an A-V fistula.

HIV INFECTION IN HEMODIALYSIS (HD) CENTERS IN THE USA. N. Mittman, L. Berkowitz*, S. Kahlam*, and M.M. Avram. The Long Island College Hospital, Brooklyn, New York

We surveyed 1,412 HD centers across the US. This report describes the first 617 responses, representing a total ESRD population of 44,613. Forty-two percent of centers were university-affiliated (33% of all pts). Seventy-one percent were urban (64% of pts), 22% suburban (23% of pts) and 7% rural (13% of pts). A total of 228 pts with HIV infection are currently being treated (71% urban, 22% suburban, 7% rural) in 209 centers (34% of all centers). Special infection control procedures are used by 82% of centers, involving separate machines in 75%. Three percent of centers offer only home dialysis (CAPD in most) for HIV+ pts. "High risk" pts without documented HIV serology are treated with precautions in 28% of centers. This was true independent of university affiliation, setting or size of facility (χ^2 p=NS). Fifty-eight percent of centers reuse dialyzers, 10% in HBV+ pts and 7.6% reuse in HIV+ pts. Fifty-six percent of centers screen for HIV infection, 16% screening all pts and 40% only "high risk" pts. (also unaffected by university affiliation, setting or size) (χ^2 p=NS). Only 54% of centers screening all pts obtain informed consent, while 73% who screen "high risk" obtain prior informed consent.

As expected, this survey reveals a heterogeneous approach to HIV infection in HD centers across the US, perhaps reflecting lack of satisfaction with CDC recommendations. Although state regulations certainly explain some of the differences (especially with regard to HIV testing), a unified approach from within the nephrology community is needed.

THE DISSOCIATION OF COMPLEMENT ACTIVATION AND DIALYSIS MEMBRANE BIOCOMPATABILITY. B. Moore*, T. Holmes*, G. Schulman, L. Arbeit, SUNY-Stony Brook, NY.

The mechanism of dialysis membrane bioincompatibility is related to complement activation. Sheep plasma(P), exposed to a cuprophane membrane(C), produces pulmonary artery hypertension(PAH) when reinjected back into a sheep(S). P exposed to polyacrylonitrile membrane(PAN) does not produce PAH. [table]. In fact, P exposed to PAN before (PAN+C) or after (C-PAN) exposure to C does not produce PAH [table]. C activates complement. PAN binds complement and its activated components, possibly exerting a protective effect.

We developed a hemolytic assay for directly measuring complement activation in P. A significant decrease was seen in the CH_{50} value of P exposed to C. This plasma injected into S produced PAH [table]. There was a further significant decrease found in the CH_{50} value of P exposed to PAN. PAH was not seen upon reinjection of this plasma. Double-membrane exposed plasma, both C to PAN and PAN to C, resulted in a decreased CH_{50} value and did not produce PAH.

	CH_{50}	Mean PAP (mm Hg)
n=16 baseline	15.1 ± 2.4	16.04 ± 5.2
n=10 C	11.6 ± 1.4 *	38.6 ± 9.2 *
n=8 PAN	8.5 ± 1.1 **	14.1 ± 4.4
n=7 C-PAN	8.83 ± 0.8 *	17.5 ± 1.8
n=7 PAN-C	7.49 ± 1.1 *	16.3 ± 2.9

*p<.01, **p<.001. Our data suggests a dissociation between complement activation and PAH. Other plasma factors may play a role in membrane bioincompatibility.

PLATELET (PLT) ANGIOTENSIN II RECEPTORS (AII R) IN CHRONICALLY HYPOTENSIVE (LBP) HEMODIALYSIS (HD) PATIENTS. T. Moore*, J.M. Lazarus, R.M. Hakim, Brigham & Women's Hospital, Boston, MA; Vanderbilt University Medical Center, Nashville, TN.

Approximately 10% of HD patients have LBP, defined as pre-dialysis systolic BP <100 mm Hg, without obvious cardiac abnormalities. We previously showed that human Plt possess AII R which modulate with salt intake and plasma AII levels, a response similar to that reported for vascular smooth muscle. We therefore investigated pre-dialysis Plt AII R, plasma AII, renin, and catecholamine levels in 8 LBP patients and compared them to 9 HD patients who were normotensive (NBP) without B.P. medications.

Using % specific binding of ^{125}I -AII incubated with platelets, Plt AII R was significantly reduced in LBP patients (mean \pm SEM) (0.816 ± 0.43 for LBP vs. 3.63 ± 1.08 for NBP, $p \leq 0.03$). There was no statistical difference in plasma levels of renin, AII or catecholamines between the two groups. In addition, we investigated the BP response of both groups of patients to a graded AII infusion (1, 3, 10 and 30 ng/Kg/min) and found a blunted response to AII in the LBP group (e.g. at 3 ng/Kg/min, there was a 1.2 ± 0.4 mm Hg rise in BP of LBP patients and 6.9 ± 1.1 mm Hg in NBP, $p \leq 0.03$). However, the decrease in heart rate in response to AII infusion was similar in both groups and not statistically significant, suggesting equivalent sympathetic system. These data support the concept that chronic hypotensive patients have reduced AIIR and have a blunted response to ambient AII.

USE OF A SILICONE CATHETER WITH A DACRON CUFF AS LONG-TERM HEMODIALYSIS ACCESS. A.H. Moss, M.M. McLaughlin* and J.L. Holley. West Virginia University Medical Center, Morgantown, WV.

We evaluated a dual lumen silicone catheter with a Dacron cuff (C) as a long-term vascular access in 17 hemodialysis patients who had delayed maturation of an arteriovenous fistula or refused further access surgery. In each case, C was initially placed as a temporary access. The mean age of the patients was 60 yrs and 59% were diabetic. The cumulative C experience totaled 11.9 patient-years. Each C was used a mean of 8.4 months (range of 4.5-17) for a mean of 97 dialysis treatments. Average heparin dose per treatment was 6,000 U. Nineteen exit site infections occurred resulting in a rate of 1.6 infections/patient-year. Eighteen of these resolved with parenteral antibiotics; C-related bacteremia developed from one exit site infection (0.08 bacteremic episodes/patient-year). Urokinase was required for intraluminal clot dissolution prior to dialysis in 3.5% of treatments and blood lines were reversed to achieve a minimal blood flow rate of 200 ml/min in 7% of treatments. Recirculation with blood lines reversed was 7.8%. A 12 hour streptokinase infusion to restore catheter function was necessary in 3 patients. No additional clotting problems occurred when the heparin dose was adjusted to keep the activated clotting time > 225 seconds. No C required removal due to thrombotic complications. We conclude that the C can be used as a long-term vascular access for hemodialysis. Higher doses of heparin to minimize thrombotic complications and parenteral antibiotics to treat C-associated infections may be necessary.

EFFECT OF ALUMINUM ON THE SET POINT OF CALCIUM FOR PARATHYROID HORMONE SECRETION IN DIALYSIS PATIENTS WITH ALUMINUM BONE DISEASE. S. Nematzadeh*, A. Felsenfeld, M. Rodriguez*, and F. Llach. Dept. of Med., Univ. of Okla. Health Sciences Ctr. and VA, OKC, OK.

Dialysis patients (pts) with aluminum (AL) toxicity have a relative deficiency of parathyroid hormone (PTH) which may be secondary to high serum and/or tissue AL levels. In this study, 16 hemodialysis pts with AL-associated osteomalacia (OM) or aplastic bone disease (bone AL stain > 25%), serum AL levels 149 ± 26 μ g/L and basal PTH levels 148 ± 41 pg/ml (as compared with pts with osteitis fibrosa, mean 750 pg/ml; OM and aplastic bone disease, mean 165 pg/ml) were evaluated. They were dialyzed with a calcium (Ca) free and high Ca (4 mEq/L) dialysate. Thus, both the level of Ca which maximally stimulated PTH (Ca min) and the level of Ca which maximally inhibited PTH (Ca max) were evaluated. The Ca min, Ca max and the Ca level corresponding to 50 percent reduction (Ca₅₀) of PTH (set point of Ca) were evaluated. A positive correlation was observed between Ca min, Ca max, and Ca₅₀ and serum AL ($p < .001$, $p < .03$, and $p < .001$, respectively). The PTH values obtained from maximal stimulation with Ca free dialysis and maximal inhibition with a high Ca dialysis did not correlate with serum AL levels.

In summary, in pts with aluminum associated OM or aplastic bone disease and a relative PTH deficiency, serum AL levels correlate with Ca max, Ca min, and Ca₅₀ but not with absolute changes in PTH. In conclusion, these results suggest that serum AL levels may directly affect the set point of calcium in hemodialysis pts with AL bone disease and existing relative PTH deficiency.

LONG TERM DIALYSIS WITH A NEW SUBCLAVIAN CATHETER. Bernard D. Nidus, Steven Vaccarezza, Denise Rodriguez, New York VA Medical Center, NYU School of Medicine, New York, NY

Seven outpatients and four inpatients were dialyzed with a new subclavian catheter from 90 to 370 days (average 193 days). 48 cannulations were performed. 20 catheters had to be replaced over a wire-guide because of clotted side holes. Ignoring replacements, average catheter duration was 44 days. Catheters were removed or lost because of septicemia (7), fever (13), skin site infection (6), broken skin suture (3), and creation of A-V access (8). Many patients had other causes of sepsis; 3 had AIDS. Clinical evidence of subclavian vein stenosis was not encountered in any patient even after creation of A-V access on the same side. The polyurethane, single lumen, 7 french catheter with 8 side holes (Cook Inc.) was very pliable and was 15 or 20 cm in length. To minimize dead space, external tubing was eliminated. During catheter placement, insertion of the wire-guide to its full length identified cephalad misplacement and thus obviated the need for fluoroscopy. A 9 french dilator facilitated insertion. Skin incision and subcutaneous tunneling were not used. Maximum blood flow was 300 ml/min. Blood was usually returned to a peripheral vein. When a unipuncture double pump system was used, recirculation was 5%. Catheter patency was maintained by instilling 2 ml heparin (1000 U/ml) post dialysis. A special dressing was applied and generally not changed until the next dialysis.

Our incidence of complications is less than with large bore double lumen catheters. To date, no patient has failed on dialysis with this single lumen catheter.

AN ELECTRON MICROSCOPIC STUDY OF HEMODIALYSIS (HD) ASSOCIATED AMYLOIDOSIS. Shinichi Nishi* Sojiro Ogino† Yuichiro Maruyama† and Masaaki Arakawa* (intr. by F.Marumo). Niigata Univ. Med. School, Dept. of Med. (II). Niigata, Japan

Carpal tunnel syndrome and osteoarthropathy seen frequently in long-term HD patients have recently recognized as amyloidosis, of which the structural protein was determined to be β_2 -microglobulin (β_2 -MG) by our group. We studied the ultrastructural characteristics of amyloid fibers taken from the perineural nodules and the cystic lesions of carpal bones, compared with those of other primary or secondary amyloidosis.

The most striking feature was well-demarcated, fusiform or rod-shaped amyloid nodules adjacent closely to collagen fiber bundles in the interstices. One single fiber, consisted of two elementary filaments, was approximately 7-10nm in width, wider than that of other amyloidosis (6-8nm), several of which gathered in one direction and constituted one bundle. These bundles were strongly PAM-positive and distributed irregularly in all directions in the nodule. On the other hand, the fibers of other amyloidosis were PAM-negative and distributed separately around the vascular endothelial and epithelial cells. The mesenchymal cells such as synovial cells or fibrochondrocytes became atrophic and degenerative, although these surface coats contained few isolated amyloid fibers. Some macrophages showed intracellular amyloid fibers, representing phagocytic activity.

These findings showed that β_2 -MG has strong affinity to collagen fibers, and that therefore amyloid fibers were formed close to collagen fiber bundles.

RECOMBINANT ERYTHROPOEITIN (r-EPO) IMPROVES BRAIN FUNCTION IN CHRONIC HEMODIALYSIS (CHD) PATIENTS (PTS). AR Nissen-son, JT Marsh*, WS Brown*, S Schweitzer*, DL Wolcott*, UCLA School of Medicine, Los Angeles, CA.

Uremia is a neurobehavioral syndrome that can be studied using brain event-related potentials (ERP) as a measure of CNS functional status. We measured ERP in 13 pts before and after at least 12 weeks treatment with r-EPO in order to assess the hypothesis that anemia is a cause of brain dysfunction in uremia. One ERP measure, the latency of the P3 wave has proven to be a sensitive and objective measure of the speed and efficiency of information processing; short latencies indicate rapid, efficient CNS function. Two paradigms were used in this study, tone pulses and a spoken vowel. Studies were performed 24 hours following a routine HD. Mean Hct. was 22.7% at the time of the first study and had risen to 36.6% ($p < .001$) by the second study. 9 of 13 pts had a decrease (improvement) in P3 latency with the tone stimulus (375 msec \rightarrow 336 msec, $p = .08$). 8 of 13 had an improvement with the vowel stimulus (441 msec \rightarrow 420 msec, $p = .15$). Given the small sample size, these results look promising. They indicate that correction of anemia with r-EPO leads to measurable improvement in the functional status of the CNS in CHD pts and further suggest that a component of the uremic syndrome may be attributable to the effects of anemia on the CNS.

TREATMENT OF RENAL ANEMIA BY RECOMBINANT HUMAN ERYTHROPOIETIN (Ep): THE EFFECT ON PERIPHERAL HEMODYNAMICS AND OXYGENATION. B. Nonnast-Daniel, A. Creutzig, K. Kuehn, E. Reimers, R. Brunkhorst, L. Caspary, K.M. Koch. Med. School, Hannover, FRG. (intr. by G.M. Eisenbach).

Treatment of renal anemia with Ep may not only result in a rise of hemoglobin (Hb) but may also affect peripheral hemodynamics. Thus, an elevated peripheral vascular resistance has been suggested as a cause for an increase of blood pressure (BP) observed under Ep therapy. We studied in 9 regular hemodialysis patients (HD pts) regional blood flow (BF) of the calf by plethysmography and transcutaneous oxygen pressure ($tcPO_2$) in the skin of the forefoot with a platinum electrode prior and 3 months (mo) after start of Ep treatment (40-120 U/kg iv. 3 times/week). Results: (mean \pm SEM) Whereas Hb and mean arterial BP increased significantly (6.8 \pm 0.1 vs 10.7 \pm 0.2 g/dl, $p < 0.001$; 95 \pm 2 vs 100.5 \pm 3.6 mm Hg, $p < 0.05$) regional BF of the calf decreased significantly (5.59 \pm 0.7 vs 3.5 \pm 0.4/100 ml tissue/min, $p < 0.05$). Accordingly calculated regional vascular resistance (RVR) per 100 ml tissue increased (14.5 \pm 1.1 $\times 10^5$ vs. 25.9 \pm 3.7 $\times 10^5$ dyn. sec cm^{-5} , $p < 0.01$). $TcPO_2$ increased significantly after 3 mo Ep treatment (5.9 \pm 0.8 vs 11.2 \pm 1.3 mm Hg, $p < 0.01$). These data demonstrate that Ep treatment in anemic HD pts is associated with a considerable increase of RVR and a decrease of regional BF. In spite of these changes an improved tissue oxygenation was achieved. This suggests that the reduced Hb oxygen affinity described for anemic HD pts does not increase proportionally with rising Hb levels under Ep therapy.

CALCITONIN GENE RELATED PEPTIDE (CGRP) IS INAPPROPRIATELY RELEASED DURING HEMODIALYSIS (HD). I. Odar-Cederlöf*, E. Theodorsson*, B. Hamberger*, B. Tidgren*, C. Kjellstrand. Depts. Med., Clin. Chem., Surg. and Phys., Karolinska Hospital, Stockholm, Sweden.

Vasodilatation (VD) occurs during HD and may result in severe hypotension (HT).

We studied CGRP, the most potent vaso-dilatory peptide known, during HD by means of a RIA -method in acid ethanol extracted plasma. Dialysis was divided into pure ultrafiltration (UF) mixed UF + HD and pure HD. CGRP, atrial natriuretic peptide (ANP), NPY-NKA-like immunoreactivities, plasma renin activity (PRA) and catecholamines were measured before and after UF, UF + HD and HD. The changes in plasma levels were tested for correlation to each other, bloodpressure (BP), BUN and bodyweight.

CGRP rose during UF, UF + HD and HD from 98 \pm 28, pmol/l to 135 \pm 30, pmol/l ($p = 0.01$), ANP fell from 200 \pm 90 to 116 \pm 53 pmol/l, NPY, NKA dopamine and adrenalin did not change. Noradrenalin rose during UF (2.9 \pm 0.5 to 4.1 \pm 0.7 pmol/l; $p = 0.002$) and fell during HD (to 2.2 \pm 0.5; $p = 0.003$). PRA rose from 1.7 \pm 0.7 pmol/l to 2.6 \pm 1.1 pmol/l; ($p = 0.057$) during UF an did not change during HD. The CGRP-rise was not correlated to PRA or noradrenalin, weakly correlated to changes in weight ($r = 0.31$) and BUN ($r = 0.36$), but strongly correlated, negatively, to post-HD BP ($r = 0.87$, $p = 0.02$).

CGRP, a potent vaso-dilatory peptide, is present in normal concentrations in most patients before dialysis, inappropriately rises during UF, UF + HD and HD, behaves opposite to ANP and independent of noradrenalin. It is strongly, negatively correlated to postdialysis BP and may be an important cause of dialysis hypotension.

THE DELETERIOUS EFFECT OF ACIDOSIS AND SECONDARY HYPERPARATHYROIDISM (2° HPT) REFLECTED BY CHANGES IN GLA PROTEIN AND BONE FILMS IN HEMODIALYSIS PATIENTS (HDP). C.W. Oettinger, J.C. Oliver*, R.L. Mars, and D. Greene*. Emory University and Dialysis Clinic, Inc., Atlanta, Georgia.

We have found that a significant acidosis occurs in HDP. However, it is unclear whether the acidosis observed in HDP is harmful to bone. Thus, we 1) determined the bone base deficit of HDP undergoing acetate dialysis (ACD) and 2) quantified changes in metabolic bone parameters after HDP were changed from ACD to HCO₃ dialysate (BD). 9 HDP after 6 weeks dialysis on ACD were switched to high BD (40 mEq/L) in order to achieve a steady state predialysis HCO₃ of 24 mEq/L. HCO₃ flux to the patient was calculated for each dialysis treatment. The cumulative HCO₃ load was determined. 34 HDP on ACD for at least 3 years were switched to BD (35 mEq/L). GLA, PTH, PO₄, HCO₃, and alkaline phosphatase (AP) were measured when the HDP were on ACD and then at 1 and 3 years after switching to BD. High resolution hand films were taken on AD and BD. RESULTS: 1) After 6 weeks of ACD in 9 HDP, a total of HCO₃ deficit of 502 \pm 62 mEq was present (62% Bone, 38% TBW). 2) 1 year after 34 HDP were switched from AD to BD, HCO₃ increased from 15.7 \pm 1.7 to 18.0 \pm 3.3 ($P < 0.001$), GLA decreased from 16.0 \pm 10.2 to 7.2 \pm 5.4 ($P < 0.001$), PO₄ decreased from 6.5 \pm 1.8 to 5.7 \pm 2.3 ($P < 0.02$), AP increased from 144 \pm 105 to 188 \pm 197 ($P < 0.05$), PTH was unchanged. Radiographic bone changes improved in 53% of patients who initially had changes of 2° HPT. After 3 years of BD, GLA increased to 17.5 \pm 15.7 and PTH increased from 24,912 to 29,437 \pm 29,887 ($P < 0.02$). PO₄ and HCO₃ remained unchanged. CONCLUSIONS: 1) A significant bone base deficit is caused by ACD. 2) Improvements of acidosis leads to a decrease in GLA and improvement in bone films with no change in PTH. This is consistent with chemical and radiographic bone improvement due to a partial correction of acidosis. 3) If PTH increases further, the skeletal benefits of partial improvement in acidosis may be reversed.

TRANSMISSIBILITY OF HIV INFECTION IN CHRONIC HEMODIALYSIS, Carmen Ortiz*, Maria De Medina*, Eugene Schiff*, Jacques J. Bourgoignie and Guido Perez. Univ. of Miami and VA Med. Ctr., Dept. of Med., Miami, Florida.

The prevalence, modes of transmission, and natural history of HIV infection in regularly dialyzed patients remains undefined. Eighty one chronic dialysis patients were tested for HIV antibodies in July, 1985, and their clinical and HIV status were reevaluated two years later. On initial testing, 9 patients were HIV positive (both Elisa and Western Blot), two were false positive (positive Elisa, negative Western Blot), and 70 were negative. Of the 9 HIV positive patients, 8 were carriers and one had ARC. At followup, five had expired (2 of AIDS and 3 of other causes) and 4 were alive (3 carriers, 1 ARC). One false positive patient died of a myocardial infarct, the other died of sepsis. Only one seroconversion was documented in the 45 HIV negative patients retested two years later. The latter was a Haitian woman who had received several blood transfusions. Two patients were lost to follow-up, and the other 23 expired of causes unrelated to HIV infection. We conclude that the incidence of HIV infection in our dialysis population is very high (11%). Nevertheless, we found no evidence that the infection is highly transmissible in the dialysis unit.

A PROSPECTIVE STUDY OF DILATED CARDIOMYOPATHY IN DIALYSIS PATIENTS. Parfrey PS, Harnett JD, Barre PE, Griffiths SM, Taylor R, Hand J, King T. Memorial Univ, St. John's and McGill Univ, Montreal, Canada.

Dilated cardiomyopathy (DC), hypertrophic hyperkinetic disease (HH) and ischemic heart disease predispose to congestive heart failure in dialysis patients (Parfrey et al, Arch Int Med, in press). In a cross-sectional study we observed DC (ejection fraction < 55% and LVEDD \geq 5.5 cm) in 28 of 150 (19%) dialysis patients. Review of echos, performed before DC was diagnosed, revealed 10 patients diagnosed at or within 2 years of starting ESRD therapy and 18 with late onset DC. None of the latter group went through a HH phase. Both groups were followed prospectively with serial echocardiograms for 3-5 years. In those with DC at start of ESRD therapy 80% had persistent DC and 70% died. In the late DC group 72% had persistent DC and the remainder reverted to hypertrophic disease (LV wall thickness \geq 1.2 cm in diastole). Fifty% died. In the group (N=30) who had normal echocardiograms in the initial study 60% remained normal, 37% developed hypertrophic disease, 1 developed mild DC, and 23% died during the 3-5 year follow-up. There was no difference in age, duration of ESRD treatment, any index of hyperparathyroidism or serum cholesterol between the late onset DC and normal groups; LV wall thickness and diastolic BP were significantly higher, as were proportion of smokers, those with high weight gains between dialyses, and those who had myocardial infarct after starting ESRD therapy. We conclude that DC does not evolve from HH, does not usually remit and has a bad prognosis.

CONTINUOUS ARTERIOVENOUS HEMODIALYSIS (CAVHD) IS AN EFFICIENT RENAL REPLACEMENT THERAPY. T.L. Pallone*, S. Hyer*, and J Petersen Palo Alto VA Hospital, Palo Alto, Ca. and Univ. of Rochester, Rochester, N Y

Low dialyate (D) to blood (B) flow rate (Qb) ratios are a unique feature of CAVHD. We evaluated its efficiency by measuring urea clearances (Cl) using the Hospal AN69S parallel plate dialyzer (acrylonitrile, 4300 cm²) designed for this modality. The CAVHD circuit was perfused with bovine B (hct = .28, [urea] = 50 mg/dl) at a perfusion pressure of 66 mmHg in three modes: pure ultrafiltration (UF) (H column height, H = 40.6 cmH₂O); zero net UF (O-UF) (H = 0 cmH₂O) and D flows, Qd = 10, 20, or 30 ml/min, or combined UF + dialysis (UF + D). Qb was 89.5 \pm 9 ml/min and the following Cl (ml/min) were obtained (mean \pm S.E.). N = number of circuits (4-9 measurements/circuit);

Qd	UF (N=3)	O-UF (N=5)		UF + D (N=3)
	(M)	(M)	(C)	(M)
0	7.56 \pm .9	---	---	---
10	---	9.50 \pm .4	9.04	14.9 \pm .7
20	---	17.8 \pm .4	15.9	23.5 \pm 1.2
30	---	24.7 \pm .5	21.8	30.3 \pm .6

M = Measured, corrected for plasma water; C = Cl calculated for perfect (diffusive only) D-B equilibration;

D dextrose concentration (1.5, 2.5, or 4.25%) had no effect on Cl or UF. Filter hydraulic permeability fell minimally over 4-8 hours. We conclude: CAVHD with this hemofilter is highly efficient. For the above conditions, Cl is D flow limited. Due to solvent drag near the B inlet-D outlet, UF may contribute to Cl even when net filtration is zero. Cl > 25 liter/day can be achieved.

EFFECTS OF ACTIVATED COMPLEMENT (C) C3 AND C5 ON PULMONARY ARTERY PRESSURE IN THE SWINE. Charles J. Parker*, Alfred K. Cheung, and Linda Wilcox,* VA Med. Cntr. and Univ. of Utah, Salt Lake City, Utah.

Hemodialysis with cuprophane membranes is associated with C activation, and consequently, with formation of the anaphylatoxins, C3a and C5a. Infusion of cuprophane-activated plasma into swine produces pulmonary hypertension, and previous studies have suggested that this effect is mediated by the anaphylatoxins. It has been unclear, however, whether C3a, C5a or both mediate the hypertension. To investigate this problem, we have exploited the properties of two types of cobra venom factor (CVF) which have different effects on the activation of C5 but not on C3.

Using an assay which quantifies C3 consumption, the CVF from *Naja Naja* (CVF_N) and *Naja Haje* (CVF_H) were found to activate porcine C3 with similar efficacy. In contrast, using a hemolytic assay which depends on C5 activation, CVF_H was found to lack the capacity to support porcine C5 activation whereas CVF_N was found to be a potent C5 activator. Intravenous infusion of either 3 μ g/kg BW of CVF_N or CVF_H into anesthetized swine produced increases in mean pulmonary artery pressure of 163 \pm 46% and 73 \pm 37% respectively ($p < 0.02$, CVF_N vs CVF_H). Significant leukopenia was induced by CVF_N but not by CVF_H.

The results of these experiments suggest that in vivo activation of either C3 or C5 can produce significant pulmonary hypertension, and that the effects of anaphylatoxins C3a and C5a on pulmonary artery pressure are additive. Dialysis-related leukopenia, however appears to be mediated by C5a but not by C3a. These observations indicate that both C3a and C5a which are generated as a result of C activation are, at least in part, responsible for the pulmonary hypertension associated with hemodialysis using cuprophane membranes.

REDY COMPUTE (RC): EVALUATION OF A COMPUTER GUIDE TO SORBENT DIALYSIS. J.A. Pederson, C. Williams*, and W. Shapiro. Dept. of Med., U. of Okla. and VAMC, Okla. City, OK and Brookdale Hospital Med. Center, Brooklyn, NY.

Redy compute is an interactive soft ware guide to dialysis with the Redy system. Urea volume (UV) is calculated from body surface area. Other required data are; blood flow (QB), dialysate flow, venous line pressure, pre-dialysis BUN, dialyzer constants for urea clearance and ultrafiltration rate (UFR). RC estimates the duration of dialysis (DD) to achieve a target post dialysis weight and BUN. Alternatively, the BUN expected may be calculated for a target DD. Size of sorbent cartridge and the hourly UFR are also calculated. Pre-dialysis serum sodium (Na) and bicarbonate (HCO_3^-) permit projection of post dialysis Na and HCO_3^- if NaHCO_3 or NaCl are added to the dialysate.

Predicted BUN and DD were evaluated in 62 dialysis sessions of 3.7 ± 0.07 hrs. (M+SD) among 5 women and 9 men. All had fistulae (AVF) or dual lumen venous catheters. Expected BUN, 45.7 ± 1.98 mg% did not significantly differ from actual BUN, 49.5 ± 2.26 mg%. DD was underestimated by 12.1% ($p < 0.01$). Correcting for recirculation reduced the differences by 37% for BUN and 33% for DD. In 14 sessions at minimal recirculation, projected vs actual post dialysis Na and HCO_3^- were 141.7 ± 1.33 vs 141.1 ± 1.48 mEq/L and 22.8 ± 1.32 vs 23.2 ± 2.02 mEq/L ($p > 0.6$).

In summary: Redy Compute needs minimal laboratory data. The program underestimates DD. This improves as does estimated BUN if QB is corrected for AVF recirculation. Expected Na and HCO_3^- did not differ from actual values.

REMOVAL OF β_2 -MICROGLOBULIN DURING HIGH FLUX HEMODIALYSIS: A COMPARATIVE STUDY Jeffrey Petersen Danny Choy* and Isabella Yeh*. Palo Alto Vet. Admin. Center and Stanford Univ., Palo Alto, CA.

The elevation in β_2 microglobulin ($\beta_2\text{M}$) levels in long term hemodialysis patients has been implicated in the development of dialysis associated amyloid osteoarthritis. $\beta_2\text{M}$ can be effectively removed during dialysis with some but not all high flux dialyzers. We have studied $\beta_2\text{M}$ levels pre, post ($\Delta\beta_2\text{M}$, %pre) and rebound (% post) within 1 h of discontinuation of high flux dialysis with a polysulfone (PSF80) and polymethylmethacrylate membrane (PMMA). To further characterize the removal, the in-vivo simultaneous blood clearance (Cb) and dialysance (Cd) at 15min of dialysis and the in-vitro adsorption of 125-I- $\beta_2\text{M}$ to PSF80, PMMA and cuprophane (CU) were measured. Results are expressed as mean \pm SE:

	PSF80	PMMA
$\Delta\beta_2\text{M}$, % pre	- 43 ± 4 (n=8)	- $26 \pm 2^*$ (n=8)
rebound, % post	+ 21 ± 4 (n=11)	+ 7 ± 1 (n=7)
Cb, ml/min	84 ± 4 (n=8)	50 ± 10 (n=4)
Cd, ml/min	$55 \pm 3^\dagger$	0

* $p < 0.05$ vs PSF80, $\dagger p < 0.05$ vs Cb

$\beta_2\text{M}$ levels fell in an exponential fashion during high flux dialysis but rebounded in the post dialysis phase. Clearance from blood was greater than dialysance during dialysis with PSF80 membranes. No $\beta_2\text{M}$ was detected in the dialysis fluid with the PMMA membrane in contrast to PSF80 where 258 ± 7 mg was collected during dialysis. In-vitro adsorption of 125-I- $\beta_2\text{M}$ was highest with the PMMA membrane compared to PSF80 or CU ($9 \pm 1 > 6 \pm 1 > 2 \pm 1$, % of total $\beta_2\text{M}$ bound at 1h, $p < 0.05$ respectively).

We conclude that following high flux dialysis the post dialysis rebound of $\beta_2\text{M}$ suggests a multicompartmental model to describe removal. $\beta_2\text{M}$ is removed during high flux dialysis with the PSF80 membrane by a combination of diffusion and adsorption. In contrast to dialysis with PSF80, adsorption appears to be the main removal mechanism with PMMA. This is supported by significant in-vitro binding.

TERMINATION OF DIALYSIS THERAPY AS A CAUSE OF DEATH. Friedrich K. Port, Robert A. Wolfe,* Victor M. Hawthorne,* C. William Ferguson,* University of Michigan Schools of Medicine and Public Health, Ann Arbor, Michigan.

Termination of life sustaining dialysis therapy led to death in 282 of 5,208 patients who started therapy for end-stage renal disease in Michigan during 1980-85 with a follow-up through 1986. Based on life table estimates at 60 months after initiation of therapy, 10% of patients overall died due to termination of dialysis, 12% of females versus 9% of males ($p=0.01$), < 4% for ages < 49 versus 56% for > 80 years, 13% for white versus 4% for black patients ($p<0.001$) and 16% for diabetic versus other diagnostic groups ($p<0.05$). The Cox regression model confirms these findings for race, (B/W=0.28, $p<0.0001$), diabetes (DM/glomerulonephritis=3.3, $p<0.001$) and age ($p<0.0001$), but not for sex. A separate analysis of all 2,564 deaths in dialysis patients according to prevalence cases for 1980-84 revealed that 8.9% were due to termination of dialysis. Prior transplant failure or prior CAPD therapy did not significantly influence the percent of patients dying due to dialysis withdrawal. There was a 60% increase in overall withdrawals for the years 1980 to 1985 ($p<0.02$, Cox). The findings of a significant increase of dialysis withdrawal over time and the marked racial difference indicate the need for exploration by further studies.

A NEW APPROACH TO THE MEASUREMENT OF THERMAL ENERGY BALANCE IN HEMODIALYSIS PATIENTS. R. Provenzano*, B. Sawaya*, H. Polaschegg*, T. Roy*, and N.W. Levin. Henry Ford Hospital, Detroit, Michigan.

The measurement of thermal energy balance during hemodialysis offers an opportunity to investigate the physiologic response to external stimuli affecting heat production. A self contained computerized autocalibrating blood temperature monitoring system has been developed (Fresenius AG) which enables accurate non-invasive measurement of in-line arterial and venous temperatures and of blood flow during hemodialysis. Thermal energy balance is calculated by the equation: $dE=(T_a-T_v) \times Q_b \times C \times R$, where dE =energy change (calories/min); T_a =arterial temperature ($^{\circ}C$); T_v =venous temperature; Q_b =blood flow(l/sec); C =specific heat of blood (3.64 KJ/K.Kg) and R =density of blood (1.052 Kg/L). e.g. energy loss on a polysulfone dialyzer:

	Duration of dialysis (min)						
	1	30	60	90	120	150	180

T_a	35.58	35.38	35.47	35.35	35.33	35.24	35.26
dE	173.8	157.2	131.0	109.6	59.5	64.3	69.1

By varying the dialysate temperature to maintain constant core temperature, this device will enable evaluation of the effects of new and reprocessed dialyzers (of different degrees of biocompatibility) and of dialysate composition and sterility on energy balance.

ADEQUACY OF SHORTENED HEMODIALYSIS TIME USING A HIGH EFFICIENCY DIALYZER. F. Raudales, B. Stinebaugh and G. Dolson. Baylor College of Medicine and V.A. Medical Center, Houston, Tx.

The optimum hemodialysis time as indicated by $Kt/V = 1$ and the actual high efficiency dialyzer urea clearance for each treatment were determined for a dialyzer (CA 170) in stable chronic hemodialysis patients. Treatments were conducted for 120 min. at a maximum blood flow rate of 400 ml/min on a Travenol SPS 450 machine with computer controlled ultrafiltration. Pre and post hemodialysis weights and BUN were obtained for 2 sequential hemodialyses, and using a computer program developed by this laboratory, the Kt/V for the 120 min treatment as well as the hemodialysis time required to achieve $Kt/V = 1$ were calculated. In addition, actual high efficiency dialyzer urea clearance and the patient's protein catabolic rate, which is equal to protein intake in stable patients, were also determined. These calculations were based on the patient's actual urea balance as derived from the laboratory parameters and taking the patient's body habitus into consideration. The mean (\pm SE) values for the 8 male patients hemodialyzed on the CA-170 were; Kt/V (120 min), 0.75-0.07; hemodialysis time for $Kt/V = 1$, 174-12 min; high efficiency dialyzer urea clearance, 263-24 ml/min (published value of 299 at blood flow rate 400); the protein catabolic rate 0.89-0.12 g/kg, a sufficient protein intake. No patient became hypotensive, and only one patient experienced leg cramps. We conclude that for stable patients eating sufficient protein, adequate hemodialysis can be administered in 3 hours using shortened hemodialysis and a high efficiency dialyzer.

HIV TESTING IN CHRONIC HEMODIALYSIS PATIENTS
I. Reiser, W. Shapiro and J. Porush. Brookdale Hospital Medical Center, Brooklyn, New York.

Since January 1, 1986 all the patients (pts) already in our maintenance hemodialysis unit and all new pts entering our program were tested for HIV antibody using the ELISA and Western Blot methods. Of the 155 patients screened, 16 (10%) were positive for HIV antibody (HIV+). Eleven of these 16 (69%) pts were intravenous drug abusers (IVDA), which represents 65% (11/17) of the IVDA population in our unit, similar to previous data reported for New York City. The 5 HIV+, non-IVDA pts may have been exposed secondary to: transfusions prior to HIV antibody testing of blood donors (n=2), multiple sexual partners (n=2) or homosexuality (n=1). One patient had clinical signs of AIDS prior to the onset of dialysis. Seven HIV+ pts expired during the study (6 IVDA), 3 with clinical AIDS. The remaining 9 HIV+ pts (5 IVDA) are alive for 11 ± 3 mos (mean \pm SEM) (range 2-18 mos) since testing positive. One is the patient with clinical AIDS prior to starting hemodialysis and one has Mycobacterium TB. The remainder are asymptomatic. Thus, of 16 HIV+ pts in our program 7 (44%) are asymptomatic for periods of up to 18 mos without unusual complications. To our knowledge these are the first data on HIV positivity in hemodialysis pts. This relatively high incidence of HIV positivity, almost one-half of which are asymptomatic for relatively long periods of time, makes it mandatory that HIV testing be performed on a routine basis in hemodialysis units, particularly those serving an inner city population.

CORRECTION OF PHAGOCYTTIC DEFICIENCY IN HEMODIALYSED PATIENTS BY A NEW CEPHALOSPORIN. S. Ringoir,* N. Van Landschoot,* R. Vanholder,* (intr. by R. Hakim). Renal Division, University Hospital, De Pintelaan, 185, 9000 Ghent, Belgium

Chronic renal failure is characterized by increased susceptibility for infections. In vitro experiments suggest that Cefodizime^R (C), a new cephalosporin, causes an increase in phagocytic capacity. Therefore, the in vivo effect of a treatment with C on phagocytosis was studied in 10 hemodialysed patients by measuring the ¹⁴CO₂ production during the metabolism of radioactive glucose by phagocytic cells, in the resting state and after activation by Latex or Zymosan. Two grams of C were injected IV after each dialysis during 2 weeks, phagocytosis was studied before and after these two weeks. In the table, the production of ¹⁴CO₂ (Dpm x 10³) is represented in normal subjects and in the hemodialysed patients (HD) under study:

	Normals (n=27)	HD before C (n=10)	HD after C (n=10)
Resting state	12.6 ± 4.6	5.6 ± 1.4 ^a	6.3 ± 1.9 ^a
After Latex	40.9 ± 19.5	17.9 ± 11.9 ^a	27.4 ± 7.8 ^b
After Zymosan	94.1 ± 34.6	48.4 ± 24.9 ^a	78.8 ± 15.1 ^b

a: p < 0.01 vs normal; b: p < 0.01 vs before C. Treatment with C improved the response of the depressed phagocytosis after the challenge by Latex or Zymosan. In conclusion, these results reveal a stimulation in vivo of the depressed phagocytic system of the uremic dialysis patient by the new cephalosporin Cefodizime^R.

STUDIES ON BLOOD FLOW DYNAMIC AND ULTRAFILTRATION KINETICS DURING C.A.V.H. Claudio Ronco*, Juan P. Bosch, Susie Lew. Nephrology Div. Vicenza, Italy and G.W. University, Washington, DC.

Recent studies have demonstrated that filtration pressure equilibrium may occur inside the hemofilter during CAVH. In vivo and in vitro experiences are carried out and described in this paper in order to clarify the possible way to optimize the CAVH circuit. Blood flows at different hydrostatic pressures have been tested using 3 different catheters (1.5-2.0-3.0mm Ø), blood lines of different length (45-90 cm) and different hemofilters (AMICON D-30; D-20; D-10). The venous pressure was maintained at 10 mmHg while the arterial pressure, simulated by a bag raised above the filter at different heights was adjusted at 60-90-120 and 150 mmHg. Pressures were measured in different points of the circuit to evaluate the specific resistance of each component of the system and to measure the pressure drop inside the filter. Ultrafiltration rates and filtration fraction were also measured in each condition. The same parameters were studied in 10 patients treated with CAVH with different blood accesses and hemofilters in order to compare the results obtained in vitro with the ones achieved in vivo. The results suggest the use of a particular blood access, and a specific hemofilter according to the patient's blood pressure, hematocrit and protein concentration. With high blood flows a D-30 can be used while with low flow a short-fat geometry should be preferred (D-10).

COMPARISON AMONG FOUR DIFFERENT SHORT DIALYSIS TECHNIQUES: TECHNICAL AND CLINICAL EVALUATION.

Claudio Ronco*, Aldo Fabris*, Mariano Feriani*, Stefano Chiaramonte*, Alessandra Brendolan*, Luisa Braganti*, Giuseppe La Greca* (intr. by Paul Kimmel), Dept of Nephrology, St. Bortolo Hospital, Vicenza, Italy.

Reduction of dialysis treatment time can be achieved with different techniques. We have compared in terms of efficiency, clinical tolerance, technological investment and costs four different short dialysis treatments: 1) Rapid bicarbonate dialysis with 1.5sqm cuprophane membrane; 2) High flux biofiltration with 1.2sqm AN69S hollow fiber membrane; 3) Hemodiafiltration with 1.2-1.9sqm polysulphonic hollow fiber hemodiafilters, and 4) High flux hemodiafiltration with two serial hemodiafilters with AN69S membrane (total=2.4sqm). Hydraulic properties and solute clearances at different blood flows (300-500 ml/m²) were tested for each technique. Once the optimal operative level was established three patients were treated with each technique for at least three month period. Since BUN clearance averaged 310 ml/m², the treatment duration varied from 120 to 180 minutes/session with a KT/V always higher than 1. The average PCR was 0.9 g/Kg/24hr. The clinical tolerance was generally good but slightly better in treatments with high convective component. Despite the higher efficiency of treatment n°4, the technological requirements and costs are such to make the other techniques more usable for clinical routine. The other treatments on the contrary, can safely be used for a clinical program of rapid dialysis.

EVIDENCE FOR AMYLOIDOSIS IN ASYMPTOMATIC DIALYSIS PATIENTS DIAGNOSED BY FINE NEEDLE ASPIRATION (FNA) OF ABDOMINAL FAT. Edward A Ross, Gaurang M Shah, Omar Moussabeck*, Jozef Kollin*. Long Beach VAMC, UC Irvine-Long Beach VA Medical Program, Long Beach, CA.

Amyloidosis, especially that caused by beta-2-microglobulin, has recently been appreciated to be a significant problem in chronic hemodialysis (HD) pts and typically presents with synovial involvement. Still controversial, however, is the incidence and extent of non-articular involvement in asymptomatic HD and chronic peritoneal dialysis (CPD) pts. To address this issue we screened our dialysis population: 34 pts underwent FNA of the abdominal fat pad with Congo Red staining of the tissue fragments. Amyloid was identified by characteristic birefringence under polarized light.

A total of 25 HD pts were studied, 4 of whom also had chronic spinal cord injury (SCI). All were men using only cellulose membranes for HD of 61 ± 12 months (mean ± SE). Of the 21 non-SCI pts, 3 (14%) had positive amyloid staining and had been on HD 77-183 months: although 1 of these 3 pts did have articular complaints, there had been no clinical suspicion of amyloid in the other 2 (10%) pts. Of the 4 SCI pts, 3 had positive staining for amyloid: all of these were at risk for secondary amyloidosis because of chronic decubitus infections. 9 male pts on CPD for 26 ± 8 months were studied: none had evidence of amyloid by FNA.

We conclude that 1) HD pts can have systemic as well as articular involvement by amyloid, 2) previous studies of pts identified by articular complaints underestimate the incidence of what can be occult disease, and 3) characterization of the type of amyloid obtained by FNA is under study.

TWO YEARS' EXPERIENCE WITH RAPID BLOOD FLOW SHORT DIALYSIS. Jack E. Rubin* and Geoffrey M. Berlyne. Brooklyn VA Medical Center, Brooklyn, New York.

Twelve chronic hemodialysis patients were started on a 3 hours-3 times/week dialysis schedule using rapid blood flow rates (BFR) of 400 ml/min, polyacrylonitrile dialyzers and volumetrically controlled Monitral dialysis monitors. Dialysate Na was 145 mEq/l, Qd was 500 ml/min and acetate was used as the buffer.

After 24 months, 7 patients were on the protocol. One was removed after he contracted AIDS, while the 4 others died of non-dialysis related causes; a 2 year survival rate of 73%. All accesses were able to deliver enough blood to allow a BFR of 400 ml/min using 16 gauge fistula needles.

At the end of 24 months, biochemical, as well as clinical, parameters were not significantly different from control values. We noted no increase of intradialytic complications despite the use of high BFR's, efficient dialyzers and acetate dialysate.

We conclude this type of high BFR short dialysis, using high efficiency dialyzers with volumetrically controlled dialysis monitors can be routinely used in all dialysis patients, as ours did not suffer any clinical or biochemical deterioration after 24 months of this type of dialysis.

ACUTE MORBIDITY DURING SHORT-TERM HEMODIALYSIS. Stephen Sandroni and Betty Dillman*, University Hospital, Jacksonville, Florida.

Widespread application of new dialysis techniques should be preceded by demonstration that morbidity is not increased. We retrospectively reviewed acute morbid events during six months of short-term hemodialysis, at a single center, and compared them to a six month period of conventional hemodialysis. We studied two types of events: 1) morbid events requiring physician intervention, and 2) symptomatic hypotension/cramping requiring saline infusion, as well as attendance and admissions.

Conditions:	CONVENTIONAL	SHORT-TERM
# of patients	34	34
Dialysate	Acetate	Bicarbonate
Duration	4 hours	3 hours
Pre-Tx BUN	93.3	81.8
Membrane	Various	CA-170 (Travenol)

Results:

Morbid events requiring physician	152	146
Saline infusions	1038	974
Admissions	24	18
No shows	15	3

Initial experience with short-term dialysis was accompanied by no change in overall acute dialysis morbidity and by improved patient attendance and chemistries. The need for close observation of patients during treatments remains essential as symptomatic hypotension was not reduced despite addition of ultrafiltration monitoring equipment.

THE STRIKING EFFECT OF A NEW POLYAMIDE-HEMOFILTER ON THE SERUM BETA-2-MICROGLOBULIN IN HEMODIALYSIS PATIENTS. K. Schaefer, J.-P. Kaiser*, D. von Herrath*, H. G8hl*, J. Hagemann*, Med. Abt. 11, Joseph-Krankenhaus 1, Bäumerplan 24, 1000 Berlin 42, Germany

The association of beta-2-microglobulin (B2M) with dialysis amyloidosis is now well established. Although there is apparently no correlation between the serum concentration of B2M and the appearance of the amyloidosis syndrome, it is reasonable to assume that dialysis strategies which remove efficiently B2M are especially desirable. As few reports indicated that blood purification methods as hemofiltration (HF) or hemodiafiltration were capable of removing up to 190 mg of B2M per treatment, we were interested in studying the effect of a modified hydrophilic polyamide-hemofilter (PoHF) (surface area of 2.0 m², fiber diameter 215 µm) on serum B2M prospectively. The investigations were performed in 4 stable patients being maintained on chronic HF since years. The new PoHF decreased the serum B2M from 49.5 ± 3.6 mg/L to 15.8 ± 4.9 mg/L. In addition, 253 ± 16 mg B2M could be found in the hemofiltrate after one treatment. As additional studies revealed that further 100 mg were adsorbed by the membrane, it is evident, that more than 350 mg B2M can be removed during one treatment session. - In conclusion, HF performed with a new PoHF is presently the most efficient blood purification technique to remove B2M. It remains to be established, whether the occurrence of B2M-amyloidosis could be prevented or at least modified by such a membrane.

CORRECTION OF THE ANEMIA OF CHRONIC HEMODIALYSIS PATIENTS WITH RECOMBINANT HUMAN ERYTHROPOIETIN. R.M. Schaefer, B. Kürner, M. Zech, and A. Heidland (Introd. by Shaul G. Massry), Univ. of Würzburg, Dept. of Int. Med., Würzburg, FRG

Since recombinant human erythropoietin (r-HuEPO) became available for clinical trials, we conducted a study to evaluate the effects of r-HuEPO (AMGen, Thousand Oaks, CA) in 15 hemodialysis patients suffering from severe but stable anemia. The initial dose of r-HuEPO was 24 IU/kg thrice weekly given as an intravenous bolus at the end of each hemodialysis session. This dose was doubled every 2 weeks if the increment of hemoglobin was less than 2 g/100 ml. The mean reticulocyte count increased from 31 ± 5 × 10³/µl to 72 ± 7 × 10³/µl after 4 weeks of r-HuEPO and to 152 ± 1 × 10³/µl following 16 weeks of treatment. The mean baseline hematocrit in these patients was 23.7 ± 1.2 %. After 16 weeks of treatment the mean hematocrit reached 35.7 ± 0.2 %. Baseline hemoglobin values were 7.3 ± 0.3 g/100 ml and these values rose to 11.3 ± 0.2 g/100 ml after 16 weeks of r-HuEPO. As for side effects, 3 patients developed frank hypertension that made antihypertensive therapy necessary. Platelets increased significantly during the first 10 weeks of treatment from 199 000 ± 16 000/µl to 242 000 ± 18 200/µl. During the following 6 weeks the mean platelet count decreased again almost reaching baseline values. Two patients suffered from occlusions of their arteriovenous fistulas. One patient had low grade fever and 2 patients reported bone pain after administration of the hormone. Taken together, our data demonstrate that r-HuEPO is readily capable to correct the anemia of end-stage renal disease.

MUCORMYCOSIS: A LIFE-THREATENING COMPLICATION OF DEFEROXAMINE THERAPY IN LONG-TERM DIALYSIS PATIENTS. R Segal,* KA Zoller, DJ Sherrard, & JW Coburn. VA Med Ctrs, WLA & Seattle, Cedars-Sinai Med Ctr, Los Angeles & St Mary Med Ctr, Long Beach, CA

Mucormycosis (M) due to *Rhizopus* occurs with diabetes mellitus or untreated uremia with acidosis, malignancy, and immunosuppression but not with long-term dialysis. Five patients on long-term hemodialysis (HD) developed M during deferoxamine (DFO) therapy for aluminum toxicity. M was

Case #	1	2	3	4	5
Age/sex	45/M	60/M	47/F	36/F	70/M
HD (yrs)	12	17	15	15	6
1 M Form*	RC	C	RC+P	D	P
Diagnosis	Sinus Bx	Skin Bx	PM&	PM	PM
DFO Dose#	3x18	3x12	4x12	4x6	6x<1
Ferritin@	15	83	11,000	479	330
WBC (K/mm ³)	6.7	normal	19.3	1.5	5.6
Hct (%)	23	46	29	20	27
HCO ₃ - (mEq/L)	23	--	18	26	14

* M Form: RC, rhinocerebral; C, cutaneous; P, pulmonary; D, disseminated. # Dose: g/wk x mos; @ Ferritin, ng/dl; & PM = post-mortem; Bx = biopsy fatal in 4 of 5 cases, being recognized in life only in cases #1 & #2; they received amphotericin (#1 1.5 g over 13 wks; #2 approx 150 mg in 6 days) and surgical debridement. Only case #1 survives. The degree of acidosis, iron stores, DFO dose & duration, and hematologic indices varied widely; DFO Rx & long-term acetate HD were the only common factors. We are aware of 13 other cases making this association very real. Deferoxamine, a bacterial siderophore, could enhance *Rhizopus* pathogenicity, as occurs with *Yersinia*. Thus, DFO should be used cautiously in dialysis patients and only with definite indications for therapy.

POSTPRANDIAL BLOOD PRESSURE (BP) CHANGES DURING HEMODIALYSIS (HD). Richard A. Sherman, Francisco Torres,* Ronald P. Cody.* UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

The effect of eating on BP during HD was examined in a prospective controlled crossover study of 125 treatments in 9 non-diabetic ESRD patients (pts). Pts ranged in age from 30-69 (mean 51) years and had received HD for 20-125 (mean 58) months. No pt suffered from symptomatic peripheral neuropathy or orthostatic hypotension. A standard meal (turkey sandwich, pound cake, 4 oz. cranberry juice) was given at the midpoint of HD (62 treatments) or following completion of HD (63 treatments). Diastolic (P=0.01) and mean (P=0.03) BP fell significantly faster in the 45 min. postprandial period in the fed treatments compared with equivalent times in the fasting treatments. Diastolic (69.6 vs 76.6 mmHg, P<0.05) and mean (86.2 vs 94.1 mmHg, P<0.05) BP were significantly lower 0.5 hours postprandially in the fed treatments compared with the equivalent time in the control treatments. Symptomatic hypotension occurred postprandially 13 times in 5 pts fed during HD compared with 2 episodes in 1 pt in the fasting group (P<0.05). Eating during HD increases the risk of symptomatic hypotension. Consumption of meals during HD should be avoided in pts at risk for hypotension.

COMPUTERIZATION OF REDY SORBENT DIALYSIS. W. Shapiro, T. Schilb* and J. Porush. Brookdale Hospital Medical Center, Brooklyn, New York.

The REDY sorbent system (Organon Teknika) is an efficient means of treating patients with renal failure. Recently, the manufacturer has marketed kits to produce dialysates for treatment (Tx) of acid-base disorders complicating renal failure. Kits 1-3 contain HCO₃ (30,60,100 mEq/l) as the base with standard acetate infusate to treat acidosis and kit 4 contains Cl only (120 mEq/l) with acetate infusate to treat alkalosis. In order to develop a computer program for predicting the effects of these kits on post-Tx Na, HCO₃ and BUN, we performed in-vitro studies in which 32 liters of artificial "sera" with BUN 40 or 80 mg/dl and HCO₃ equal to 13,18,22,26 or 28 mEq/l were dialyzed 4 hrs against dialysates prepared from each of the kits. Samples of "sera", and pre and post-sorbent cartridge dialysate were analyzed for Na, HCO₃ and BUN at 15 min intervals. These data were used to derive formulae which form the basis of the computer program. Given inputs of age, sex, dialyzer clearance and pre-Tx weight, BUN, Na and HCO₃ the program was then utilized to predict post-Tx values in 13 patients treated with the REDY system (REDY 2000) and either kit 1 (n=6), 2 (n=5), 3 (n=1) or 4 (n=1). Mean (±SEM) predicted vs actual post-Tx values were: BUN 31.2±1.8 vs 25.7±2.8 (mg/dl); Na 143.6±1.0 vs 143.4±1.3 (mEq/l) and HCO₃ 24.7±0.6 vs 26.4±1.4 (mEq/l). For each parameter the differences between the means did not differ from zero. These data demonstrate that the computer program accurately predicts the outcome of REDY dialysis. It can be run on a PC or pocket calculator and alerts the user to questionable entries.

CORRELATES OF RENAL RECOVERY AND SURVIVAL IN ACUTE RENAL FAILURE (ARF) PATIENTS (pts) DIALYZED IN ICU'S. D. Spiegel,* G. Zerbe,* M. Ullian,* and T. Berl. Univ. Colorado Sch. Med., Denver, CO.

The survival rate of critically ill pts who develop ARF is extremely low, despite sophisticated support systems, including dialysis. Therefore, it would be advantageous to identify, early in the disease course, those few survivors. We reviewed the clinical course of 43 consecutive critically ill pts who developed ARF and were first dialyzed in an intensive care unit setting to define co-morbid conditions, present at the time of first dialysis, that were predictive of outcome. Mortality rate was 88%. Although 10 (23%) recovered renal function, 5 subsequently died during the same hospitalization. Adult respiratory distress syndrome (ARDS) (p<0.05), requirement for antibiotics (p<0.01) or intubation (p<0.01) impacted negatively on recovery of renal function. 10/10 pts with ARDS, 9/9 on pressors, 10/10 with gastrointestinal bleeding, 5/5 with liver failure, and 11/11 who were comatose, died. However, the most powerful predictor of mortality was the need for ventilatory assistance. Of 31 pts who were intubated, none survived, whereas of 12 non-intubated pts, 5 survived (p<0.001). The presence of either ventilatory support or coma yielded a predictive value for mortality of 100% (33/33); and in their absence, of 50% (5/10). The presence of either of these co-morbid conditions predicts an 89-100% mortality with 95% confidence. We conclude that initiation of dialysis in comatose or intubated pts does not alter the uniformly fatal outcome. The advisability of undertaking dialysis in this setting requires careful evaluation.

USE OF RECOMBINANT ERYTHROPOIETIN (rHuEPO) WITH HIGH FLUX DIALYSIS (HFD) DOES NOT WORSEN AZOTEMIA OR SHORTEN ACCESS SURVIVAL. J. Stivelman, D. Van Wyck*, L. Kirlin*, D. Ogden; U. of Az. Health Sci. Ctr; Tucson VAH, Tucson, AZ.

Because correcting anemia in chronic hemodialysis patients (PX) may impede dialysis efficiency in HFD by lowering effective plasma flow, and clot A-V accesses by raising blood viscosity, we evaluated the effect of increased HCT after rHuEPO on selected chemistries and access survival in 18 PX on HFD. Age 56.2±2.8 (SEM) yrs, blood flow rate 371±11 ml/min, HFD time 2.6±0.1 hrs 3x/wk using high flux dialyzers and HCO₃ dialysate. Chemistries for each PX were measured over 4 months pre-rHuEPO; during induction (ACUTE) with 150u/kg rHuEPO until HCT reached 35 (59.9±4.1 da); and during maintenance (CHRONIC) of HCT 35 thereafter with 75u/kg rHuEPO (82.5±7.5 da). Mean results during ACUTE and CHRONIC for each PX were compared with 4 month mean pretreatment values, and expressed as percent change in BUN, Cr, and HCO₃. For all PX (paired-t):

	BUN	Cr	HCO ₃
ACUTE	-20.0±2.7 (p<.001)	-6.9±2.4 (p<.02)	+6.7±2.3 (p<.01)
CHRONIC	-12.8±3.0 (p<.001)	+1.0±2.8 (p=NS)	+9.1±3.7 (p<.05)

No significant changes were seen in P, K, Ca, or HFD time between pretreatment, ACUTE, or CHRONIC. The number of clotted accesses in all PX in CHRONIC (6/18) was not different from all other PX (10/41) at this center over the treatment interval (p=NS). Rapid rises in HCT with rHuEPO in PX on HFD do not worsen chemistries or shorten access survival, and may even improve urea and acid-base balance by mechanisms still to be defined.

EVALUATION OF MUSCLE METABOLISM IN HEMODIALYSIS (HD) PATIENTS USING ³¹P MAGNETIC RESONANCE SPECTROSCOPY (MRS). H. Szerlip, K. McCully*, B. Chance*. Depts. of Med. and Biochem/Biophys. Univ. of Penn., Phila., PA.

HD patients often complain of muscle weakness and fatigue. Utilizing ³¹P MRS we examined metabolism in forearm muscles of 6 "healthy" male hemodialysis patients during rest, increasing levels of exercise and recovery. The metabolic parameters were compared to 4 controls. After a resting spectra, subjects performed increasing levels of work by depressing a lever coupled to an ergometer until they could no longer match the target value. Spectra were obtained each minute during exercise and throughout the recovery period. Measurements were made of work, free phosphate (Pi), phosphocreatine (PCr), final intracellular pH, and ATP. Comparisons were made of recovery of PCr/Pi·min, final pH, and end-exercise level as a % of maximum determined before the start of graded exercise.

	PCr/Pi·min	pH	%Max
normals	2.19 ±.36	6.67 ±.07	90 ±5
dialysis	0.88 ±.01*	6.42 ±.09**	71 ±3*
X ± SEM	*P<.05	**P<.06	

As compared to normals, HD patients 1) have lower end-exercise pH, and 2) take longer to recover phosphocreatine despite exercising at a lower level of their maximum work effort. These findings demonstrate an impairment in intracellular energy metabolism which may explain the fatigue and weakness of patients on maintenance hemodialysis.

HIGH FLUX DIALYSIS USING A POLYSULFONE MEMBRANE: 10 MONTHS OF CLINICAL EXPERIENCE. Jerome S. Tanenbaum, Richard L. Gibson, John G. Pearson, Jeffrey L. Hymes, Steve Bannister, Division of Nephrology, St. Thomas Hospital, Nashville, TN.

This report summarizes High Flux dialysis in 61 patients who received a total of 5,216 treatments over 6.7 months. F-60 (polysulfone) dialyzers were used at an avg. blood flow of 390 ml/min. The average duration was 3.2 hrs; kinetic modeling revealed an avg. P.C.R. of 0.9 and KT/V of 1.1. Hypotensive episodes complicated less than 10% of the treatments. Avg. inter-dialytic wt. gain was 2.25 kg; 12.7% of the treatments were associated with muscle cramps, 3% of the treatments were complicated by nausea and vomiting. At 6 months pre-dialysis chemistries were:

	BUN	Creat	Calcium	Phosph.	Potassium	HCO ₃	Hct(%)
	72±	10.5±	9.7±	5.4±	5.0±	19.8±	27.5±
+SE	3.2	0.5	0.1	0.2	0.1	1.5	0.2
n=	60	60	60	60	60	60	635.

High Flux dialysis is associated with a low morbidity rate. Patients in the study spent an average of 5.44% of their days in the hospital. The most frequent cause of hospitalization was a primary cardiac event (52%) followed by infections (14.2%) and clotted grafts (10.8%). GI bleeding accounted for only 3.8% of the total days spent in the hospital. Conclusion: High Flux dialysis with a polysulfone membrane and bicarbonate dialysate is associated with very few intra-dialytic complications and allows a short dialysis time without compromising dialysis efficiency, based on: kinetic modeling, routine laboratory evaluation, subjective self assessment by the patient, and hospitalization rates as an index of morbidity.

COMPARISON OF THREE METHODS FOR THE ESTIMATION OF UREA KINETICS IN HEMODIALYSIS. R. Vanholder*, P. Van Trimpont*, S. Ringoir* (intr. by R. Hakim). Nephrology Department, University Hospital, Ghent, Belgium.

It has been claimed that computed urea kinetic (UK) modelling in hemodialysed patients for the estimation of protein intake leads to overestimation of protein catabolic rate (PCR). In the present study, three different methods of kinetic modelling for the determination of PCR are compared in 15 patients. The first method was the computed urea kinetic modelling method (UK) as described by Sargent and based on an iterative calculation. Dialyzer clearances were however measured directly and not estimated by theoretical extrapolation. The second method used was the direct quantification method (DDQ) based on the collection of all urea eliminated from the body. The third method is based on the indirect calculation of urea distribution volume (VU) according to Watson and of dialyzer clearances from this VU and from pre- and post-dialysis urea concentration. All three methods resulted in PCR's that were not significantly different (UK: 1.04±0.20; DDQ: 0.98±0.21; Watson: 1.07±0.24 mg/Kg BW.24 hrs; p>0.05). When the results were correlated, the following results were obtained: UK vs Watson: r=0.64, p<0.01; DDQ vs Watson: r=0.76, p<0.001; UK vs DDQ: r=0.65, p<0.01). In conclusion, all methods seem equally reliable in determining mean PCR. Our data, obtained with directly measured dialyzer urea clearances, do not confirm the earlier held opinion that computed modelling results in an overestimation of PCR.

A CONTROLLED STUDY OF RECOMBINANT ERYTHROPOIETIN (EPO) IN CHRONIC HEMODIALYSIS PATIENTS. JC Van Stone, ME Jones* and CE Hires*, Department of Medicine, University of Missouri Medical School, Columbia, MO.

The effects of the intravenous administration of EPO to 33 chronic hemodialysis pts (CHD) were compared to 27 control CHD pts. Prior to study the HCT was less than 30 in all EPO pts and 30 or greater in all control pts. Nineteen of 33 EPO pts and 3 of 27 control pts were female otherwise there were no significant differences between the two groups. EPO pts initially received 150 U/kg after each dialysis and the dose was adjusted to keep the HCT between 32 and 40%. The mean maintenance dose of EPO was 103+48 (SD) U/kg. All EPO pts had an increase in HCT (mean 23+3 to 33+5). There was no significant change in the control pts hematocrit (31+5 to 31+6). There were no significant differences in either group in the routine biochemical determinations, blood pressure, hospitalization rate, access problems, intradialysis complications, or dialyzer reuse rate. There was a significant decrease in both pre and post dialysis weight in the EPO pts (-2.3 kg, $p > 0.001$) and no significant change in weight in the control pts. The decrease in weight appears to be secondary to decreases in target weight for blood pressure control. The majority of pts receiving EPO reported a marked increased state of well-being, improved exercise tolerance and decreased fatigue, especially at the end of the day.

We conclude that EPO is effective in treating the anemia of chronic renal disease and results in marked subjective improvement.

ALUMINUM EXCESS POSES MODEST RESISTANCE TO RECOMBINANT HUMAN ERYTHROPOIETIN (rhEPO) FOR DIALYSIS ANEMIA. DB Van Wyck*, J Stivelman, J Ruiz*, MA Katz and DA Ogden. Univ. of Arizona Departments of Medicine and Geology, VA Medical Center, Tucson, Arizona

To determine whether aluminum overload, a potential contributor to dialysis-related anemia, impairs response to rhEPO, we examined chelatable aluminum burden and hemoglobin (Hgb) rise in 20 iron-replete chronic hemodialysis patients undergoing acute (150mg/kg tiw) treatment for anemia (pretreatment Hgb 7.84±.22, mean±sem). Before treatment, plasma aluminum (Al) was measured by ICP-quadrupole mass spectroscopy before and 48 hours after a single 40mg/kg iv injection of deferoxamine (DFO) and the difference expressed as chelatable Al. After starting treatment, Hgb was serially determined in each patient and response expressed as time (Tflex) and Hgb increment (Hflex) at the inflexion point of maximum rate of Hgb rise (dHgb/dt), maximum predicted equilibrium Hgb (H_{eq}) and time to achieve 90% of H_{eq} projected by logistic regression analysis ($r^2 = .92 \pm .01$). We found a negative correlation between chelatable Al (range 11 to 534 ppb) and the logarithm of H_{eq} ($r = -.625$, $p = .003$), dHgb/dt ($r = .571$, $p = .009$), and Hflex ($r = .648$, $p = .002$), but no relationship between chelatable Al and Tflex, T90, or pretreatment Hgb. Nevertheless, no patient required dose augmentation or chelation therapy to achieve target Hct (≥ 35) within 90 days. Thus, aluminum-mediated rhEPO resistance, though detectable, is modest, and impedes but does not totally block response to therapy.

MUSCLE HIGH-ENERGY METABOLITES DURING HEMODIALYSIS AGAINST ACETATE. P. Vinay, E. Shoubridge, D. Arnold, M. Cardoso, Neurological Institute and Nephrology Service, Notre-Dame Hospital, Montreal, Canada.

The rapid metabolism of acetate by the muscle (and the heart) may reduce the ATP content of these tissues. In order to examine the possible effects of acetate-dialysis on the muscle energy metabolism, three patients "tolerant" to acetate were hemodialyzed against acetate using standard conditions while in a magnetic resonance spectrometer (1.5T). Phosphate containing metabolites were monitored (^{31}P NMR) in the gastrocnemius muscle using a surface coil. Measurements were obtained under fully relaxed conditions (32 transients in 16 min) at the beginning, serially during and after the cessation of the dialysis. No change in ATP and phosphocreatine was observed and no PP_i accumulation was detected. Phospho-monoesters and diesters were also unaffected. Intra (i) and extra (e) cellular P_i fell progressively as expected. Intracellular pH was calculated from the chemical shift of $\text{P}_i(i)$ which is readily distinguished from $\text{P}_i(e)$ in these patients with high total body P_i . Both the pH_i and pH_e increased with time respectively from 7.03 to 7.08 (pH_i) and 7.38 to 7.45 (pH_e). The pH_e calculated by NMR agreed ± 0.05 pH unit with the simultaneous electrode measurement (arterial blood). It is concluded that dialysis against acetate does not affect significantly the energy metabolism in the skeletal muscle of acetate-tolerant patients studied under resting conditions.

INFLUENCE OF DIALYSIS MEMBRANES ON POLYMORPHONUCLEAR (PMN) CELL LIPOXYGENASE ACTIVITY IN HEMODIALYZED PATIENTS. Denis Vincent*, Jean-Pierre Charmes*, Michel Rigaud*, Claude Leroux-Robert* (intr. by K.F. Badr). Hôpital Dupuytren, Limoges, France.

Leukopenia occurs in uremic patients during hemodialysis sessions. It is well known that PMN cell lipoxigenases are responsible for producing substances which increase neutrophil chemotaxis. We studied the in vitro production of leukotriene B₄ (LTB₄), 5 hydroxyeicosate-traenoic acid (5-HETE) and 15 hydroxyeicosate-traenoic (15-HETE) from PMN cells in hemodialyzed patients (n = 18). PMN cells were incubated with or without dialysis membranes, (polyacrylonitril -PAN-, cuprophane -CU- and cellulose acetate -AC-) for 15 mn at 37°C ; 73 μM of arachidonic acid (AA) was then added to the medium. The reaction was stopped after 5 mn. Analysis of AA metabolites was performed by HPLC and by gas chromatography/mass spectrometry. Statistical analysis employed two way variance analysis and the Kruskal-Wallis test.

	LTB ₄	5-HETE	15-HETE
Controls	33	276	426
PAN	30	325** +	412*** +
CU	17	149**	304 +
AC	26	196 +	255***

(Mean ng/ml) + $p < 0,05$ ** $p < 0,01$ *** $p < 0,001$

These results suggest that CU and AC dialysis membranes partially inhibit in vitro production of 5-HETE and 15-HETE whereas PAN does not. It can be postulated that these variations of exogenous AA metabolites may participate in hematologic disturbances observed during hemodialysis sessions.

ON-LINE STERILE RINSE OF REPROCESSED HIGH-FLUX DIALYZERS. B. von Albertini,* V. Barlee,* J.P. Bosch, George Washington University, Washington, DC.

More efficient, shorter hemodialysis with large surface, high permeability dialyzers is economically unfeasible at present without dialyzer reuse. Concern about the risks of exposure to the chemical desinfectants of reprocessing mandates optimal elution prior to clinical use. Conventionally, this is attempted by prolonged single-pass rinse of the dialyzer's dialysate compartment (DC), followed by recirculation (rc) and dialysis of sterile priming solution in the blood compartment (BC).

A novel approach for high-volume, single-pass rinsing of both compartments was introduced by adapting equipment modified for on-line hemodiafiltration (Fresenius 2008). Sterile, pyrogen-free dialysate, obtained from passage through permanently installed serial polysulfone filters (2x 1.25m²), rinses in single-pass the BC via the blood lines, while the DC is rinsed conventionally.

The dialyzers studied (5 Fresenius F-80, 5 Travencol CA 210) had been reprocessed automatically after single use and stored in 4% formaldehyde (F) at room temperature for 44 hrs before rinse. Residual F was determined semiquantitatively with sulfurdioxide at the venous end after the rinse cycle and again after 10' rest (rebound). Q_D=800ml/min

	residual F	Rebound F
conventional rinse	2 ppm	10 ppm
(20' DC + 15' rc BC, DC)		
single pass rinse	<2 ppm*	5 ppm*
(20', BC 10L + DC 6L)		

(* = p<.05)

Conclusion: High-volume, single pass rinsing with sterile, pyrogen-free dialysate provides improved elution of formaldehyde from reprocessed high-flux dialyzers.

DOWN-REGULATION OF NEUTROPHILS DURING HEMODIALYSIS REQUIRES THEIR PASSAGE THROUGH THE DIALYZER. J.P. Wauters, P. Neveceral, M. Markert. Division of Nephrology, University Hospital, Lausanne, Switzerland.

The decrease of neutrophil metabolism at the maximum neutropenia during cuprophan (CU) hemodialysis has been attributed to systemic down-regulation of the cells by activated complement. To further unravel this mechanism, neutrophils were simultaneously isolated from the arterial (A) and venous (V) sites at 1 and 15 min after the start of dialysis on CU and polyacrylonitrile (PAN). Neutrophil oxygen radical production stimulated with opsonized zymosan was measured by luminol-amplified chemiluminescence (CL): (mean ± SEM, peak CL in mV, * = p<.02 versus A)

	0	1 min		15 min	
		A	V	A	V
CU (n=4)	47±6	62±11	59±18	43±10	20±5*
PAN (n=1)	31	40	58	60	59

In spite of a similar decrease in circulating leukocytes on CU (V 1,2 ± 0,2; A 1,0 ± 0,2 x 10⁹/l), only cells collected from the venous site at the maximum leukopenia were found to be significantly less responsive. Conclusion: using CU, the down-regulation of neutrophils occurs only transiently and within the dialyzer suggesting that, besides systemic complement activation, the membrane plays an additional role.

LONGITUDINAL ASSESSMENT OF CURRENT PERCEPTION THRESHOLD VS. NERVE CONDUCTION VELOCITY IN ESRD PATIENTS. S.A. Weseley, B. Sadler*, J. Katims*, A.I. Goodman, N.Y. Medical College, Dept. of Med., Valhalla, NY.

The criteria of adequate dialysis(D) has been complexed and controversial. Quantitative evaluation can include hematocrit, serum albumin, BUN, cognitive tests, and Nerve Conduction Velocity(NCV). The advent of new therapeutic methods eg. erythropoietin, kinetic modeling of urea, liberalization of the diet has negated most of the above criteria. The health of the peripheral nerve is one of the few remaining quantitative measures of adequacy in the D pt. With the introduction of short time/high flux D, enhanced neurophysiological monitoring will be advantageous to augment the clinical evaluation.

We have established that Current Perception Threshold, (CPT) a noninvasive measurement, to be equivalent to invasive NCV in measuring peripheral nerve integrity. CPT is a test which pts. find acceptable as opposed to NCV (98% vs. 25% compliance). HD pts. have been followed by CPT, and NCV for 1 yr. (n=15). Correlations between the 2 measures were initially r=0.83 and r=0.80 (p<.001) a yr. later. During this period both measures remain stable in 8 pts., improved in 4, deteriorated in 1, and diverged slightly in 2. Additionally, carpal tunnel compression was demonstrated. Routine CPT should replace NCV.

FISTULA RECIRCULATION (AVR): A 2 NEEDLE METHOD. C. Williams*, J. A. Pederson, R. Dunlay and F. Llach. Dept. of Med. U. of Okla. HSC and VAMC, Okla. City, OK.

Expected dialyzer clearance is impaired by AVR resulting in sub-optimal dialysis. BUN dilution at the dialyzer inlet (A) by BUN from the outlet (V) estimates AVR. This requires an extra needle puncture for a systemic (S) BUN. AVR = S-A/S-V. BUN in A at 15 minutes post and that in S at 5 minutes before dialysis ends is similar.

This report compares %AVR at 5 minutes before ending dialysis (%MAVR) to that when BUN from A at 15 minutes post dialysis is used for S BUN in the AVR formula (%EAVR). Both calculations use BUN values from A and V at 5 minutes before dialysis ends. Data were collected from 5 patients during 20 routine treatments.

Results are:

	+15min	-5min	-5min	-5min		
	A BUN	S BUN	A BUN	V BUN	CB	MAVR EAVR
	mg%	mg%	mg%	mg%	ml/min	% %
M	60.2	59.1	53.5	23.5	209	16.8 13.9
SE	2.23	2.33	2.39	1.41	2.28	3.08 3.35

BUN from A at 15 minutes after and from S at 5 minutes before dialysis ends as well as MAVR and EAVR do not differ (p > 0.2). The linear relation of EAVR to MAVR follows:

$$\%EAVR = 1.1 \%MAVR + 2.0 \pm 0.11 (r = 0.98).$$

Conclusion: The BUN from A at 15 minutes post dialysis closely matches that from S at 5 minutes before dialysis ends. This permits estimation of AVR using only the 2 needles employed for dialysis.

RECOMBINANT ERYTHROPOIETIN (r-EPO) IMPROVES COGNITIVE FUNCTION (CF) AND QUALITY OF LIFE (QL) OF CHRONIC HEMODIALYSIS (CHD) PATIENTS (PTS). DL Wolcott*, S Schweitzer*, JT Marsh*, AR Nissenon, UCLA School of Medicine, Los Angeles, CA.

Anemia is thought to be one of the causes of impaired QL and to contribute to poor CF in pts on CHD. To assess this hypothesis we studied CF, subjective health status, mood state, dialysis-related stresses, self-esteem and social/leisure activities in CHD pts before and after correction of anemia with r-EPO. 13 pts have completed the psychosocial instruments prior to receiving r-EPO (T1) and after Hct had stabilized, 12-16 weeks later (T2). 9 pts have completed CF testing at both data points. Hct increased from 23.1% at T1 to 36.0% at T2. Modality specific stress decreased significantly from T1-T2, and improved profile of mood states (POMS), vigor score, increased physical activities, and decreased POMS fatigue and general treatment stress scores approached statistical significance. There was a clear trend for improved CF on tests of visual, conceptual and visuomotor tracking and auditory-verbal learning, with some T1-T2 improvements reaching statistical significance. These findings suggest that correction of anemia with r-EPO in CHD pts is associated with improvements in some aspects of QL and CF. They further implicate a role of anemia in the brain dysfunction seen in uremic pts.

UREA KINETICS (UK) IN PATIENTS ON RDT TREATED WITH RECOMBINANT ERYTHROPOIETIN (EPO)

E. Zehnter, M. Pollok, F. Longere, P. Bramslepe, D. Ziegenhagen, C.A. Baldanus
Dept Nephrol, Med Clin I, Univ. Cologne, FRG
(introduced by K.F. Kopp)

Treatment of RDT patients with EPO increases well being and activity. The concomitant rise in serum urea (U) can be attributed either to increased protein intake and catabolism or to less efficient dialysis (HD). To test this, UK were performed for 3 weeks pre EPO and during a steady state phase (SSP) at a rised stable hematocrit (HCT).

8 patients (3m, 5f) at a mean age of 44 y, stable on HD for > 1y were treated with 80 (n=4) and 120 (n=4) IU/kg HD EPO (Boehringer Mannheim, FRG). Pre (C₀) post (C_t) serum urea (mg/dl), HCT and BW (kg) were determined before EPO and during SSP. Urea generation rate (UGR; mg/min/kg BW) and protein catabolic rate (PCR; g/kg BW/d) were calculated. Total body potassium (TEP) as indicator for lean body mass was measured before EPO and will be repeated at 1/2 y SSP. Results: (* : p<0,05, paired t-test):

	n	BW	HCT	C _t /C ₀	UGR	PCR
pre	8	65,7	23	.27	13,2	1.0
SSP	8	66,2	32*	.28	15,3*	1,2*

HCT increased in all patients to the target value and was maintained by 30-50 IU/kg BW/HD. PCR in EPO treated ESRD patients increases, they eat more and are more active than before. The fact that dry BW had to be increased supports anabolism more than catabolism. TEP will help to discriminate.

THE UTILITY OF A HUMAN IMMUNODEFICIENCY VIRUS (HIV) STAGING SYSTEM IN END-STAGE RENAL DISEASE (ESRD) PATIENTS (PTS). William G. Wortham*, David Burleson*, John B. Copley. Nephrology Svc, Brooke Army Med Ctr, Ft Sam Houston, TX.

HIV screening of asymptomatic individuals often results in disease staging of those found positive. Because of immune system alterations associated with renal failure, we assessed the applicability of the Walter Reed Staging System (WRSS) to ESRD PTS. This system relies heavily on T-cell subsets and delayed hypersensitivity status (DHS) for PT classification into the first 4 of 6 total stages. Stable dialysis PTS and age, race and sex matched controls (CT) were studied. T-cell subsets were accomplished by separating blood with a Ficoll-Hypaque gradient, staining with Ortho™ monoclonal antibodies, and counting with dual laser flow cytometry. DHS was determined by assessing the number of positive reactions (0 to 4) to an intradermally applied anergy panel consisting of 4 antigens (mumps, monilia, trichophyton and tetanus toxoid). No individual tested positive to a simultaneously drawn ELISA for HIV. Results = mean + SD. *per mm³

	N	T lymphs*	T4 cells*	T8 cells*	T4/T8	DHS
PTS	37	1434+972	565+362	185+151	3.9+2	0.8+1
CT	40	2155+764	1067+456	295+149	4.5+3	2.0+1
P		0.001	0.001	0.01	NS	<0.001

Nine of 37 PTS and 1 of 40 CT demonstrated T helper cells <400/mm³. Thus PTS had significant abnormalities of both T-cell subsets and DHS when compared to controls. We conclude that immune system alterations inherent to ESRD preclude the utilization of the WRSS in this group of PTS.

OCCURRENCE OF BETA 2-MICROGLOBULIN (β₂-M) AMYLOID DEPOSITS IN STERNOCLAVICULAR SYNOVIUM OF CHRONIC HEMODIALYSIS (HD) PATIENTS (pts). J Zingraff,* Thomas Bardin,* LH Noël,* C Dubost,* D Kuntz,* and T Drüeke,* (intr. by DA McCarron). Necker, St Louis and Lariboisiere Hospital, Paris, FR.

Dialysis-related amyloidosis of the β₂-M type has been observed with increasing frequency in chronic HD pts. Its incidence appears to vary greatly from one center to the other. Its precise evaluation is still hazardous because of the difficulty in obtaining biopsy material. In order to address this question more accurately, we performed systematic synovial biopsies in the sterno-clavicular joint of 22 HD pts (13 women, 9 men) who underwent neck surgery for severe secondary hyperparathyroidism. Nine out of the 22 samples contained Congo red-positive amyloid deposits that reacted only with antiserum directed against β₂-M but not with antisera against AA, A1 or prealbumin. Mean age of β₂-M (+)ve pts was 55.4 years versus 43.1 years in β₂-M (-)ve pts (p=NS); mean length on HD treatment was 12.6±1.1 (SEM) versus 8.5±1.1 years (p<0.02). An excellent concordance was found between histological and x-ray findings: juxta-articular, subchondral bone cysts existed in all 9 β₂-M (+)ve pts except one who had destructive spondylarthropathy. In contrast, none of the 13 β₂-M (-)ve pts had such x-ray lesions. No clear-cut effect of the type of dialysis membranes used was apparent. In conclusion, the incidence of biopsy-proven β₂-M amyloid deposits in articular synovium increases with increasing time on dialysis. Moreover, x-ray evidence of subchondral bone cysts appears to be a valid criterion of β₂-M amyloidosis.

DIALYSIS: PERITONEAL

INCREASED INCIDENCE OF EXIT-SITE INFECTIONS (ESI) IN CAPD PATIENTS WITH NASAL CARRIAGE OF STAPHYLOCOCCUS AUREUS (SA). E.R. Ahrens, S.W. Zimmerman, J. Leggett*, C.A. Johnson*, M. O'Brien*, S.C. Engeseth*, W.A. Craig*, University of Wisconsin, Madison, Wisconsin.

Nasal carriage of SA occurs with increased frequency in patients who experience frequent skin breaks, (ie. diabetes, hemodialysis patients, etc.) and is associated with both increased skin colonization and infection with SA. We have previously shown that CAPD patients have an increase in nasal SA carriage at a rate comparable to other high risk groups (39%). In addition, nasal carriage of SA is associated with both colonization of the exit-site with SA and a history of prior SA ESI. We have prospectively followed a cohort of CAPD patients with nasal SA carriage (N=34) for a total of 271 patient months. Non-SA carrier CAPD patient controls (N=31) were followed for 253 mos. Patients with SA nasal carriage had a significantly higher incidence of exit-site infections, 1/13.6 vs. 1/50.6 mos ($p < .005$). ESI caused by SA were also more frequent in the SA nasal carriers (1/18.1 mos) than the non-carriers (1/63.3 mos) ($p < .02$). While SA peritonitis was more frequent in the SA nasal carriers (1/45.2 vs. 1/126.5 mos), *S. epidermidis* peritonitis was more common in the non-SA carriers (1/54.2 vs. 1/25.3 mos). The differences in staphylococcal peritonitis rates for these groups did not reach statistical significance however ($p < .15$ and $p < .08$). These data provide further support for the importance of nasal SA carriage as a risk factor for subsequent SA exit-site infections.

ABSORPTION OF RECOMBINANT HUMAN ERYTHROPOIETIN (EPO) FROM THE PERITONEAL CAVITY IN RABBITS. J.M. Bargman, A. Breborowicz*, H. Rodela*, G. Abraham*, D.G. Oreopoulos. Toronto Western Hospital and University of Toronto, Toronto, Ontario, Canada.

The purpose of this study was to determine whether EPO can be absorbed from the peritoneal cavity.

Uremic rabbits received intraperitoneal (IP) infusion of 125-I-human EPO by 4 protocols, simulating different dialysis regimens. Group 1 received EPO and vehicle only, without dialysate. Group 2 received EPO mixed with 35 ml/kg dialysate. In groups 3 and 4 EPO and vehicle were infused alone followed by dialysate after 1 hour (group 3) and 2 hours (group 4). Data was collected for 24 hours in group 1 and after a 6 hour dialysate dwell in groups 2, 3 and 4.

At 24 hours 98±.4% (mean±SEM) of administered EPO was absorbed in Group 1. In group 2 60±7% was absorbed after a 6 hour dwell. EPO alone for 1 hour followed by infused dialysate for 6 hours in group 3 did not change total absorption (59±3%). Finally, absorption of EPO in group 4 was 76±5% after 8 hours. In groups 2, 3 and 4 there was a strong correlation between EPO absorption and dialysate absorption ($r = 0.89$, $p < 0.001$).

We conclude that EPO is almost completely absorbed from the empty peritoneal cavity after 24 hours in uremic animals. When EPO is given in a CAPD-like regimen, absorption is less and correlates significantly with fluid absorption. The findings suggest IP EPO can be given at the end of each IPD. In CAPD, EPO absorption without fluid absorption could be achieved by instilling EPO overnight in the dry peritoneal cavity.

HETEROPOROSITY MODEL OF PERITONEAL TRANSPORT IS NOT SUPPORTED BY HYDRAULICALLY-DRIVEN CONVECTIVE TRANSPORT (HCT). J.L. Bell*, J.K. Leyboldt*, R.P. Frigon*, and L.W. Henderson. Nav Hosp, VA Med Ctr, Univ of Cal, San Diego, CA.

The peritoneum exhibits open diffusive transport (DT) yet tight osmotically-driven convective transport (OCT). One model of heteroporosity relegates DT to larger venular pores and OCT to smaller arteriolar pores. To test this model we have contrasted HCT with OCT. Open transport through large pores should greatly predominate in HCT and sieving coefficients ($1-\sigma$) should approach unity.

Thirteen eviscerated rabbits had 20 mmHg negative intraperitoneal pressure applied for three 30 min study periods. Concentrations of creatinine (C), PAH (P), 4000 mw dextran (D4) and 20000 mw dextran (D20) were measured in ultrafiltrate and bracketing plasma samples. $1-\sigma$ was calculated (±SEM):

	C	P	D4	D20
$1-\sigma$.72(.03)	.67(.05)	.51(.03)	.41(.04)

Control hypertonic exchanges followed in 6 animals. Volume at 2 hours was $1.21 \pm .09$ SEM times the initial volume ($p < .05$). The permeability area product (PA) and $1-\sigma$ were calculated (±SEM):

	C	P	D4	D20
$1-\sigma$.65(.17)	.45(.10)	.006(.04)	.010(.05)
PA(ml/min)	.51(.13)	.31(.06)	.090(.02)	.044(.01)

PA and σ values compared with previously studied noneviscerated rabbits suggest no fundamental membrane alteration.

Sieving coefficients are less than one during HCT for all solutes tested. We cite this as evidence against the heteroporosity model of peritoneal transport.

IMPROVED DIAGNOSIS OF CAPD EXIT SITE INFECTIONS (ESI) WITH CATHETER MANIPULATION (CM) AND THE USE OF A GRADING SYSTEM. K. Boyrer*, J. Holley, A. Moss. WV Health Care Coop., WV Univ., Morgantown, WV.

ESI is a major cause of morbidity with CAPD. The lack of a standardized definition and means of diagnosing ESI compromises comparison of CAPD techniques and ESI treatment outcomes. We developed a protocol for grading exit sites based on inspection and CM (squeezing the ES followed by tugging on the catheter then both techniques applied simultaneously to express purulent drainage). Exit sites were graded from 0 to 4 with 4 indicating severe infection and grade was compared with results of Gram stain and culture. The protocol was applied to 48 patients on 212 visits over a 96 day period and resulted in the diagnosis of 27 ESI in 14 pts. Erythema and crusting were not predictive of ESI (33 and 7.4% respectively) nor was tenderness over the exit site (present in 14.8%). The tug-squeeze CM detected 14 (52%) ESI missed by the squeeze technique. Grade predicted WBC on Gram stain (WBC present on 85% of grade 3 vs 61% of grade 2 exit sites $\chi^2 p = .0005$). Cultures were also confirmatory; 100% of grade 3 ESI had positive cultures vs 62% of grade 2. We conclude that inspection and CM provide criteria for exit site grading which accurately predict Gram stain and culture results. If uniformly adopted, this system would improve diagnosis and reporting of ESI.

SUCCESSFUL TREATMENT OF EXPERIMENTAL CANDIDA PERITONITIS WITH TUFTSIN. C. Chaimovitz*, R. Levi*; Z. Kaim*, S. Segal*, M. Elkan*, M. Friskin*. (Intr. by J. Levi). Soroka Medical Center, Beersheva, Israel.

Peritonitis due to candida (PCA) is a serious complication of CAPD. Since the response to antibiotics is poor, catheter removal is usually mandatory. Treatment of PCA by enhancement of host defences to infection has not yet been investigated. The immunoregulatory IgG-Fc derived tetrapeptide, Tuftsin, was found to activate phagocytic and antigen presenting cells of the RES of mammals. In this study, the beneficial effect of Tuftsin was investigated in mice with experimental PCA. PCA was induced by injecting candida cells (6×10^8 cells) suspended in 10ml of dialysis solution (1.5% Dianeal) into the peritoneal cavity of mice. To simulate CAPD, 10ml of Dianeal were injected into the peritoneal cavity daily. Tuftsin (50ug/mouse), given in a single dose before induction of PCA improved the survival of mice from 10% to 50%. If Tuftsin treatment was continued after induction of PCA, survival rose to 70%. Two days of treatment with Tuftsin, given only after induction of PCA with sublethal doses of candida (10^8 cells), decreased the number of viable candida recovered from the peritoneal fluid from (mean \pm SD) 700 ± 190 colony forming units (CFU) to 17.4 ± 8 CFU/ml ($p < 0.001$). Fewer animals were found to have fungemia, and the CFU decreased from 100 ± 46 to 17 ± 6 CFU/ml blood ($p < 0.01$). These results suggest that stimulation of the phagocytic activity of peritoneal macrophages by Tuftsin is a potential mode of treating PCA in CAPD patients.

IMMUNOLOGIC ASSESSMENT OF CAPD EFFLUENT. John Chapman*, Fred Aono*, and Cliff Holmes* (intro. by Sarah K. Webster). Baxter Healthcare Corporation, Round Lake, Illinois.

Limited knowledge exists on risk factors for the occurrence of peritonitis in CAPD patients. The purpose of this study was to quantitate immunologic factors in CAPD effluent related to opsonization of bacteria associated with peritonitis. Test samples were peritoneal effluents collected after overnight dwells from 7 patients all of whom displayed no sign of infection (length of time on dialysis 6 weeks to 4 years). ELISA to quantitate specific antibody to *Staph. epidermidis* (SE) demonstrated peritoneal effluent samples contained 15 to 273 IgG antibody activity units (AAU/ml) as compared to normal sera which had 28,371 IgG AAU/ml. ELISA for *Pseudomonas aeruginosa* (PA) specific antibody found peritoneal effluent to contain 0-123 IgG AAU/ml as compared to normal sera which had 6,722 IgG AAU/ml. IgM antibody to both organisms was not detectable (< 10 IgM AAU/ml) in peritoneal effluent samples as compared to normal sera which contained 6,521 and 2,298 IgM AAU/ml for SE and PA, respectively. ELISA to measure complement fixation activity (C3b deposition) on SE demonstrated minimal activity in peritoneal effluent (1/300 of normal serum). The deficiency in complement fixation could not be reversed by precoating bacteria with purified gamma globulin indicating a lack of complement components in the effluent. These data provide the first direct measure of specific immunologic factors in peritoneal effluent samples that are of relevance to host resistance to pathogens associated with CAPD peritonitis.

PERITONITIS IN CAPD PATIENTS—A RANDOMIZED CLINICAL TRIAL (RCT) OF TRIMETHOPRIM-SULFAMETHOXAZOLE (TMP/SMX) PROPHYLAXIS. David N. Churchill, Dimitrios G. Oreopoulos, D. Wayne Taylor*, Stephen I. Vas, M. Arifie Manuel, George Wu*, McMaster University & University of Toronto, Canada.

A double-blind RCT compared the effectiveness of prophylactic oral TMP/SMX, compared to placebo (P), in preventing peritonitis in CAPD patients. A daily TMP/SMX dose of 160/800 mg gives a steady state dialysate TMP/SMX of 1.07/4.35 mg/l in the final dwell of each dosing interval. Identification of a 40% reduction in annual peritonitis probability (0.60 to 0.36) with an 80% statistical power and a type I error of 0.05 (one-tailed) requires 52 per group. Among 4 CAPD programs, with stratification by previous peritonitis, 56 were allocated to TMP/SMX and 49 to P. For TMP/SMX there were 5 deaths and 7 catheter losses. For P there were 3 deaths and 9 catheter losses. There were 20 withdrawals from TMP/SMX; 9 from P. There was no difference between TMP/SMX and P with respect to time to peritonitis ($p = 0.19$). At 6 months, 64.1% of TMP/SMX and 62.5% of P were peritonitis free; at 12 months 41.9% of TMP/SMX and 35.0% of P were peritonitis free. There was no effect ($p > 0.05$) of age, sex, catheter care technique, spike or luer, or dialysate additives. Previous peritonitis increased the risk of peritonitis by 2.06, (5% CI, 3.61-1.18) while frequent (6 weekly) extension tubing changes increased the risk by 1.79, (95% CI, 3.04-1.02). Adjusting for these baseline variables does not improve the effect of TMP/SMX. TMP/SMX appears ineffective in prevention of CAPD peritonitis.

INTERMITTENT AMBULATORY PERITONEAL DIALYSIS (IAPD) B.H. Cohen, S. Alexander*, T. Sudhakar, M. Somerstein, M. Raza and J. Gerepka*, Helene Fuld Medical Center, Trenton, N.J.

A limiting factor in CAPD is "burn out" from the need for uninterrupted daily RX. An IAPD schedule was initiated which permitted medically stable, compliant pts. to refrain from RX. 1 day/wk. These pts. were taught, previously, to disconnect themselves using Delmed Safelock System. Nine of 36 pts., with a total of 35 pt. Rx. months, have been followed on a 6 day/wk. - 4 exchange/day program without dietary or fluid restriction. Some pts. utilized a higher osmotic solution prior to and following the "off day". Lab. studies, fluid balance and physical exams were performed and evaluated the a.m. following the "off day" X 2 consecutive wks., then monthly. The control BUN, Creat. and K^+ means were 55.2mg%, 10.65mg% and 4.55mEq/L respectively, changing to 68.4mg%, 11.1mg% and 4.67mEq/L after the non-dialysis day. The maximum individual change was 20% for BUN and 14% for Creat. BUN's never rose > 84 mg% and one K^+ 6.1mEq/L was controlled by diet. Peritonitis has not occurred. Cultures of the effluent from the 1st exchange and transfer set following the "off day" showed no growth despite haziness of fluid (the empty abdomen for 24hrs. may protect against infection as previously suggested). Fluid balance and physical exam has remained stable. In conclusion, IAPD appears safe and may reduce "drop out" in selected pts.. Decrease protein loss and better nutritional status may be added benefits. Overwhelming pt. acceptance and continual medical stability may lead to consideration of a 5 day/wk. schedule.

CAN THE INCIDENCE OF PERITONEAL CATHETER TUNNEL INFECTIONS BE REDUCED? C. Cruz, A. Melendez†, M. Faber†, R. Provenzano†, B. Sawaya† Henry Ford Hospital, Detroit, Michigan.

Since June of 1986, 90 (10 straight and 80 curled) Tenckhoff catheters have been peritoneoscopically inserted in 96 patients (55 males and 35 females, mean age 52 yrs) using a modified peritoneoscope (Needlescope[®]), local anesthesia, prophylactic cefazolin and a paramedian incision with an inferolateral catheter exit site.

After a mean follow-up period of 200±117 days, outcomes were compared to those of the previous 183 catheters placed by standard surgical techniques:

	Standard n=183	Peritoneoscopic n=90	
Inadequate outflow or Leakage	22 (12%)	2 (2%)	P=.0063
Tunnel infection	13 (7%)	0 (0%)	P=.0096

Six minor staphylococcal exit site infections occurred within the first postoperative month. These resolved with oral antibiotics and local care consisting of povidone iodine washing, drying with a blow dryer and thrice daily exposure to an incandescent lamp.

These data illustrate the advantages of this technique over conventional catheter insertion methods, viz, little perioperative morbidity, immediate availability for use without leakage or outflow obstruction and remarkably reduced incidence of tunnel infection.

MULTICENTER EVALUATION OF THE "O" SET IN CAPD PATIENTS. James W. Dobbie, Rosalie Villano,* Baxter Healthcare Corporation, Deerfield, Illinois.

The "O" Set was developed as a reusable Y-Set system, providing the patient with the ability to flush before fill and 0.5% sodium hypochlorite antiseptics. The patient is also released from the constant impediment of carrying a bag with tubing next to the body. 171 patients were started on the "O" Set in 12 centers. Their clinical performance was tracked for an average period of 8.3 months to a total of 1413 patient months. 75% of the patients were Caucasian, 14% Black, 11% other races. The mean age was 60.3 years. Peritonitis rate was 1 episode per 22.8 patient months. 48% of the organisms were Staph epi, 11% were Staph aureus. 6% were other gram positive organisms. 18% of the organisms were gram negative, 5% were mixed growth and no growth was obtained on culture in 11%. There were 19 episodes of exit site infection (1 episode per 74.4 patient months). Accidental infusion of 0.5% sodium hypochlorite occurred once in 33.6 patient months. (4,380 exchanges). Although the data is derived from a non-randomized study with no control group, the extended duration of the study and the large number of patients has provided a useful insight into the long term success and peritonitis reducing qualities of the "O" Set system for CAPD.

PSEUDOMONAL AND FUNGAL PERITONITIS IN HIV INFECTED INDIVIDUALS ON C.A.P.D. Robert Dressler*, Alexandra Peters*, Robert Lynn. Albert Einstein College of Medicine, Bronx, NY

At our center, HIV infected patients treated with C.A.P.D. more frequently developed pseudomonal (Ps) and fungal (F) peritonitis (P) when compared to the remainder of our C.A.P.D. population. Over the past 22 months we followed 74 patients for 768 patient months (pt mos): 7 were confirmed HIV antibody positive; 7 others were high risk (HR) for HIV infection (bisexual, intravenous drug abuser, known sexual contact), but were not tested; and 60 were low risk (LR).

	P per 100 Pt Mos					
	Pt Mos	Tot P	Gm+*	Ps Other	Gm-\$ F	
HIV+	50.8	41.3	21.7	11.8	2.0	7.9
HR	77.8	50.9	33.4	2.6	0.0	0.0
LR	639.8	20.6	17.3	0.5	1.9	0.9

*-gram positive \$-gram negative
Compared to the LR group, the increase in the total peritonitis rate in the HR group was primarily due to more Gm+ infections. This was not unexpected because these patients were felt to have poor aseptic technique. In contrast, the HIV+ group's two-fold rise in total peritonitis over the LR group was attributable to marked increases in the pseudomonal and fungal infection rates. These infections were invariably associated with catheter loss and frequently resulted in permanent conversion to hemodialysis. We conclude that the increased occurrence of pseudomonal and fungal peritonitis may limit C.A.P.D. as an effective dialysis modality in the HIV+ population.

VANCOMYCIN IS SUPERIOR TO CEFAZOLIN FOR TREATMENT OF CAPD PERITONITIS. MJ Flanigan, RM Freeman, WJ Lawton, Univ. of Iowa Hosp. & Clinics, VAMC Iowa City, IA, and Fresno VAMC, Fresno, CA.

CAPD peritonitis is a common dialysis complication, yet the choice of antibiotics and proper length of treatment remain controversial. A randomized prospective trial was constructed to compare the effectiveness of intraperitoneal vancomycin & cefazolin in the treatment of CAPD and CCPD peritonitis. Between 1981 and 1986 patients receiving continuous peritoneal dialysis were randomly assigned to use either intraperitoneal vancomycin 25mgm/L or cefazolin 50mgm/L for the initial treatment of peritonitis episodes. When laboratory results were consistent with peritonitis, patients initiated rapid peritoneal lavage with two exchanges containing antibiotic and resumed a routine dialysis schedule adding antibiotic and heparin to each exchange for 14 days.

During the trial there were 255 episodes of peritonitis in 131 patients; 121 episodes were treated initially with intraperitoneal vancomycin and 134 with intraperitoneal cefazolin. Primary cure (remaining infection free for 14 days following antibiotic use) was 83% following vancomycin and 67% following cefazolin treatment (p=0.003). The likelihood of successful treatment without hospitalization was also improved by vancomycin use (p=0.037). The frequency of catheter removal following cefazolin treatment was 1.75 times that following vancomycin. This did not reach statistical significance (p=0.37). The cure rate achieved through the initial use of intraperitoneal vancomycin is superior to that obtained using cefazolin.

FUNCTIONAL AND HISTOLOGICAL CHANGES OF PERITONEAL MEMBRANE (PM) AFTER PLASTICIZERS EXPOSURE (PLS). Agostino Fracasso, Giorgio Bazzato*, Sandra Sabatini and Neil A. Kurtzman, Nephrology and Dialysis Dpt., Umberto I Hospital, Venice-Mestre, Italy and Texas Tech University, Lubbock, Texas.

PLS have been recently proposed as one of the major factors responsible for sclerosis of PM in the long term CAPD. We have studied the functional and histological behaviour of PM in 2 groups of rats with normal renal function (S) and CRF in basal condition and after exposure to 3 different PLS during PD (1.5 and 4.25 mg% glucose). The UF rate as well as the UF coefficient in CRF-PLS groups was significantly lower than controls, while no significant changes were found in the S-PLS animals. Urea clearance changes didn't reach statistical significance among all the groups while blood glucose resulted significantly higher in the CRF-PLS animals. At LM and SEM, PM of CRF-PLS animals appeared thickened with massive disappearance of microvilli. At TEM we have found three different aspects: 1) mesothelial cells degenerated and fibrosis; 2) fibrosis and disappearance of mesothelial cells; 3) normal mesothelial cells and massive submesothelial oedema. In the S-PLS animals at LM the PM was normal with thickness of the wall in scanty areas or the specimen (5%); at SEM the surface was almost completely covered by microvilli while the TEM showed a membrane normal with only a light submesothelial oedema. These data obtained in an animal model suggest that PLS exert a toxic metabolic effect on PM. This mechanism can be postulated also in human pts, where it is advisable plasticizer-free material for PD.

ACINETOBACTER PERITONITIS (AP) COMPLICATING CHRONIC PERITONEAL DIALYSIS (CPD). C. Galvao*, R. Swartz, L. Rocher, J. Reynolds*, B. Starmann*, D. Wilson. Univ. of Michigan Medical Center, Division of Nephrology, Ann Arbor, Michigan.

Among gram negative bacilli isolated during peritonitis in CPD, *Pseudomonas* species are most common but *Acinetobacter* species are increasingly encountered. Survey of more than 450 patient-yr experience with CPD reveals 20 episodes of AP, making this the second most common form of gram negative peritonitis. No predilection for age or underlying diagnosis was detected, technical error was highly suspect in only 5 cases, and concomitant tunnel/exit infection, all non-*Acinetobacter*, occurred in only 3 cases. AP appeared as the first peritonitis in 5 cases and as the second in 5 cases, with only 1 case in 20 experiencing recurrent AP. Duration of CPD at the time of AP ranged from less than 1 to more than 56 months; however, AP was noted to appear shortly after therapy of non-AP or shortly after CPD access placement, within 2 mos in 8 cases (40%) and within 3 mos in 12 cases (60%). Treatment with IP antibiotic succeeded in 19 cases (95%) without CPD interruption or catheter removal: tobramycin alone in 12 cases, gentamicin alone in 1, and combined aminoglycoside with a penicillin or cephalosporin in 6. Catheter removal in one case resulted from concomitant tunnel infection with dialysate leak. In conclusion, although AP appears opportunistic because of a temporal occurrence suggesting superinfection during vulnerable periods, AP also appears amenable to IP antibiotic therapy alone without interruption of the CPD routine.

ACID-BASE STATUS OF ADULTS WITH END-STAGE RENAL DISEASE TREATED BY CHRONIC PERITONEAL DIALYSIS. David A. Goodkin* and Rasib M. Raja. Kraftsow Division of Nephrology, Albert Einstein Medical Center, Philadelphia, Pennsylvania.

Pts with end-stage renal disease commonly manifest high anion gap metabolic acidosis. Recent work has shown, however, that pts on hemodialysis in fact may be hyperchloremic, and that the most common pattern of acidosis is a mixed high anion gap/hyperchloremic picture (Am J Kidney Dis, 1986, NKF Abstracts, A4). This prompted us to investigate the effects of peritoneal dialysis on acid-base status. The following data (mean \pm SEM) were obtained from 12 stable peritoneal dialysis pts, ages 25 to 90: venous pH 7.33 ± 0.01 , venous PCO_2 45.0 ± 1.5 mmHg, serum Na^+ 142.7 ± 0.9 mEq/L, Cl^- 102.1 ± 1.3 mEq/L, K^+ 4.1 ± 0.2 mEq/L, TCO_2 23.2 ± 0.5 mEq/L, anion gap 17.4 ± 1.0 mEq/L, blood lactate 1.00 ± 0.19 mEq/L. Nine pts had anion gaps greater than 14, but none had a decrement in TCO_2 of magnitude equal to the increment in anion gap. Two subjects had venous PCO_2 less than 41 mmHg, and 2 had venous PCO_2 greater than 53, but the respiratory disturbance was not severe enough to perturb the pH beyond the normal range in any of the 4. These data suggest that the most common acid-base pattern in our peritoneal dialysis pts is a high anion gap metabolic acidosis, offset by a concomitant metabolic alkalosis. The net result is preservation of TCO_2 concentration and normal systemic pH. Absorption of alkali from the dialysate in the form of lactate (which is subsequently converted to bicarbonate) is known to exceed the dialytic loss of serum bicarbonate, and most likely, accounts for the metabolic alkalosis.

EXPERIENCE WITH INTRAPERITONEAL INSULIN (IPI) FOR DIABETIC PATIENTS ON CAPD. C.A. Johnson*, S.W. Zimmerman, S.C. Engeseth*. Center for Health Sciences, Univ. of Wisconsin, Madison, Wisconsin.

Experience with IPI in long-term survivors (LTS) on CAPD (>4 yr) is limited. We examined 10 LTS on IPI to compare initial and long-term IPI requirements, glycosylated hemoglobin ($HgbA_1$), dialysate glucose exposure and weight. The Wilcoxon signed-rank test was used. The following mean changes were observed:

	Change From 1 mo. Values	
	4 yr	5 yr
Dialysate glucose (gm/day)	+24.0 (p<.05)	+30.7 (p=.06)
Weight (kg)	+6.0 (p=.07)	+6.3 (p<.05)
IPI dose (U/day)	+22.8 (NS)	+1.4 (NS)
	Change From Pre-CAPD	
$HgbA_1$ (%)	-2.4 (p=.05)	-2.2 (NS)

Data from all other current users of IPI for <4 yr were combined with LTS data (total n=22) to compare current IPI dose to daily glucose exposure and pre-CAPD S.C. insulin dose, and to assess recent blood glucose control with IPI. Current IPI dose correlated with dialysate glucose (p<.01, n=22) and with pre-CAPD S.C. insulin dose (p<.01, n=21). The mean blood glucose from the last 10 clinic visits was 188 ± 47 mg/dl (n=22). The mean of the last 4 $HgbA_1$ determinations was 9.8 ± 1.4 (n=22). Hypoglycemia was less frequent with IPI in 14/22 patients and more frequent in only 2/22. IPI was preferred by 20/21 patients. These data show that IPI is safe and effective. While dialysate glucose needs increase in diabetic LTS, insulin needs remain unchanged.

WHY IS ULTRAFILTRATION LOWER IN CHILDREN ON CAPD?

R. Khanna, R.A. Mactier,* K.D. Nolph and T. Groshong* Depts. of Med. and Pediatr.*, Univ of MO and H.S. Truman V.A. Hosp., Columbia, MO.

Net ultrafiltration (ml/m² body surface area) after long-dwell peritoneal dialysis is lower in children than adults. Net UF is, in effect, the difference between total net transcapillary UF (TC UF) into and lymphatic absorption (L) out of the peritoneal cavity during the exchange. Four hour exchanges with 40ml/Kg, 2.5% Dianeal^R containing albumin were performed in 6 children (0.94 ± 0.11 m² body surface area). Similar exchanges using 2L volumes were performed in 10 adults (1.89 ± 0.07 m²). L was estimated from the net removal of dialysate albumin and net TC UF was calculated from the dilution of the initial dialysate albumin concentration.

	Children*	Adults*	
Infusion Volume	1333 ± 156	1254 ± 63	
Total TC UF	406 ± 61	430 ± 42	
Lymph Flow	271 ± 48	180 ± 36	
Measured Net UF	111 ± 52	237 ± 26	p < 0.05

*Results (mean ± SEM), expressed ml/m² body surface area, were compared by Student's t test.

During exchanges with similar dialysis mechanics, L represented 73 ± 10% of total net TC UF in children and 40 ± 6% in adults (p < 0.01). We conclude that lower net UF (ml/m²) after long-dwell exchanges in children is due to relatively higher L during the dwell time. This explains why short-dwell cyclical dialysis (CCPD or nightly PD) is especially useful in achieving desired daily net UF in children on PD.

PHOSPHATIDYLCHOLINE ENHANCES THE EFFICIENCY OF PERITONEAL DIALYSIS BY REDUCING LYMPHATIC REABSORPTION. R.A. Mactier,* R. Khanna, H. Moore,* Z. Twardowski and K. Nolph. Dept. of Med., Univ. of MO and H.S. Truman V.A. Hosp., Columbia, MO.

Net ultrafiltration and solute clearances (Cl) in CAPD are reduced by peritoneal lymphatic absorption (L). The mechanism of increased net UF and solute Cl after long-dwell exchanges with intraperitoneal (IP) phosphatidylcholine (PC) is not established. We performed 4 hour exchanges using 20ml, 4.25% Dianeal^R solution containing 1.5% albumin in 12 rats. 50mg/L PC was added to the infused dialysate of one of each pair of rats. L was estimated from the rate of removal of IP albumin and total transcapillary UF (TC UF) by adding L and measured UF.

	CONTROL	PC	*
Measured UF (ml/4hrs)	4.2 ± 3.3	10.8 ± 2.3	p < 0.01
Lymph Flow (ml/4hrs)	14.3 ± 3.0	8.0 ± 3.4	p < 0.01
TC UF (ml/4hrs)	18.4 ± 2.2	18.8 ± 2.6	N.S.
Urea Cl (ml/min)	0.08 ± 0.01	0.10 ± 0.01	p < 0.01
D/P urea (4hrs)	0.88 ± 0.05	0.84 ± 0.05	N.S.
Phosphate Cl (ml/min)	0.05 ± 0.01	0.07 ± 0.01	p < 0.05
D/P phosphate (4hrs)	0.54 ± 0.00	0.53 ± 0.04	N.S.

*Mean ± 1 SD were compared by Student's t test. These data indicate that PC augments net UF and solute Cl in the rat by decreasing lymphatic reabsorption and without increasing transperitoneal transport of water and solutes into the peritoneal cavity. Reduction in L with PC offers an alternative means of enhancing the efficiency of long-dwell peritoneal dialysis.

THE EFFECT OF DIALYSATE VOLUME ON LYMPHATIC FLOW (LF) IN PERITONEAL DIALYSIS (PD). Bruce Z. Morgenstern and Geoffrey A. Patrissi*. USAF Med Cen Keesler, Keesler A.F.B., Mississippi

The role of lymphatic absorption from the peritoneal cavity during PD has gained increasing importance in kinetic studies. Larger molecular weight molecules (>39,000) are predominantly absorbed by means of these lymph channels. Increasing dialysate volume has been shown to result in greater solute removal, except for proteins, leading to the theory that greater LF results in more protein convection away from the dialysate. To determine the effect of intraperitoneal volume on LF, 8 male New Zealand rabbits underwent two dialysis exchanges. The rabbits were randomly selected to receive either the small volume (SV, 40ml/kg) or the large volume (LV, 80ml/kg) as the first exchange. Dialysate was 2.5% dextrose commercially available fluid with 0.5mcCi of C¹⁴ dextran (M.W. 70,000) per 100ml added as a volume marker. Four hour exchanges were performed with dialysate sampling every thirty min and blood sampled at 0, 120, and 240 min. LF was calculated over each dialysate sampling interval and then a mean LF was determined. LF remained relatively constant throughout the exchanges. The mean LF for the SV exchange was 3.03 ml ± 0.28 (SD). The mean LF for the LV exchange was 3.06 ± 0.24. These values were not statistically different. As expected, there was a greater ultrafiltrated volume in the LV group vs the SV. It is concluded that intraperitoneal volume has no effect on LF. An alternative theory must be sought to explain the lack of an increase in protein loss with an increase in dialysate volume.

SERUM PROTEIN BINDING OF A HIGH-AFFINITY CEPHALOSPORIN DURING CAPD. Gene Morse,* Mark Sinnott,* and J. Joseph Walsh. SUNY at Buffalo, Depts. of Pharmacy and Medicine, Buffalo, New York.

Cephalosporins are considered a first-line therapy for CAPD related infections. Cefonicid (Cef), a long-acting, highly protein bound cephalosporin, may be a useful antibiotic for intermittent therapy (1-2 weekly) of peritonitis, due to its prolonged half-life in renal failure and the resultant high plasma concentrations. However, since drug activity correlates with the unbound rather than total drug concentration, the fractional binding (Fb) of Cef was examined.

Cef was added to serum (100 mcg/ml and 50 mcg/ml from normals (n=10) and patients undergoing CAPD (n=8) and hemodialysis (HD, n=7). Fb was studied via equilibrium dialysis and Cef was measured by HPLC. The mean Fb at 100 mcg/ml was 0.98 ± 0.01 in normals, 0.93 ± 0.04 in CAPD patients (p < 0.05), and 0.87 ± 0.11 in HD patients (p < 0.05). The mean Fb at 50 mcg/ml was 0.95 ± 0.03 in normals, 0.90 ± 0.01 in CAPD patients (p < 0.05), and 0.88 ± 0.11 in HD patients (p < 0.05).

These data confirm the high degree of Cef binding in normals. However, Fb was significantly reduced in both dialysis groups. Decreased serum binding in CAPD patients may well translate into enhanced antimicrobial activity. However, drug toxicity may also be a potential problem in this patient group, if the prolonged half-life and altered binding of Cef is not taken into account.

SUPPRESSION OF POLYMORPH SUPEROXIDE PRODUCTION BY CONVENTIONAL PERITONEAL DIALYSIS SOLUTION BECAUSE OF ITS LOW pH. Z.M. Nawab, U. Wadood*, D. Gupta*, M. Rahman*, F. Manahan*, J. 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 commercially available peritoneal dialysis solution containing 1.5% or 4.25% dextrose on polymorph superoxide formation as determined by reduction of cytochrome C. Polymorphs from 9 normal subjects were incubated in either dialysis solution containing added cytochrome C. Dialysis solution pH was adjusted to 5.8, 6.8, or 7.4 by use of NaOH. Results were calculated as nanomoles of superoxide produced per million cells.

With 1.5% or 4.25% dialysis solution at pH 5.8, superoxide production was 3.20 ± 0.85 (SEM) or 4.45 ± 1.33 respectively; after correction of pH to 6.8, superoxide formation increased ($p < 0.001$) to 18.1 ± 2.0 or 16.1 ± 2.3 , respectively. Further increase to pH 7.4 resulted in a slight additional rise ($p < 0.01$) in superoxide production, to 21.7 ± 2.6 or 18.3 ± 2.4 . There was no difference in superoxide generation between the 1.5% and the 4.25% solutions.

Our results suggest that conventional peritoneal dialysis solutions inhibit polymorph superoxide production due to their low pH.

PHOSPHATIDYLCHOLINE SYNTHESIS BY RAT PERITONEUM. Thomas Pavlina,* John Lloyd,* Robert Johnson,* J.W. Dobbie. Renal Therapy, Baxter Healthcare Corporation, Round Lake, Illinois.

Following the detection of phosphatidylcholine (PC) in peritoneal dialysis effluent (Grahame, G.R. et al., Bull. Perit. Dial. 5:109-111, 1985), there has been much interest in the possible role of PC in fluid and solute transfer in peritoneal dialysis. This study investigated the possibility that the peritoneum is capable of synthesizing PC. The synthesis of PC by rat lung (positive control), liver (negative control), and transparent mesentery (test tissue) was determined by in-vitro incubation of these tissues in the presence of (methyl- ^{14}C) choline chloride for 3 hours at 37°C in Warburg flasks. All lipid material present in tissue and incubation media was extracted from each sample using the Folch technique. Carrier egg PC was added to each sample and total PC isolated using thin-layer chromatography. The PC fractions were then counted for total radioactivity. The mean radioactive value for liver when compared to those of lung and mesenteric tissue was significantly lower ($P < 0.001$). No statistically significant difference was observed between the mean radioactive value for lung compared to mesenteric tissue. Thus, under the conditions of the experiment, we have shown for the first time that the peritoneal mesenteric tissue is capable of synthesizing PC, and in amounts equivalent to lung. The production of PC, a known surface active agent, by peritoneum has significant implications for the physiology of peritoneal dialysis.

LIPOPROTEINS IN PEDIATRIC PATIENTS (PTS) TREATED WITH CAPD/CCPD. Uwe Querfeld*, Renee C. LeBouef*, Isidro B. Salusky, Richard N. Fine. UCLA Sch. of Med., Dept. of Peds and VA Wadsworth Med. Ctr., Div. of Lipid Research, Los Angeles, CA.

Plasma lipoproteins and apoproteins were studied in 34 pts, aged 13+6.6 (0.7 to 23) yrs, treated with CAPD/CCPD for a period of 2.0+1.2 (0.2 to 4.9) yrs. After an overnight fast, we measured total plasma cholesterol (CHOL) and triglycerides (TG), cholesterol in the VLDL (VLDL-C), LDL (LDL-C) and HDL (HDL-C) fraction and apoprotein (apo) AI and apo B. Apo AI and apo B were also measured in the dialysate of 13 pts. Results (mean+SD; mg/dl) were compared to 19 aged/matched control subjects.

Plasma	Pts	Controls	P (T-test)
TG	162+69	71+32	<0.001
CHOL	189+46	144+38	<0.001
VLDL-C	24+20	9+7	<0.001
LDL-C	126+32	94+24	<0.001
HDL-C	38+14	41+12	NS
APO AI	111+31	116+29	NS
APO B	93+28	66+17	<0.001
C:HDL-C	5.7+2.3	3.8+1.2	<0.001
APO AI:B	1.34+0.55	1.89+0.7	<0.005

Apo AI and apo B in the dialysate averaged 14.5+7.5 and 2.6+3.7 mg/kg/day, respectively. We conclude that these pts have "atherogenic" lipoprotein profiles, cholesterol ratios and apoprotein ratios but normal apo AI and HDL-C levels in spite of considerable apo AI losses in the dialysate.

EFFECT OF DRAINAGE BAG SIZE ON SOLUTE TRANSPORT (C) AND ULTRAFILTRATION IN PERITONEAL DIALYSIS. Rasib M. Raja, Mark S. Kramer, Stephen J. Goldstein, Maria Mendez and Sidney M. Kobrin*. Albert Einstein Medical Center, Kraftsow Division of Nephrology, Philadelphia, Pennsylvania.

Since the introduction of plastic bags, the peritoneal dialysate bags are commonly used for drainage. Two liters of dialysate are supplied in bags with 2 and 3 L capacity. The effect of bag size on solute transport and UF has not been evaluated. We measured UF and C for urea (U), creatinine (Cr) and inulin (I) with 4.25% Dianeal using 2 L exchanges with 2 and 3 L plastic bags in 8 pts during 1 hr exchange. Each pt received 4 consecutive exchanges with each bag. The results are:

BAG	C_U	C_{Cr}	C_I	UF
	ml/min			ml/EXCHANGE
2 L	18.2+1.8*	14.5+1.2	6.7+0.6*	390+34*
3 L	16.1+1.0	13.1+1.1	5.8+0.5	506+45

* $P < 0.05$ (paired t test)

The ratio of dialysate/plasma conc for U, Cr and I was higher for 2 L than 3 L bag ($P < 0.01$). The intrabag pressure was determined. In 2 L bag, the pressure was 2, 24 and 45 while in 3 L bag, 0.0, 1.0 and 5.5 cm of H₂O with bag vol of 2, 2.5 and 3.0 L respectively. The data suggest that drainage bag size may have marked effect on UF and peritoneal C. UF is lower with 2 L than 3 L bag and may be due to higher intrabag pressure and incomplete drainage which, in turn, may increase intraperitoneal pressure. C is higher with 2 than 3 L bag and may be due to residual dialysate and increased effective surface area. The use of 2 L bag for pts requiring high C and low UF while 3 L bag for pts requiring high UF may be preferred.

CHARACTERISTICS AND CLINICAL OUTCOME OF CAPD RELATED GRAM NEGATIVE (GN) INFECTIONS (I). Padma Reddy,* Christine Krol,* and J. Joseph Walshe. SUNY at Buffalo, Dept. of Med., Buffalo, New York.

A prospective study of CAPD associated I was carried out from 1984-86, with 103 patients (pts) experiencing 180 episodes of peritonitis (P) and/or exit-tunnel I, for a rate of 1 episode per 8 Pt months. Data on Staphylococcal P have already been reported (J Clin Micro 1986;23:809-812).

In 31 Pts, 47 episodes of I (26%) were due to GN organisms. Of the Pts who developed P, 6 had associated exit-tunnel I, while 8 had GN I elsewhere. Mean age was 55.5 years and time on CAPD was 21.8 months. Fifteen Pts (48.4%) were diabetic and 6 (19%) had chronic glomerulonephritis. Abdominal pain, cloudy effluent and fever were the major presenting symptoms. Mean serum and dialysate WBC were 9600 and 4525/mm³ respectively. Four of the 19 Pts (21%) had positive blood cultures. An aminoglycoside was the therapy of choice and treatment period averaged 19.3 days. Thirteen Pts (27.7%) required hospitalization, with an average stay of 10.4 days.

Pseudomonas species were isolated in 12 (25.5%) *Acinetobacter* in 8 (17%), *Serratia* in 7 (15%), *Klebsiella* in 7 (15%) and *E. coli* in 4 (8.5%). Persistent or relapsing I was the reason for removal of 12 catheters (C). This loss of C was significantly higher in diabetics (9) and Pts with *Pseudomonas* (4). Age, sex, race and time on CAPD did not increase Pt risk for GN I.

In comparison to our data on Staphylococcal P, diabetics suffered proportionally more GN I. While there was no mortality directly associated with the GN I, morbidity was increased.

LONG TERM DIALYSIS AND TECHNIQUE SURVIVAL. J Rubin, J. D. Bower. University of Mississippi Medical Center, Jackson, Mississippi

We studied survival with dialysis therapy and the technique success of CAPD, home hemodialysis (HHD) and hemodialysis (LCD). The service base was 60% of MS. There were 1216 patients from 1/67 to 6/86 available for analysis: 230 to CAPD; 150 to HHD, and the remainder to LCD. The median patient survival was 9.4 years. Neither race, sex, or marital status altered survival. Patients starting dialysis under 40 yrs had a survival of 70% at 10 yrs versus 3.6 yrs for over 60. Except for diabetics under 40, median = 5.7 yrs, etiology of renal failure did not alter survival. Forty-six % of CAPD and 18% of LCD mortality occurred to diabetics. We addressed whether patients once entered into a home dialysis technique, remain on home dialysis? LCD patients transferred to HHD or CAPD after 180 days of therapy, LATE, assume the risk of the new therapy. HHD, median=7.5 yrs, and LCD, median=9.9 yrs, technique survivals were similar and both greater than CAPD, 1.9 yrs. The groups differed with respect to number of diabetics (least in HHD), mean age and number dying from a presumed cardiovascular cause (both greater in CAPD). In summary, patient survival differs between therapies, but the patient populations are not comparable. Survival on LCD and HHD are close enough to not allow dogmatic recommendations. The only way CAPD can be compared to these therapies, and its suitability as a general dialysis technique confirmed, is by a randomized prospective trial.

ISOLATED PERFUSION OF HUMAN PERITONEUM: A NEW MODEL TO STUDY PERMEABILITY TO WATER AND SOLUTES OF THE PERITONEAL MEMBRANE. Claudio Ronco*, Aldo Fabris*, Mariano Ferianni*, Stefano Chiamonte, Luisa Bragantini*, Alessandra Brendolan* and Giuseppe La Greca* (intr. by Paul Kimmel), Dept of Nephrology, St. Bortolo Hospital, Vicenza, Italy.

The study is designed to evaluate an experimental model utilizing an intestinal loop surgically isolated from patients operated for G.I. Cancer. 5 cms of bowel are isolated close to the neoplasia and an artery and a vein are cannulated. The loop, placed in a graduate container with 2.5% glucose PD solution, is perfused by gravity at different pressures (60-80-100 mmHg) with thermostated blood. 5 experiments gave the following result: The priming volume of the blood compartment ranged from 0.75 to 2.1 cc. The blood flows obtained were proportional to the applied pressure and varied from 1 to 1.9 ml/m'. In all cases filtration fraction remained stable at about 55% showing a constant relationship between Ultrafiltration and plasma flow. UF rate varied from 0.25 to 0.75 ml/m'. Solute clearances always approached the values of plasma flow with average ratios of 85 ± 10%. The model partially reproduces the peritoneal dialysis system with very high dialysate flows. The results achieved suggest that the model operates under conditions of filtration pressure equilibrium and that solute clearances are mainly limited by the low blood flow more than membrane surface area or permeability.

AMINOGLYCOSIDE-RELATED NEUROSENSORY HEARING LOSS (NSHL) IN PEDIATRIC DIALYSIS PATIENTS. Richard F. Salmon* and Billy S. Arant, Jr. UT-Southwestern Medical School, Dept. of Pediatrics, Dallas, TX.

Aminoglycoside (AG) therapy must be modified for patients with end-stage renal disease (ESRD) if not for its nephrotoxicity, then its ototoxicity. The relative risk of neurosensory hearing loss (NSHL) in children with ESRD has not been reported previously. To determine if NSHL were an unrecognized clinical problem and attributable to previous AG therapy, audiograms were performed on 25 ESRD patients (19 on PD, 4 on HD, 2 treated by PD+HD). No patient had Alport's disease or hereditary deafness. Five patients had conductive hearing losses explained by recurrent otitis media or congenital otologic malformation. Seven/25 (28%) patients had NSHL from 8000 (1), 4000 (3), 2000 (2) hz, and 1 was deaf. Eighteen patients had received AG therapy: 1 intravenously (iv) only, 4 intraperitoneally (ip) only, 13 both iv+ip; 6 (33%) had NSHL. Seven patients had never received AG: 1 had a NSHL which was unilateral only from 8000 hz. Of the 4 patients who had received AG ip only, none had NSHL, but of the 14 patients given AG iv only or in addition to ip, 6 (43%) exhibited NSHL. We conclude that NSHL occurs frequently in children with ESRD treated with iv AG even when the initial dose/interval is modified for renal failure and subsequent therapy determined by serum drug levels. We suggest audiometric testing of children with chronic renal insufficiency to identify unsuspected NSHL and subsequent testing following iv AG. Moreover, iv AG therapy should be modified further to avoid high transient serum drug levels associated with ototoxicity.

BIOAVAILABILITY OF CALCITRIOL: COMPARISON OF ORAL, INTRAVENOUS AND INTRAPERITONEAL ROUTES OF ADMINISTRATION IN CAPD PATIENTS. IB Salusky, WG Goodman, KC Norris, R Horst,* RN Fine, & JW Coburn. Depts Peds & Med, UCLA Sch Med & VA Med Ctrs, WLA & Sepulveda, LA, CA

Calcitriol (1,25) can lower serum (S) PTH levels in dialysis patients, and intravenous (IV) 1,25 may be more effective than oral (PO) 1,25. To evaluate possible mechanisms, 6 adolescent patients with osteitis fibrosa, age 16±3 (SD) yrs, on CAPD/CCPD for 22±12 mos received single doses of 1,25, 4.0 ug/70 Kg BW, via the IV, PO and intraperitoneal (IP) routes on 3 separate occasions. S-1,25 and Ca were measured 2, 15, and 30 min after IV 1,25 and after 1, 2, 3, 6, 12 and 24 hrs in all studies. Basal S-1,25 levels were 6±5 pg/ml (normal, 20-80); peak levels were 477±306 pg/ml at 2 min for IV; 100±49 at 3 hrs for IP; and 99±39 at 6 hrs for PO (IV>PO & IP, both p<0.01). S-1,25 levels were higher for 6 hrs after IV 1,25 vs. PO and IP, but did not differ thereafter; 24 hr values were 56±18, 54±20, and 56±15 after IV, IP, and PO. The area under the curve (AC) for S-1,25 vs time was 50-62% greater after IV than after PO or IP 1,25 (IV, 2340±523 pg/ml/hr; PO, 1442±467; and IP, 1562±477, (IV > PO & IP, p<0.05) due to greater values from 0 to 6 hrs. AC beyond 6 hours did not differ among groups. Neither peak S-1,25 nor AC differed after PO and IP doses. Baseline S-Ca, 9.4±0.1 mg/dl, was unchanged after 1,25 given by each route. Thus, IV 1,25 provides higher peak levels and greater bioavailability of 1,25 than PO or IP; IP route offers no advantage over PO. The higher peak levels and greater bioavailability of 1,25 could account for the enhanced biologic effects after IV administration.

COMPARISON OF TWO PERITONEAL DIALYSIS CATHETERS. Gaurang M. Shah, George Juler*, Anna Sabo*. VA Medical Center and UCI-Long Beach Medical Program, Long Beach, CA 90822.

The longevity of peritoneal catheters remains crucial to the long-term management of patients on peritoneal dialysis. In this report we outline our experience with peritoneal dialysis access, using 2 different two-cuff catheters: Tenckhoff (T) and Column-disc (CD) catheters. 57 catheters were placed in 39 patients (age 26-76 yrs, 37 men and 2 women) in the VA Medical Center peritoneal dialysis program over 5 years. All catheters were placed in the operating room using standard surgical technique by the same surgical team. The results are analyzed by Chi-square method.

	T (n=35) 563	CD (n=22) 314	p value
Patient-months			
Complications:			
cuff extrusion	3 (9%)	0 (0%)	ns
infection	16 (46%)	7 (32%)	ns
hernia	5 (14%)	2 (9%)	ns
leakage	5 (14%)	1 (5%)	ns
Catheter Loss:			
leakage	4 (11%)	0 (0%)	ns
drainage failure	1 (3%)	5 (23%)	<0.05
infections	6 (17%)	8 (36%)	ns

ns = not significant.

Of the 16 infected T catheters, the rate of infection/catheter was 2.4, whereas for the 7 infected CD catheters, it was 1.8 (ns). The rate of infection did not appear to be related to the location of the exit site. We conclude that both T and CD catheters are comparable with respect to incidence of complications and catheter loss experience, with the exception of higher incidence of drainage failure associated with CD, requiring catheter removal.

HISTAMINE AND ANTAGONISTS INFLUENCE PERITONEAL PERMEABILITY. Avshalom Shostak*, Przemyslaw Hirszel and John F. Maher, Dept. Med., Uniformed Services Univ. Hlth. Sci., Bethesda, MD.

To examine effects of histamine on peritoneal fluid and mass transfer rates 7 alert intact NZW rabbits underwent control peritoneal dialyses and dialyses with 2 mg/Kg of histamine (H) added intraperitoneally. Percutaneously 75 ml/Kg of standard dialysis fluid was instilled with autologous ^{203}Hg protein without drugs or with H, an H_1 antagonist, diphenhydramine (B), an H_2 antagonist, cimetidine (T) or ranitidine (Z), each with or without H. Dialysate was sampled every 12 min for 1 h; ultrafiltration rate (UF) by ^{203}Hg dilution, clearances (C) and protein excretion were measured. H raised UF slightly (260 to 330 $\mu\text{l}/\text{Kg}/\text{min}$), an effect abolished by TH and ZH, but not BH. UF/osmotic gradient changed similarly. T and Z alone did not lower UF. C urea, C K^+ and C glucose were minimally affected by the drugs, but most drugs alone or combined raised C PO_4 without an agonist/blocker pattern. H increased protein excretion from 1.6 to 2.9 mg/Kg/min; this was fully blocked by TH and ZH and partially by BH. T and Z alone did not lower protein excretion. The data suggest little effect of H on peritoneal blood flow, i.e. small solutes, but considerable impact on permeability, i.e. protein excretion. The peritoneal capillaries have H receptors that can be blocked by antagonists. Sterile peritonitis caused by intraperitoneal desoxycholate raised C and protein excretion. Z did not reduce these effects, indicating H does not mediate them.

QUANTITATIVE (Q) SOLUTE (S)-SYMPTOM (Sx) RELATIONSHIPS IN RENAL FAILURE (RF) AND SOLUTE-SPECIFIC DIALYSIS (SSD). P. Teschan, J. Lipman, P. Lawrence, D. DeBoer, D. White, D. Sulser. Vanderbilt University, Nashville, TN.

This study addresses mechanisms by which RF produces, and D reverses, the mainly neurobehavioral (NB) uremic Sx. Extracellular fluid (ECF) S concentrations, [S], were controlled for 9-16d (avg 13d) in bilaterally nephrectomized (Nx) conscious, ambulatory laboratory rats by daily peritoneal D (vol=10% body wt/exchange (E), 8 E/day, 30 min dwell). As in human RF, rat Q.EEG power spectra revealed slowing in the higher ratios (R) of theta (T, 3-7 Hz) to alpha (A, 7-13 Hz) power (TAR) following Nx, and reversal following therapeutic D (TD) using 1.5% Inpersol. In Series I: Post-Nx TAR responses to TD and UD (a mock "uremic dialysate" containing 9 conventionally measured S--urea, Cr and 7 electrolytes (phosphate [Pi] = 16 mg/dl) in distilled H_2O --to match [ECF] of preterminal Nx rats with typical Q.EEG changes) were compared: while TAR-TD remained at control levels (1.6 avg) avg TAR-UD rose 50% to 2.5 avg and avg survival declined to 5.5d. In Series II: Post-Nx TAR responses to TD and SSD using TD + [Pi=16 mg/dl] were compared: while [time-averaged] of Pi, TAC-Pi, differed (TD rats, 11 mg/dl; UD rats, 16.3 mg/dl, p<.001) neither the avg TARs nor the avg latencies of auditory-evoked potential peaks in the 2 groups differed. We conclude: (1) SSD selectively modifies ECF [S] by which its linkage to Q indices of NB Sx may be established; (2) The TAR responses to SSD in Series II indicate that the TD-UD differences in TARs in Series I cannot be attributed solely to their absolute or comparative ECF Pi concentrations.

DIALYSANCE OF CORTISOL AND CORTICOSTEROID BINDING GLOBULIN (CBG) DURING CAPD. PG Zager and HJ Frey*. Univ. of New Mexico Sch. Med., Albuquerque, NM.

We postulated that significant quantities of cortisol and CBG are removed during CAPD. To test this hypothesis, we studied 7 CAPD patients. Plasma cortisol and CBG were normal. Dialysate appearance rates of cortisol and CBG were 68 ± 7 ug/24h and 87 ± 18 mg/24h respectively. The CBG:cortisol molar concentration ratio was higher in dialysate (6.3 ± 0.6) than in plasma (1.6 ± 0.2) ($p < 0.001$).

To characterize the kinetics of cortisol binding, we performed a series of equilibrium dialyses *in vitro*. In plasma, the distribution of cortisol among CBG bound ($78 \pm 1\%$), albumin bound ($13 \pm 0.5\%$) and unbound ($9 \pm 0.3\%$) fractions was normal. The affinity (K_a 2×10^7 M⁻¹) and the cortisol binding capacity (20.2 ug/dl) of CBG were normal. In dialysate however, cortisol binding to CBG was markedly decreased. The affinity (K_a 1.1×10^7 M⁻¹) and cortisol binding capacity (0.6 ug/dl) of CBG were significantly lower than in plasma. The fractional occupancy of cortisol binding sites on CBG was lower in dialysate (0.03) than in plasma (0.55) ($p < 0.001$). Therefore, the distribution of dialysate cortisol among CBG bound ($23 \pm 1\%$), albumin bound ($6 \pm 1\%$) and unbound ($70 \pm 2\%$) fractions was markedly different from that of plasma cortisol.

Significant amounts of cortisol and CBG are removed during CAPD. The amount of free cortisol in dialysate effluent approaches that excreted in the urine of normal subjects. The low fractional occupancy of cortisol binding sites on dialysate CBG is due to the decreased affinity of CBG for cortisol.

LONG-TERM EXPERIENCE OF DIABETIC PATIENTS RECEIVING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). S.W. Zimmerman, F. Wiedenhoef*, C.A. Johnson*, J. Feyzi*, M. O'Brien*. Center for Health Sciences, Madison, Wisconsin.

CAPD has been accepted as an adequate short-term therapy for diabetic patients, but long-term outcome is still unknown. We examined the outcome of 74 diabetic patients starting CAPD from 1979 to August 1986. Patients were followed from 1-83 mos ($18 > 3$ yrs, $11 > 4$ yrs). Patient survival on CAPD was 80%, 55% and 40% at 1, 3 and 5 yr, respectively. Twenty-four remained on CAPD, 27 died, 19 received transplants and 7 transferred to hemodialysis. By using Cox's proportional hazards model, we determined pre-dialysis cardiac and vascular disease and lower pre-dialysis serum creatinine were risk factors for patient survival. Compared to pre-dialysis, CAPD improved blood pressure and glucose control, and was associated with stabilized vision. There was a low rate of non-CAPD related infections (0.1/year) and amputations (5/130 pt. yrs.). CAPD was associated with stable serum albumin, improved hematocrit, and serum levels of calcium and phosphorous. Serum cholesterol and platelets increased. There was one episode of peritonitis/pt year. *S. aureus* was the most common pathogen. Gastrointestinal symptoms increased on CAPD and bone fractures were common (15). Patients were hospitalized for 18.1 days/yr., 3.9 days/yr were for peritonitis. In conclusion, CAPD remains an adequate treatment for diabetic patients. Long-term survival is possible, but pre-dialysis cardiovascular disease is a major risk factor.

HORMONES/AUTACOIDS

THE PRESENCE OF HEYMANN NEPHRITIS (HN) ANTIGEN (330 KD) ON CULTURED RAT GLOMERULAR MESANGIAL CELLS (MC): STIMULATION OF EICOSANOID SYNTHESIS FOLLOWING ANTIBODY BINDING. N. Alavi. Rush Medical College, Chicago, IL.

The presence of a HN antigen (330 kD) in the rat proximal tubular cells, glomerular epithelial cells and in other non-renal tissues is well known. However, the molecular nature of the mesangial cell antigen and its function is unknown. 330 kD antigen was demonstrated on MC by immunofluorescent and immunoblot techniques using anti-Fx1A antiserum (a gift from Dr. A.K. Singh). Specific binding of purified Ab or antiserum stimulated synthesis of immunoassayable PGE₂, 6-keto-PGF_{1α} (PGI₂) and TxB₂ in a time and dose dependent manner, expressed as ng/mg prot/hr.

	Control	Anti-Fx1A			
		1:10	1:20	1:40	1:80
E ₂	6.6±1	*84±13	*63±8	*52±6	*8.8±2
PGI ₂	2.6±1	*8.6±3	*10.9±3	*4.4±.9	3.4±1
TxB ₂	1.7±.1	*15±2	*14.4±1	*6.7±.9	1.8±.2

Mean±SEM, n=8, *p<.01 compared to control

PGE₂ synthesis by MC was diminished in the presence of increasing concentrations of purified exogenous Fx1A antigen reaching the control level at an antigen concentration of 100 ug/ml, showing that the stimulation was generated by immunoreaction on the surface of the cells. PGE₂ stimulation was abolished in the presence of 10⁻⁶M cyclooxygenase inhibitor, sulindac sulfide and phospholipase A₂ inhibitor, dexamethasone (10⁻⁶M), demonstrating that the stimulation effect was due to de novo synthesis of PGE₂. It is concluded that MC may play a mediatory role in the hemodynamic changes taking place in passive Heymann nephritis.

RENAL PROSTAGLANDINS: CRITICAL DETERMINANTS OF INTRINSIC ANTIDIURETIC ACTIVITY OF VASOPRESSIN ANTAGONISTS IN VIVO. C.R. Albrightson-Winslow*, N. Caldwell*, B. Brickson*, F. Stassen, W. Huffman, and L.B. Kinter, Smith Kline & French Labs., Dept. of Pharmacology, Swedeland, PA, USA

Renal prostaglandins (PG) diminish vasopressin (VP) antidiuretic activity *in vivo* and *in vitro*. Cyclooxygenase inhibition potentiates VP antidiuresis in rats and dogs, and to a lesser extent, humans [Clin. Sci. 61:493, 1981]. In rats and dogs [Pmp¹⁰-Tyr(Et)²Val⁴desGly⁹]VP (SK&F 101926) is a potent VP antagonist, but in humans it is an agonist. In conscious water-loaded dogs, free water clearance (C H₂O; ml/min) was determined before ($3.6 \pm .3$) and after administration of SK&F 101926 (100 ug/kg) in the absence ($0.31 \pm .2$) or presence of indomethacin (indo; $-0.34 \pm .05$). With indo, SK&F 101926 (3 ug/kg) agonist activity appeared to be receptor-mediated since it was abolished by 100 ug/kg SK&F 105494 ([Pas^{1,6}D-Tyr(Et)²Val⁴Arg⁷D-Arg⁸desGly⁹]VP) which has minimal agonist activity itself (Uosm, mOsm/kg H₂O, *p<.05 vs. basal):

Drug	Basal		Treatment	
	U _{osm}	C H ₂ O	U _{osm}	C H ₂ O
SK&F 101926	57±3	3.1±.3	1266±22*	- .46±.1*
SK&F 105494	57±5	2.6±0.1	82±2	1.5±.2
In combination	58±10	3.2±.6	65±12	2.6±.6

Thus, in dogs, cyclooxygenase inhibition unmasks agonist activity of the VP antagonist, SK&F 101926. This activity is antagonized by SK&F 105494 which has less intrinsic agonist activity. Low renal PG tone, as evident by poor indo potentiation of VP antidiuresis in humans, may be responsible for their supersensitivity to the antidiuretic activity of SK&F 101926.

MECHANISM OF NEUROPEPTIDE Y INHIBITION OF VASOPRESSIN IN RAT COLLECTING TUBULE. RJ Anderson and MA Dillingham. VA Med. Cntr. and Univ. of Colo., Denver, Colorado.

Neuropeptide Y (NPY) is a unique 36-aminoacid polypeptide which often co-localizes with neurotransmitters known to regulate renal tubular transport. We therefore examined the effect of NPY on arginine vasopressin (AVP)-stimulated hydraulic conductivity (Lp, cm/atm·sx10⁻⁷) in rat cortical collecting tubules (CCT) perfused at 37° C in vitro. Addition of 10⁻⁸M NPY to bathing fluid significantly and reversibly decreases AVP (50μU/ml) stimulated Lp (300±30 to 213±21, P<.02, n=6). By contrast, 10⁻⁸M NPY does not reduce Lp response to 10⁻⁴M ClPheScAMP (330 ± 29 to 374 ± 31, NS, n=5). Since CCT's have an α₂ adrenergic receptor which can inhibit AVP-stimulated adenylate cyclase activity, we studied the role of this receptor in NPY inhibition of AVP action. Both α₂ receptor blockade with yohimbine (10⁻⁵M, n=5) and α₂ receptor occupancy with clonidine (10⁻⁴M, n=4) prevent NPY inhibition of AVP-stimulated Lp. To delineate if an inhibitory G protein (Gi) participates in NPY action, CCT were pre-treated with pertussis toxin which also prevents NPY inhibition of AVP-stimulated Lp (n=4). These physiologic studies suggest that NPY acts through an α₂ adrenergic receptor coupled to a pertussis toxin sensitive Gi protein to inhibit AVP-stimulated cyclic AMP formation and hydro-osmosis in rat CCT.

EFFECT OF DEMECLOCYCLINE (D) ON THE cAMP SYSTEM IN VASOPRESSIN (AVP) SENSITIVE SEGMENTS OF THE RAT MEDULLA. M. Anger,* J. Mansour,* and T. Berl. Univ. Colorado Sch. Med., Denver, CO.

D is the most widely employed AVP antagonist but the mechanism of this antagonism is unknown. We studied the effects of D on microdissected medullary collecting ducts (MCD) and ascending limbs (MAL) and cultured inner medullary collecting tubule (RIMCT) cells. In MCD, D (100 μg/ml) inhibited AVP stimulated adenylate cyclase (AC, fm/mm/30') (n=8) at 10⁻⁸ M AVP (36.7±5.1 vs 76±13, p<.02), 10⁻⁷ M AVP (72±8 vs 112±11, p<.01) and 10⁻⁶ M AVP (107±25 vs 266±47, p<.005). Likewise, forskolin (F) (10 μM) stimulated AC was decreased (1382±158 vs 1987±114, p<.01) suggesting a post-receptor effect. To further define the site of the defect, RIMCT cells were studied. D exposed cells had decreased cAMP formation (fm/μg) (n=10) at both 10⁻⁸ M AVP (54.0±7.2 vs 72.6±6.7, p<.02) and 10⁻⁷ M AVP (81.9±7.3 vs 127.0±11.6, p<.005). D-mediated inhibition was not seen in pertussis toxin exposed cells (10 μg/ml, 16 hr) as 10⁻⁸ M AVP stimulated cAMP was restored to 148±21. Cholera toxin (20 μg/ml, 18 hr) stimulated cAMP was not inhibited by D (512±37 vs 505±51.7). Since the MAL is responsive to AVP in the rat, the effect of D was tested. D inhibited the AC response to 10⁻⁷ M AVP (58.1±10 vs 97±23, p<.005) but not to F (329±79 vs 285±76 fm/min). The effect on AVP is not specific as 10⁻⁸ M glucagon stimulated cyclase was also inhibited by D (55±17 vs 118±21, p<.05). We conclude that D antagonizes AVP action by inhibiting AC in the collecting duct at a post-receptor site, most likely G_i, as well as by a receptor mediated effect on the MAL.

PHORBOL MYRISTATE ACETATE (PMA) AND A23187 ARE ADDITIVE BUT MECHANISTICALLY SEPARATE INHIBITORS OF VASOPRESSIN (AVP)-INDUCED HYDRAULIC CONDUCTIVITY (Lp) IN CORTICAL COLLECTING TUBULE (CCT). Y. Ando*, HR. Jacobson and MD. Breyer. Div. Nephrol., Vanderbilt Univ., and V.A. Medical Center, Nashville, TN.

Activation of protein kinase C (PKC) and elevation of cytosolic free Ca⁺⁺ concentration ([Ca⁺⁺]_i) result from polyphosphoinositide breakdown. We have previously shown that a PKC activator, PMA, and a Ca-ionophore, A23187, inhibit AVP-induced peak Lp in rabbit CCT perfused *in vitro* at 37°C (JCI 80:590, Int Congr Nephrol:247,1987). The preferential site of action of PMA and A23187 was post- and pre-cAMP, respectively. We further explored the mechanism of inhibition by these agents (See table). By using 0-Ca bath medium instead of normal 1.8mM Ca medium, the peak Lp suppression by A23187 was reduced from 73.0±2.6% to 38.3±3.5% (p<.001) while PMA suppression was unaffected. Bath indomethacin (IND) attenuated the suppression by A23187 (p<.01 vs. without IND) but not that by PMA. The combined effect of 1nM PMA and 20nM A23187 was greater than that of either of these agents alone (p<.02).

	10μM AVP-induced peak Lp (x10 ⁻⁷ cm/atm/s)			
	CONTROL	0-Ca	5μM IND	1nM PMA
CONTROL	166.0±5.9	223.6±14.5	166.4±20.5	59.3±7.5
20nM A23187	44.8±4.4	138.0±7.9	105.5±13.4	24.0±3.5
100nM PMA	18.6±4.8	33.0±1.8	14.6±2.0	-----

We also examined the effect of bath amiloride (AML, 0.5mM) on PMA suppression of 0.1mM chlorophenylthio-cAMP (CcAMP)-induced peak Lp. AML, which had no effect on CcAMP-induced peak Lp, attenuated the suppression by 100nM PMA; the PMA-treated peak Lp (x10⁻⁷cm/atm/s) was 113.8±12.6 with AML and 25.7±2.9 without AML (p<.01). We conclude: 1)PKC activation and increased [Ca⁺⁺]_i are additive in their suppression of AVP effect on water transport in CCT. 2)The Ca effect is largely cyclooxygenase dependent, while the PMA effect is not. 3)The PMA effect may partly be secondary to stimulation of AML-sensitive Na⁺/H⁺ exchange on basolateral membrane.

REGULATION OF AROMATIC L-AMINO ACID DECARBOXYLASE (L-AADC) ACTIVITY BY CHANGES IN DIETARY SODIUM INTAKE IN THE RAT. A. Aperia, I. Seri, BM Brenner, BJ Ballermann. Harvard Med. Sch., Boston MA and Karolinska Institute, Stockholm.

Extraneuronal dopamine (DA), thought to enhance Na⁺ excretion during Na⁺ loading, is formed in the kidney by uptake of L-dopa (D) into proximal convoluted tubules (PCT), where D is converted to DA by L-AADC. To determine whether regulation of L-AADC is involved in the adaptation to changes in dietary Na⁺, and to the increase in FE_{Na} after uninephrectomy (Nx), we measured renal cortical cytoplasmic L-AADC activity using ³H-D as substrate. Two groups of rats (n=3/group) were fed the same low-Na⁺ diet. The high-salt (HS) group received 0.9% saline to drink, while the low-salt (LS) group was given tap water. After 9-13 d Nx was performed, and the respective diets were continued. L-AADC activity was determined in the removed kidneys, and in the remaining kidneys 11-18 d after Nx. DA degradation (±.3%/min) did not differ in LS and HS rats.

	V _{max} (μmol/g/min)		K _m (mM)	
	Pre-Nx	Post-Nx	Pre-Nx	Post-Nx
LS	0.89±.1	0.94±.1	3.9±.3	3.7±.1
HS	1.58±.1**	1.62±.2*	4.6±.2	4.3±.2

** = p<0.01, * = p<0.05 vs LS

Thus, without change in K_m, V_{max} was 78% higher in HS compared to LS, with similar results after Nx. Conclusions: regulation of L-AADC may play a role in the renal adaptation to a high salt intake. Although no additional change in L-AADC was observed per unit wt after Nx, total cortical L-AADC activity increased as a result of compensatory renal hypertrophy.

ASSESSMENT OF THE FUNCTIONAL ROLE OF ENDOGENOUS ATRIAL NATRIURETIC PEPTIDE (ANP) BY PURIFIED ANTI-ANP ANTIBODY: STUDY IN A RAT MODEL OF CONGESTIVE HEART FAILURE (CHF). M Awazu*, T Imada*, V Kon, T Inagami* & I Ichikawa. Depts. of Ped. & Biochem., Vanderbilt Univ., Nashville, TN.

CHF and ECF volume depletion are remarkably similar in the mechanisms of homeostatic adjustments of renal sodium excretion. In addition to low renal plasma flow rate and GFR, both conditions are characterized by high ADH and renin-angiotensin-aldosterone levels, activated adrenergic system and enhanced prostaglandin actions. On the other hand, these conditions contrast in cardiac chamber pressures and ANP levels: while abnormally elevated intracardiac pressures and high ANP level are well recognized in CHF, these indices are subnormal in ECF depletion. To ascertain the functional role of the high circulating ANP level in CHF, we studied anesthetized rats subjected to surgical myocardial infarction 4 weeks earlier (MI), rats deprived of water for 48 hours (WD), and normal control rats (NL), before (C) and after (E) iv administration of purified rabbit anti-rat ANP IgG in a volume of 1.2 μ l/gBW (titer: anti-ANP IgG required for 50% neutralization of 1 μ g ANP = 1 μ l): [P<0.05 vs NL-C (*); vs C (†)]

	ANP pg/ml	GFR ml/min/100gBW	V' μ l/min	UNaV' μ Eq/min	MAP mmHg
MI (C)	366±63*	0.31±.05	5.7±0.7	0.75±.21	102±7
(n=4) (E)	-	0.29±.08	3.9±0.8	0.25±.08†	101±8
WD (C)	75±10*	0.32±.07	3.8±1.2	0.13±.05	107±1
(n=4) (E)	-	0.25±.06	3.3±0.7	0.18±.03	106±1
NL (C)	145±29	0.37±.04	7.1±2.3	1.10±.51	124±9
(n=4) (E)	-	0.32±.01	7.0±1.8	1.17±.44	124±9

While baseline of both MI and WD tended to have depressed urine flow (V'), sodium excretion rates (UNaV') and GFR, arterial plasma concentration of ANP was elevated only in MI and not WD. In response to anti-ANP IgG, V' decreased and UNaV' fell markedly in MI without a significant change in GFR or mean arterial pressure (MAP), contrasting to the pattern seen in WD and NL. The study demonstrates an important physiologic effect of ANP: high circulating ANP in CHF promotes sodium excretion which may serve to prevent avid sodium retention, further rise in cardiac chamber pressure, and hence progression of CHF.

CHARACTERIZATION OF LEUKOTRIENE D₄ (LTD₄) BINDING TO RAT GLOMERULAR MESANGIAL CELLS (MCs): DEMONSTRATION OF LTD₄-STIMULATED INOSITOL TRIPHOSPHATE (IP₃) SYNTHESIS AND ³H-THYMIDINE (³H-T) INCORPORATION. KE Badr, J Ebert*, RL Hoover*, S Mong*, and RC Harris*. Vanderbilt Univ. Nashville, TN and Smith, Kline, and French Labs., Philadelphia, PA.

LTD₄ contracts MCs in culture and depresses the glomerular capillary ultrafiltration coefficient in vivo. The latter effect may mediate the reduction in GFR during experimental glomerulonephritis. To further define LTD₄-MC interactions, we studied LTD₄ binding to cultured MCs, and attempted to characterize the signal transduction mechanisms.

Following incubation of MCs with ³H-LTD₄, equilibrium binding was reached at 20 min. Specific binding at 4°C was 62±5% (n=12) with an estimated K_d of 10⁻⁸ M and an R₀ = 30 fmol/10⁶ cells. Binding was inhibited by 40% in the presence of a 100-fold concentration of the LTD₄ receptor antagonist, SK&F 104353. Competition experiments revealed the following rank order of potency for ³H-LTD₄ binding: LTD₄>LTE₄>5R,6S-LTD₄>LTB₄.

MCs were also incubated x48 hrs in myo-inositol-free media +10% dialyzed fetal calf serum + 4 μ Ci/ml 3H-myo-inositol. They were then stimulated with 10⁻⁹, 10⁻⁸, 5x10⁻⁸, and 10⁻⁷ M LTD₄. Fifteen sec later, IP₃ generation was measured by anion exchange chromatography and compared to that of vehicle-stimulated controls. The above concentrations resulted in stimulation of IP₃ synthesis by 26±16%, 47±14%*, 93±8%*, and 40±6%*, respectively. Finally, dose-dependent (10⁻¹⁰M to 10⁻⁸ M) LTD₄ stimulation of ³H-T incorporation by MC was also observed, with the 10⁻⁸M concentration providing 50% stimulation. *: p<0.05 vs. control.

These studies: 1. Suggest strongly the presence of specific membrane receptors for LTD₄ on rat MCs. 2. Support the notion that the signal transduction pathway for LTD₄-induced MC contraction likely involves receptor-stimulated phosphatidylinositol turnover. 3. Suggest a role for LTD₄ as a MC mitogen, thereby extending its pro-inflammatory potential.

SECRETION OF ATRIAL NATRIURETIC FACTOR (ANF) FROM FISH ATRIAL AND VENTRICULAR MYOCYTES IN TISSUE CULTURE. Robert L. Baranowski and Christof Westenfelder, V.A. Medical Center and Univ of Utah, Salt Lake City, Utah.

Adult mammalian atrioocytes grown in culture store and secrete high molecular weight pro-ANF into the culture medium. However, data concerning the molecular form of ANF that ventricular cardiocytes secrete in culture have been conflicting. Certain species of fish have been shown by immunohistochemistry to contain ANF-immunoreactive material (ir-ANF) in both the atria and the ventricle. The purpose of this study was to determine the nature of secreted ANF from adult fish atrial and ventricular cells grown in culture. The Utah chub (*Gila atraria*) was selected because it adapts to springs of different salinity and was shown by us to contain typical atrial and ventricular secretory granules (by electron microscopy) similar to those found in mammalian atrioocytes. Atria and ventricles from four adult fish were dissected, minced and treated with a mixture of 0.025% trypsin and EDTA. Cells derived from such treatment were placed in primary culture at 27°C and 5% CO₂ using Dulbecco's modified Eagles medium supplemented with 10% fetal calf serum. In another experiment the atria and ventricles of 2 fish were minced and put into organ culture using the above media and conditions. Media from all cultures were collected and analyzed by RIA using an antibody directed to human ANF (Peninsula Labs). Serial dilution of the media in the RIA yielded parallel curves to standard ANF. Selected media were also subjected to high performance liquid chromatography (Vydac C18, gradient 10-60% Acetonitrile in 0.1% trifluoroacetic acid). Culture media from both atria and ventricle produced substantial quantities of ir-ANF (4.54 μ g and 4.48 μ g respectively). Chromatography of the media from both atria and ventricle exhibited properties similar to authentic human ANF (MW 3000). We therefore conclude that fish atrial and ventricular cells secrete a predominance of low molecular weight ir-ANF.

ATRIAL NATRIURETIC FACTOR (ANF) ANTAGONIZES ANGIOTENSIN II (ANG II)-MEDIATED PHOSPHOINOSITIDE (PI) CHANGES IN CULTURED RAT MESANGIAL CELLS (MC). R. Barnett, P. Ortiz, G. Lollo* and L. Ransammy, SUNY-Stony Brook, NY

The vasorelaxant properties of ANF have been demonstrated in a variety of preparations including isolated glomeruli and MC. While the mechanism of action remains undefined, Hassid et al. (FASEB 1987) reported the ability of ANF to antagonize the ANG II-mediated rapid rise of intracellular Ca⁺⁺ in MC.

In a series of six experiments we examined MC subcultures grown in replicate 6 well culture plates prelabelled with [³H]myo-inositol. The effects of ANF (10⁻⁷M) on ANG II (5x10⁻⁷M) stimulation of the PI cascade was examined.

ANG II increased inositol trisphosphate (IP₃) release from 218±49 (CPM/100 ug protein) to 473±51. 10 min pretreatment with ANF blunted this increase (283±30 p<.02). The small ANG II-mediated loss of label from phosphatidyl inositol bisphosphate (PIP₂) (813±39 to 715±42) was attenuated by ANF (795±56 p<.05). ANG II mediated an increase in inositol phosphate (IP) (2647±595 to 4302±688) which was inhibited by ANF (3219±579 p<.05). A small but significant attenuation of ANG II-mediated loss of label from phosphatidyl inositol (PI) was also observed. MC phospholipids were also prelabelled with [¹⁴C]arachidonic acid. ANF pre-incubation partially inhibited the ANG II-mediated loss of label from phosphatidyl choline, phosphatidyl ethanolamine, PI and PIP₂ (p<.05). We conclude from these results that ANF antagonizes ANG II-stimulated phospholipid metabolism in MC.

ROLE OF PROTEIN KINASE C (PKC) AND Na^+/H^+ EXCHANGER IN THE STIMULATION OF GLOMERULAR EPITHELIAL CELL (GEC) PROLIFERATION BY LEUKOTRIENES (LT) C₄ and D₄. Laurent Baud, Gisèle Cherqui, Joelle Perez, Edward J. Cragoe, Jr, and Raymond Ardaillou (Intr. by K.F. Badr). INSERM U. 64, Hôpital Tenon, Paris, France.

In addition to possessing potent contractile actions on mesangial cells, LTC₄ and LTD₄ (1-100 nM), are also mitogenic for cultured human GEC. Our aim was to determine if activation of PKC and Na^+/H^+ exchange, which have been shown to control cell growth, was required for LT-induced GEC proliferation. Progressive removal of extracellular Na^+ (Na^+_o), using N-methylglucamine⁺ as a substitute, inhibited LT-induced DNA synthesis by 54 % at 50 mM Na^+_o and 100 % at 5 mM Na^+_o . Addition of amiloride to a medium containing 135 mM Na^+ reduced LT-induced DNA synthesis in a dose-dependent manner ($K_i=12.5 \mu\text{M}$). Two analogues with a higher affinity for the Na^+/H^+ exchanger, 5-(N-ethyl-N-isopropyl) amiloride and 5-(N-methyl-N-isobutyl) amiloride exhibited a greater inhibitory potency ($K_i=0.1 \mu\text{M}$). Activation of phospholipase C-coupled G protein and PKC were involved in the stimulation by LTD₄ of Na^+/H^+ antiport-dependent cell proliferation since the G-protein blocker pertussis toxin (500 ng/ml) and the PKC inhibitor 1-(5-isoquinolyl-sulfonyl)-2-methylpiperazine (100 μM) reduced LTD₄-induced DNA synthesis by 95 and 69 % respectively without decreasing basal DNA synthesis. In addition, exposure of GEC to 100 nM LTD₄, as well as to phorbol esters, resulted in time-dependent PKC mobilization. In summary, LTC₄ and LTD₄ stimulate DNA synthesis and growth of human GEC via mechanisms which include activation of both PKC and Na^+/H^+ exchanger.

INCREASED PLASMA ATRIAL NATRIURETIC FACTOR (ANF) IN POORLY CONTROLLED DIABETES MELLITUS.

Gordon M Bell, Richard K. Bernstein, Steven A. Atlas, Gary D. James, Mark S. Pecker, Jean E. Sealey*, John H. Laragh*. Cornell Univ. Med. Coll., NY, NY.

To investigate ANF and its relationship to the renin system in diabetes, we measured plasma immunoreactive ANF and plasma renin activity (PRA) in 27 non-ketotic diabetics without evidence of cardiac or overt renal disease, and in 26 age- and sex-matched normal subjects. Diabetics were divided into those with poor (PGC) or moderate (MGC) glycemic control depending on their concurrent HbA1c levels (>9 or \leq 9 PCT, respectively).

	n	ANF (fmol/ml)	PRA (ng/ml/hr)	HbA1c (pct)	CCr (ml/min)
PGC	14	15.7 \pm 1.8	1.7 \pm 0.4	10.8 \pm 0.5	116 \pm 6
MGC	13	9.9 \pm 0.8	3.3 \pm 0.6	7.7 \pm 0.3	120 \pm 9
Normals	26	10.1 \pm 1.3	2.7 \pm 0.3	-	97 \pm 6

Plasma ANF was elevated in PGC diabetics compared to MGC diabetics ($p<0.001$) and normal subjects ($p<0.05$). In contrast, PRA was lower in the PGC diabetics compared to the other groups ($p<0.05$). Among the diabetics, ANF was directly related to HbA1c ($r=0.49$, $p<0.005$) and urinary albumin excretion ($r=0.40$, $p<0.02$), and was inversely related to PRA ($r=-0.36$, $p<0.04$) and plasma creatinine ($r=-0.42$, $p<0.02$). However, CCr was elevated in both diabetic groups ($p<0.05$). The opposite changes in ANF and renin in poorly controlled diabetes may reflect plasma volume alterations related to glycemic control. These hormonal changes may be factors in the early renal dysfunction of diabetes mellitus.

URINARY EXCRETION OF BOTH THROMBOXANE B₂ (TxB₂) AND ITS METABOLITE 2,3-DINOR-TXB₂ ARE INCREASED IN RATS TREATED WITH CYCLOSPORIN A (CyA). Ariela Benigni*, Chiara Chiabrando*, Antonella Piccinelli*, Norberto Perico*, Marco Gavinelli*, Mauro Abbate*, Tullio Bertani* and Giuseppe Remuzzi* (intr. by M.J. Dunn). Mario Negri Institute for Pharmacological Research, Bergamo and Milan, Italy.

CyA administration to rats is associated with a selective increase in urinary excretion of immunoreactive TxB₂ (i-TxB₂) negatively correlated with the decrease in glomerular filtration rate (GFR) (Am J Physiol 1986; 251:F581). The present study was designed to get further insight into the origin of the abnormal i-TxB₂ urinary excretion. Rats given orally CyA (50 mg/kg/d) for 30 d had a significant ($p<0.01$) increase in the urinary excretion of both TxB₂ (CyA: 11.86 \pm 0.17; vehicle: 1.35 \pm 0.64 ng/d) and 2,3-dinor-TxB₂ (CyA: 6.92 \pm 1.30; vehicle: 1.80 \pm 0.59 ng/d) measured by technique of capillary column gas chromatography-negative ion chemical ionization mass spectrometry. Urinary TxB₂ is more likely to reflect the renal synthesis of the parent compound, whereas 2,3-dinor-TxB₂ is considered to reflect the amount of TxB₂ formed in circulation. Ultrastructural studies on kidney specimens from animals given CyA showed focal glomerular endothelial damage together with a marked infiltration of blood-borne cells of monocyte-macrophage type in the glomerular tuft. It is suggested that the cause of increased urinary excretion of 2,3-dinor-TxB₂ is the consequence of intrarenal platelet and macrophage activation probably triggered by the endothelial damage. The parallel increase in urinary excretion of unmetabolized TxB₂ is likely to reflect a concomitant activation of resident renal cell arachidonic acid metabolism induced by CyA.

REVERSIBLE RENAL FAILURE AND CHANGES IN GLOMERULAR PROSTANOIDS INDUCED BY SALT DEPLETION (LNa) AND CAPTOPRIL (CEI). J. Bernheim, E. Podjarny, A. Pomerantz and H. Mathaus. Meir Hosp., Kfar-Saba and Tel-Aviv University, Israel (Intr. by M. Chambellan).

Ten days CEI treatment (captopril 30 mg/kg/d), induces renal failure in LNa rats. This is associated with reduced glomerular PGE₂. To evaluate the time course and reversibility of renal failure in this model, creatinine clearance (CCL, ml/min/100 g bw), systolic blood pressure (SBP, mmHg), glomerular PGE₂ and 6-keto PGF_{1 α} and TXB₂ (pg/mg prot/30 min) were measured in controls (n=10), LNa and LNa-CEI rats in groups of 5 at days 4, 9, 15 and 35. Other five 35 days LNa-CEI rats were also studied after 10 days of Na replacement, while continuing CEI.

In LNa rats, CCL remained normal. In LNa-CEI, CCL decreased to 0.40 \pm 0.04 at day 9, 0.21 \pm 0.02 at day 15, ($p<0.01$ vs control), and remained stable till day 35. After Na replacement, CCL increased to 0.70 \pm 0.02. SBP decreased only in LNa-CEI animals. Glomerular PGE₂ and 6-keto PGF_{1 α} were increased in LNa rats at day 15 (2625 \pm 1372 vs 478 \pm 67, $p<0.05$ and 193 \pm 20 vs 32 \pm 9, $p<0.01$ respectively) and returned to normal at day 35. TXB₂ did not change. In LNa-CEI rats, PGE₂ and 6-keto PGF_{1 α} were not different from control but TXB₂ was increased (150 \pm 43 at day 9, 85 \pm 14 at day 15 and 90 \pm 9 at day 35 vs 34 \pm 4 in control, all $p<0.01$). In Na-repleted LNa-CEI rats, the synthesis of all glomerular prostanoids was above normal values.

In summary, LNa-CEI, in the rat, induces severe stable renal failure, reversible after Na replacement. Abnormal glomerular prostanoids synthesis probably plays a pathogenetic role in this model.

ADH INCREASES GLYCEROPHOSPHORYLCHOLINE (GPC) IN INNER MEDULLA (IM) OF BRATTLEBORO RATS (DI). J.D. Blumenfeld*, SC Hebert, JA Balschi*, & SR Gullans. Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

We have shown previously that IM concentration of the osmolyte GPC increases in WKY rats made maximally antidiuretic with 3 days of water restriction. In this study, DI rats were used to determine the independent effects of ADH and dehydration on GPC levels in IM. GPC was measured in perchloric acid extracts of IM using H-1 NMR spectroscopy. Water intake was either ad lib (C) or partially restricted for 72 hrs (-H2O). The latter resulted in a 20% reduction in body weight, similar to that observed in the previous WKY study. -H2O had higher Uosm and Posm but lower GPC than C. An additional set of studies were done to determine if ADH, in the absence of dehydration, alters GPC accumulation. Osmotic minipumps infused either 0.9% NaCl (S; 14ul/d) or +ADH (3ug/d) for 14d with water intake ad lib. +ADH had higher Uosm and GPC than S, with similar Posm.

	Uosm	Posm	GPC (umol/gm wet wt)
C (4)	242±11	305±3	16.7±2.4
-H2O (6)	1200±32*	336±4*	9.2±1.2*
S (5)	174±15	297±2	5.6±1.1
+ADH (5)	1782±135**	295±2	25.3±3.4**

p<.05 ** vs S * vs C

In summary, marked dehydration, in the absence of ADH, did not increase GPC levels. In contrast, ADH replacement, without accompanying dehydration, resulted in a 4.5-fold increase in GPC concentration. These data suggest a role for ADH in the modulation of GPC in the IM of DI rats.

ENERGY METABOLISM DURING VASOPRESSIN (VP) STIMULATED WATER FLOW. A.S. Brem, M.A. Pacholski*, K.C. Inman*, and R.G. Lawler*. Brown Univ., Depts. of Pediatrics and Chemistry, Providence R.I.

In the toad bladder, both the onset and offset of the hydroosmotic response to VP appear to be energy dependent processes (AJP 211:1175,1966 & J Membr Biol 48:237,1979). We chose to study relative changes in high energy phosphates in the toad urinary bladder before, during, and after recovery from VP (20 mU/ml) stimulated water flow using ³¹P NMR. Bladders were mounted on glass pipets using the Bentley bag technique. The mucosa of the bladder was exposed to water while the serosal bath consisted of a standard amphibian Ringer aerated with compressed air. Spectra were obtained at 25°C on a Bruker AM spectrometer at a frequency of 162 MHz for ³¹P. Each mounted hemibladder was studied with 600 scans in a 10 mm tube before, during peak water flow (30 min after VP added) and 30 min after VP was removed from the bath. Tissues were only exposed to Ringer containing glucose (10 mM) when they were in the spectrometer; at other times they were bathed in aerated glucose free Ringer. NMR peaks from creatine phosphate, γ-ATP, α-ATP/NAD and β-ATP were easily identified and did not appear to change in intensity or configuration with VP stimulation or recovery. Interestingly, the α-ATP peak developed a noticeable up-field shoulder consistent with a rise in NAD/NADH on recovery (offset) from VP stimulation. Such a finding could be associated with an increase in oxidative phosphorylation.

EGF BINDS TO SPECIFIC EGF RECEPTORS AND STIMULATES MITOGENESIS IN RENAL MEDULLARY INTERSTITIAL CELLS. J. Breyer* and R. Harris* (int. by M Breyer). Vanderbilt Univ, Nashville, TN.

Medullary interstitial cells have been demonstrated to play an important role in renal arachidonate metabolism. Recently medullary interstitial cells relative to other cell types in the kidney have been found to be rich in lipocortin, a 35 Kd protein which is a major phosphorylation substrate for the EGF-EGF receptor complex. Not only is EGF found in high concentrations in the urine and is likely of renal origin but also the levels of mRNA for the EGF precursor, preproEGF, are orders of magnitude greater in the kidney than in other organs in the body. EGF effects are mediated through EGF receptors and the specific localization of EGF receptors in the kidney is unknown. We therefore characterized EGF binding to rabbit medullary interstitial cells in culture. We demonstrated greater than 80% specific binding at 4°, 23° and 37° of ¹²⁵I EGF. Equilibrium binding studies done at 40° and 37° revealed saturation of binding sites and an approximate Kd of .3nM. Receptor density was estimated at 1.7 x 10⁴ receptors/cell. EGF binding was not inhibited by ADH, ANF or bradykinin (10⁻⁶M). There was concentration dependent inhibition of ¹²⁵I EGF binding by alpha transforming growth factor a protein known to compete with EGF for binding to the EGF receptor. EGF increased thymidine incorporation threefold over serum free controls an effect equal to that seen with 10% calf serum. Medullary interstitial cells in addition to containing the 35 Kd protein have EGF receptors and proliferate in response to EGF.

EPIDERMAL GROWTH FACTOR (EGF) INHIBITS BOTH CHLOROPHENYLTHIO-CYCLIC AMP (CcAMP) AND VASOPRESSIN (VP) STIMULATED HYDRAULIC CONDUCTIVITY (Lp) IN THE RABBIT CORTICAL COLLECTING TUBULE (CCT). M.D. Breyer, H.R. Jacobson, and J. Breyer*. Div. of Nephrol., Vanderbilt Univ. and V.A.M.C., Nashville, TN.

EGF is a 53 amino acid polypeptide with potent mitogenic effects on cultured cells. Recently the thick ascending limb has been identified as a major site of synthesis for preproEGF. Renal clearance of EGF far exceeds that of creatinine suggesting that it is secreted into the urine. The kidney is rich in specific EGF receptors, however, the role of EGF in the kidney remains uncharacterized.

We have recently reported that 10⁻⁸M EGF inhibits VP stimulated Lp (10⁻⁷cm/atm/sec) in isolated microperfused Rabbit CCTs (37°C)(ICN,257,1987). We further characterized this effect and the mechanism EGF action. A dose-response study showed that pretreatment of the CCT with peritubular EGF from 10⁻⁸ to 10⁻¹²M results in a reduction in peak Lp response to 10μU/ml VP from control values of 232.4±9.6 (n=15) to 142.9±20.3 (n=4) at 10⁻¹²M EGF (p<0.0005). Below 10⁻¹²M EGF no effect was seen. 10⁻⁸M luminal EGF had no effect on VP stimulated Lp (mean 239±31.1, p>0.1 vs. control). Preincubation with 5μM indomethacin failed to reverse the inhibitory effect of 10⁻¹⁰M EGF on VP action (peak Lp, EGF+INDO=145.8±20.0 (n=8), vs EGF alone=124.4±16.3 (n=4)(NS), vs control 232.4±9.5 (n=15) (p<0.0005). 0.1mM CcAMP stimulated Lp was also inhibited by 10⁻¹⁰M EGF from 225.8±25.2 to EGF+CcAMP Lp of 132.2±17.4 (p<0.01).

These results demonstrate potent effects of EGF on a non-cultured renal epithelium-- microdissected CCTs. The lack of an effect of luminal EGF argues against a downstream role for urinary EGF in the CCT. Since this effect was not blocked by indomethacin, it is unlikely that prostaglandins mediate this action of EGF. EGF inhibits both VP and CcAMP stimulated Lp, distinguishing it from other humoral modulators of VP action in the CCT including alpha adrenergic agents and bradykinin.

SYNTHETIC HUMAN GROWTH HORMONE-INDUCED SODIUM RETENTION IN THE ISOLATED PERFUSED RAT KIDNEY. ME Brier*, GR Aronoff, and DP Henry*. Depts. of Medicine and Pharmacology, University of Louisville Sch. of Med. Louisville, KY and Lilly Lab. for Clin. Res., Indianapolis, IN.

Growth hormone has antinatriuretic properties in man and experimental animals. To test the hypothesis that synthetic human growth hormone (GH) directly effects sodium reabsorption by the kidney, we perfused the right kidney from male Wistar rats randomly assigned to three groups. Rats were given GH 1 mg/kg subcutaneously 4 hours prior to perfusion or 1 mg as a bolus to the perfusion medium at the beginning of the 90 minute experiment. Creatinine clearance (GFR), fractional sodium (FENa), and fractional potassium (FEK) excretion were compared between groups and to controls. The data are below (mean +/- sd).

	GFR ml/min	FENa %	FEK %
Control(9)	1.06 (0.13)	0.92 (0.44)	16.7 (6.2)
Pretreat(7)	0.96 (0.13)	0.46 (0.21)#	9.6 (3.9)#
Bolus(7)	0.99 (0.19)	0.70 (0.42)	19.0 (7.0)

different from control (p<0.05)
Pretreatment with GH decreased sodium excretion 50 percent and potassium excretion 42 percent. Bolus GH administration did not change FENa or FEK. GH caused retention of sodium and potassium independent of GFR in the absence of renal nerve activity and other circulating hormones. Although the mechanism for sodium retention is unknown, the need for GH pretreatment suggests this direct effect on the kidney requires protein synthesis.

CHARACTERIZATION OF THE MACULA Densa SIGNAL FOR RENIN SECRETION. Josephine P. Briggs and Ole Skótt*, Department of Internal Medicine, University of Michigan, Ann Arbor, Mich.

We have recently developed a technique for study of renin secretion from the isolated perfused juxtaglomerular apparatus (JGA). The tubule segment containing the macula densa was perfused, using the isolated perfused tubule technique with the modification that the specimen was also superfused. Secreted renin was measured in collected superfusate using the "antibody trapping" ultra-micro renin assay of Lykkegaard and Poulsen. Twelve 10 min collections were made from each perfused JGA, 5 in the control period and 7 after changing the tubular perfusate. Renin content of the single JGA, measured at the termination of each experiment, averaged 350 ± 110 μ Goldblatt Units (GU) per JGA. In an initial series of studies we found that decreasing macula densa NaCl concentration resulted in a tenfold stimulation of single JGA renin secretion rate, (Skótt and Briggs, *Science*, in press, 1987). In time control studies renin release averaged 3.93 ± 1.0 nGU/min in the control period and 2.98 ± 1.1 nGU/min after a mock perfusate change (n=10). Fractional release rate was $0.78 \pm 0.23\%$ per hour. Furosemide produced a significant stimulation of renin release from 2.94 ± 1.1 to 13.54 ± 2.3 nGU/min per JGA (p < 0.05, n=5). In conclusion: furosemide stimulates renin secretion directly through action on the macula densa. The results suggest that renin secretion is controlled by the rate of NaCl transport at the macula densa, and that the transport mechanism utilizes the Na-K-2Cl co-transporter.

AUTORADIOGRAPHIC LOCALIZATION OF A₁ ADENOSINE RECEPTORS TO TUBULES IN THE RED MEDULLA AND PAPILLA OF THE RAT KIDNEY. M.L. Brines and J.N. Forrest Jr, Dept. Med. Yale Univ. Sch. Med., New Haven, Ct.

Adenosine has diverse effects on renal function including modulation of NaCl and water transport. However, adenosine receptors have not been localized precisely in the intact kidney. We defined the intrarenal distribution and affinity of adenosine receptors using *in vitro* autoradiography with the A₁ receptor ligand [¹²⁵I]hydroxy-phenyl isopropyladenosine (¹²⁵I-HPIA). Frozen sections (16 μ m) of male Sprague-Dawley rat kidneys were incubated with ¹²⁵I-HPIA for 2 h with or without unlabelled agonists and antagonists and adenosine deaminase (1U/ml). Sections were apposed to LKB ultrafilm at 4° C for 1-7 days and films analyzed by computerized quantitative densitometry. Specific binding was highest in the inner (red) stripe of the outer medulla and the inner papilla (50 and 75% of total binding, respectively). No binding was present in the outer portion of the inner medulla and high non-specific binding was present in the cortex. Specific binding in the RM and P was saturable with an apparent K_d of 250 pM, consistent with an A₁ receptor. Competition by adenosine analogs and antagonists in RM and P was also consistent with an A₁ receptor with cyclohexyladenosine > 2 chloroadenosine = NECA > theophylline. An identical order of potency was observed in filtration binding studies on membranes prepared from the papilla. Combined autoradiographic and histologic analysis of fixed sections of kidney using liquid emulsion revealed the density of grains to be greatest over thick ascending limbs (TAL) in the RM and papillary collecting ducts (PCD) in the P. These studies provide the first autoradiographic localization of adenosine receptors in the kidney and indicate substantial nephron heterogeneity. The highest density of A₁ receptors occurs in medullary segments identified as TAL and PCD.

HUMAN CEREBROSPINAL FLUID CONCENTRATIONS OF ATRIAL Natriuretic Factor: IS THERE A RELATIONSHIP TO CSF PRESSURE OR PLASMA ANF? JP Broderick*, TR Schwab, DM Heublein*, JP Whisnant*, JC Burnett, Jr., Mayo Medical School, Rochester, MN.

A role for atrial natriuretic factor (ANF) in central nervous system volume regulation is suggested by recent studies demonstrating the presence of ANF in the brain and receptors for ANF at the site of cerebrospinal fluid (CSF) production. The present study was designed to determine 1) whether ANF is measurable in CSF in humans, 2) the relationship between CSF-ANF concentration and CSF pressure, and 3) the relationship between ANF concentrations in CSF and plasma. Human subjects ages 18 to 80 (n = 70; 34 males, 36 females) were referred for outpatient lumbar puncture. Following measurement of CSF opening pressure, subjects had simultaneous collections of plasma and CSF for ANF determinations. ANF levels were measured in extracted plasma and CSF by radioimmunoassay to alpha-hANF (1-28) with a lower limit of detectability of 3 pg/ml.

Mean CSF pressure was 172 ± 6 mmHg. ANF concentration in CSF (14.1 ± 0.3 pg/ml) was significantly lower than plasma (39.2 ± 2.7 pg/ml, p<0.001). No significant relationship was observed between ANF concentrations in CSF and plasma (r = 0.12) or CSF-ANF concentration and CSF pressure (r = -0.06).

We conclude that ANF is detectable in CSF of humans albeit at lower concentrations as compared to plasma. This observation and the lack of a relationship of cerebrospinal fluid ANF to plasma ANF concentration suggests the central nervous system may be the principal source of atrial natriuretic factor in the cerebrospinal fluid.

ANGIOTENSIN II (ANG II) RECEPTORS AND EICOSANOID PRODUCTION IN RABBIT PREGLOMERULAR VESSELS.

G.P. Brown and R.C. Venuto. SUNY at Buffalo, Schools of Nursing and Medicine, Buffalo, NY.

Ang II directly constricts preglomerular (pregl) vessels. However, the constrictor response may be modulated by eicosanoids (eic.) These studies evaluated 125 I-Ang II binding and eic. production (prod.) in pregl. vessels. Arcuate and interlobular arteries with proximal afferent arterioles were dissected from rabbit renal cortices. Membranes were prepared and utilized in a radio-receptor assay for Ang II or the vessels were incubated in media which was analyzed for eic. by RIA. Specific binding was saturable and reversible. Linear Scatchard plots gave a K_d of 1.5 ± 0.1 nM and number of sites (N) of 59 ± 7 fmol/mg protein (n=9). The order of binding inhibition (BI) potencies of analogs was: (Sar, Ile⁸) Ang II > (Sar, Ala⁸) Ang II > Ang II = Ang III >>> Ang I, consistent with in-vivo reports of the effects of analogs on renal blood flow. In-vitro eic. prod. (ng/mg protein/15 min) is shown below. Prod. was inhibitable with meclofenamate and proportional to protein content.

	(n)	6-Keto-PGF _{1α}	PGE ₂	TXB ₂
Control	(6)	17.2 \pm 2	0.7 \pm .1	0.3 \pm .03
AA, 10ug	(3-4)	35.6 \pm 3*	2.8 \pm .4*	0.6 \pm .04*
Ang II, 1nM	(5-6)	15.7 \pm 2	0.8 \pm .1	0.3 \pm .04

(TXB₂ = thromboxane B₂; AA = arachidonic acid; *p < 0.001 vs control. We conclude: 1) Pregl. vessels have a single class of Ang II receptors. 2) The K_d and N are similar to, but the BI potencies of Ang analogs differ from that reported for extrarenal vessels. 3) Pregl. vessels increase intrinsic eic. prod. in response to AA. The data support a direct pregl. constrictor effect of Ang II which may be modulated by locally produced eic.

COMPARISON OF THE EFFECTS OF α -H ATRIAL NATRIURETIC PEPTIDE (ANP) INFUSION WITH PERITONEOVENOUS SHUNTING (PVS) IN CIRRHOSIS. P. Campbell*, W. Leung*, A. Logan, L. Blendis*, and K. Skorecki. Univ. of Toronto, Toronto, CANADA.

We have previously reported that the immediate natriuresis following PVS is associated with a 6 fold rise in ANP and an increase in urinary cGMP excretion. However, a cause and effect relationship remains unproven. Therefore, in a separate group of 5 patients we examined the effect of a 2 hour infusion of ANP, designed to reproduce pANP levels comparable to those seen post PVS.

On a constant 20 mM sodium diet, mean UnaV was 7 \pm 3 mM/day. Following an initial bolus, ANP was infused at 15 ng/kg/min for 2 hours; Inulin/PAH were infused for 2 hours pre, and 2 hours during ANP; urine was collected hourly.

Results: HR and BP did not change. Mean \pm SEM

	Pre	ANP Infusion		Peak Post PVS
		1 hour	2 hours	
UnaV mmol/hr	0.5 \pm 3	2.0 \pm 0.1	*3.8 \pm 1.5	6.5 \pm 2
UV ml/hr	69 \pm 15	*173 \pm 41	*173 \pm 23	194 \pm 38
UcGMP nmol/hr	77 \pm 19	160 \pm 48	*227 \pm 36	140 \pm 20
C ₁ PAH ml/min	596 \pm 92	371 \pm 29	507 \pm 84	
C ₂ ml/min	68 \pm 6	58 \pm 4	79 \pm 12	
ANP pg/ml	46 \pm 7	**228 \pm 33	**219 \pm 26	280 \pm 20

*p < 0.05 **p < 0.01 compared to preinfusion baseline.

The peak natriuresis, induced by ANP was 3.8 \pm 1.5 mM/hr, compared to 6.5 \pm 2 mM/hr post PVS. Indeed, 2 of the infused patients had a poor natriuresis (<2.0 mM/hr) despite elevated ANP and increased UcGMP. Furthermore there was no correlation between UnaV and UcGMP. In conclusion, simply greatly increasing the pANP may not be sufficient to overcome the sodium retaining lesion in some cirrhotic patients.

A PERTUSSIS TOXIN-SENSITIVE VASOPRESSIN V₁ RECEPTOR IN RABBIT CORTICAL COLLECTING TUBULE (RCCT) CELLS. Maria A. Burnatowska-Hledin* and William S. Spielman. Michigan State University, East Lansing, Michigan.

The effect of arginine vasopressin (AVP) on cytosolic free calcium concentration ($[Ca^{+2}]_f$) was examined in freshly immunodissected rabbit cortical collecting tubule (RCCT) cells using the fluorescent Ca^{+2} indicator fura-2. The addition of AVP to a cell suspension resulted in a rapid, transient, and dose-related (ED₅₀: ~50 nM) increase in the $[Ca^{+2}]_f$. 1-deamino-8-D-arginine VP (dDVP), a V₂ receptor agonist of AVP which stimulated adenosine 3',5' cyclic monophosphate (cAMP) production in these cells, had no effect on $[Ca^{+2}]_f$, and did not affect AVP induced increase in $[Ca^{+2}]_f$. The AVP induced increase in $[Ca^{+2}]_f$ but not the increase in cAMP was blocked by the V₁ receptor antagonist, d(CH₂)₅Tyr(Me)AVP. The AVP stimulated increase in $[Ca^{+2}]_f$ appeared to be largely due to Ca^{+2} release from intracellular stores as reduction of extracellular Ca^{+2} with EGTA had no effect on the AVP-induced increase in $[Ca^{+2}]_f$. Finally, the AVP-stimulated increase in $[Ca^{+2}]_f$ appears to involve a guanine nucleotide binding protein (G), as the pretreatment of cells with pertussis toxin for 4-6 hrs inhibited this effect. These results demonstrate that cells of the cortical collecting tubule possess two distinct receptor systems for vasopressin, the well-known V₂ receptor coupled to adenylate cyclase, and a pertussis toxin-sensitive V₁ receptor system that leads to the mobilization of cytosolic Ca^{+2} , presumably coupled through a G protein to a phospholipase C-dependent production of inositol phosphates.

EFFECTS OF PREGNANCY AT TERM AND DELIVERY ON ANF, PLASMA ALDOSTERONE (A) AND PLASMA VOLUME (PV) IN NORMAL PREGNANT WOMEN. A Dal Canton*, G Romano, F Uccello, P Veniero, M Altomonte, C Esposito, M Sabbatini, A Di Liato, VE Andreucci. Univ. of Catania* and Univ. of Naples, Italy. (introduced by Giuseppe Andres)

This study was performed to understand whether ANF plays a role in setting PV and antagonizing A in pregnancy at term and in the early postpartum period. h- α -ANF and A were measured by radioimmunoassay and PV was measured by Blue Evans' method in 16 pregnant women 1-3 days before and 1-3 days after delivery. Control consisted of 8 age-matched non-pregnant women. In pregnant women before delivery ANF averaged 43.4 pg/ml (control 12.7, p < 0.05), PV was 3.97 L (control 2.50, p < 0.01), A averaged 336.0 pg/ml (control 182.2, p < 0.01). After delivery, both average PV and A fell significantly, to 2.98 L and 156.7 pg/ml, respectively. These values were not different from non-pregnant control. By contrast, ANF was unchanged after delivery (mean, 55.0 pg/ml), remaining significantly higher than in control. These results indicate that increased PV and stimulated renin-aldosterone system are associated with high ANF levels in pregnancy at term. Persistent elevation of ANF after delivery, while PV and A become normal, suggests a role of ANF in setting volume homeostasis to non-pregnant normality in the early post-partum period.

β -RECEPTOR MEDIATED REGULATION OF C-AMP IN CULTURED RABBIT RENAL MICROVASCULAR ENDOTHELIAL CELLS (RMVEC). A. Chaudhari, A. Pedram*, S. Gupta*, M. Patel* and M.A. Kirschenbaum, VA Med Ctr and UCI-Long Beach Med Prog, Long Beach, CA 90822.

We have shown that a β -agonist increases intracellular cAMP levels in preglomerular RMVEC suggesting the presence of β -receptors in these cells (The Pharmacologist 29:165, 1987). Further studies were designed to examine: a) if endogenous prostanoids (PG) contribute to the isoproterenol (IP)-induced rise in cAMP previously noted, b) if the IP-induced accumulation of cAMP can be blocked by a β -antagonist, and c) if increased intracellular Ca^{2+} affects IP-induced cAMP accumulation. Confluent monolayers of RMVEC were incubated with IBMX (1 mM, a phosphodiesterase inhibitor) or IBMX + aspirin (ASA, 1 mM, a cyclooxygenase inhibitor) for 10 min prior to test substance addition. Cells were further incubated for 5 min after addition of A23187 (10 μ M), IP (10 μ M) or IP + propranolol (P, 10 μ M). After incubation, the medium was removed, the cells lysed and the released cAMP was measured by RIA. ASA pretreatment decreased cAMP accumulation in IBMX-treated cells. A23187 or IP had no effect in the absence of IBMX pretreatment, however, both increased cAMP (6X and 28X, respectively) in IBMX pretreated cells. IP produced a dose-dependent increase in ASA + IBMX pretreated and non-treated cells. P blocked the IP-induced cAMP accumulation with or without ASA pretreatment. Since ASA pretreatment lowered IP and IP + IBMX induced cAMP accumulation, the data suggest that a major portion of the increased cAMP seen with IP may have resulted from stimulation by endogenous PG. The Ca^{2+} -ionophore provoked fall in IP-induced cAMP accumulation suggest that the fall may have been due to Ca^{2+} -dependent phosphodiesterase activation. These data further support the presence of β -receptors in RMVEC.

RENAL EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) INFUSION IN YOUNG (Y) AND ADULT (A) RATS. R. L. Chevalier, R. A. Gomez, R. M. Carey*, M. J. Peach*, J. Linden*. University of Virginia School of Medicine, Charlottesville, Virginia.

Decreased renal response to circulating ANP has been proposed to explain limited natriuresis following volume expansion in the neonate. To delineate the effects of growth on the response to ANP, rats were anesthetized for study at 31-41 days of age (Group Y, N=10), or in adulthood (Group A, N=6). Kidney weight (KW) was 0.64 ± 0.04 g in Y and 1.42 ± 0.09 g in A. Synthetic rat ANP (28 amino acid peptide) was infused intravenously and plasma ANP concentration ([ANP], pg/ml), urine sodium (UNaV, μ Eq/min/gKW) and urine cyclic GMP (UcGMPV, pmol/min/gKW) excretion were measured at each rate. Results (mean \pm SE):

		ANP infusion rate (ug/kg/min)				
		0.0	0.1	0.2	0.4	0.8
[ANP]	Y	20 \pm 3	86 \pm 27	373 \pm 95	788 \pm 190	1339 \pm 358
	A	42 \pm 6	164 \pm 59	752 \pm 225	2211 \pm 744	3180 \pm 1133
UNaV	Y	.2 \pm .1	.6 \pm .3	1.0 \pm 0.4	1.8 \pm 0.5	2.2 \pm 0.6
	A	.6 \pm .4	1.2 \pm .7	2.8 \pm 1.0	3.5 \pm 0.9	3.1 \pm 0.6
UcGMPV	Y	17 \pm 3	16 \pm 3	31 \pm 4	69 \pm 10	116 \pm 21
	A	9 \pm 2	23 \pm 7	80 \pm 20	156 \pm 34	172 \pm 22

The slope of [ANP], UNaV, or UcGMPV to ANP infusion rate was higher for A than Y ($p < 0.05$), while there was no difference in the ratio of [ANP] to UNaV or UcGMPV. We conclude that the apparent increase in renal response to exogenous ANP with growth is due to decreasing ANP clearance with age. The response of UNaV and UcGMPV to circulating ANP does not change from 30 days to adulthood.

DONOR SPECIFIC TRANSFUSION (DST) PRESERVES FUNCTION AND REDUCES THROMBOXANE (TX) PRODUCTION BY RAT RENAL ALLOGRAFTS WITHOUT AFFECTING INFLAMMATORY CELL INFILTRATION. T.M. Coffman, P. Ruiz*, F. Sanfilippo, W.E. Yarger, and P.E. Klotman. Duke Univ. and Durham VA Medical Centers, Durham, NC

DST is associated with improved graft survival following renal transplantation. In order to investigate the mechanisms by which DST might improve transplant outcome, we evaluated the effects of DST on both the characteristics of cellular infiltrates and production of the vasoconstrictor eicosanoid TXA₂ by rat renal allografts. PVG strain recipients were transfused with 0.5 ml of whole blood from autologous PVG (ABT) or donor ACI rats (DST). One week later, transfused PVG recipients received kidneys from ACI donors. 6 days following transplantation, C_{IN} (4.54 ± 0.60 ml/min/kg) and C_{PAH} (17.54 ± 2.43 ml/min/kg) in the DST group were significantly increased compared to the ABT group (0.30 ± 0.20 and 1.17 ± 0.67 ml/min/kg; $p < 0.0005$). TXB₂ production by *ex vivo* perfused allografts was significantly reduced in the DST group (272 ± 66) compared to the group which received ABT (1798 ± 462 pg/min; $p < 0.01$). Despite these marked differences in allograft function and eicosanoid metabolism, there were no differences in the prevalence or pattern of infiltrating inflammatory cell subsets including OX-42 (rat monocyte/macrophage), OX-1 (rat common leukocyte), OX-8 (cytotoxic/suppressor T lymphs), and W3/25 (rat helper/inducer). Similarly, there were no differences between the DST and ABT groups in the phenotypic profiles of cells isolated from allografts as assessed by flow cytometry. Thus, DST induces a preservation in renal allograft function and is associated with reduced TX production. A portion of the beneficial effects of DST may be mediated by TX inhibition. Furthermore, this TX inhibition is not associated with significant alterations in the number or phenotypic characteristics of cells infiltrating the allograft.

NATRIURESIS AND ANF SECRETION INDUCED BY WATER DRINKING IN HEALTHY HUMANS. Elle Cogan*, Marie-France Debiève*, Piedad Calderon*, Joëlle Nortier* and Maurice Abramow* (intr. by J.J. Grantham) Dept. of Physiol. and Pathophysiol., Free Univ. of Brussels (U.L.B.), Belgium.

Atrial natriuretic factor (ANF) is secreted by the atria under various hypervolemic stimuli. In addition, an increased natriuresis may be associated to water retention in inappropriate antidiuresis. We thus studied ANF secretion and natriuresis in 7 healthy humans submitted to a standard oral water loading test (20 ml bottled water/kg body weight). Blood and urine were collected before and hourly (4 times) after water loading for determination of electrolytes, urea, creatinine and osmolality. Plasma renin activity (PRA), plasma angiotensin II (pAll), aldosterone (pAldo) and ANF (pANF) were determined by RIA. Water loading induced a transient 3.5% increase in body water and was associated to an increase in pANF (from 4.07 ± 0.39 to 8.12 ± 1.53 fmoles/ml; $p < 0.01$) and in natriuresis (from 91 ± 13 to 182 ± 23 μ moles/min; $p < 0.01$). After 4 hours, 105% of the ingested water were excreted; pANF (5.09 ± 1.27) was not significantly different from control values. The natriuresis decreased but remained above preloading value (142 ± 23 ; $p < 0.02$). Water loading tests were performed in the same subjects after intranasal administration of desmopressin. In these conditions, only 14% of the ingested water were excreted after 4 hours. A persistent water retention occurred and was associated with a sustained increase in pANF (C: 3.64 ± 0.26 ; peak: 7.13 ± 1.24 ; $p < 0.01$; after 4 hours: 5.73 ± 1.16 ; $p < 0.02$). The natriuresis increased from 89 ± 11 to 196 ± 21 ($p < 0.01$) and remained $139 \pm 19\%$ above the control value after 4 hours ($p < 0.02$). In addition, a delayed decrease in PRA, pAll and pAldo was also observed. When the data of all the tests were pooled, a significant relationship was noted between the increases in pANF and the corresponding natriuresis ($r = 0.49$; $p < 0.001$). It is concluded that water absorption is a physiological stimulus for ANF secretion in humans. We suggest that during water expansion, a small increase in pANF may contribute to volume adaptation in part by increasing transiently the urinary sodium output.

*Spa Reine®, Belgium (Na content: less than 0.13mmoles/L)

EFFECTS OF COMBINED ATRIAL NATRIURETIC PEPTIDE (ANF) AND DOPAMINE (D) ON ACUTE RENAL ISCHEMIA. J.D. Conger, S.A. Falk,* and R.W. Schrier. V.A. Med. Ctr. and U. Colorado Sch. Med., Denver, CO.

ANF corrects the decrease in glomerular filtration rate (GFR) induced by renal artery (RA) clamping. However, a decline in systemic blood pressure (BP) accompanies the improved GFR. Also, the glomerular mechanism of GFR improvement is unclear. In this study ANF (0.2 µg/kg/min) and D, sufficient to maintain BP >100 mmHg were infused i.v. for 240 min after 50 min RA clamp in Munich-Wistar rats. Control (C) rats were infused with saline vehicle. BP, renal blood flow (RBF), GFR, urine flow (V), glomerular plasma flow (QA), single nephron GFR (SNGFR), transcapillary glomerular hydraulic pressure (ΔP), transcapillary colloid osmotic pressure (ΔΠ), and glomerular ultrafiltration coefficient (K_f) were measured during the final 60 min of infusion.

Whole kidney and micropuncture results:

	BP mmHg	RBF ml/min	GFR ml/min	V µl/min
C	110±9	4.0±1.5	.362±.12	10±10
ANF-D	102±5	7.2±2.6*	1.06±.34*	87±57*

	QA nl/min	SNGFR nl/min	ΔP mmHg	ΔΠ mmHg	K _f nl/s/mmHg
C	174±114	32±13	33±4	16±2	.0388±.0215
ANF-D	250±17	57±5*	50±5*	16±1	.0306±.0042

The rise in P was the result of a markedly elevated glomerular capillary pressure (P_{GC}) (69±7 in ANF-D vs 49±1 mmHg in C, p<.001).

Conclusions: (1) ANF-D protects BP while improving post-ischemic GFR. (2) The mechanism for increase in GFR is a rise in P_{GC}.
*Different at minimum of p<.05.

INHIBITION OF ANGIOTENSIN I CONVERTING ENZYME PREVENTS THE POSTPRANDIAL INCREASE IN GLOMERULAR FILTRATION RATE. Corman B.* and J.B. Michel* (introd. by D.Z. Levine). Dept of Biologie, CEN Saclay, Gif-sur-Yvette and INSERM U36, Paris, France.

The role of the renin-angiotensin system in postprandial hyperfiltration was investigated in 3 month-old conscious rats which were regularly fed a 17% protein diet or fasted for 24h. In controls the glomerular filtration rate was 45% greater in fed than in fasted animals but plasma renin concentration did not significantly differ (17.7 ± 1.6 in controls and 15.7 ± 4.7 ng AI/ml/h, n=5 in fasted animals). In a second experimental series, converting enzyme activity was chronically inhibited by daily administration of perindopril. Plasma renin concentration rose to 407 ± 95 and 774 ± 67 ng AI/ml/h (n=6) in fed and fasted conditions respectively and converting enzyme activity fell to 6.1 ± 1.7 and 5.7 ± 0.5 mU/ml. Inulin clearance was similar in the fed and fasted treated animals.

	Control (n=6)	Converting enzyme inhibition (n=6)
Fed	1.73 ± 0.11	1.25 ± 0.05
Fasted	1.17 ± 0.08	1.22 ± 0.03

glomerular filtration rate (ml/min/g. kidney)

We conclude that the renin-angiotensin system is involved in the renal response to food intake and that postprandial hyperfiltration can be prevented by converting enzyme inhibition.

LACK OF L-DOPA EFFECT ON Na-K PUMP ACTIVITY OF CULTURED RAT RENAL PROXIMAL TUBULAR CELLS (RPTC). M. Grabos,* A. Aperia and C. Lechene. Harvard Medical School, Boston and Karolinska Institute, Stockholm.

Dopamine is synthesised in proximal tubule cells. Because dopamine inhibits ATP hydrolysis by Na-K ATPase in rat microdissected proximal tubules, this suggests that it may be regulating Na-K pump activity in RPTC. Na-K pump rate was measured using electron probe analysis as ouabain sensitive K influx and Na efflux on 3 day cultures of RPTC incubated in the presence or absence of l-dopa, a dopamine precursor. Studied were performed at several levels of Na-K pump rate. Steady state pump rates were varied by increasing intracellular Na concentration (Na_i) by preincubation in medium lacking K. Rapid changes in pump rates were obtained by using the cation ionophore monensin (10µg/ml, 5 min.) or by activating Na-H exchange using preincubation in NH₄Cl (15mM) followed by its abrupt removal. Some cells were cultured from chronically salt-loaded rats to increase decarboxylase, an enzyme which converts l-dopa to dopamine. Some experiments were performed in the absence of amino acids to increase a possible Na/l-dopa cotransport. Among all these conditions, Na-K pump rate increased by a factor of 5, essentially in parallel to increase in Na_i between extremes from 0.022 to 0.117 (mM K/mM P/min) in cells maximally Na loaded. In none of these conditions did l-dopa addition (10⁻⁴M, 5 to 60 min) change pump rates obtained in l-dopa absence. In normal, low Na_i cells, mean pump rate values were 0.041 ± .004 without vs. .036 ± .004 (n=14) with l-dopa, and over a range of increased Na-K pump rates, mean values were .066 ± .007 vs .063 ± .009 (n=15) (mM K/mM P/min) ± SE. In conclusion, in RPTC, Na-K pump rate, which may vary broadly with Na_i, remains insensitive to l-dopa. This lack of effect on Na-K pump rate may be due to lack of intracellular l-dopa entry, major decrease in decarboxylase, or lack of regulatory effect of dopamine on the Na-K pump of cultured RPTC.

ATRIAL NATRIURETIC PEPTIDE (ANP) RESPONSE TO PHYSIOLOGIC MANEUVERS IN CARDIAC TRANSPLANT PATIENTS. Howard M. Cushman, John P. Mulrow*, John B. Copley, Rick L. Latham*, Steven Bailey*, and Terrance A. Fried. Brooke Army Med. Ctr. and Univ. Tex. Hlth. Sci. Ctr., San Antonio, Texas.

ANP levels, reportedly, are elevated in cardiac transplant patients. The effect, however, of physiologic maneuvers known to increase ANP release in normal subjects has not been fully characterized in these patients.

Five stable patients 5-11 months post-transplant were brought into balance on a 200 mEq Na diet and then evaluated with high fidelity cardiac catheterization. Right atrial pressure (RAP), and peripheral (PR) and pulmonary artery (PA) ANP levels were measured prior to and during supine submaximal exercise. (Data shown as Mean ± SE)

	RAP (mmHg)	PR (pg/ml)	PA (pg/ml)
REST	5.6 ± 0.9	77 ± 10	144 ± 31
EXER	15.8 ± 2.7	135 ± 23	366 ± 100
P	< .01	< .05	< .05

In addition, four of these patients were brought into balance on a 20 mEq Na diet and then evaluated during central monitoring with acute volume expansion (normal saline 15 ml/kg). The ANP levels increased from (PR) 50 ± 9 and (PA) 78 ± 6 (baseline) to (PR) 96 ± 15 and (PA) 144 ± 29 (volume expansion).

These data suggest that cardiac transplant patients have the ability to respond to both exercise and acute volume expansion with an increase in ANP release. This indicates that despite partial denervation and elevated basal levels, their ANP reserve remains.

COBALT ACTION ON THE HYDROSMOTIC RESPONSE TO ANTIDIURETIC HORMONE IN TOAD URINARY BLADDER. K. Danechi* and M. Bergeron. Univ. of Montreal, Dept of physiology, Montréal, Québec.

To investigate the role of extracellular Ca^{++} in the osmium impregnation of the endoplasmic reticulum (Cell Tissue Res. 247:215, 1987) in parallel with the hydrosmotic response to ADH, the effect of cobalt, an inhibitor of Ca^{++} entry, was examined *in vitro* in the toad urinary bladder. Each lobe of the bladder was mounted before excision as a sac on a glass tube. Bladders were stimulated with 10^{-6} M ADH or 10 mM cAMP, 10 min before creating an osmotic gradient by reducing the mucosal [NaCl] to 5.6 mM. The addition of 4 mM $CoCl_2$ to the mucosal bath along with the gradient induction increases the maximal hydro-osmotic response to ADH and cAMP ($30 \pm 7.2\%$ with ADH; $32 \pm 5.5\%$ with cAMP). The addition of 4 mM $CoCl_2$ to the serosal bath decreases the maximum effect of ADH by 40%; when 4 mM $CoCl_2$ is added to the serosal side after the effect of ADH or cAMP had reached the maximum value, the water flow rate drops (within 5 to 20 min) to the baseline whereas addition of cobalt to the mucosal bath creates a transient increase of water flow. These cobalt effects are reversible with washing. These results suggest that the extracellular Ca^{++} (mucosal) has an inhibitory effect on ADH-induced maximum water flow but a stimulatory effect when present on the serosal side. Serosal Ca^{++} is essential for the maintenance of the ADH or cAMP-induced water flow in toad bladder.

OSMOREGULATION IN PREGNANT WOMEN: THE ROLE OF CHORIONIC GONADOTROPIN (hCG). JM Davison,* EA Sheills,* PR Philips,* MD Lindheimer: MRC Reproduction Group, Univ. of Newcastle, Newcastle upon Tyne, UK and Univ. of Chicago, Chicago, IL

In order to characterize changes in the osmotic thresholds (T) of thirst and vasopressin (AVP) release throughout pregnancy (P) 8 women underwent serial hypertonic saline infusion tests starting prior to conception (PC); then during gestational weeks 5-8, 10-12, 28-33, and 10-12 weeks postpartum (PP). Body tonicity already decreased by weeks 5-8 ($P < 0.001$), remained 10 mOsm/kg below PC and PP throughout P. T_{AVP} (defined as the abscissal intercept of regression lines relating P_{AVP} to P_{osm}) and T_{Thirst} (from analogue scales relating degrees of thirst to P_{osm}) were also decreased throughout P, and decrements in T_{Thirst} appeared to precede those of T_{AVP} resulting in a transient increase in 24h urine volumes at 5-8 weeks ($P < 0.05$). Slopes of the regression equations defining P_{AVP} vs P_{osm} (r 's 0.79-0.99) were reproducible PC and PP, and similar at weeks 5-8 and 10-12 compared to non-pregnant values, but were markedly reduced ($P < 0.001$) in late P. These volunteers had also been tested PC after receiving 10,000 IU hCG over 5d, resulting in levels 10 fold lower than in P. Still T_{AVP} and T_{Thirst} decreased 3 and 4 mOsm/kg ($P < 0.01$). Also, one patient with a hydatiform mole manifested decreased P_{osm} , T_{AVP} , and T_{Thirst} which failed to normalize postevacuation until P_{hcg} was barely detectable (at 90d). In contrast, T usually normalizes within 2 weeks after normal P. Conclusion: T_{AVP} and T_{Thirst} decrease during the very first weeks of P, the apparent osmotic sensitivity ($\Delta P_{AVP}/\Delta P_{osm}$) for AVP release decreases in late P, and hCG may influence osmoregulation.

EFFECT OF DIETARY PROTEIN INTAKE ON VASOACTIVE HORMONES. Barbara S. Daniels and T. H. Hostetter, University of Minn. Minneapolis, MN.

Dietary protein intake influences glomerular filtration rate, proteinuria, and the progression of chronic renal disease. However, the humoral mechanism(s) through which these effects occur in humans remains largely unknown. Therefore, we sought to determine the response of several vasoactive hormones to two different levels of dietary protein intake. Seven normal volunteers, two women and five men, ages 22-49 were randomly placed on one of two dietary protein intakes, .55 g protein/kg/day (LOW) or 2 g/kg/day (HIGH) and studied after five days. Indomethacin (INDO) was then administered for one day and subjects were re-studied. After a washout period of at least 7 days, the subjects were studied after five days on the other diet. The diets were identical in calories, sodium, potassium, calcium and phosphate. Blood pressure, pulse and weight were not different on the two diets. (Results mean \pm SEM, * $p < 0.05$) (PRA=Standing plasma renin activity, Aldo=standing plasma aldosterone, AVP=plasma arginine vasopressin, NE=plasma norepinephrine).

	AVP (pg/ml)	NE (pg/ml)	PRA (ng/ml/min)	ALDO (ng/dl)	C_{CREAT} (ml/min)
LOW	1.6 ± 0.2	311 38	3.2 0.7	9.6 1.4	104 6
HIGH	4.3* ± 0.9	244* 13	5.5* .8	18.1* 3.7	147* 13

Thus, dietary protein augments AVP and renin secretion, but is associated with a lower NE. After INDO, PRA remained higher with the high protein diet (PRA $2.32 \pm .56$ on Low, $3.85 \pm .72$ on High) suggesting that the effects of protein on PRA may not depend on prostaglandin synthesis.

GLOMERULAR MICROCIRCULATORY RESPONSES TO PLATELET ACTIVATING FACTOR (PAF) IN THE RAT.

DK DeBoer*, K. Takahashi*, HR Jacobson, and KF Badr. Vanderbilt University, Nashville, TN.

A role for PAF, a potent lipid-derived inflammatory mediator, has been proposed in some forms of experimental glomerular diseases. PAF is known to contract cultured mesangial cells, stimulate mesangial cell PGE2 synthesis, decrease GFR, and increase protein excretion. Its precise effects on the glomerular microcirculation, and its interactions with other pro-inflammatory mediators, however, remain controversial. We therefore examined the effects of selective intrarenal administration of PAF in doses of 12.5, 25, and 50 ng/Kg/min in euolemic male Munich-Wistar rats in the absence or presence of indomethacin (I, 2mg/Kg). Under these conditions, PAF administration was not associated with significant changes in arterial pressure or hematocrit. Mean control (C) values for GFR and RPF (ml/min) versus those obtained during PAF infusion are shown below. *: $p < 0.025$ vs. C.

Dose:	12.5(n=3)	25(n=3)	50(n=9)	25+I(n=3)	50+I(n=4)
GFR C:	1.16	1.20	1.05	1.12	1.18
PAF:	1.13	0.84*	0.61*	1.04	1.06
RPF C:	4.15	3.86	3.66	4.86	4.56
PAF:	3.86	3.09	2.88*	4.52	5.15

Micropuncture measurements (50 ng/Kg/min dose) revealed that PAF infusion induced increases in pre- (Ra) and post (Re) glomerular arteriolar resistances (2.32 ± 0.14 to $2.73 \pm 0.19^*$ and 1.32 ± 0.13 to $1.45 \pm 0.10^*$ 10^{10} dyn·s·cm $^{-5}$, respectively) resulting in a decrease in glomerular plasma flow rate (126 ± 7 to $101 \pm 6^*$ nl/min). The fall in SNGFR, however, (40.6 ± 2.1 to $21.5 \pm 2.5^*$ nl/min) was due mainly to a dramatic reduction of the glomerular ultrafiltration coefficient, Kf [0.058 ± 0.012 to $0.020 \pm 0.003^*$ nl/(s·mmHg)]. Single nephron FF also fell from 0.33 ± 0.03 to $0.21 \pm 0.03^*$.

Thus, PAF increases Ra and Re and decreases Kf, thereby compromising glomerular perfusion and filtration rates. The abrogation of its effects by I strongly suggests the involvement of a vasoconstrictor cyclooxygenase product in the mediation of these biological effects.

DIRECT MEASUREMENT OF ACTIVE (AR) AND TRYPSIN ACTIVATABLE INACTIVE (IR) RENIN IN HYPERTENSIVE INFANTS AND CHILDREN WITH COARCTATION OF THE AORTA: EFFECT OF CAPTOPRIL ADMINISTRATION. Dechaux M*, Blazy I*, Sidi D*, Laborde K*, Sachs C. Necker-Enfants Malades Univ. Paris, France.

As the role of the RAS in the hypertension of coarctation of the aorta is still debated, we studied the effect of captopril (C) on arterial blood pressure (AP) and plasma renin concentration in 20 infants (less than 1-year old) and in 4 children (3-9 year old). They were all hypertensive for age and the diagnosis was assumed on pressure recording during cardiac catheterisation. C treatment, 1mg/Kg body weight, was attempted with a view to delaying surgery. AP was recorded every 5 min, 1 hr before and 2 hrs after C. Blood was sampled before and 1 hr after C. AR and IR were measured using a monoclonal antibody specifically directed against AR. Mean AP decrease after C was observed only in few cases and was then insufficient to normalize AP.

	AR		IR	
	Basal	C +	Basal	C +
INFANTS				
Patients, mean	231 ^{n.s.}	1026 ^{***}	435 ^{n.s.}	614
range	30-1200	83-5200	77-648	10-1630
Controls, mean	133	----	284	----
range	10-308	----	40-592	----
CHILDREN				
Patients, mean	58	339 ^{***}	138	178
range	19-118	78-820	86-216	69-320
Controls, mean	43	----	240	----
range	10-143	----	122-509	----

***, p < 0.001, C+ vs basal ; n.s. patients vs controls

In conclusion, these data do not support the hypothesis of a RAS role in the hypertension of the coarctation of the aorta.

VESICLES, RATHER THAN AGGREGATES, DELIVER WATER-CONDUCTING PARTICLES IN FROG BLADDER. G. Ding,* N. Franki,* J. Bourguet,* and R. M. Hays. Albert Einstein College of Med., Bronx, N.Y. and C.E.N. de Saclay, Gif-Sur-Yvette, France.

The current model of ADH action is based largely on studies of the toad urinary bladder, where long cytoplasmic organelles (aggrephores) fuse and deliver water-conducting particles to the luminal cell membrane. Fused aggrephores can be identified by examining multiple transmission electron microscopic sections of epithelial cells; in toad bladder, 6.0±2.1 fused aggrephores per 100 μm² of luminal membrane surface are present 15 minutes after vasopressin stimulation.

We now report a system in which ADH does not produce aggrephore fusion. In the frog bladder (Rana esculenta), while particle-containing aggrephores are present in the cytoplasm, only 0.1±0.1 fused aggrephores per 100 μm² were seen following oxytocin. In Rana catesbeiana, no fused aggrephores were seen. Although aggrephore fusion was absent, a study in R. esculenta showed an increase in the frequency of luminal membrane pits (an index of vesicle exo- and endocytosis), from 4.2±0.2 in control to 9.3±0.1 in oxytocin-treated bladders (p<0.01). Freeze-fracture of the luminal membrane showed the area of individual particle aggregates to be small, again consistent with vesicular delivery.

We conclude that vesicles, rather than aggrephores, mediate particle delivery in frog bladder. The aggrephore appears to function solely as a post-Golgi particle storage organelle. Upon hormonal stimulation, vesicles which "bud" from the aggrephores, the Golgi, or other sites are released to fuse with the luminal membrane.

INCREASED PLASMA VOLUME AND ATRIAL NATRIURETIC PEPTIDE (ANP) BUT REDUCED DIURESIS FOR AN INTRARENAL DEFECT IN ADRIAMYCIN (ADR) -INDUCED NEPHROTIC SYNDROME (NS). F. Delaini*, N. Perico*, C. Lupini*, M. Tagliaferri* and G. Remuzzi* (intr. by M.J. Dunn). Mario Negri Institute for Pharmacological Research, Bergamo - Italy.

NS was induced in male S.D. rats (n=8) by iv injection of ADR (7.5 mg/kg). 21 days after ADR plasma volume and plasma immunoreactive ANP (i-ANP) were determined. Plasma volume, measured by the dilution principle using ¹³¹I-serum albumin, and plasma i-ANP were significantly higher in nephrotic animals (NS) than in controls (C)

	NS	C
Plasma volume (ml/kg)	69.61 ± 15.02 ^{**}	47.05 ± 5.32
Plasma i-ANP (pg/ml)	104.22 ± 36.41 [*]	59.94 ± 20.88

mean ± S.D.; *p < 0.05, ** p < 0.01 vs C.

Unilateral disease was induced in rats by clamping the left renal artery, injecting iv ADR (3.75mg/kg) and removing the clamp 8 min later. In this model only right kidney (RK) was proteinuric. Marked reduction of diuretic and natriuretic response to 40-min infusion of exogenous atrial extract (AE) occurred in proteinuric RK not in the contralateral one (CK) (urine flow, RK: 6.32±1.02 to 68.51±21.09 μl/min; CK: 5.09±1.56 to 11.74±4.74 μl/min. Na⁺ excretion, RK: 0.70±0.14 to 7.41±3.14 μEq/min; CK: 0.47±0.24 to 1.37±0.42 μEq/min). Comparable increase in GFR during AE infusion was found in both RK and CK (RK:43%; CK:42%). These results show that plasma volume and plasma i-ANP are significantly higher in experimental NS than in normal rats; the blunted diuretic and natriuretic response to exogenous AE observed previously in nephrotic animals (Am J Physiol 1987;252:F654) is a consequence of a renal defect and does not reflect systemic changes.

PGE2 SYNTHESIS AND INTRACELLULAR Ca²⁺ ARE STIMULATED BY BRADYKININ IN CULTURED SMOOTH MUSCLE CELLS OF RENAL CORTICAL ARTERIOLES. Jean-Claude Dussaulte, Josée Sraer, Laurent Baud, Joelle Perez and Raymond Ardaillou. (Intr. by K.F. Badr) INSERM 64, Hôpital Tenon, Paris, France.

Smooth muscle cells were cultured from an arteriole-rich fraction of the rabbit renal cortex and characterized by electron microscopy and by their high content of creatine kinase (60 times that of the initial preparation). Cells were studied after 2-4 passages. As shown by radio-metric HPLC and specific RIA, they produced mainly PGE2 and, to a lesser extent, PGF_{2α}. Basal PGE2 synthesis (7±0.81 ng/mg protein/5 min; m±s.e.m., n=25) increased 60-fold with arachidonic acid and 6-fold with A 23187 Ca²⁺ ionophore (2 μM). Increasing concentrations (0.1 nM-1 μM) of bradykinin (BK) stimulated progressively PGE2 production. The effect of BK (1 μM) was more marked after 5 min (30-40 times basal value) than 60 min (x 15-20) or 24 hr (x 7-9) incubation periods. 50 % maximum stimulation was obtained at 1.6 nM. Des Arg⁹BK was inactive at 10 nM and slightly potent (2 times basal value) at 1 μM. BK also stimulated intracellular Ca²⁺ (Ca²⁺ i). Basal value (133±14 nM) reached 209±37 nM in the presence of 10 nM BK. The specific anti-B2 receptor, Thi 5,8-D Phe 7 BK, markedly inhibited PGE2 synthesis induced by 10 nM BK. Inhibition was 33.5, 84 and 94 % in the presence of 0.1, 1 and 10 μg/ml. The same antagonist abolished the stimulatory effect of BK on Ca²⁺ i. These results demonstrate that renal cortical arteriolar smooth muscle cells possess BK receptors (B2 type) linked to PGE2 production and Ca²⁺ i stimulation, thereby supporting a role for BK in the control of cortical microcirculation.

ATRIAL NATRIURETIC FACTOR IN ACUTE AND CHRONIC CONGESTIVE HEART FAILURE: VARIATION IN STIMULUS/RELEASE RELATIONSHIP AND TARGET ORGAN EFFECTS. Brooks S. Edwards*, Wayne L. Miller*, Robert S. Zimmerman, Michael D. McGoon*, and John C. Burnett, Jr., Mayo Clinic, Rochester, MN

Preliminary studies in a model of acute congestive heart failure (CHF) produced by rapid ventricular pacing (PACE) have demonstrated that atrial natriuretic factor (ANF) is increased and activation of the renin-angiotensin-aldosterone system (RAAS) does not occur despite reductions in arterial pressure. The present studies were designed to investigate the relationships between 1) atrial pressures and ANF, and 2) the RAAS and ANF in both an acute (45 min PACE) and a chronic (3 weeks PACE) model of CHF. Responses to acute and chronic CHF are as follows:

	ACUTE CHF(n=9)	CHRONIC CHF(n=5)
MAP mmHg	92±3	94±7
RAP mmHg	2.2±0.2	12.4±1.7*
PCWP mmHg	11.2±0.7	24.2±2.9*
ANF pg/ml	269±24	316±25
Renin ng/ml/hr	3.54±.33	14.0±3.7*
Aldo ng/dl	4.3±.4	81.1±12.7*

*p<.05 acute versus chronic CHF

The present studies demonstrate that 1) ANF is not increased in chronic CHF beyond that observed in acute CHF despite further increases in atrial pressure, and 2) despite plasma levels of ANF which in the acute state may inhibit activation of the RAAS, the inhibiting action of ANF on the RAAS is not apparent in chronic CHF. We conclude that chronic CHF is characterized by an altered stimulus/release relationship. Further, the RAAS escapes from the inhibiting action of increased circulating atrial natriuretic factor.

VASOPRESSIN SELECTIVELY CONTRACTS RABBIT EFFERENT ARTERIOLES IN VITRO. R.M. Edwards, W. Trizna and L.B. Kinter, Smith Kline & French Labs., Dept. of Pharmacology, Swedeland, PA, USA.

Previous studies suggest that vasopressin (VP) may selectively increase post-glomerular resistance. The purpose of this study was to assess the direct effects of VP on afferent and efferent arterioles *in vitro*. Arterioles were dissected from the cortex and mounted on micro-pipettes. Changes in lumen diameter were recorded videometrically. In afferent arterioles, VP, over a concentration of 10 fM to 10 μM, had no significant effect on lumen diameter. In contrast, significant reductions in lumen diameter (-26.4 ± 4.9%) of efferent arterioles were detected with VP concentrations as low as 1 pM. Half-maximal responses to VP were observed at 0.4 nM as compared to 34 nM for NE. The maximum response to VP was similar to that observed with NE, 83.1 ± 4.6 and 86.7 ± 6.7% decrease in lumen diameter, respectively. Unlike VP, dDAVP, a V₂ agonist, had no significant effect on the efferent arteriole. The response to VP was antagonized in a concentration-dependent but non-competitive manner by the V₁ antagonist, [d(CH₂)₅Tyr(Me)]AVP, and the V₁/V₂ antagonist, [d(CH₂)₅-D-Tyr(Et)Val desGly]AVP. The maximum response to VP was reduced by 64% and 88% by 10 nM of the V₁ and V₁/V₂ antagonists, respectively. The V₂ antagonist, [d(CH₂)₆D-Ile-Val]AVP, had no significant inhibitory effect on VP-induced contractions. The results demonstrate that VP in physiologic concentrations selectively contracts the rabbit efferent arteriole via V₁ receptors. This suggests that VP may play a role in regulating post-glomerular blood flow.

PHARMACOKINETICS OF RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo) ADMINISTERED TO HEMODIALYSIS (HD) PATIENTS. J.C. Egre, J.W. Eschbach*, T. McGuire, and J.W. Adamson. AMGen, Thousand Oaks, CA and Univ. of Washington, Seattle, WA.

The pharmacokinetics of rHuEpo were determined in 11 HD patients. Serum rHuEpo levels, determined by RIA, were compared as a function of route of administration, dose level, and time after start of therapy. Six patients received rHuEpo by an IV route (2 each at doses of 15, 150 and 500 U/kg). rHuEpo was cleared from circulation in an exponential fashion, with an average (±SD) half-life for all dose groups after the first administration of 9.3±3.2 hr. The average calculated volume of distribution was 5.5% of body weight. After 7 treatments, the half-life decreased for 5 of 6 patients, with the average (±SD) being 6.2±1.8 hr. Continued treatment with rHuEpo did not result in a further shortening of half-life after 3 months of therapy. The half-life of rHuEpo after one year of therapy will be presented. Serum rHuEpo levels were examined in 5 patients following subcutaneous administration (2 at 15 U/kg and 3 at 150 U/kg). Following the first administration, peak serum concentrations, which were only 10% of those attained by IV injection of the same dose, were achieved within 8-12 hr and maintained at the peak level for at least the next 12-16 hr. After 7 treatments, peak concentrations were reached and sustained over the same time course, but were only 40-70% of the levels after the first dose. *In vivo* bioassays on selected serum samples confirmed that for all time points tested circulating rHuEpo levels measured by RIA reflected fully biologically active hormone.

ANDROGEN REGULATION OF RAT ANGIOTENSINOGEN MESSENGER RNA DURING DEVELOPMENT.

Kristin E. Ellison*, Julie R. Ingelfinger, Victor J. Dzau. Brigham and Women's Hospital, Division of Vascular Medicine and Atherosclerosis, and The Children's Hospital, Division of Nephrology, Boston, MA.

Previous studies have demonstrated that angiotensinogen (ang-n) mRNA in the male WKY rat increases significantly during puberty. Renal angiotensinogen mRNA levels in the adult female WKY rat are considerably lower than those in the male. To investigate further the mechanism involved in ang-n mRNA induction, male WKY were castrated two weeks prior to sacrifice at prepubertal, pubertal, and postpubertal ages. Quantitative Northern blot analysis with α-p³² labeled ang-n cDNA (pRang 3) demonstrated that castration attenuated the levels of ang-n mRNA in the kidney by >60% at all ages compared to control. In addition female WKY rats were implanted with slow releasing testosterone or control pellets to deliver 2.5 mg testosterone or vehicle per day, and sacrificed twenty days after treatment. A significant increase in ang-n mRNA levels was seen in the testosterone treated female rats as compared to controls. (p<.05) Taken together, these data suggest that androgen regulates intrarenal ang-n mRNA expression. Since it has been shown that androgen influences the structure of the proximal tubule and the expression of transport proteins (e.g., Na⁺/H⁺ antiporter) at this site, our observation may have important physiological implications in the regulation of tubular function during ontogeny.

DOPAMINE-1 REGULATED SODIUM TRANSPORT IN RAT RENAL BRUSH BORDER MEMBRANE VESICLES. Christian C. Felder, Melvin M. Blecher, and Pedro A. Jose. Georgetown University Medical Center, Wash, DC

To determine a tubular mechanism for the natriuretic effect of dopamine-1 agonists we measured the uptake of $^{22}\text{Na}^+$ in rat renal cortical brush border membranes vesicles (BBMV) prepared by differential centrifugation and MnCl_2 precipitation. The BBMV marker gamma glutamyl transpeptidase was enriched 11 fold while the basolateral membrane marker $\text{Na}^+-\text{K}^+-\text{ATPase}$ was decreased 5 fold. The DA-1 agonist SKF 82526 but not the DA-2 agonist LY 171555 inhibited the uphill Na^+ accumulation induced by imposition of a H^+ in H^+ out transmembrane gradient with an IC_{50} of 28.5 μM . The inhibitory effect was stereo selective (R)-SKF38393 > (S)-SKF 38393. The decreased uptake of $^{22}\text{Na}^+$ into BBMV was not due to increased permeability, or vesicle size (sodium and glucose space in BBMV during equilibrium were unaffected). An immediate effect of DA-1 agonists on the Na^+/H^+ exchanger was also evident when the drug was added directly to the BBMV. In addition, the effect of DA-1 agonist on sodium transport persisted when the drug was preincubated with renal tubular cells for 15 min and washed off during BBMV preparation (verified by agonist tracer studies) before the sodium uptake studies. The DA-1 inhibitory effect on BBMV Na^+ uptake was partially blocked by DA-1 antagonist, SCH 23390 (10^{-9} - 10^{-7}M) but not by alpha adrenergic blocker, phentolamine. These studies are compatible with an indirect receptor mediated inhibitory effect of DA-1 agonist on the Na^+/H^+ exchanger; a direct competitive process is also present.

BIPHASIC EFFECT OF LITHIUM ON ADH-STIMULATED WATER FLOW IN TOAD BLADDER: EVIDENCE FOR A MODULATION OF VASOPRESSIN ACTION BY THE PHOSPHOINOSITIDE PATHWAY. Bruno Flamion, Renaud Beauwens, Elie Cogan and Maurice Abramow (Intr. by J.J. Grantham) Dept. of Physiol. and Pathophysiol., Free Univ. of Brussels (U.L.B.), Belgium.

The time - course of the effects of lithium (Li) on vasopressin (VP)-induced water flow in toad urinary bladder was reexamined accurately. Paired toad hemi-bladders bathed in Ringer's solution were incubated with a mucosal hypotonic solution of NaCl or LiCl (11 meq/L) during 3 hours before VP, desmopressin or forskolin (FK) were introduced into the bath. Water flow ($\mu\text{l}/\text{min}/\text{cm}^2$) was determined gravimetrically every 10 min. Li clearly inhibited VP (20mU/ml) - induced water flow : a 17% inhibition detectable 30 min after the VP challenge (Li: 2.96 ± 0.15 ; Na: 3.58 ± 0.25 ; $n=10$; $p<0.05$) increased with time to 30% after 120 min ($p<0.001$). Li similarly inhibited the hydrosmotic action of desmopressin (20 mU/ml) and FK (10 μM). During the first 10 min, however, Li unexpectedly stimulated VP water flow by 39% (Li: 2.73 ± 0.15 ; Na: 1.97 ± 0.22 ; $p<0.02$). In contrast, Li failed to stimulate the early hydrosmotic effect of either FK, which directly stimulates the catalytic unit of adenylate cyclase (AC), or desmopressin, a specific V2 agonist. In the toad bladder, stimulation of V1 receptors is thought to inhibit the AC through the activation of protein kinase C by diacylglycerol (DAG), a second messenger of the phosphoinositide pathway. The suppression of that inhibitory pathway by Li - a well known inhibitor of the phosphoinositide metabolism - may explain the early stimulation of VP action we observed. In order to prevent the depletion by Li of DAG content, we preincubated the bladders with myo-inositol. In these conditions, the early hydrosmotic effect of VP was not significantly affected by Li. Similarly, exposure to phorbol-myristate-acetate, which mimics the action of DAG, suppressed the Li stimulatory effect on VP action. In conclusion, the early stimulatory effect of Li on VP-induced water flow in toad bladder may explain why its inhibitory effect could not be demonstrated previously by all authors. It brings further evidence that VP is able to modulate its own action on AC by stimulating V1 receptors and activating the phosphoinositide pathway.

THE EFFECT OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN PATIENTS WITH CHRONIC RENAL FAILURE. Thomas Fleischhauer, Terrance A. Fried, and Jay H. Stein. University of Texas Health Science Center, San Antonio, Texas.

ANP has been reported to increase glomerular filtration rate and sodium excretion rate in a rat model of chronic renal failure. In the present study, we investigated the effect of 0.5, 1.5, and 3.0 $\mu\text{g}/\text{kg}$ intravenous boluses of anaritide, a 25-amino acid synthetic analogue of ANP, in six patients with chronic renal failure. The mean basal creatinine clearance for these patients was $18.3 \pm 3.0 \text{ ml}/\text{min}/1.73\text{m}^2$.

The effect of anaritide on urine flow rate and sodium excretion rate varied considerably from patient to patient, with some demonstrating no response and others a definite response. Overall, there was no significant effect of anaritide on urine flow rate, sodium excretion rate or creatinine clearance to any of the doses tested. There was, however, a significant positive correlation between the basal creatinine clearance and the change in urine flow rate ($R = .69$, $P = .04$) and the basal creatinine clearance and the change in sodium excretion rate ($R = .86$, $P = .03$).

These data suggest that atrial natriuretic peptide is not an effective diuretic or natriuretic in patients with advanced renal insufficiency. This may be a consequence of these patients having maximized their ANP sensitive sodium and water excretory mechanisms.

INTRATUBULAR EFFECT OF ADENOSINE A1 ANALOG ON TUBULOGLOMERULAR FEEDBACK (TGF) MECHANISM. M. Franco, P. D. Bell and L. G. Navar. University of Alabama at Birmingham, Birmingham, Alabama.

To evaluate further the role of adenosine on the TGF mechanism, we studied the effects of intratubular administration of an adenosine analog and an antagonist on feedback mediated changes in stop flow pressure (SFP). Retrograde microperfusion experiments were conducted in anesthetized rats using the A1 receptor agonist, N6 cyclopentyladenosine (CPA, $n=8$). In control experiments, SFP decreased by $10 \pm 0.8 \text{ mmHg}$ during perfusion with an isotonic solution and by $1.8 \pm 0.4 \text{ mmHg}$ during perfusion with a hypotonic solution (40 mOsm). Addition of 360 nM CPA to the hypotonic solution, markedly enhanced feedback responses; SFP decreased from 37.8 ± 0.7 to $24.6 \pm 1.6 \text{ mmHg}$ during retrograde perfusion with CPA. The antagonist, 1,3-dipropyl-8-sulphophenylxanthine (PSPX) was used to block adenosine receptors. In this series ($n=9$), perfusion with the hypotonic solution containing 360 nM CPA alone decreased SFP by $11.1 \pm 0.7 \text{ mmHg}$. Addition of 10mM PSPX to the CPA containing solution, attenuated SFP feedback responses to less than 1 mmHg ($\Delta = 0.44 \pm 0.5$); values similar to those obtained with the hypotonic solution alone. PSPX also inhibited feedback responses obtained with an isotonic solution alone ($n=9$), SFP responses were decreased from the control value of $10.7 \pm 1.2 \text{ mmHg}$ to $1.1 \pm 0.4 \text{ mmHg}$. These data demonstrate that intraluminal administration of an adenosine A1 agonist stimulates TGF mediated transmission of vasoconstrictor signals and therefore supports a role for adenosine receptors in the signal transmission pathway.

ABNORMAL cAMP CATABOLISM IN COLLECTING TUBULES OF MICE WITH HEREDITARY NEPHROGENIC DIABETES INSIPIDUS (NDI-mice). Susan Gapstur*, Sumiko Homma*, Aline Coffey*, Heinz Valtin, and Thomas P. Dousa, Mayo Clinic, Rochester, Minnesota and Dartmouth Med. School, Hanover, New Hampshire.

We previously found that abnormally high cAMP hydrolysis by phosphodiesterase (PDE) in medullary collecting tubules (MCT) of NDI-mice prevents the increase of cAMP accumulation in response to vasopressin (AVP). Now we studied the effects of PDE inhibitors on the cAMP accumulation in cortical collecting tubules (CCT), papillary collecting ducts (PCD) as well as in MCT microdissected from control mice (C-mice) and NDI-mice. The PDE activity was significantly higher not only in MCT, but also in CCT and PCD of NDI mice. The cAMP accumulation in response to 10^{-6} M AVP was much lower (3-15X) in CCT, MCT and PCD of NDI-mice than in C-mice. Addition of 1-methyl-3-isobutyl-santhine (MIX), a nonspecific inhibitor of PDE, caused cAMP elevation in tubules of both C-mice and NDI-mice. However, addition of 0.1 mM rolipram (RP) with 0.1 mM cilostamide (CS), selective inhibitors of cAMP-specific PDE isozymes (PDE-III type), had little or no effect on the AVP-dependent cAMP accumulation in MCT and PCD of controls. In contrast, addition of RP with CS greatly increased (10 X) the AVP-dependent cAMP in MCT or PCD of NDI-mice, up to the levels found in C-mice. Results suggest that excessive activity of cAMP-PDE, probably PDE-III type, in all subsegments of collecting tubule system of NDI-mice is a major cause of failure to accumulate cAMP in response to AVP. Specific inhibitors of PDE-III isozymes can correct completely this anomaly in vitro.

GLOMERULAR PROSTAGLANDINS (PGs) PRODUCTION IN COMPLEMENT DEPLETED ANTI-GLOMERULAR BASEMENT MEMBRANE (AGBM) GLOMERULONEPHRITIS (GN) IN RATS. J. Garcia-Esteban*, R.J. Roman, J. Garancis and E. Lianos. Medical College of Wisconsin, Milwaukee, WI.

Renal prostanoids participate in the changes in renal hemodynamics produced by AGBM antibody. Cobra venom factor (CVF) improves the course of the acute GN induced by AGBM antibody. We examined whether this effect was due to changes in glomerular PG production. Control (n=14) and CVF-pretreated (n=14) Munich-Wistar rats were given AGBM (n=7) or non immune serum (n=7) and changes in RBF and GFR were measured for 2 hours. GFR decreased from a control of 1.31 ± 0.13 ml/min/g by 45% and 25% respectively 1 and 2 hr after injection of AGBM. RBF increased 26% 1 hr after the injection of the immune serum from a control value of 5.83 ± 0.48 ml/min/g and returned to control during the second hour. Complement depletion with CVF did not alter control GFR (1.56 ± 0.05 ml/min/g) but blunted the fall in GFR after AGBM. GFR was reduced by only 20% 1 and 2 hours after AGBM injection in CVF pretreated rats. CVF had no effect on changes in RBF produced by AGBM. Production of PGE₂ by isolated glomeruli was not elevated by AGBM in control or CVF treated rats. Glomerular thromboxane B₂ production was increased three-fold after AGBM in both the control and CVF treated groups. These data suggest that changes in glomerular PGE₂ or TXB₂ production are not the mechanism by which CVF blunts the fall in GFR after AGBM. Moreover, the change in RBF produced by AGBM is not affected by complement depletion.

IS VASOPRESSIN INVOLVED IN COMPENSATORY RENAL HYPERTROPHY? Geelen G.* and B. Corman* (introd. by D.Z. Levine). Université Claude Bernard Lyon and Dept of Biol. CEN Saclay, Gif-sur-Yvette, France.

Since chronic administration of vasopressin increases kidney size, a role of antidiuretic hormone in compensatory renal hypertrophy seemed possible. This possibility was investigated as follows: in an initial series of experiments, changes in vasopressin plasma concentration following ablation of one kidney were investigated in Wistar rats. Unilateral nephrectomy was performed under ether anesthesia and plasma vasopressin concentration was determined in the second week following surgery. Results were compared with that of control intact animals: the values did not differ in the two groups being 1.8 ± 0.5 ng/ml (n=8) in the operated and 1.9 ± 0.4 ng/ml (n=8) in the control animals. In a second series left kidney was removed from Brattleboro homozygote rats with hereditary hypothalamic diabete insipidus, i.e. which lack circulating vasopressin and in heterozygote Brattleboro rats with circulating vasopressin. Three weeks later, the remaining kidney was compared to kidneys of sham-operated rats. After uninephrectomy, the right kidneys of homozygote Brattleboro rats were 32% larger than those of sham-operated animals. Furthermore, the amplitude of the hypertrophy was similar to that obtained in Brattleboro heterozygote rats with circulating vasopressin.

These results indicate that vasopressin is not essential to compensatory renal hypertrophy in the rat.

PERTUSSIS TOXIN BLOCKS ALPHA₂-ADRENOCEPTOR-MEDIATED INHIBITION OF VASOPRESSIN ACTION. M. Gellai* and R. Edwards, Smith Kline & French Labs., Dept. of Pharmacology, Swedeland, PA, USA.

In vivo and in vitro studies were performed to assess the mechanism of inhibition of the antidiuretic effect of vasopressin (VP) by the selective alpha₂-agonist, B-HT 933. In conscious Brattleboro homozygous (DI) rats (n=4), the antidiuretic effect of sustained VP infusion (10 pg/min, i.v.) was completely inhibited by infusion of 25 µg/min of B-HT 933 (control). Pretreatment of the same DI rats with pertussis toxin (PT), 2 µg/kg, i.v. 4-5 days prior to testing, abolished the inhibitory effect of B-HT 933. Values for urine flow (µl/min.100 g) were (mean ± SEM):

	Baseline	VP	VP + B-HT 933
Control	81.5 ± 7.1	11.9 ± 1.3	103.9 ± 11.4
PT	99.5 ± 2.1	16.6 ± 5.5	$8.9 \pm 5.1^*$

*p<0.05 vs. control

In medullary collecting tubules (MCT) of DI rats, B-HT 933 produced a significant inhibition of VP-stimulated cAMP formation. This effect was competitively inhibited by rauwolfscine (alpha₂-antagonist), but not by prazosin (alpha₁-antagonist). In the MCT taken from the PT-treated DI rats used in the in vivo studies, the inhibitory effect of B-HT 933 was abolished. The results indicate that B-HT 933-induced diuresis is due, at least in part, to the inhibition of the tubular action of VP by alpha₂-adrenoceptor stimulation. Furthermore, it appears that this inhibition is due to the coupling of alpha₂-adrenoceptors to adenylate cyclase via the inhibitory guanine nucleotide binding protein.

MECHANISM OF INHIBITION BY LITHIUM (Li) OF VASOPRESSIN (VP)-SENSITIVE ADENYLATE CYCLASE. H. Goldberg*, P. Clayman*, and K. Skorecki. Membrane Biology Group and Dept. Medicine, Univ. of Toronto Toronto, CANADA.

We have investigated the mechanism for Li induced inhibition of VP-stimulated cAMP production in the renal epithelial cell line, LLCPK₁. In LLCPK₁ membranes, Li caused direct inhibition of hormone stimulated adenylate cyclase activity by competing with magnesium (Mg). 50% inhibition occurred at 20 mM Li. The V_{max} but not K_m for activation by VP was altered. Activation by GTP and its non-hydrolyzable analogs was also inhibited by Li. Furthermore, kinetic studies revealed that the lag phase in the activation of adenylate cyclase by Gpp(NH)p was prolonged from 1 to 3 min. suggesting an effect of Li on Mg dependent activation of the stimulatory guanyl nucleotide regulatory protein, G_s. The function of the corresponding inhibitory guanyl nucleotide regulatory protein, G_i, was assessed by GTP-inhibition of VP-stimulated adenylate cyclase, in the absence and presence of pertussis toxin, and was unaffected by Li.

Intact LLCPK₁ cells incubated in 10 mM Li (approximate urinary concentration in Li treated patients) attained an intracellular Li concentration of 17 mM which led to a 40% reduction in cAMP formation. Magnesium loading of intact cells reversed the inhibitory effect of Li.

It is concluded that Li directly inhibits the activation of VP-sensitive adenylate cyclase in renal epithelia by competing with Mg for activation of G_s. This direct effect on G_s activation accounts for the inhibitory effect of Li on cAMP production in the intact cell.

HYDROXYEICOSATETRAENOIC ACIDS (HETES), MESANGIAL CELLS (MC), AND PROSTAGLANDIN PRODUCTION. Joel A. Gordon, Russell F. Husted, John B. Stokes, and Arthur A. Spector* Depts. of Internal Med and Biochemistry, University of Iowa College of Medicine, Iowa City, IA.

In glomerulonephritis, inflammatory cells invade the glomerulus and upon activation, release mediators such as the HETES in the vicinity of MC. To determine if the macrophage (12- and 15-HETE) and/or neutrophil (5-HETE) lipoxygenase derivatives of arachidonic acid (AA) interact with MC, primary cultures of rat MC were grown until confluent and incubated for 0.5 to 4 hr with 1.0 μM [³H] 5₃, 12- or 15-HETE and for comparison, 1.0 μM [³H] AA. At 4 hr, uptake was greatest for AA (12.6 nmol/mg protein) followed by 5-HETE (8.9 nmol/mg protein). Uptake of 12- and 15-HETE was less (1.8 and 1.4 nmol/mg protein). Thin layer chromatography revealed the majority (>60%) of the radioactivity of all 3 HETES and AA was incorporated into cellular phospholipids, predominantly the choline phosphoglycerides. However, AA and 15-HETE were also substantially incorporated into the inositol phosphoglycerides (AA 21% and 15-HETE 19% at 4 hr). To assess possible functional consequences of this interaction, we measured PGE₂ production by RIA. After a 2 hr incubation with 5.0 μM 5-HETE and a 20 min stimulation by 2.0 μM A23187, PGE₂ production was decreased by 47%. These findings suggest that all 3 lipoxygenase derivatives are capable of biochemical interaction with MC. The reduction in PGE₂ production by 5-HETE further suggests a potentially important role for this inflammatory mediator in modifying glomerular function.

ENHANCED ATRIAL NATRIURETIC PEPTIDE RELEASE IN DOGS WITH REDUCED RENAL MASS DURING CHRONIC SODIUM LOADING. J.P. Granger, M.J. Solhaug*, J.W. Scott*. Depts. of Physiology and Pediatrics, Eastern Virginia Medical School, Norfolk, Va. 23501

The importance of atrial natriuretic peptide in the maintenance of sodium (Na) balance during chronic increases in Na intake is unclear. The purpose of this study was to determine the response of the ANP system to chronic increases in Na intake in normal dogs and in dogs with reduced renal mass --5/6 nephrectomy, (n=7). Na intake was increased from 5 to 50 to 275 mEq of Na per day by progressive increases in isotonic saline infusion. The animals were maintained on each intake for 7 days. At a Na intake of 5 mEq/day, glomerular filtration rate (GFR) and mean arterial pressure (MAP) averaged 59±9 ml/min and 99±4mmHg, respectively, and did not change significantly at intakes of 50 and 275 mEq/day. Increases in Na intake in normal dogs had no significant effect on plasma ANP. In contrast, increases in Na intake in dogs with reduced renal mass resulted in a 120% increase in plasma ANP (28.0±1.3 to 61.0±3.3 pg/ml). In addition, MAP increased by 14% during increases in Na intake in dogs with reduced renal mass. GFR did not change significantly in the reduced renal mass group, averaging 15.7±4.5 ml/min throughout the study. Thus, under normal conditions, increases in plasma ANP do not appear to be essential for the maintenance of Na balance during chronic increases in Na intake. However, in animals with reduced renal mass, increases in plasma ANP as well as MAP may be important mechanisms for maintaining Na balance.

PHORBOL MYRISTATE ACETATE ENHANCES PHOSPHOLIPASE A₂ ACTIVITY IN GLOMERULAR MESANGIAL CELLS. Joseph H. Gronich*, Raphael A. Nemenoff* and Joseph V. Bonventre. Mass Gen. Hosp. and Harvard Medical School, Boston, MA.

Phospholipase A₂ (PLA₂) is an important enzyme in mesangial cell prostaglandin synthesis. We have examined the effects of protein kinase C activation with phorbol myristate acetate (PMA) on PLA₂ activity. Arachidonic acid (AA) release was monitored in cells stimulated with the Ca²⁺ ionophore, A23187 (0.1 μM), PMA (300 nM), or A23187+PMA, and results compared to those obtained with vasopressin (AVP, 100 nM). The levels of free AA (% total radioactivity) in response to each agonist were as follows: Control, 3.0±0.4; A23187, 5.3±1.4; PMA, 2.9±0.3; PMA+A23187, 10.5±1.7; AVP, 11.0±2.7. Therefore while PMA alone had no effect, it acted synergistically with A23187 to enhance PLA₂ activity. The effects of AVP were mimicked by A23187+PMA, suggesting that under normal conditions of stimulation with AVP both the associated increase in cytosolic free Ca²⁺ concentration and the stimulation of protein kinase C activity are important for PLA₂ activation and prostaglandin synthesis. To further explore these actions of PMA we stimulated cells for 10 min with PMA and then assayed PLA₂ activity in crude homogenates and supernatants of 200,000 g sedimentation. PLA₂ activity was assayed in the presence of EGTA or 1 mM [Ca²⁺]. Ca²⁺ dependent PLA₂ activity was enhanced in preparations from cells previously stimulated with PMA.

In conclusion, PMA enhances Ca²⁺ stimulated PLA₂ activity in glomerular mesangial cells presumably by phosphorylation of PLA₂ or PLA₂ modulatory proteins.

ATRIAL NATRIURETIC PEPTIDE (ANP) RECEPTOR-MEDIATED PARTICULATE GUANYLATE CYCLASE (PGC) ACTIVITY IN RABBIT INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. **M Gunning***, P Silva, BM Brenner, ML Zeidel. Harvard Med. Sch. Boston, MA.

Our previous characterization of equilibrium binding kinetics of ANP to the surface of IMCD cells suggested the existence of a single class of high affinity receptors, functionally coupled to increases in cellular cyclic GMP. We have now sought to identify the structural and functional characteristics of this receptor, by covalently binding ^{125}I ANP to it, and by assessing associated GC activity. Covalent binding was carried out with 1mM Disuccinimidyl suberate under equilibrium binding conditions. Following solubilization, polyacrylamide gel electrophoresis demonstrated two specifically labeled bands with M_r 128 \pm 5KD and 78 \pm 6 KD (Mean \pm SD, n=5). Because the higher M_r band has been associated with PGC, IMCD cells were homogenized, and a membrane pellet, made by high speed centrifugation, was assessed for PGC activity. GC activity with and without ANP was linear up to 5 min and was stimulated by ANP (143 \pm 38 vs 38 \pm 12 pMoles/mg prot: n=3). V_{max} increased >3 fold with ANP (116 \pm 35 vs 35.4 \pm 8.6 pMol/min/mg prot: n=4) without significant change in the K_m (.55 \pm 1.7 vs .67 \pm .34 mM: n=4) of the enzyme. K_m Half maximal stimulation of GC activity occurs at 5×10^{-10} M ANP, a concentration consistent with our binding data, and with physiological effect. ANP did not stimulate enzyme activity in the supernatant. We conclude that binding to the ANP receptor results in stimulation of PGC, increasing its V_{max} and thereby elevating intracellular cGMP max , the likely mediator of ANP action in these epithelial cells.

DIMINISHED RELEASE OF ATRIAL NATRIURETIC FACTOR IN SALINE-EXPANDED NEWBORN DOGS. **A. Haramati** and S.E. Mulroney *, Dept. of Physiology, Georgetown Univ. School of Medicine, Washington, DC

The natriuretic response to volume expansion (VE) is known to be attenuated in most, but not all, neonates. To examine whether this may be related to differences in the release of atrial natriuretic factor (ANF), clearance experiments were performed on 24 mongrel puppies (1-38 days of age) that underwent VE at 2 ml/kg.min for 15 min and 0.5 ml/kg.min for the duration of the experiment. Plasma levels of ANF (pANF) and fractional excretion of sodium (FENa) were determined during control (C) and VE. Most animals (n=16) exhibited a blunted natriuretic response to VE (FENa<3%)(termed: NONRESPONDERS), whereas in 8 rats FENa was >3% (termed: RESPONDERS). VE resulted in similar decreases in the hematocrit (17%) in both groups and there were no differences in blood pressure or GFR. * = p<0.05.

	RESPONDERS		NONRESPONDERS	
	C	VE	C	VE
FENa(%)	0.8 \pm 0.2	7.6 \pm 1.0*	0.3 \pm 0.1	1.4 \pm 0.2*
pANF (pg/ml)	49 \pm 9	219 \pm 47*	42 \pm 6	57 \pm 9

The increase in FENa during VE in the RESPONDERS was associated with a significant rise in pANF levels. However, in the NONRESPONDERS there was no significant change in pANF. Also, urinary cyclic GMP excretion, a marker for ANF action, increased significantly in RESPONDERS (23 \pm 5 to 50 \pm 5 pmol/ml, p<0.05), but not in NONRESPONDERS (21 \pm 3 to 27 \pm 4 pmol/ml). We conclude that the diminished release of endogenous ANF may contribute to the blunted natriuretic response to VE in some newborn dogs.

EPIDERMAL GROWTH FACTOR (EGF) BINDS SPECIFICALLY TO RAT MESANGIAL CELLS AND INDUCES CYTOPLASMIC ALKALINIZATION. **R.C. Harris***, R. L Hoover*, H.R. Jacobson and K.F. Badr. Departments of Medicine and Pathology, Vanderbilt University, Nashville, TN

We have recently shown (Clin. Res. 35:542, 1987) that selective renal infusion of EGF decreases GFR, due mainly to a fall in the glomerular capillary ultrafiltration coefficient, K_f. Since the latter may be mediated through mesangial cell contraction, the present study examined in vitro effects of EGF upon cultured rat mesangial cells. Following incubation of these cells with ^{125}I -EGF, equilibrium binding was seen at 4°C and 23°C. There was 73 \pm 8% specific binding at 4°C (n=4) and 83 \pm 4% at 23°C (n=9). Scatchard analysis showed both a high affinity binding site, with K_d of 1×10^{-10} M and R_o of 1.8×10^3 binding sites/cell, and a low affinity binding site with K_d of 1.7×10^{-9} M and R_o of 8.8×10^3 binding sites/cell. EGF binding was not inhibited by ADH or by AII (10^{-6} M), but there was concentration dependent competition by transforming growth factor β , a known functional analogue of EGF. Intracellular pH (pH_i) was monitored in individual mesangial cells grown on glass coverslips and loaded with the pH-sensitive dye, BCECF, using a microfluorometer. In nominally HCO₃⁻ free medium, pH_i was 7.04 \pm 0.04 (n=19). Removal of external Na⁺ or addition of amiloride led to cytoplasmic acidification. Addition of EGF (1-30 nM) led to cytoplasmic alkalization of 0.12 \pm 0.01 pH units (n=11), which occurred only in the presence of external Na⁺ and was amiloride inhibitable.

Thus, cultured rat mesangial cells possess specific EGF receptors and EGF activates Na⁺/H⁺ exchange in these cells. These studies strongly suggest that the observed in vivo effects of EGF upon glomerular hemodynamics, particularly K_f, are a result of direct receptor-mediated interaction of EGF with mesangial cells. Activation of Na⁺/H⁺ exchange and intracellular alkalization appear to constitute a component of the resultant signal transduction mechanism.

VASOPRESSIN AND THE RENAL NERVES ALTER ATRIAL PEPTIDE-INDUCED NATRIURESIS. **D. Hartupee**, A. Trapani*, J. Koepke, L. Graczak*, M. Blaine*, and E.H. Blaine*. Searle Research and Development, Washington Univ., St. Louis, MO

We investigated the interaction of arginine vasopressin (AVP) and the renal nerves with atrial peptide (AP)-induced natriuresis. In 5 anesthetized dogs, IV infusion of AVP (1.2 mU/kg.min) during IV infusion of AP24 (103-126) (0.36 nmol/kg.min) augmented the AP-induced natriuresis from 46 \pm 16 (AP24 alone) to 301 \pm 75 μ Eq/min (p<0.001). This augmentation by AVP is not due to an intrarenal effect, since intrarenal AVP infusion (0.12 mU/kg.min) in a second group (n=3) did not alter the AP24-induced natriuresis. To determine if the effect of AVP is mediated by the renal nerves, the left kidney was denervated prior to IV AP24 and AVP infusions in a third group (n=5). AP24 alone (0.36 nmol/kg.min) caused a larger natriuresis in the left, denervated kidney compared to the right, innervated kidney (Table I). Subsequent IV AVP infusion (1.2 mU/kg.min) did not further increase sodium excretion in the left kidney, as it did in the right kidney (Table I).

TABLE I - SODIUM EXCRETION (μ EQ/MIN)

	Basal	AP24	AP24 & AVP
Left Kidney	33 \pm 5	303 \pm 38*	266 \pm 40
Right Kidney	35 \pm 17	146 \pm 49*	213 \pm 48**

*p<0.001 vs Basal, **p<0.025 vs AP24

We conclude that the renal nerves inhibit full expression of the natriuresis caused by AP24 alone and, further, that intravenous arginine vasopressin infusion augments the AP24-induced natriuresis by blocking this inhibition.

DISSOCIATION OF RENAL RENIN CONTENT (RRC) AND PLASMA RENIN CONCENTRATION (PRC) IN CYCLOSPORINE A (CyA)-TREATED TWO-KIDNEY, ONE-CLIP HYPERTENSIVE RATS. Udo Helmchen*, Dieter Bach, Charlotte Rohland, and Hermann-Josef Groene. Institute of Pathology, University of Goettingen, West Germany (intr. by M. J. Dunn).

The effects of CyA on PRC, on RRC in both kidneys (l=left clipped; r=right untouched), and on systolic blood pressure (BP) were examined in 3 groups of Wistar rats with two-kidney, one-clip hypertension. The groups were untreated hypertensive (A; n=23), oil vehicle-treated hypertensive (B; n=25), and CyA-treated hypertensive rats (C; n=24). After a 6 week hypertensive period CyA (25 mg/kg/d) and the vehicle were given by a gastric tube for 3 weeks. Thereafter, the following data were obtained (mean±SEM; *p<0.01 compared to group A):

groups	PRC (ng/ml/h)	RRC _l (mcg/100mg/h)	RRC _r (mcg/100mg/h)	BP (mmHg)
A	78.4±5	11.0±1	2.8±0.8	183±6
B	82.2±5	11.9±1	1.7±0.4	179±4
C	48.6±3*	49.2±8*	23.3±3.1*	140±4*

In accordance with the enhanced RRC the areas of the epitheloid juxtaglomerular cells were morphometrically enlarged and the number of their secretory granules, studied by electron microscopy, was significantly increased within the clipped and the untouched kidneys of group C. Thus, in two-kidney, one-clip hypertensive rats the chronic treatment with CyA caused a significant fall of PRC and BP. The low PRC probably reflects an impaired renin secretion in the presence of an increased renal renin storage.

EFFECTS OF ATRIOPEPTIN III (AP) AND cGMP ON RENIN RELEASE (RR) FROM ISOLATED JUXTAGLOMERULAR (JG) CELLS. W. Henrich, E. McAllister*, P. Smith*, and W. Campbell*, U of Tx HSCD and Dallas VAMC, Dallas, TX.

The ability of ANP and cGMP to affect RR stimulated by the B-adrenergic agent isoproterenol (ISO, 10⁻⁵M) and the calcium channel blocker diltiazem (D, 10⁻⁴M) was studied in JG cells isolated using methodology of Kurtz et al (PNAS 83:4769, 1986). JG cells were placed in primary cell culture for 48h; 45 min incubations were performed. In the control incubations, RR increased by 21% and renin secretory rate (RSR) by 35 ng/mg/h. ISO significantly increased both RR (by 58%) and RSR (to 122 ng/mg/h); D also increased RR significantly (by 50%) and RSR (to 84 ng/mg/h). The addition of ANP (2.1 x 10⁻⁸M) decreased ISO-stimulated RSR by 34% D-stimulated RR by 27%. cGMP (10⁻⁶M) also ablated the increase in RSR to both ISO and D. Finally, the guanlylate cyclase inhibitor methylene blue (MB, 10⁻⁶M) completely reversed the renin inhibitory effects of ANP: when both MB and ANP were included in the media with ISO, RSR was restored to 107.4 ng/mg/h. These results in isolated JG cells show a direct inhibitory action of ANP and cGMP on RR stimulated by ISO and D. Further, the data strongly implicate cGMP as an important mediator of renin inhibition.

14,15-DIHYDROXYEICOSATRIENOIC ACID INHIBITS VASOPRESSIN (AVP)-INDUCED HYDRAULIC CONDUCTIVITY (Lp) IN CORTICAL COLLECTING TUBULES. D. Hirt*, J Capdevila, J Falk, M Breyer, and H Jacobson, Nephrology, Vanderbilt Univ., Nashville, TN and Molecular Genetics, UTHSC, Dallas, TX.

Arachidonic acid is metabolized by a cytochrome P450 NADPH-dependent epoxygenase to 4 regio-isomeric epoxides: epoxyeicosatrienoic acids (EETs). EETs are further enzymatically hydrated to their respective vicinyl diols: dihydroxyeicosatrienoic acids (DHETs). We have previously shown that all 4 DHETs suppress 10μU/cc AVP-induced hydraulic conductivity (Lp 10⁻⁷ cm/atm/s ±SE) in rabbit CCTs perfused *in-vitro* at 37°C (ASCI 635A, 1987); the 14,15-DHET isomer, applied 1μM in the bath, produced the most potent inhibitory effect.

Having shown these compounds modulate CCT function we demonstrated their local production by subjecting microdissected CCTs to gas chromatography/mass spectroscopy and found EETs in the phospholipid fraction. A dose-response study of 14,15-DHET for its inhibition of AVP-induced Lp shows significant (p<.005) suppression of peak Lp (control = 235 ±10.2, n=17) present to 10⁻⁹M (171 ±17.9, n=5). Prostaglandin E₂, a known inhibitor of AVP-induced water flow, at 10⁻⁷M suppressed peak Lp to 88 ±13.4, n=5; 10⁻⁷M 14,15-DHET reduced Lp to 114 ±6.2, n=5. The hydroosmotic effect of AVP is mediated through its second-messenger: cAMP. Purportedly, the suppressive effect of PGE₂ on AVP-responsive epithelia is inhibition of cAMP production. Using the permeant cAMP analogue, 8-chlorophenylthio-cAMP at 10⁻⁴M, peak Lp (189.6 ±11, n=7) was reduced upon pretreatment with 1μM 14,15-DHET (132 ±13.4, n=5, p<.005). We conclude: 1) epoxygenase products are made in CCTs; 2) 14,15-DHET inhibits AVP-induced Lp in a dose dependent fashion which is significant at nanomolar concentrations; 3) at 10⁻⁷M the inhibition of Lp by 14,15-DHET and PGE₂ is comparable; 4) DHETs inhibit vasopressin-induced water flow at least, in part, post-cyclic AMP.

CATABOLISM OF cAMP AND cGMP IN COLLECTING TUBULES OF NORMAL MICE AND MICE WITH HEREDITARY NEPHROGENIC DIABETES INSIPIDUS (NDI). Sumiko Homma*, Susan M. Gapstur* and T.P. Dousa. Neph. Res. Unit, Mayo Clinic and Foundation, Rochester, Minnesota.

Enzymatic hydrolysis of cAMP and cGMP by phosphodiesterases (PDE) was investigated in the three major subsegments of collecting tubule system: cortical (CCT), medullary (MCT) and papillary duct (PCT), microdissected from kidneys of control mice (C-mice) and mice with NDI (NDI-mice). The cyclic-3',5'-nucleotide diesterase (PDE) activity was assayed at low (1 μM) substrate concentration with or without 10 μM Ca²⁺. Activity of cAMP-PDE, but not cGMP-PDE, was lower in PCD than in MCT and CCT. Addition of Ca²⁺ increased cAMP-PDE in MCT, but not in PCD of C-mice; no effect of Ca²⁺ on cAMP-PDE was found in tubules from NDI mice. The cAMP-PDE was significantly higher in CCT (+ 52%), in MCT (+ 90%) and in PCD (+ 101%) of NDI mice compared to C-mice, and this difference was more expressed in the absence of Ca²⁺. In contrast, activity of cGMP-PDE did not differ between C-mice and NDI-mice. Rolipram and Cilostamide, selective inhibitors of the high-affinity cAMP-specific PDE (PDE-III type), caused a dose-dependent inhibition of cAMP-PDE, but had no effect upon cGMP-PDE. In NDI mice, all subsegments of collecting tubule systems have anomalously high cAMP-PDE, but not cGMP-PDE, and the activities are inhibited by selective PDE-III inhibitors. Results thus suggest that cells of collecting tubule system of NDI mice contain anomalously high activity of cAMP-PDE with properties analogous to PDE-III type isozyme.

PROSTAGLANDINS OF MESANGIUM ORIGIN INHIBIT MESANGIAL CELL (MC) PROLIFERATION AND MATRIX SYNTHESIS. T. Homma*, I. Ichikawa and R. L. Hoover*. Dpts. of Pediatrics & Pathology, Vanderbilt University, Nashville, TN.

It has been suggested in several experimental models of primary and secondary glomerulopathies that prostaglandins may play an important modulatory role in the expression of glomerular lesions. In the first step of the study, the effect of prostaglandin E₂ (PGE₂) on cultured rat MC was investigated. MC seeded at the density of $0.5 \times 10^4/\text{cm}^2$ were growth-arrested by serum-depletion, and were then induced to proliferate with 10% fetal bovine serum. Addition of PGE₂ at concentrations $\geq 10^{-12}\text{M}$ caused significant inhibition of MC proliferation in a dose-dependent manner with $60 \pm 5\%$ inhibition induced at 10^{-7}M on Day 1 (n=6). From Day 2 until MC reached confluency, cell doubling time was identical for control and PGE₂-treated cells (20 ± 1 vs 21 ± 1 hours) unless PGE₂ was added on a daily basis, where a dose-dependent growth inhibition was observed. The agents known to stimulate prostaglandin synthesis in MC, namely arachidonic acid (AA), angiotensin II (AII), and arginine vasopressin (AVP), also resulted in a dose-dependent inhibition with $\geq 50\%$ inhibition achieved at $1 \mu\text{g}/\text{ml}$ of AA, 10^{-7}M of AVP and 10^{-7}M of AII. The action of AII was antagonized by saralasin, and that of these three agents was abolished by indomethacin ($5 \mu\text{M}$), which alone was without effect on MC growth. Prostaglandins appeared to modulate the cell size and the matrix production in MC: when MC were exposed to PGE₂ daily at 10^{-9}M , there was $\sim 20\%$ increase in cell protein content (from 5.02 in control to 6.08 μg protein/ 10^4 cells, $P < 0.0005$) whereas cell layer protein (=total layer protein minus cell protein content) was reduced (from 0.91 in control to 0.44 μg protein/ 10^4 cells, $P < 0.005$). Exposure to AA at $2 \mu\text{g}/\text{ml}$ resulted in similar changes (cell protein content to 6.01 and cell layer protein to 0.49 μg protein/ 10^4 cells). Indomethacin ameliorated the action of AA. These results indicate that prostaglandins synthesized by MC are capable of suppressing their own proliferation and matrix production. This action of prostaglandins of mesangial cell origin may play an important role in controlling mesangial expansion and proliferation, which often are the predominant histopathologic features of glomerulopathies.

PROSTAGLANDIN E₂ (PGE₂) INHIBITS Na/K-ATPase IN RABBIT INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. Kathy Jabs*, Patricio Silva, and Mark L. Zeidel, Harvard Medical School, Boston, Massachusetts.

PGE₂ inhibits collecting duct Na transport. To determine the mechanism of this inhibition, we studied the effect of PGE₂ on transport-dependent O₂ consumption (QO₂) and ouabain-sensitive rubidium (Rb) uptake in fresh suspensions of IMCD cells, as well as Na/K-ATPase in papillary membranes. PGE₂ (10^{-5}M) reduced QO₂ by $16.9 \pm 1.5\%$ (SE, n = 20) in IMCD cells but not in outer medullary collecting duct cells ($1.0 \pm 3.9\%$). Half maximal inhibition of QO₂ in IMCD cells occurred at 10^{-9}M PGE₂. PGE₂ failed to inhibit QO₂ in the absence of sodium or in the presence of ouabain and blunted the increase in QO₂ in response to amphotericin B. These results suggested that PGE₂ directly inhibited Na/K-ATPase activity. Inhibition of pump activity was confirmed by measurements of Rb uptake: PGE₂ (10^{-5}M) reduced ouabain-sensitive Rb uptake by $40.8 \pm 4.7\%$ at 1 min and $21.0 \pm 1.7\%$ at 5 min without altering equilibrium uptake. Furthermore, in rabbit papillary homogenates, PGE₂ reduced Na/K-ATPase activity in a concentration-dependent manner with maximal inhibition at 10^{-6}M ($69.4 \pm 5.4\%$, n = 5). PGE₂ did not significantly alter QO₂, Rb uptake, or Na/K-ATPase activity. These results demonstrate that PGE₂ directly inhibits IMCD Na/K-ATPase activity and suggest a role for this inhibition in the natriuretic effect of PGE₂.

MODULATION OF VASOPRESSIN (AVP)-SENSITIVE cAMP METABOLISM BY CALCIUM IN RAT PAPILLARY COLLECTING DUCT (PCD). Brian A. Jackson, Dept. of Physiology, Univ. of Kentucky, Lexington, KY.

Calcium is considered an important modulator of vasopressin action at both pre- and post cAMP loci. The present study was designed to determine the extent to which calcium modulation of cAMP levels results from effects on adenylyl cyclase (AdC) and/or cAMP-phosphodiesterase (cAMP-PDIE) activities in microdissected PCD.

	cAMP (fmol / 20 min/mm length)		
[Ca ²⁺]:	0.0 mM	1.2 mM	5.0 mM
-MIX:	55.2	29.2	19.4
(n=6)	± 6.3	± 2.9	± 2.4
+MIX:	325.3	178.5	172.6
(n=4)	± 31.6	± 22.2	± 17.8

Compared to controls (1.2 mM Ca^{2+}), a calcium-free medium increased AVP dependent cAMP accumulation by 89% ($p < 0.01$) in the absence of the phosphodiesterase inhibitor MIX, and similarly by 82% ($p < 0.01$) in the presence of MIX. High medium calcium (-MIX) reduced AVP dependent cAMP levels by 34% ($p < 0.05$), but had no effect in the presence of MIX. Increasing intracellular calcium with A23187 reduced AVP-dependent cAMP levels by 69% in the absence of MIX (28.7 ± 3.4 vs 9.0 ± 1.0 fmol cAMP/20 min/mm; $p < 0.01$) and by a lower 47% ($p < 0.05$) in the presence of MIX (240.7 ± 26.8 vs 127.4 ± 16.0 ; $p < 0.01$). These data demonstrate that calcium can modulate AVP-dependent cAMP accumulation in intact PCD via effects on either AdC or cAMP PDIE or both, depending on the direction and extent to which intracellular calcium is altered.

GLOMERULAR FILTRATION RATE (GFR), EXTRACELLULAR FLUID VOLUME (ECV) AND ATRIAL NATURETIC FACTOR (ANF) IN INSULIN-DEPENDENT DIABETICS.

S.L. Jones, N. Perico, A. Benigni, G. Remuzzi and G.C. Viberti. Introduced by Francesco Pugliese. Unit for Metabolic Medicine, UMDS, Guy's Hospital, London, UK. and Mario Negri Institute, Bergamo, Italy.

The interrelationships of GFR, ECV and ANF in human diabetes are unknown. We have studied ECV, measured as volume of distribution of ⁵¹CrEDTA in 2 groups of 23 non-proteinuric insulin-dependent diabetics matched for age, mean (range) 28 (16-44) vs 29 (17-48) yrs, sex (18m, 5f) and duration of diabetes, 8 (1-19) vs 9 (2-17) yrs selected for GFR (⁵¹CrEDTA clearance) either in the supranormal range, mean (range) 153 (135-196) ml/min/1.73m², or in the normal range 118 (93-134) ml/min/1.73m². Sixteen healthy, sex and age matched individuals served as controls. ANF was measured in 20 diabetics (6 hyperfilterers, 14 normofilterers) and 6 controls. Mean (range) ECV was significantly elevated in diabetics with hyperfiltration, 16.4 (13.6-19.6) litre/1.73m² compared with diabetics with normal GFR 14.7 (11.7-19.2) litre/1.73m², ($p < 0.0006$), who were similar to normal controls 15.0 (10.9-18.0) litre/1.73m². Median (range) ANF was elevated in diabetics, 45 (13-135) pg/ml compared to controls 31 (24-50) pg/ml. In the diabetic group there was no correlation between ANF and GFR, ECV, mean BP or 24hr excretion of sodium or albumin. A weak correlation was found with plasma glucose ($r = 0.27$).

ECV is only elevated in those diabetics with glomerular hyperfiltration. In diabetic ANF levels are higher than controls and tend to be associated with blood glucose but not with GFR or ECV.

SK&F 101926 IS A V1 RECEPTOR ANTAGONIST IN MAN. Bernard E. Ilson,* Nancy L. Allison, Dianne F. Tatoian,* Robert G. Familiar* and Robert M. Stote. Smith Kline and French Clinical Research Unit, Presbyterian Hospital, Philadelphia, PA.

SK&F 101926 is a synthetic peptide which in preclinical studies is a potent inhibitor of vasopressin at both the V2 renal tubular receptor and the V1 vascular receptor. In initial clinical studies, however, SK&F 101926 acts as a V2 agonist in water-loaded subjects. The purpose of the present study is to evaluate effects of SK&F 101926 on AVP-induced changes in hemodynamic parameters. A dose-response curve was constructed in 8 normal subjects with incremental bolus IV doses of AVP (1 to 20 $\mu\text{g}/\text{kg}$) given at 20 minute intervals. Placebo (n=4) or SK&F 101926 1.0 $\mu\text{g}/\text{kg}$ (n=4) was then infused and the dose-response curve with AVP was repeated.

AVP dose	Δ Mean BP (mmHg)	Δ SVR (dynes.sec.cm ⁻⁵)
0	Placebo	101926
1	6.9 4.5 NS	128 96 NS
2	10.5 6.7 NS	160 160 NS
5	11.9 5.3 NS	232 128 NS
10	9.4 10.7 NS	208 240 NS
20	7.5 10.3 NS	144 160 NS

We observed blunting of the pressor response to AVP after SK&F 101926 infusion, but not after placebo. Systemic vascular resistance (SVR) tended to decrease with successive doses of AVP after SK&F 101926 infusion (data not shown). No changes in hemodynamic parameters were noted after SK&F 101926 infusion alone (doses of 0.5, 1.0, and 10.0 $\mu\text{g}/\text{kg}$). We conclude that SK&F 101926 is a V1 receptor antagonist in man.

LOCALIZATION OF THE INTRARENAL RENIN ANGIOTENSIN SYSTEM (RAS) BY IN SITU HYBRIDIZATION OF RENIN AND ANGIOTENSINOGEN (ANG-N) mRNAs. Julie Ingelfinger, Edward A. Fon,* Kristin E. Ellison,* Victor J. Dzau,* Brigham and Women's Hospital, Division of Vascular Medicine and Atherosclerosis and The Children's Hospital, Division of Nephrology, Boston, MA

Increasing evidence supports the existence of a complete intrarenal RAS, but cellular localization is unclear. Localization would lead to important clues as to the sites of formation of intrarenal AII and its possible function. To determine if renin and ang-n mRNA are expressed in the same or different cell types, we used the technique of *in situ* hybridization in male Sprague-Dawley rats using 35S cRNA probes to rat renin and ang-n, as well as anglerfish insulin as a control probe. Testis and liver were used as tissue controls. Ang-n mRNA was present primarily in the proximal renal tubule, while renin mRNA was present primarily in the juxtaglomerular apparatus and glomerular tuft. Ang-n mRNA was also seen in renal vessels and in the renal medulla. Our data, taken together suggest that the bulk of intrarenal renin and ang-n mRNA's are expressed in differing cell types, and that the proximal tubule is a major site of intrarenal AII formation. Given the effects of AII on proximal tubules, these findings imply that endogenous AII formed in the proximal tubule via the intrarenal RAS may be important in local regulation of tubular transport processes.

THE EFFECT OF THE VALSALVA MANEUVER (VM) ON ATRIAL NATRIURETIC FACTOR (ANF) RELEASE IN NORMAL HUMANS. C.Kablitz, F.M.Birch, R.L.Baranowski, A.O. Haakenstad*, A.K. Cheung, C.Cuny*, and C.Westenfelder. Univ. of Utah and VA Med.Centers, Salt Lake City, Utah.

An increase in right atrial pressure (RAP) is a well recognized stimulus of ANF release. In the present study, we examined whether the VM, which also raises RAP, could be used to elicit an acute increase in circulating ANF levels. A vigorous VM was executed in 6 normal volunteers under control conditions, after 14 hrs of furosemide (FS) -induced volume contraction (1 mg FS /kg BWt), after acute re-establishment of euvoemia (i.v. replacement of FS-induced salt loss), and again after acute volume expansion (20 cc NS /kg BWt over 45 min). Mean \pm SE circulating ANF levels [pg/ml] showed a tendency to rise immediately following completion of the VM (see Table). This rise in ANF levels post VM was statistically significant (*, P < 0.05) only when the individuals were hypervolemic.

In conclusion, these data demonstrate that a marked increase in RAP (as induced by the VM), is not per se a sufficient stimulus for ANF release. It appears that actual atrial stretch, which the VM does not normally produce, is the critical signal for ANF release. Additionally, abrupt changes in autonomic discharge induced by the VM may blunt ANF release. Finally, it seems that the VM may induce more atrial stretch when volume expansion pre-exists.

	CONTROL		CONTRACTED		EUVOLEMIC		EXPANDED	
VM	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Mean	368	404	403	438	319	345	287	331*
\pm SE	34	26	22	13	32	15	9	12

EVIDENCE THAT THE WATER PERMEABILITY OF LUMINAL MEMBRANE AGGREGATES IN TOAD BLADDER IS CONSTANT. W.A. Kachadorian and K.R. Spring. NIH: GRC,NIA, Baltimore, MD and LKEM, NHLBI, Bethesda, MD

ADH stimulation of toad bladder leads to the appearance of aggregates of intramembrane particles in the luminal membrane (LM) of the granular cells. We have shown that the number of these is a direct measure of the water permeability of the LM, but not always an index of total tissue water permeability. In the present study we compared effects of ADH (20mU/ml), cAMP (10mM), and forskolin (F) (50 μM) on LM water permeability. Effects of these agents on total tissue water permeability and aggregate frequency were known from previous parallel experiments by us. The luminal solution was Ringer diluted fivefold (40 mOsm); the serosal solution was Ringer (220 mOsm) to which the relevant stimulant was added. LM water permeability was measured by quantitative light microscopy from the rate of cell volume change when luminal osmolality was instantly changed from 40 to 260 mOsm. Our findings indicate that LM water permeability and aggregate frequency after cAMP treatment are identical to values observed with ADH, while total tissue water permeability is only half as great. F causes aggregates and LM water permeability to rise to twice the extent observed with ADH, however, tissue water permeability becomes only equal to the level with ADH treatment. These observations suggest that apparent water permeability per aggregate remains constant with ADH, cAMP, and F treatments. Accordingly, a second, post-luminal site appears to limit tissue water permeability under conditions in which LM water permeability is increased by cAMP or F.

CRITICAL ROLE FOR ALDOSTERONE (ALDO) IN ADAPTATION TO HIGH POTASSIUM (K) INTAKE IN THE RAT. S. Kaehny*, B. Dixon*, S. Anderson*, R.J. Anderson, and W.D. Kaehny. V.A. Med. Ctr. and Univ. of Colo., Denver, Colorado.

Rats adapt to an increase in dietary K intake with an immediate kaliuresis that peaks at 48h and then stabilizes. We studied conscious control and high K intake rats (n=8-12 in each group) to delineate the role of physical and humoral factors in the mechanism of this kaliuresis. Restriction of water intake or sodium intake blocked the polyuric and natriuretic response to high K intake but not the kaliuresis. Plasma aldo (29 ± 4 control; 123 ± 17 1d; and 74 ± 8 pg/ml 7d, $p < .01$) but not insulin, norepinephrine or epinephrine levels, rose with high K intake. To test the physiological role of this increase aldo, adrenalectomized rats were given fixed aldo in basal replacement doses by minipumps. The inability to increase aldo did not affect the kaliuresis of adaptation but did increase plasma K (4.5 ± 0.1 with high aldo 5.9 ± 0.3 mEq/l with fixed aldo, $p < .02$). Either water or sodium restriction plus fixed aldo replacement reduced the kaliuresis with resultant severe hyperkalemia (>8.0 mEq/l). These results demonstrate a key role for aldo in maintaining normal plasma K response to high K intake. Reducing either urinary flow rate or sodium excretion also unmask a critical role for aldo in mediating kaliuresis with high K intake.

ADENOSINE RELEASED DURING HORMONAL STIMULATION IS A FEEDBACK INHIBITOR OF CHLORIDE TRANSPORT IN THE SHARK RECTAL GLAND. G.G. Kelley, O.S. Aassar, J.N. Forrest Jr., Dept. Medicine, Yale Univ. Sch. Med. New Haven, CT and MDI Bio. Lab., Salisbury Cove, ME.

We previously provided evidence for an A_1 adenosine (Ado) receptor that inhibits chloride transport in the shark rectal gland. The present studies examined the physiological role of this receptor in the isolated perfused rectal gland. Interaction of endogenous Ado with extracellular A_1 receptors was prevented by agents that altered the degradation, transport and binding of adenosine. ADA (0.1U/ml) had no effect on basal secretion but increased the secretory response to forskolin (F; $1\mu M$) by 2-fold (Cl secretion 463 ± 28 $\mu Eq/hr/g$ with F alone and 833 ± 105 with F+ADA, $p < 0.001$). Nitrobenzylthioinosine (NBTI), an Ado transport inhibitor that prevents the cellular efflux of Ado, also had no effect on basal secretion but increased the response to F 2-fold. ($592 = 119$ to 1122 ± 186 $\mu Eq Cl/hr/g$, $p < 0.02$). The A_1 receptor antagonist 8-phenyltheophylline (8PT) also had no effect on basal secretion but increased the F response 2-fold (420 ± 84 to 858 ± 81 , $p < 0.01$). These results suggested that Ado is present at inhibitory concentrations only during hormonal stimulation. We therefore measured Ado concentrations in the venous effluent by HPLC with simultaneous measurements of Cl transport. Basal Ado levels were 3 ± 0.5 nM and basal Cl secretion was 90 ± 10 . When secretion was stimulated with F, the Ado concentration increased in a dose dependent manner from 30 ± 5 nM at $1\mu M$ F to 750 ± 23 at $10\mu M$ F while Cl secretion increased in parallel from 450 ± 10 to 2191 ± 138 . The secretagogue vasoactive intestinal peptide (VIP), also increased Ado concentrations in parallel with increased Cl secretion. In glands stimulated with F, the addition of $1\mu M$ NBTI decreased Ado concentrations to basal levels in parallel with the increased secretion of Cl. These findings provide the first demonstration in epithelia that endogenous Ado is released during hormonal stimulation of ion transport. In the shark rectal gland model, endogenous adenosine is an important feedback inhibitor of chloride transport via extracellular A_1 adenosine receptors.

ARGININE VASOPRESSIN (AVP) GENE EXPRESSION IN CONGESTIVE HEART FAILURE (CHF). J.K. Kim, J.-B. Michel,* F. Soubrier,* J. Durr, P. Corvol,* and R.W. Schrier. INSERM, U36, Paris, France; and Univ. Colorado Sch. Med., Denver, CO.

Non-osmotic release of AVP has been shown to be involved in the water retention associated with CHF in both man and animals. There is, however, no information about the effect of CHF on AVP biosynthesis. In the present study, therefore, AVP-messenger RNA (mRNA) was examined in hypothalamus from control (Cont) and coronary ligated CHF rats. The effects of the CHF were assessed after 3 months of coronary artery ligation. At that time, plasma atrial natriuretic factor (ANF) was higher (554 ± 56 vs 205 ± 36 pg/ml, $p < .001$), blood pressure was lower (113 ± 4 vs 131 ± 3 mmHg, $p < .05$, and heart weight was greater (1346 ± 22 vs 929 ± 19 mg, $p < .001$) in the CHF than Cont rats. AVP-mRNA was determined by solution hybridization. Antisense AVP-RNA was used as the probe and a pure sense AVP-mRNA was used as the standard, both were produced by *in vitro* transcription from a rat AVP-cDNA. The AVP-mRNA in the hypothalamus from rats with CHF was significantly higher than in those from Cont rats (20.2 ± 1.5 vs 14.3 ± 1.5 fmoles/hypothalamus, $p < .02$). These results therefore indicate that chronic CHF is associated with increased stimulation of AVP gene expression. Thus, increased hypothalamic AVP biosynthesis as well as increased non-osmotic release of AVP from the neurohypophysis are involved in the abnormality in water excretion associated with CHF.

ATRIAL NATRIURETIC PEPTIDE (ANP) IN TYPE 1 DIABETES (DM-1): RELATIONSHIP WITH GLOMERULAR FILTRATION RATE (GFR), EXTRA-CELLULAR FLUID VOLUME (ECF), AND PLASMA RENIN ACTIVITY(PRA). Kindermans C*, Laborde K*, Thiriet I*, Levy Marchal C*, Sachs C and Dechaux M*. Necker Univ., Dpt. of Physiol. & Pediatric Diabetology, Paris, France.

Increase in GFR and decrease in PRA are reported as physiological effects of ANP. To evaluate the possible role of the peptide in the glomerular hyperfiltration (HF) and low PRA levels reported in DM-1, plasma ANP, PRA and urinary GMPc have been measured in 23 DM-1 children aged 13.5 ± 3.3 yrs (diabetes duration 5.1 ± 4 yrs). Twelve patients were hyperfiltering (HF), GFR >160 ml/min/1.73m² and 11 normofiltering (NF), GFR ≤ 160 . GFR and renal plasma flow (RPF) were measured by inulin and PAH clearances respectively. ECF by inulin distribution volume, plasma ANP, PRA and UGMPc by RIA. ANP was significantly correlated to GFR ($p < 0.02$) to RPF ($p < 0.001$), to UGMPc ($p < 0.02$) but neither to ECF nor to PRA.

	HF	NF
GFR(ml/min/1.73m ²)	177 \pm 18 ***	132 \pm 16
RPF (" ")	837 \pm 148 **	631 \pm 75
ECF (% body weight)	18 \pm 3.1	17 \pm 1.3
PRA (ng/ml.h)	1.8 \pm 1.1 *	4.0 \pm 2.5
ANP (pg/ml)	62 \pm 24	47 \pm 9
UGMPc(pM/min/1.73m ²)	944 \pm 186 **	658 \pm 21

(***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.02$)

In conclusion, in DM-1 patients, HF is associated to elevated ANP, UGMPc and low PRA. These data are consistent with the previously suggested role of these vasoactive hormones in the hyperfiltration observed in DM-1.

SEGMENTAL NEPHRON CHLORIDE HANDLING DURING EUGLYCEMIC INSULIN ANTIURETIC IN THE RAT. Kent A. Kirchner, Dept. of Med., Univ. of Mississippi Med. Ctr., Jackson, Mississippi.

To determine where insulin (INS) reduces urinary chloride (Cl) excretion, late proximal (LP), early distal (ED), and late distal (LD) micropuncture was performed during sustained volume expansion (10% body wt) in rats (N=8) before and during euglycemic INS infusion (85 μ g/kg load and 8 μ g/kg/min). A second group (N=6) were time controls (TC) and received INS-vehicle. INS reduced urinary Cl excretion by 50% (FeCl: 6.2 \pm 0.8 to 3.2 \pm 0.5%; P<0.02). Urinary Cl excretion was constant in TC rats. GFR, arterial pressure, and plasma Cl and glucose were unaltered by INS and not different from TC. SNGFR and fractional (Fract) Cl delivery to LP was not different before and during INS or between INS and TC. Fract Cl delivery to ED was reduced (18.6 \pm 1.7 to 10.9 \pm 0.9%; P<0.001) during INS and lower than TC (19.6 \pm 2.1%; P<0.005). Absolute Cl delivery showed a similar pattern. ED TF/PI was not different (P=NS) during INS or between groups. LD Cl delivery was lower during INS (P<0.01) and less (P<0.05) than TC. INS had no effect on calculated proximal Cl uptake but increased (P<0.001) loop Cl uptake (47.7 \pm 4.6 to 60.0 \pm 3.9%). Distal tubule (DT) Cl uptake was reduced during INS. Thus INS reduces urinary Cl excretion in volume expansion by direct tubular effects. In superficial nephrons, INS primarily affects Cl uptake in the loop. As INS increases superficial nephron loop Cl uptake by 12% but reduces urinary Cl excretion by 50%, INS may also have major effects beyond the DT or in deep nephrons.

A23187-INDUCED PROSTACYCLIN (PGI₂) PRODUCTION IN RABBIT RENAL MICROVASCULAR ENDOTHELIAL CELLS (RMVEC) IN CULTURE IS INDEPENDENT OF cAMP. M.A. Kirschenbaum, S. Gupta*, A. Pedram*, A. Chaudhari, Nephrology Section, VA Medical Center and UCI-Long Beach Medical Program, Long Beach, CA.

It has been proposed that cAMP affects PGI₂ generation by regulating intracellular (IC) free Ca²⁺. We previously reported that decreased A23187-induced PGI₂ production in RMVEC was cAMP dependent (KI, 31:264, 1987). In these studies IBMX (a phosphodiesterase inhibitor) was used to elevate IC cAMP levels. The present study examined whether agents which stimulate endogenous cAMP generation (via adenylate cyclase activation) would decrease RMVEC PGI₂ production. Confluent monolayers of RMVEC were preincubated with IBMX (1 mM) or buffer for 10 min prior to a 5 min incubation with test substances (each 10 μ M): A23187, forskolin (FSK), and isoproterenol (IP). After incubation, the medium was removed for PGI₂ assay (RIA) and the cells were lysed and the released cAMP was measured (RIA). Both in the absence and presence of IBMX, IP (a receptor-mediated cAMP stimulator) and FSK (a nonreceptor-mediated, direct stimulator of adenylate cyclase) produced no effect on A23187-induced PGI₂ production while they both increased cAMP accumulation in RMVEC. The increase in cAMP levels in the IP and FSK treated cells was greater in the presence, than in the absence, of IBMX. However, neither IP nor FSK produced any additional decrease in A23187-induced PGI₂ production over that noted in our previous studies in which IBMX decreased A23187-induced PGI₂ production. These data indicate that there was no inhibition of A23187-induced PGI₂ production associated with IP- or FSK-induced increased IC cAMP levels. Thus, these studies suggest that A23187-induced PGI₂ production may not be regulated by IC cAMP in RMVEC.

DISSOCIATION OF cAMP AND Ca²⁺ SIGNALING SYSTEMS IN PROSTAGLANDIN-STIMULATED UMR-106 CELLS.

C. Kleeman, D. Yamaguchi, and S. Muallem*. Veterans Administration Medical Center, West Los Angeles, Cedars-Sinai Med. Ctr., Los Angeles, CA.

Prostaglandins (PG) have a diverse action on bone where both enhanced bone formation and resorption have been described. PG action may be mediated by cAMP, but recent reports indicate that PG can also stimulate an increase in free cytosolic Ca²⁺ ([Ca²⁺]_i). We investigated the relationship between the cAMP and Ca²⁺ signaling systems in the osteoblast-like cell line, UMR-106. PGF₂ induced a rapid, transient increase in (Ca²⁺)_i that was mainly due to Ca²⁺ release from intracellular stores (EC₅₀ for PGF₂=7nM). PGE₂ similarly increased (Ca²⁺)_i but with an EC₅₀=1.8 μ M. PGE₂ increased cAMP by 80-fold (EC₅₀=73 nM) while 25 μ M PGF₂ increased cAMP by 5-fold. Both PGs were able to further increase (Ca²⁺)_i in cells stimulated by maximal doses of either PG. Both PGs increased phosphatidylinositol (PI) turnover. Phorbol 12-myristate-13-acetate (PMA) pretreatment inhibited PG-mediated PI turnover as well as the increase in (Ca²⁺)_i. However, PMA pretreatment had no effect on PGE₂-mediated cAMP increase. PGE₂ caused a dose-dependent decrease in ³H-thymidine incorporation (TdI) in the cells. PMA pretreatment while not changing TdI by itself, augmented the inhibitory effect of PGE₂ on TdI. PGF₂ alone or with PMA pretreatment did not inhibit TdI. Conclusions: 1) PGE₂ and PGF₂ second messenger signaling systems are independent of each other and appear to involve separate receptors for each PG; 2) There are probably two subclasses of PGE₂ receptors; 3) The PGE₂-cAMP system is inhibitory to proliferation; 4) The PGF₂-Ca²⁺ system may temper the negative proliferative effect of cAMP.

NONVASORELAXANT ATRIAL PEPTIDE LIGAND ON BLOOD PRESSURE AND RENAL FUNCTION. John P. Koepke, Philippe Bovy and Edward H. Blaine. Searle R&D, Dept. of Pharmacol., Washington Univ. Sch. Med., St. Louis, MO.

SC46542 [(des Phe¹⁰⁶, Gly¹⁰⁷, Ala¹¹⁵, Gln¹¹⁶) AP(103-126)] selectively binds to nonvasorelaxant-linked atriopeptin receptors, which may have a clearance function (Maack et al., Am. Soc. Hypert. Abs. pg. 184, 1987). We examined the effects of SC46542 on mean arterial pressure (MAP), urine flow (V), and sodium excretion (U_{Na}^V), and whether SC46542 potentiates or prolongs the duration of responses to AP(103-126) in conscious rats. In part I of table, V (ul/min) and U_{Na}^V (uEq/min) are measured during 20 min control (C), SC46542 (16 μ g/kg/min; E1, E2, E3) and recovery (R) periods. In part II of table, the MAP (mmHg) responses to AP(103-126) (0.05 μ g/kg/min) during E1, E2 and E3 with SC46542 (16 μ g/kg/min; started 60 min before C) and without SC46542 (CONTROL) are shown. Data are mean \pm SE; *p<0.05 compared to C; †p<0.05 compared to CONTROL.

	C	E1	E2	E3	R
I. V	18 \pm 3	22 \pm 3	31 \pm 6*	37 \pm 8*	28 \pm 5
U _{Na} ^V	1.2 \pm 0.3	2.2 \pm 0.6	3.7 \pm 1.1*	4.1 \pm 1.1*	3.4 \pm 1*
II. CONTROL	132 \pm 3	129 \pm 3	127 \pm 3	126 \pm 3	128 \pm 3
SC46542	129 \pm 3	123 \pm 3	118 \pm 3 [†]	112 \pm 4 [†]	121 \pm 4

SC46542 increased V and U_{Na}^V, but had no effect on MAP. A potentiation of the MAP response (but not V or U_{Na}^V) to AP(103-126) was produced by SC46542; the duration of action of AP(103-126) was not prolonged. Conclusion: Binding of clearance receptors with SC46542 increases V and U_{Na}^V, and potentiates the MAP response to AP(103-126) in conscious rats.

ROLE OF DIACYLGLYCEROL-PROTEIN KINASE C IN FLUID AND PHOSPHATE TRANSPORT IN PROXIMAL STRAIGHT TUBULE (PST). E. Lederer, R. Weeks*, W.N. Suki, Baylor College of Medicine, Houston, Texas.

Parathyroid hormone is known to modulate phosphate transport via cAMP and possibly Ca^{++} . Evidence for the presence of a second messenger system involving phosphoinositide breakdown-protein kinase C (PKC) activation has been demonstrated in proximal tubule cells but its role is not clear. These studies investigate the effect on Na and H_2O (Jv) and on phosphate (Jp) transport in *in vitro* microperfused rabbit proximal straight tubule (PST) of: 1) Phorbol 12-myristate, 13-acetate (PMA) which putatively activates PKC, its inactive analogue 12,13-didecanoate (PED), in concentrations of 10-6M added to the bath, 2) 1-oleoyl-2-acetyl-rac-glycerol (OAG), a diacylglycerol analogue in a concentration of 10 or 20 ug/ml added to the bath. PMA caused a significant decline in Jv from 1.15 to 0.86 ul/mm.min (n=8) while Jp was unchanged. Neither PED nor acetone vehicle exerted any effect. OAG, on the other hand, caused an increase in Jv from 0.45 to 1.06 ml/mm.min (p=0.05) while Jp was not significantly changed. We conclude: 1) the phosphoinositide system does not appear to regulate Jp in the PST, 2) OAG increases fluid absorption in PST, a finding consonant with reported activation of the Na^+/H^+ antiporter by this system, and 3) Phorbol ester may have actions on PST other than activation of PKC.

ROLE OF EICOSANOIDS IN THE CONTROL OF RENAL HEMODYNAMICS IN EXPERIMENTAL LIVER CIRRHOSIS. D.J. Leehey, M.T. Uckerman*, M.A. Rahman*, Hines-Loyola Medical Center, Hines IL.

Although there is considerable evidence that vasodilator eicosanoids such as prostaglandin E_2 (PGE_2) are important in the regulation of renal hemodynamics in liver cirrhosis, the role of the vasoconstrictor eicosanoid thromboxane A_2 (TXA_2) is controversial. We measured effective renal plasma flow (CpAH), glomerular filtration rate (C_{IN}), and glomerular synthesis of PGE_2 and TXA_2 (the latter estimated by measuring the metabolite TXB_2) in cirrhotic and control rats. CpAH, C_{IN} , and basal glomerular PGE_2 and TXB_2 were similar in both groups of rats. Indomethacin 5 mg/kg, a dose sufficient to result in a 68% inhibition of glomerular PGE_2 synthesis (from 1060 + 142 to 342 + 61 pg/mg glomerular protein, p < .01) caused significant decreases in both CpAH (from 6.59 + .69 to 4.52 + .67 mL/min, p < .025) and C_{IN} (from 1.34 + .16 to .68 + .07 mL/min, p < .025) in cirrhotic rats. However, thromboxane synthesis inhibition with UK-38485 1 mg/kg, despite causing an 84% inhibition of glomerular TXB_2 production (from 782 + 103 to 122 + 22 pg/mg glom prot, p < .01) had no effect on renal hemodynamics. Neither drug caused significant renal hemodynamic effects in control rats.

We conclude that vasodilator prostaglandins but not the vasoconstrictor thromboxane are important in the control of renal hemodynamics in rats with experimental cirrhosis.

ENDOGENOUS ATRIAL NATRIURETIC FACTOR PREVENTS SODIUM RETENTION DURING ACUTE CONGESTIVE HEART FAILURE. Mu E. Lee*, Brooks S. Edwards, Wayne L. Miller*, and John C. Burnett, Jr., Mayo Medical School, Rochester, MN

While circulating atrial natriuretic factor (ANF) is increased in acute congestive heart failure (CHF), the physiologic significance of this elevation is unclear. This study was designed to test the hypothesis that increased circulating ANF in acute CHF prevents sodium retention despite reductions in renal perfusion pressure (RPP). To test this hypothesis, renal perfusion pressure was reduced by 15% in three experimental models in anesthetized dogs: Group I, acute CHF produced by rapid right ventricular pacing (RRVP, n=5) with increased right atrial pressure (RAP) and ANF; and Group II, thoracic inferior vena caval constriction (TIVCC, n=5), with decreased RAP and ANF. (p < .05 CHF vs TIVCC)

	ΔRPP mmHg	ΔRAP mmHg	$\Delta U_{Na}V$ $\mu Eq/min$	ΔANF pg/ml
CHF	-18±2	+3.1±0.6	+12.5±9.2	+325±61
TIVCC	-17±1	-1.8±0.2	-31.5±13.4	-0.5±4.2
†p values	NS	p<0.001	p<0.05	p<0.05

In a third group, ANF was infused in dogs with TIVCC to produce circulating concentrations of ANF observed during acute CHF produced by RRVP. In this group with TIVCC and ANF infusion, a decrease in sodium excretion was prevented. We conclude that the role of increased endogenous ANF in acute CHF is to serve as a homeostatic hormone to prevent sodium retention and intravascular volume overload despite decreases in renal perfusion pressure.

ROLE OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN THE NATRIURETIC RESPONSE TO HEAD OUT WATER IMMERSION (HWI) IN SODIUM RETAINING CIRRHOTICS. WM Leung,* KL Skorecki, PJ Campbell,* TE Debowski,* LM Blendis,* AG Logan. Dept. of Medicine, University of Toronto, Toronto, Canada.

In cirrhotics with documented sodium retention, the natriuretic response to HWI is highly variable, ranging from a blunted response in decompensated patients to an exaggerated response in relatively compensated patients. In order to discern whether differences in ANP release or biochemical responsiveness served as a determinant of natriuresis, we measured plasma ANP, urine sodium excretion ($U_{Na}V$) and urine cGMP excretion (U_{cGMPV}) during 3 hour control (C) and HWI periods in 12 cirrhotic patients who had been maintained for 7 days on a 20 mmol/d sodium diet. Responses during the 3 hours were divided into 2 groups depending upon whether the patient went into negative sodium balance during HWI. Data are expressed as mean ± SEM.

		$U_{Na}V$ (mmol/hr)	ANP (pg/mL)	U_{cGMPV} (pmol/hr)
R	C	0.047±0.014	21.3± 4.4	33.7± 6.8
n = 6	HWI	6.922±2.191	95.1±22.9	115.6±29.8
NR	C	0.011±0.001*	21.9± 2.1	32.9± 3.5
n = 6	HWI	0.103±0.048**	73.7±15.0	132.8±46.8

where R=responders and NR=non-responders

* p<0.05 (Wilcoxon) compared to corresponding RC

** p<0.01 (Wilcoxon) compared to corresponding RHWI

All cirrhotic patients studied had a rise in plasma ANP and demonstrated biochemical responsiveness to ANP (rise in U_{cGMPV}), whether or not they had a natriuretic response. Therefore we conclude that other factors associated with the decompensated cirrhotic state overcame the effectiveness of ANP in inducing a natriuresis during HWI.

URINARY EXCRETION OF 19-NOR-ALDOSTERONE IN MEN. Sabina Lewicka*, Sabine Koch*, David Morris*, Marcel Harnik* and Paul Vecsei* (intr. by Robert Davis). Univ. of Heidelberg, Dept. of Pharmacology Heidelberg, FRG, Brown University, Providence, R.I., Tel Aviv University, Israel. 19-Nor-aldosterone, synthetic derivative of aldosterone was recently reported as a potent mineralocorticoid and hypertensinogen as assessed from in vivo two different rat bioassays. The steroid was equipotent to aldosterone in inducing a rise in systolic blood pressure in young spontaneously hypertensive rats (SHR), as well as in induction of changes in urinary Na⁺ and K⁺ in adrenalectomized male rats. Encouraged by these findings we have undertaken studies aiming at detection and determination of 19-nor-aldosterone in human urine. Samples were extracted after pH1 hydrolysis with Sep-Pak C₁₈ cartridges with ³H-19-nor-aldosterone as recovery tracer. After subsequent RP HPLC isolation, fractions corresponding to ³H-19-nor-Aldo were quantitated with RIA applying 19-nor-Aldo calibration curve, aldosterone-antibody high crossreacting with 19-nor-Aldo and ³H-aldosterone. The control values (51.6 ± 17.8 ng/24 h), n=23, were significantly lower than those of the patients with hypertension (191 ± 165.1), n=43 or primary aldosteronism (351 ± 322.9), n=17, mean ± SD. In hypertension with normal aldosterone values, 19-nor-Aldo excretions were 133 ± 161.8, n=17; with elevated aldosterone values (suspected primary aldosteronism) -230.4 ± 158.5, n=26.

MESANGIAL CELL (MC) SYNTHESIS OF PLATELET ACTIVATING FACTOR (PAF) IN RESPONSE TO DEFINED INFLAMMATORY MEDIATORS. E.A. Llanos and A. Zanglis* Department of Medicine Medical College of Wisconsin, Milwaukee.

Rat MC synthesize PAF via an acetyl-Co A: 1-0-alkyl-sn-glycero-3-phosphocholine. We studied the effect of anaphylatoxins C3a, C5a, platelet-derived growth factor (PDGF) and of exogenous PAF on the activity of this enzyme in cultured MC. MC (≈ 2 × 10⁶) were incubated with the above agonists for 3 hrs. at 37°C to induce enzyme activity. To measure enzyme activity, MC were subsequently sonicated and incubated with lyso PAF (40 μM) and ³H-acetyl-Co A (5 μCi) in RPMI-BSA (0.25%) for 15 min. at 37°C. Products were extracted and analyzed by HPLC. Enzyme activity was expressed in dpm of synthesized ³H-PAF/10⁶ MC. To assess whether the effect of PDGF or exogenous PAF were charge or receptor mediated these agonists were also employed in the presence of heparin (Hep) or the PAF receptor antagonist, L-651,108, respectively. Results, means ± SD were:

	MC acetyl transferase activity
Medium (RPMI)	17841 ± 7564
C3a (1 μg/ml)	41421 ± 5779*
C5a (20 μg/ml)	18637 ± 2614
PDGF (3 ng/ml)	35635 ± 3692*
PDGF + Hep (10 μg/ml)	18450 ± 3263
PAF (1 μM)	36158 ± 5948*
PAF + L-651,108 (10 μM)	15478 ± 2065

(* p < 0.05, compared to medium alone) The data demonstrate that MC PAF enzymic synthesis can be enhanced following exposure to anaphylatoxins C3a, PDGF and PAF itself. This effect involves charge and receptor mechanisms and could be of importance in glomerular inflammation.

ATRIAL NATRIURETIC PEPTIDE REVERSES NOREPI-NEPHRINE (NE)-INDUCED AFFERENT ARTERIOLAR VASOCONSTRICTION IN THE ISOLATED PERFUSED HYDRO-NEPHROTIC KIDNEY. Rodger Loutzenhiser*, Koichi Hayashi*, and Murray Epstein. Nephrology Section, V.A. Med. Center and Univ. of Miami, Miami, FL

Atrial natriuretic peptide (ANP) profoundly alters renal hemodynamics, but is reported to have no effect on isolated renal resistance vessels, pre-constricted with NE (AJP, 252:F317-1987). This suggests either a) that the renal vascular response to NE is refractory to ANP or b) that microdissection techniques may alter the responsiveness of microvessels to ANP. To investigate further the intrarenal actions of ANP and NE, we utilized the isolated perfused hydro-nephrotic rat kidney, a model that allows simultaneous assessment of microvessel caliber and renal vascular resistance in a controlled in vitro setting. Following chronic hydronephrosis, kidneys were perfused in vitro with artificial medium at constant renal arterial pressure (80 mm Hg). Afferent arteriolar diameter (AA-D) was visualized with videomicroscopy and renal perfusate flow (RPF) was monitored with an electromagnetic flowmeter. NE (3 μM) reduced RPF by 55 ± 12% and AA-D by 23 ± 2%. ANP (h-ANF 4-28, anaritide) reversed both actions of NE: [ANP] Basal NE 0.0001 0.001 0.01 0.1 (μM) RPF 8 ± 2* 4 ± 2 2 ± 1 4 ± 2 6 ± 2* 7 ± 2* AA-D 20 ± 1* 15 ± 1 15 ± 1 18 ± 1* 21 ± 1* 22 ± 1* mean ± SE, n=4; RPF: ml/min; AA-D: μm; * p < 0.5 vs NE. Phentolamine caused no further increase in AA-D (22 ± 2 μm), indicating that the reversal was complete; but tended to increase RPF slightly (9 ± 2 ml/min, p > 0.2). Thus, in contrast to earlier reports, these findings demonstrate conclusively that ANP completely abolishes NE-induced afferent arteriolar vasoconstriction.

EICOSANOID PRODUCTION BY THE KIDNEY OF THE GOLDFISH (CARASSIUS AURATUS). J. Lowenstein, Dept. of Med. NYU School of Medicine, New York, N.Y.

Ferreri et al (J Pharm Exp Therap 231,441, 1984) reported that rabbit renomedullary cells incubated with arachidonic acid generate, via the cyclooxygenase P450 pathway, oxygenated products which inhibit Na⁺K⁺ATPase and exert vasoactive effects. We have examined the pattern of incorporation of radio-labelled arachidonic acid (AA) by homogenates of kidney of the goldfish, a fresh water teleost in which sea water adaptation is known to induce marked reduction in GFR and a striking increase in fractional excretion of sodium.

Goldfish, maintained in fresh water (n=10) or 1/3 sea water (n=6), were sacrificed and the kidneys homogenized and incubated with ³H-AA, with or without added indomethacin (INDO) or nordihydro-guaiaretic acid (NDGA), an inhibitor of lipoxygenase. Following extraction, thin layer chromatography, and localization by radioautography, the distribution of metabolites was measured by scintillation counting.

The major metabolites, averaging 52.5 ± 6.7% of total recovered radioactivity, co-migrated with 15-hydroxyeicosatetraenoic acid (15HETE) in 2 different solvent systems. Smaller peaks, corresponding to prostaglandin standards, averaged only 8.3 ± 2.7%. INDO had little effect but NDGA reduced the formation of the major metabolites by 84%. No significant differences were observed between the homogenates derived from fresh water and sea water adapted fish.

The findings suggest that the major eicosanoids produced by the goldfish kidney are products of either the lipoxygenase pathway or the cytochrome P450 oxidative system as described by Ferreri et al.

EFFECTS OF CYCLOSPORIN A ON ENDOTHELIUM-DEPENDENT RELAXATIONS IN THE RAT RENAL ARTERY. Thomas F. Lüscher, Erika Weber* and Fritz R. Bühler*, Departments of Medicine and Research, University Hospital, Basel, Switzerland

Cyclosporin A (CyA) induces functional changes in cultured endothelial cells. Endothelial cells may secrete relaxing and constricting factors controlling vascular tone. The present study was designed to investigate whether chronic CyA therapy alters endothelium-dependent relaxations to acetylcholine (ACh) in the rat renal artery. Male Wistar Kyoto rats (22-24 weeks of age) were treated with CyA (50 mg s.c./day or the solvent) for 1 week. Renal artery rings with and without endothelium were suspended in organ chambers for isometric tension recording. ACh induced relaxations only in rings with endothelium. The IC_{25} value and the maximal relaxation of ACh (in rings contracted with norepinephrine) was significantly reduced in rats treated with CyA. The IC_{25} value in the controls was 7.4 ± 2 and 6.4 ± 0.1 in the CyA group ($p < 0.04$, $n=12$ and 11 , resp.). The maximal relaxation (10^{-4} M ACh) averaged $71 \pm 7\%$ in controls and $35 \pm 11\%$ in the CyA group ($p < 0.01$). Indomethacin (10^{-5} M) significantly enhanced the impaired relaxations to ACh. Thus, CyA impairs endothelium-dependent relaxations in the rat renal artery. The effects of indomethacin suggest an increased production of vasoconstrictor prostanoids rather than a decreased release of endothelium-derived relaxing factor as a possible mechanism.

FURTHER STUDIES ON CYCLOSPORIN A (CSA)-INDUCED HYPERRENINEMIC HYPOALDOSTERONISM: EVIDENCE FOR A DIRECT STIMULATORY ACTION OF CSA ON RENIN RELEASE. S. Lustig*, P. Eggena*, J. Barrett*, N. Stern* and D.B.N. Lee. VA Medical Center, Sepulveda and UCLA School of Medicine, Los Angeles, California.

We have reported that CSA-treatment in the rat is associated with high renin and inappropriately low aldosterone (ALD) in the circulation. We have also noted that in vitro CSA directly inhibits ALD secretion by isolated glomerulosa cells. We now test the hypothesis that in vitro CSA causes direct stimulation of renin release (RR). Renal cortical slices from 10-14 wk old male SD rats were incubated either in DMSO (control) or 10^{-5} M CSA in DMSO. Control RR rate was 102 ± 6 and this was stimulated by CSA to 122 ± 6 ng/mg/90 min ($p < 0.05$). Propranolol (P), 10^{-6} - 10^{-8} M significantly reduced RR. At P concentration ($[P]$) of 10^{-6} M, coinubation with CSA did not stimulate RR. However, CSA was able to stimulate RR partially at $[P]=10^{-7}$ M and completely at $[P]=10^{-8}$ M. Ouabain at 10^{-3} and 10^{-4} M markedly suppressed RR and this suppression was not affected by coinubation with CSA. We conclude CSA directly stimulates renal RR through a Na, K-ATPase-dependent mechanism and that this mechanism may be related to the beta-adrenergic receptor system.

ROLE OF KININS IN MEDIATING FUROSEMIDE-INDUCED RENIN RELEASE. P. Madeddu*, O.A. Carretero and A.G. Scicli*, Hypertension Research Division, Henry Ford Hosp., Detroit, MI

The role of endogenous kinins in mediating the effects of furosemide on plasma renin activity (PRA), and on sodium (U_{NaV}) and water (UV) excretion is still unknown. We investigated in normotensive conscious rats whether the changes induced by furosemide (1 mg/kg) are altered by the kinin antagonist (K-Ant) DArg⁰-Hyp³-Thi^{5,8}-DPhe⁷-bradykinin (10 μ g/min/kg). This dose of K-Ant inhibited by 75% the vasodepressor effects of bradykinin (75-300 ng/kg). No significant difference was observed in the increase induced by furosemide of either UV (control group: 367 ± 100 ; K-Ant group: 363 ± 80 μ l/min/kg) or U_{NaV} (control group: 553 ± 60 ; K-Ant group: 672 ± 90 μ Eq/min/kg). PRA increased after furosemide in controls from 5.9 ± 0.9 to 16.4 ± 2.9 ng/ml/h, $p < 0.01$, but did not change in the K-Ant group (from 6.4 ± 2.2 to 7.9 ± 1.2 ng/ml/h, n.s.). Δ PRA in controls (10.5 ± 2.6 ng/ml/h) was significantly higher than in K-Ant treated rats (1.5 ± 1.6 ng/ml/h, $p < 0.01$). Isoproterenol (100 ng/min/kg) significantly increased PRA both in control (from 5.8 ± 1.3 to 16.7 ± 3.0 , $p < 0.01$) and in K-Ant pretreated rats (from 3.5 ± 1.0 to 16.1 ± 2.3 , $p < 0.01$). Δ PRA in controls (10.8 ± 2.3) was not higher than in K-Ant treated rats (9.6 ± 2.1 , n.s.). These data suggest that kinins may contribute to the increase in PRA induced by furosemide. The effect of K-Ant on furosemide-induced renin release appears to be specific since K-Ant did not inhibit the changes induced by isoproterenol on PRA.

MECHANISM OF EPIDERMAL GROWTH FACTOR (EGF) INDUCED INCREASE IN PROSTAGLANDIN E_2 (PGE_2) PRODUCTION BY RAT GLOMERULAR MESANGIAL CELLS (MC). BL Margolis*, S. Kremer*, BJ Holub*, and KL Skorecki, Univ. of Toronto and Univ. of Guelph, CANADA.

We have previously shown that EGF is synergistic with the calcium ionophore, A23187, and vasopressin to increase PGE_2 production in MC (Clin. Res. 35:552A, 1987). Stimulation by EGF required a rise in cytosolic calcium but was not dependent on protein kinase C. In the current study we determined whether the stimulatory effect of EGF was mediated by cell alkalization or by enhancing the rise in cytosolic calcium. Intracellular pH was clamped using the K/H ionophore, Nigericin. In the presence of Nigericin, EGF still increased PGE_2 production by $108 \pm 7\%$ at pH 7.4 and $58 \pm 4\%$ at pH 7.0 (Mean \pm SEM, $n=4$). EGF did not increase basal cytosolic calcium nor increase the calcium transient response to vasopressin or A23187.

Further investigations were undertaken to determine if the effect of EGF was at the level of arachidonic acid (AA) release. EGF had no effect on the conversion of exogenously added AA to PGE_2 , indicating it did not enhance cyclooxygenase activity. However EGF significantly increased release of ^{14}C -AA from MC as free fatty acid (FFA) without increasing diacylglycerol (DAG). Mean $DPM \pm SEM$, $n=3$.

	control	A23187 (1 μ M)	A23187 + EGF (1 μ M) (10nM)
FFA	1660 ± 150	3850 ± 380	8210 ± 820
DAG	3604 ± 912	3716 ± 780	3060 ± 690

Taken together this data indicates that EGF stimulates AA release in mesangial cells but this effect is not mediated by cell alkalization or phospholipase C activation.

RENIN SECRETION IN ISOLATED GLOMERULI FROM LOW PROTEIN FED RATS. M. Martínez-Maldonado, José Pedraza* and E. Fernández-Repollet. VA Ctr., Depts. of Pharmacol., Physiol. & Med., UPR Sch. of Med., San Juan, Puerto Rico.

Low protein (LP) diet for 2 wks., results in a lower plasma renin activity (PRA) than in rats fed a normal protein (NP) diet. The decrease in PRA is associated with higher renal renin content suggesting impaired release. Of interest, LP is a hyperadrenergic state and we have shown impaired vasoconstrictor response to exogenous NE. We evaluated basal and stimulated renin secretion in isolated glomeruli from LP rats. Wistar-Furth male rats were fed a LP (6%; n=11) or a NP (23%; n=14) diet for 2 wks. At this point, rats were sacrificed, the kidneys removed, and glomeruli isolated by sieving techniques. Renin secretion (ngAl/mg prot/hr) was assessed under basal conditions and after exposure to norepinephrine (NE) 10^{-5} M; epinephrine (EP) 10^{-4} M; isoproterenol (IPT) 10^{-5} M, and phentolamine (PHEN) 10^{-4} M, and trifluoroperazine (TFP) 10^{-4} M. Results are means \pm SEM; * $p < 0.05$ LP vs NP; + $p < 0.05$ NP or LP vs. Basal.

	BASAL	NE	EP	ISP	PHEN	TFP
NP	38 \pm 8	46 \pm 9	26 \pm 6	84 \pm 17	49 \pm 7	284 \pm 56
LP	155 \pm 29*	171 \pm 21*	62 \pm 18*	186 \pm 29*	140 \pm 26*	1179 \pm 106**

These data indicate that glomerular renin secretion is significantly higher in LP than in NP rats suggesting impaired *in vivo* secretion. The lack of response to NE and EP suggests cancelling effects of alpha and beta stimulation. The inability of LP glomeruli to respond to ISP but not to TFP suggest altered beta-adrenergic response. The mechanisms by which protein deprivation alters the relation of adrenergic receptors and renin secretion remain to be elucidated.

LEUKOTRIENE B4 (LTB4) EXACERBATES RENAL FUNCTIONAL DERANGEMENTS DURING ACUTE NEPHROTOXIC SERUM NEPHRITIS (NTSN) THROUGH LEUKOCYTE (PMN)-DEPENDENT MECHANISMS. S. Matar*, GF Schreiner, A. Fogo*, and KF Badr. Vanderbilt Univ. Nashville, TN and Washington Univ. St. Louis, MO.

Our previous studies suggested a role for LTD4, a product of PMN activation, in mediating the fall in GFR during NTSN. LTB4 is a highly potent chemotactic agent that markedly increases PMN adherence to endothelium. By increasing PMN intracellular [Ca], LTB4 also induces PMN activation and degranulation. As increased glomerular LTB4 synthesis has been demonstrated in NTSN (Lianos. Clin.Res. 34:972), we investigated its potential role in exacerbating the PMN-associated, NTS-induced, fall in GFR.

GFR, RPF (ml/min), and glomerular PMN counts (250 glomeruli examined/group) were performed 2 hrs. following i.v. administration of rabbit serum or NTS in three groups of anesthetized male rats as follows: Gp 1 (controls, n=5): received 0.4 ml of rabbit serum; Gp 2 (n=5): received 0.4 ml of NTS, a dose designed to achieve a mild reduction in GFR; Gp 3 (n=5): were given the same dose of NTS but functional measurements were preceded by a 10-min selective renal infusion of LTB4 (0.5 μ g/kg/min). Results: mean \pm SEM. * : $p < 0.025$ vs Gp 1; § : $p < 0.025$ vs Gp 2.

	GFR	RPF	FF	PMN/Glom.
Gp 1:	1.19 \pm 0.09	4.24 \pm 0.37	0.29 \pm 0.03	0.74 \pm 0.06
Gp 2:	0.86 \pm 0.07*	4.27 \pm 0.25	0.21 \pm 0.02*	0.87 \pm 0.10
Gp 3:	0.46 \pm 0.08*§	2.82 \pm 0.37*§	0.18 \pm 0.04*	1.39 \pm 0.12*§

In a fourth group of 4 rats, LTB4 infusion alone was without effect on PMN counts and resulted in mild (15%), significant, fall in GFR when compared to Gp 1. Regression analysis of pooled values for GFR vs PMN counts from Gps 2 and 3 revealed a strong inverse correlation, demonstrating dependence of the fall in GFR on the increase in PMNs/glomerulus ($r^2 = 0.75$, $p < 0.01$).

These experiments: 1. Further support a central role for infiltrating PMNs in mediating the functional impairment in NTSN. 2. Define a potential role for LTB4, through PMN recruitment and activation, in the amplification of these functional derangements.

DISAPPEARANCE OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN THE CANINE. Takashi Masuda*, and Fumiaki Marumo, Kitatsato Univ. Sch. Med., JAPAN

It is well known fact that the half-life of ANP in the plasma is very short. However, it was not determined which organ degraded ANP quickly. To clarify the disappearance of ANP in the body of dog, 2 ml blood samples were simultaneously collected from the arteries (A) and veins (V) of various organs, and plasma ANP concentrations were measured by our RIA (BBRC, 137:231, 1986). Fine catheters were fixed in both A and V of the lung, kidney, spleen and cervix; and coronary sinus, supramesenteric A (SMA) and portal V (PV) in dogs infused with exogenous ANP (28 ng/kg/min, n=7) and not infused with exogenous ANP (n=6).

The plasma ANP concentration obtained from coronary sinus was 120 \pm 27.0 pg/ml. The endogenous ANP concentrations in pulmonary A decreased to 67.3 \pm 6.0% in V, that in renal A to 66.4 \pm 5.4% in V, that in splenic A to 67.4 \pm 6.2% in V, that in SMA to 88.4 \pm 7.8% in PV and that in cervical A to 75.4 \pm 3.4% in V.

Exogenous ANP infused into right atrium. The plasma ANP concentration in pulmonary A increased to 1500.4 \pm 328.5 pg/ml and decreased to 62.8 \pm 4.7% in V. That in renal A decreased to 58.1 \pm 8.7% in V, that in splenic A to 59.3 \pm 7.5% in V, that in hepatic A to 74.0 \pm 10.5% in V, that in SMA to 66.2 \pm 7.4% in PV, and that in cervical A to 62.8 \pm 4.7% in V.

The present results suggest that the arteriole wall degrades plasma ANP or consume it in the binding sites, since the plasma ANP concentrations in V were lower than those in A in every organ examined by almost same ratio. No specific organ for ANP degradation could be estimated.

DIRECT EFFECT OF BETA ENDORPHIN ON FLUID REABSORPTION IN RABBIT PROXIMAL CONVOLUTED TUBULES. J.W. McKeown. VAMC and Div. of Nephrology, Bone and Mineral Metab., Univ. of Ky., Lexington, Kentucky.

Beta endorphin (B-end) has been shown to stimulate aldosterone production and thus indirectly influence renal sodium excretion. There is, however, very little information regarding possible direct renal or tubular effects of B-end or other opioid peptides. The present studies were designed to assess the direct effects of B-end on fluid reabsorption (J_V) in the rabbit proximal convoluted tubule (PCT).

Segments of rabbit PCT were perfused in vitro using artificial solutions. After control measurements, varying concentrations of B-end, within the physiologic range, were added to the bath. One and 5pM B-end had no effect of J_V . However, 10pM B-end significantly increased J_V (1.22 \pm 0.19 vs 0.90 \pm 0.18nl/mm.min, $p < 0.005$, n=5). An increase in the bath concentration of B-end to 100pM resulted in no further increase in J_V . In a separate series, tubules were exposed to Naloxone 200pM which alone had no effect on J_V (0.81 \pm 0.04 vs 0.80 \pm 0.09, N.S., n=5). However, Naloxone added before or after exposure to B-end either blocked or reversed the stimulatory effect seen with B-end alone.

These results demonstrate: 1) direct stimulation of J_V by B-end with a threshold between 5-10pM concentration and 2) evidence of μ -receptor action as evidenced by antagonism or blockade of the effect by Naloxone.

MODULATION OF MESANGIAL CELL PROLIFERATION BY PROSTAGLANDINS. Paolo Mene*, R. Guy Mc Dermott*, and Michael J. Dunn. Depts. of Medicine and Physiology, Case Western Reserve University and University Hospitals, Cleveland, Ohio.

Prostaglandin (PG) synthesis by resident and blood-borne cells has been implicated in the control of glomerular function and cellular interactions in inflammation. We have previously identified distinct signal transduction mechanisms for $\text{PGF}_{2\alpha}$ and PGI_2 in mesangial cells, namely polyphosphoinositide breakdown by phospholipase C and stimulation of cyclic AMP, respectively. Inasmuch as these signals mediate various cell functions including contractility and proliferation, we studied whether $\text{PGF}_{2\alpha}$ and PGI_2 regulate rat mesangial cell growth in culture. $\text{PGF}_{2\alpha}$ dose-dependently stimulated [^3H]-thymidine (TdR) incorporation into quiescent, confluent monolayers incubated for 48 hrs in the absence of serum (FBS), with threshold at 1 nM and EC_{50} at 0.1 μM ($+35.1 \pm 7.5\%$, $p < 0.002$ vs. basal incorporation). 60-min pulses or 48-hr incubations with 1 μM $\text{PGF}_{2\alpha}$ had comparable mitogenic effects ($+48.5 \pm 1.1$ and $+53.9 \pm 9.5\%$ respectively, NS). 10 μM $\text{PGF}_{2\alpha}$ stimulated a $73.3 \pm 13.6\%$ elevation in TdR uptake, with corresponding increments of cell counts, at a rate of 1.3×10^4 cells/cm²/24 hrs ($p < 0.001$ vs. control). $\text{PGF}_{2\alpha}$ also enhanced replication of cycling cells in the presence of 2% FBS, as assessed by sequential, computer-assisted image analysis microscopy (3.2 vs. 1.9×10^3 cells/cm²/24 hrs, $\text{PGF}_{2\alpha}$ 1 μM and control, respectively, $p < 0.05$). The effect of $\text{PGF}_{2\alpha}$ was reduced by removal of insulin and was additive to other mitogens such as platelet-derived growth factor (PDGF 0.1 nM $+301.8 \pm 66.1\%$, PDGF+ $\text{PGF}_{2\alpha}$ 1 μM $+470.7 \pm 40.3\%$). The stable PGI_2 analogue Iloprost, 1 μM -10 μM , inhibited basal TdR incorporation, with a maximum of $-23.7 \pm 8.1\%$ at 1 nM. We hypothesize that glomerular PG regulate mesangial turnover in various pathophysiological states, including chronic glomerular disease and compensatory growth.

EFFECT OF INTRARENAL ADENOSINE ON RENAL FUNCTION AND VASA RECTA BLOOD FLOW IN THE RAT. M Miyamoto, TS Larson, CR Robertson, RL Jamison. Depts. of Med. and Chem. Eng., Stanford Univ., Stanford, CA and Univ. of Rochester, Rochester, NY.

It has been postulated that adenosine, a vasodilator, plays a role in regulation of regional blood flow but its effect on the renal medullary circulation is unclear. Blood flow in descending (Q_{DVR}) and ascending (Q_{AVR}) vasa recta of the exposed right renal papilla of anesthetized rats was determined by fluorescence videomicroscopy before (Period 1) and during the intrarenal artery infusion (via the right suprarenal artery) of adenosine [N=5; at 6 ng/min (Period 2) and 15 ng/min (Period 3)]. Control animals (N=5) received the vehicle alone in Periods 2 and 3. Urinary sodium excretion, inulin and PAH clearances (U_{NaV} , C_{IN} and C_{PAH} , respectively) were determined in parallel studies (N=6 for both groups). Results in the experimental groups (Means \pm SE):

Period	1	2	3
Adenosine ($\mu\text{g}/\text{min}$)	0	6	15
U_{NaV} ($\mu\text{Eq}/\text{min}/\text{gKW}$)	1.0 ± 0.5	$2.2 \pm 0.5^*$	$3.4 \pm 0.6^*$
C_{IN} ($\text{ml}/\text{min}/\text{gKW}$)	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.6
C_{PAH} ($\text{ml}/\text{min}/\text{gKW}$)	4.5 ± 0.1	5.3 ± 0.7	$5.5 \pm 0.5^*$
Q _{DVR} (nl/min)	7.5 ± 1.4	8.5 ± 1.9	$15.1 \pm 2.3^*$
Q _{AVR} (nl/min)	4.4 ± 0.7	5.9 ± 0.6	$7.4 \pm 0.7^*$

* $P < 0.01$ compared to Period 1. None of the parameters changed significantly in the controls in Periods 2 or 3. The results show intrarenal adenosine increased Q_{DVR} and Q_{AVR} markedly and disproportionately more than total renal blood flow. In the absence of a change in GFR, the rise in U_{NaV} may be related to the increase in Q_{Vr}.

POSSIBLE BIOCHEMICAL PATHWAY OF EARLY ALDOSTERONE ACTION IN CULTURED RENAL COLLECTING DUCT CELLS. Will W. Minuth, Ulrike Steckelings and Peter Gross (intr. by R.J. Anderson). Depts. of Anatomy and Medicine, Univ. Heidelberg, FRG

The common theory of the aldosterone-dependent Na^+ -transport is, that the hormone raises the Na^+ -transport during the 'early' and 'late' response by inducing specific proteins (AIPs). In actual biochemical studies, AIPs were mostly found 6-18 hours after aldosterone application. Regarding the 'early' response phase this means an enormous time dissociation between the physiological and biochemical events. The discrepancy raises the question whether perhaps other biochemical events such as protein modifications are involved. Labelling of cultured collecting duct epithelia (Minuth et al., Differentiation 33:156-167, 1986; Gross et al., Pflügers Archiv 406:380-386, 1986) for 1-5 hours with the radioactive methyl group donor S-adenosyl methionine (SAM) following tissue fractionation and electrophoresis, resulted in methylations of specific cytosolic proteins. Compared to controls aldosterone dependent methylations increased constantly and were twice as much after 5 hours of labelling. The methylated proteins show a molecular weight of 220-97 and 45 kd and an isoelectric point of 4.1, 5.0-5.6, 6.0-7.6, 8.6 and 8.9-9.4. Methylation of identical proteins was obtained by incubation with unlabelled SAM in spite of aldosterone. SAM induced methylations as well as aldosterone induced methylations were inhibited by S-adenosyl-homocysteine.

TWO CLASSES OF BETA-ADRENERGIC RECEPTORS IN ISOLATED SALAMANDER PROXIMAL TUBULES.

N.S. Morgunov* (intro. by D. Hirsch). Dept. of Physiol. and Biophysics., Dalhousie Univ., Halifax, Nova Scotia, Canada.

Isolated perfused salamander proximal tubules hyperpolarize or depolarize following the addition of 0.01 mM isoproterenol to the bath superfusate. Of 45 tubules examined, 10 tubules (22%) hyperpolarized. Both responses were blocked by 1 μM propranolol, suggesting two classes of β -receptors. This possibility was investigated by incubating proximal tubules for 1 hr with the β -receptor ligand (-)[^3H]-CGP 12177. Of 200 tubules studied, 25% exhibited high affinity binding characteristics ($K_D = 12.0$ nM; $B_{\text{max}} = 3.9$ fM/mm) while the remainder were classified as low affinity ($K_D = 153.8$ nM; $B_{\text{max}} = 110.2$ fM/mm). The displacer drug was 0.1 mM timolol. Competition studies showed that (-)[^3H]-CGP 12177 behaved as a typical β -adrenergic ligand. It was displaced by (-)isoproterenol, (+)timolol and (+)propranolol but not by (+)isoproterenol or (-)phenylephrine. The binding data represent functional β -receptors since the diluting segment exhibited no significant ligand binding and no electrical response to β -agonists. In conclusion, electrical/pharmacological data confirm the presence of two distinct populations of proximal tubules possessing either high or low affinity β -receptors.

MODULATION OF VASOPRESSIN-INDUCED CALCIUM MOBILIZATION BY PROTEIN KINASE C IN GLOMERULAR MESANGIAL CELLS A.R. Morrison, D. Coyne, D. Portilla. Washington University School of Medicine, Department of Medicine and Pharmacology, St. Louis, MO 63110

Vasopressin induced mesangial cell contraction has been associated with the activation of phospholipase C, calcium mobilization and prostaglandin production. To elucidate the role of PKC in modulating vasopressin responses we studied the effect of phorbol myristate acetate (PMA) and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7), an inhibitor of PKC in rat mesangial cells loaded with fura2.

Incubation of rat mesangial cell membranes with [³H] PIP₂/PE vesicles resulted in the rapid (10 seconds) formation of [³H] inositol 1,4,5 trisphosphate in the presence of GTPγS (10 μM) and vasopressin (10⁻⁷ M). Control [³H]IP₃ was 2180 cpm/mg membrane protein vs. stimulated 7900 cpm/mg membrane protein. This demonstrates a membrane associated phospholipase C coupled to the vasopressin receptor through a G protein. Vasopressin stimulated intracellular calcium mobilization in a dose dependent manner increasing cytosolic free calcium from basal levels of 100nM to peak of 710 nM with 10⁻⁶M AVP. Pretreatment with PMA produced a dose dependent inhibition of the calcium response. Preincubation with H7 reversed in a dose dependent manner the PMA inhibition. We conclude: 1) Vasopressin stimulates a membrane bound PLC with rapid formation of inositol 1,4,5 trisphosphate and calcium mobilization, 2) PMA inhibits the vasopressin induced intracellular calcium mobilization, 3) H7 reversed this inhibition suggesting it occurs through PKC activation. Phorbol ester may inhibit IP₃ generation and Ca²⁺ responses either through activation of an IP₃ triphosphatase or through disruption of the receptor-G coupling mechanism.

CAPTOPRIL REVERSES THE ANTI-NATRIURETIC ACTION OF PROSTAGLANDIN (PG) BLOCKADE IN DENERVATED KIDNEYS. Nicholas G. Moss*, Joan D. Barber*, Alyson B. Scoltock*, Nancy W. McDonald*, and Romulo E. Colindres. Depts. of Physiol. and Med., Univ. of N. Carolina at Chapel Hill, North Carolina.

We have previously shown that the natriuresis that follows acute renal denervation (DNX) is eliminated by PG blockade. A possible role for angiotensin II (AII) in this effect has been tested in rats after acute DNX of the left kidney. PG blockade with meclofenamate (MECLO, 4mg/kg BW iv) was followed by ACE inhibition with captopril (CAP) (2mg/kg BW iv and 4mg/hr). Clearance and late proximal micropuncture data from DNX kidneys are shown. V=urine flow rate, LPV=late prox flow.

	CONTROL	MECLO	CAP
V ul/min	7.5±0.4	5.2±0.5*	7.5±0.6
UNaV (uEq/min)	2.0±0.3	1.3±0.1*	2.2±0.3
GFR (ml/min)	1.4±0.1	1.4±0.1	1.5±0.3
SNVFR (nl/min)	51.5±3.6	52.3±3.6	46.2±2.3
LPF (nl/min)	28.0±0.9	26.9±1.0	27.0±0.8

Means ± SEM, *p<.05 vs CONTROL and CAP (ANOVA). Infusion of CAP before MECLO produced an increase in sodium and water excretion which was reversed by MECLO. The pronounced effects of PG blockade and CAP were not reflected in fluid delivery out of the proximal tubule and thus the anti-natriuresis of PG blockade and its reversal by CAP take place in more distal segments and/or deeper nephrons. These data support a functional antagonism between AII and PGs at a tubular level in the denervated kidney although other actions of CAP unrelated to ACE inhibition cannot yet be excluded.

EFFECTS OF SARALASIN (S) ON CYCLOOXYGENASE INHIBITION IN FEMALE RATS. K.A. Munger* and R.C. Blantz. Dept. of Med., UCSD and VAMC, La Jolla, CA.

Previous work has shown that acute prostaglandin (PG) synthesis inhibition with indomethacin (I) increases GFR and lowers renal vascular resistance (RVR) in the female but exerts no effect on the male rat. These findings imply I is inhibiting a vasoconstrictor system. Since PGs stimulate renin/angiotensin II (AII) production, the removal of this stimulus with I may exert its effect in females by lowering AII resulting in vasodilation. To test this hypothesis, the AII receptor blocker was given (S; 5μg/kg/h) throughout the euvolemic protocol, while I was administered (S+I; 2mg/kg/h) during the second period. C denotes control values in weight matched untreated animals. Whole kidney GFR, renal plasma flow rate (RPF), RVR, and arterial blood pressure (AP) are given in C, S, and S+I. (Data: Mean±SE; *p<.05, paired t-test.)

	AP mmHg	GFR ---ml/min---	RPF ml/min	RVR mmHg
C	103±2	0.83±0.04	3.0±0.4	20.3±2.4
S	99±2	0.90±0.03	3.1±0.3	18.0±1.7
S+I	103±2	1.01±0.04*	3.6±0.3*	15.6±1.0

Baseline values in C animals are similar to those in animals treated with S. I, in the presence of S, significantly increases GFR and RPF. RVR is slightly, though not significantly, lowered and AP is unchanged. Although PGs stimulate AII production, these studies clearly indicate that the increases in GFR and RPF with I in female rats are not explained by decreased AII effects on the vasculature.

EFFECT OF ATRIAL NATRIURETIC FACTOR (ANF) VERSUS CAPTOPRIL (C) UPON RENAL FUNCTION IN TWO-KIDNEY, ONE CLIP (2K,1C) HYPERTENSION. Joseph Nally, B. Gupta, H. Clarke, M. Pisano, G. Grecos. Cleveland Clinic Found., Cleveland, OH and Medical College of Ohio, Toledo, OH.

ANF has been reported to decrease mean arterial pressure (MAP), increase global GFR and urinary sodium excretion (U_{Na}V), and suppress the renin angiotensin system in normal subjects. We studied the effect of lowering MAP with either C or ANF upon the GFR (C_{in}), U_{Na}V, and Tc-DTPA renography of the stenotic (SK) and contralateral kidney in 2K,1C hypertensive dogs.

In C-treated animals (n=9), we observed that angiotensin converting enzyme inhibition with captopril (1.5 mg/kg bolus with 1.5 mg/min infusion) reduced MAP (140±4 vs 106±4) and GFR of SK (16.0±3.1 vs 11.0±2.5 ml/min, p<.03). Captopril also strikingly altered the Tc-DTPA renogram of SK by markedly decreasing renal uptake and excretion of Tc-DTPA (Hypertension 8:685-693, 1986). In the ANF-treated animals (n=8), ANF (Auriculin A 1.0 ug/kg bolus with 0.1 ug/kg/min infusion) lowered MAP (150±4 vs 123±6 mmHg, p<.01), but did not significantly lower GFR of SK (16.2±4 vs 17.0±3 ml/min, p=NS). ANF did not adversely affect the Tc-DTPA renogram of SK. Despite the reduced MAP and unchanged GFR, ANF did significantly increase U_{Na}V of SK (.09±.02 vs .28±.06 ueq/min, p<.05).

Conclusion: 1) In contrast to captopril, ANF reduced MAP without adversely effecting GFR of SK --suggesting a differing effect upon intra-renal AII vasoconstriction. 2) Increased U_{Na}V of SK with a fixed GFR suggests a non-hemodynamic role of ANF-induced natriuresis.

STIMULATION OF NEM-SENSITIVE ATPASE ACTIVITY IN THE RABBIT INNER MEDULLARY COLLECTING DUCT (IMCD) BY ALDOSTERONE (ALDO). Neelam Narang* and Lal C. Garg. Univ. of Florida Coll. of Med., Gainesville, FL.

Aldo treatment of animals has been shown to stimulate H⁺ secretion in the outer medullary collecting (OMCD) probably by increasing H-ATPase activity in this segment. The IMCD also secretes H⁺ and has also receptors. In order to investigate if H-ATPase in the IMCD is also influenced by aldo, we determined NEM (N-ethylmaleimide)-sensitive ATPase activity in the IMCD and OMCD in two groups of adrenalectomized rabbits maintained on different doses of aldo (1.5 µg or 5 µg/100 g bw) daily for 7 days (given in an osmotic minipump, Alza Corp.). NEM-sensitive ATPase activity was determined in the presence and absence of 1 mM NEM but in presence of ouabain and oligomycin by a microfluorometric assay in which ATP hydrolysis is coupled to NADH oxidation.

Rabbit Group Number	Plasma Aldo (ng/dl)	IMCD (pmol·min ⁻¹ mm ⁻¹)	OMCD (pmol·min ⁻¹ mm ⁻¹)
I	10.4 ± 0.8	8 ± 1	9 ± 1
II	70.5 ± 7*	29 ± 7*	17 ± 3*

Values are mean ± S.E.M. of 4-7 animals.

* P < 0.05 vs group I.

There was >200% increase in NEM-sensitive ATPase activity in the IMCD as compared to <100% increase in the OMCD by an increase in plasma aldo from 10.4 ± 0.8 to 70.5 ± 7.0 ng/dl. These results suggest that aldo stimulates H-ATPase activity not only in the OMCD but also in the IMCD.

ATRIAL NATRIURETIC FACTOR INHIBITS VASOPRESSIN-STIMULATED OSMOTIC WATER PERMEABILITY IN RAT INNER MEDULLARY COLLECTING DUCT. H. Nonoguchi*, J.M. Sands, and M.A. Knepper, NIH, NHLBI, Bethesda, MD.

The inner medullary collecting duct (IMCD) has been proposed to be a site of atrial natriuretic factor (ANF) action. We carried out experiments in isolated perfused terminal IMCDs to determine whether ANF (rat ANF 1-28) affects either osmotic water permeability (P_o) or urea permeability. In the presence of a physiologic, submaximally-stimulating concentration of vasopressin (0.01 nM), ANF (100 nM) significantly reduced P_o from 161.4 ± 23.1 to 89.3 ± 24.3 µm/s. Addition of vehicle had no effect. Lower concentrations of ANF also significantly inhibited vasopressin-stimulated P_o by the following percentages: 0.01 nM ANF, 18.2 ± 15.9 %; 0.1 nM, 45.5 ± 6.2 %; 1 nM, 48.0 ± 7.5 %. ANF had no effect on P_o in the absence of vasopressin. Furthermore, ANF did not affect urea permeability either in the presence or absence of vasopressin. Addition of exogenous cyclic GMP (0.1 mM) mimicked the effect of ANF in the presence of vasopressin, decreasing P_o by 48.0 ± 5.4 %. In isolated nonperfused IMCD incubated in the presence of 0.01 nM vasopressin, ANF did not alter cyclic AMP accumulation (radioimmunoassay), either in the presence or absence of the phosphodiesterase inhibitor IBMX (0.5 mM). CONCLUSIONS: 1) Atrial natriuretic factor at physiological concentrations causes a large inhibition of vasopressin-stimulated osmotic water permeability in the rat terminal IMCD. 2) Cyclic GMP is the second messenger mediating this effect. 3) The inhibition of osmotic water permeability is not dependent on an effect on cyclic AMP metabolism, but rather appears to affect some process subsequent to cyclic AMP generation.

ANGIOTENSIN II (AII) CAUSES FORMATION OF PLATELET ACTIVATING FACTORS (PAF) IN CULTURED RAT MESANGIAL CELLS (MC). R. Neuwirth, J.A. Satriano*, S. De-Candido*, and D. Schlöndorff. Dept. of Medicine, Albert Einstein College of Medicine, Bronx, NY

AII may contribute to nonhypertensive renal disease as evidenced by beneficial effects of converting enzyme inhibitors not solely explicable by altered hemodynamics. AII stimulates phospholipase A2, which is required for PAF formation. We therefore examined whether AII causes PAF formation in MC. MC of 3rd sub-culture were incubated with buffer + AII. At the times indicated, buffer and MC were separately extracted for lipids and purified by TLC. Zones corresponding to PAF standards were tested for PAF by rabbit platelet aggregation bioassay. AII caused rapid formation of PAF, predominantly in the cells, but also in the incubation buffer, which decreases with prolonged incubation.

Time After AII Buffer (n=4)	PAF (pmol/mg protein)		
	5 mins.	15 mins.	30 mins.
Cell	3.8 ± 2.3	0.5 ± 0.3	0.8 ± 0.5
Cell	25.2 ± 8.4	8.7 ± 2.0	12.0 ± 3.0

No PAF was detected in the absence of AII. PAF generation by AII was dose dependent from 10⁻¹⁰M to 10⁻⁸M of AII. PAF was authenticated by negative ion chemical ionization mass spectrometry and indentified as a single species, i.e. hexadecyl PAF. We speculate that PAF may play a pathophysiological role during intraglomerular AII formation by causing e.g. local platelet and leukocyte activation.

SELECTIVE BREEDING FOR THE RENIN RESPONSE TO A LOW SODIUM DIET. B. Noordewier and J.K. Belknap. University of North Dakota, Department of Pharmacology, Grand Forks, ND

The purpose of this study was to measure the genetic control of the renin-angiotensin system through selective breeding beginning with a genetically heterogenous strain of rats (N:NIH strain). The test procedure consisted of feeding rats a low sodium diet for 8 days after which a blood sample was collected from the tail for measurement of plasma renin activity (PRA). Rats from each generation (n = 96 per line) were tested in a series of 6 runs of about 48 rats each. Selective breeding was by a mass selection procedure using a normalized PRA score to account for differences between runs. After initially selecting and assigning rats to the high, low and control lines, all subsequent breeding was done within lines. In each of 4 generations, the highest scoring rats in the high line were interbred as were the lowest scoring rats in the low line. Control rats were selected without regard to PRA score. Selective breeding produced a progressive separation of PRA scores in each generation. Rats in generation 4 had test PRAs (mean + S.E.) of 19.5 ± .5, 24.9 ± .5 and 27.5 ± .5 ng AI/ml/hr for the low, control and high lines respectively. These PRA values were significantly different from one another (p < 0.01). The successful breeding of three lines of rats based on PRA after a low sodium diet indicates significant genetic control of the renin response.

CLEARANCE RECEPTORS OF ATRIAL NATRIURETIC FACTOR (C-ANF RECEPTORS) IN ISOLATED GLOMERULI AND MESANGIAL CELLS IN CULTURE. D. Nussenzveig*, R. Scarborough*, J. Lewicki* and T. Maack. Dept. of Physiol., Cornell Univ. Med. Coll., New York, N.Y. and California Biotechnology, Mountain View, CA.

C-ANF receptors are the majority of renal binding sites of ANF, do not mediate any of its known renal effects and serve as clearance receptors to modulate plasma ANF levels (Maack et al., *Science*, 1987, in press). In the present study we tested for the presence of C-ANF and biological(B)-ANF receptors in isolated glomeruli(G) of the rat and mesangial cells(M) in culture. The analog RSSCFG-GRIDRIGAC-NH₂(C-ANF) was used to identify C-ANF receptors. In G, apparent affinity(K_d) for binding and density of binding sites(B_{max}) for biologically active ANF₁₋₂₈ were 49pM and 2.2 pmoles/mg protein, respectively. In M, K_d=118pM and B_{max}=81 fmoles/10⁶ cells. C-ANF did bind to 82% (K_i=350pM) and 41%(K_i=435pM) of ANF₁₋₂₈ binding sites in G and M, respectively. In M, ANF₁₋₂₈ increased cGMP in a dose-related manner: a twofold increase at 10⁻¹¹M(ΔcGMP=2.5±0.1 pmoles/10⁶cells, p<0.05) and app.fortyfold increase at 10⁻⁷M. In contrast, C-ANF did not increase cGMP even at 10⁻⁷M(ΔcGMP=-0.18±0.04 pmoles/10⁶ cells, p>0.05) and did not antagonize or potentiate the cGMP generating effect of 10⁻¹¹ to 10⁻⁷M ANF₁₋₂₈. The results demonstrate that in isolated glomeruli and mesangial cells in culture, C-ANF receptors: i) constitute a large proportion of total ANF receptors; ii) bind ANF with high affinity; iii) are uncoupled to cGMP and do not influence the cGMP generating effect of ANF. Glomerular C-ANF receptors may play an important role in the renal metabolism of ANF.

ATRIAL NATRIURETIC FACTOR (ANF) ANTAGONIZES ANGIOTENSIN II (ANG II)-MEDIATED PGE₂ RELEASE IN CULTURED RAT MESANGIAL CELLS (MC). P. Ortiz*, L. Ramsamy*, R. Barnett, SUNY-Stony Brook, NY.

We have reported that ANF attenuates ANG II-mediated changes in phosphoinositide (PI) and phospholipid metabolism in MC (Barnett et al., *ASN* 1987). These studies suggested that ANF (10⁻⁷ M) would partially blunt ANG II (5 x 10⁻⁷ M) mediated increases in MC PGE₂ production. The mechanism for this effect was evaluated by pre-incubating confluent MC subcultures for 10 min with ANF and then adding ANG II, phorbol ester(TPA 10⁻⁵M) or A23187 (5μM) for an additional 10 min.

	Cont	Agonist	Agonist+ANF
ANG II (n=26)	0.48±0.06	6.81±0.46 ⁺	3.15±41 [*]
TPA (n=11)	1.05±0.08	8.55±1.0 ⁺	8.95±1.3
A23187 (n=9)	0.45±.10	24.30±2.1 ⁺	23.10±2.5

* <.05 vs control, ⁺ p<.05 vs agonist.
Results are expressed in ng PGE₂/mg protein as determined by RIA and corroborated by EIA. This ability of ANF to diminish ANG II-mediated PGE₂ release appeared to be dose dependent (ANF 10⁻¹⁰ M to 10⁻⁷ M). It has been postulated that increases in MC cGMP modulate the effect of ANF. Preincubation with 0.5 mM 8 Br-cGMP significantly inhibited ANG II-stimulated PGE₂ release (7.58±.55 to 3.0±.47, n=12). Our studies suggest that in MC, agonist-mediated changes in PGE₂ release partially result from effects at the level of phospholipase C and may involve cGMP.

ENDOGENOUS ATRIAL NATRIURETIC PEPTIDE (ANP) AUGMENTS FRACTIONAL EXCRETION (FE) OF PHOSPHATE (Pi) AS WELL AS SODIUM (Na⁺) IN RATS WITH REDUCED RENAL MASS. FV Ortola*, BJ Ballermann, S Downes* and BM Brenner. Harvard Med. Sch., Boston, MA.

FE_{Na} and FE_{Pi} are increased with reduced renal mass, a state of circulating ANP excess. Na restriction decreases plasma ANP in this setting. We therefore pair-fed 2 groups of 5/6 nephrectomized (Nx) rats a low Na (.4 mEq/d) diet. The Na intake was adjusted to .6 mEq/d in the low Na group (LS) and to 3.8 mEq/d in the regular Na (RS) group by daily saline injection IP. Values for total GFR (ml/min), FE_{Na} (%), FE_{Pi} (%) and plasma ANP (pg/ml) were determined 2 weeks post-ablation (n=5/group, mean±SEM, * p<.05).

	GFR	FE _{Na}	FE _{Pi}	ANP
Nx,RS	0.95±0.05	1.2±0.2*	21.8±3.2*	142±6*
Nx,LS	0.91±0.14	0.2±0.1	9.1±2.6	87±6

Thus, plasma ANP levels, FE_{Na}, and FE_{Pi} were greater in RS than LS despite comparable Pi intake. To clarify whether the elevated ANP in 5/6 NxRS contributes to high FE_{Na} and FE_{Pi}, specific ANP antiserum (ANP-AS) or non-immune serum (NIS) was infused in 10 other 5/6 NxRS rats.

	GFR	FE _{Na}	FE _{Pi}
Before ANP-AS	0.96±0.1	1.0±0.1	24.6±3.0
During ANP-AS	1.03±3.4	0.3±0.1*	16.9±3.3*
Before NIS	0.82±0.1	1.0±0.1	19.9±2.5
During NIS	0.90±0.1	1.5±0.3	25.0±4.1

ANP-AS blunted FE_{Pi} and FE_{Na} without changes in GFR, whereas NIS was without effect. These data implicate endogenous ANP in promoting the adaptive increases in FE_{Na} and FE_{Pi} per nephron in chronic renal disease. Unlike diabetic rats, however, the hyperfiltration response to renal ablation was not sensitive to ANP-AS.

THE EFFECTS OF SOMATOSTATIN ON ALANINE-INDUCED GLOMERULAR HYPERFILTRATION AND THE RENAL HANDLING OF PHOSPHATE. R.C. Pabico, J.A. Truglia,* S.K. Nair,* B.A. McKenna,* D.H. Lockwood.* Univ. of Rochester Medical Center, Rochester, New York.

The rise in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) during amino acid (AA) infusion is mediated by hormonal factors, e.g. glucagon (G) and growth hormone (GH). The phosphaturia during AA infusion may reflect the increase in filtered load of P and/or the G effects on tubular reabsorption of P. To determine if the phosphaturia is independent of either GFR or G, 2 sets of studies were performed on 6 healthy male volunteers: GFR and ERPF by inulin and PAH clearances, respectively, percent tubular reabsorption of filtered P (TRP) and renal threshold P concentration (T_{mp}/GFR) following I.V. infusion of L-alanine (0.2 g/kg IBW) alone and L-alanine (A) plus Somatostatin (S), 300 μg/hr. Plasma levels of G, GH, Insulin, free fatty acids, β-hydroxybutyrate and AA were also measured. The results are expressed as mean ± SE; student t test for paired data was used and p values of .05 or less are considered significant.

	GFR	ERPF	TRP	T _{mp} /GFR
	(ml/min/1.73m ²)	(%)	(%)	(mg/dl)
Control (C)	116±11	597±47	89±2	2.9±0.2
A	148±15	716±87	69±1	1.9±0.1
A + S	119±4	527±13	64±5	2.0±0.1
p (A vs C)	<.05	<.05	<.001	<.02
p (A + S vs C)	NS	NS	<.001	<.02

A infusion increased plasma G, GFR and ERPF significantly. TRP and T_{mp}/GFR decreased. S inhibited G secretion and blunted the rise in GFR and ERPF, but phosphaturia persisted. Thus, A-induced phosphaturia is independent of GFR and G.

SODIUM CHLORIDE AND OSMOTIC WATER PERMEABILITIES OF ISOLATED RABBIT PAPILLARY SURFACE EPITHELIUM: EFFECTS OF VASOPRESSIN. R.K. Packer,* J.M. Sands, and M.A. Knepper. NHLBI, NIH, Bethesda, MD, and George Washington University, Washington, DC.

Although passive transport across the papillary surface epithelium (PSE) has been proposed to play an important role in the concentrating mechanism, little is known about its permeability properties. We isolated the PSE from rabbits and mounted it in a small Ussing chamber. Apparent Cl permeability (P_{Cl}) of the PSE was determined by imposing a 116 mM NaCl gradient and measuring the resulting Cl flux and transepithelial voltage. P_{Cl} calculated using the Goldman-Hodgkin-Katz equation averaged $3.1 \pm 1.1 \times 10^{-5}$ cm/sec (n=9) and decreased to $2.2 \pm 0.8 \times 10^{-5}$ cm/sec when the tissue was exposed to vasopressin (AVP, 0.1 or 100 nM). The effect was reversible. The apparent Na/Cl permeability ratio (Goldman equation) was 0.75 ± 0.06 and was not significantly affected by AVP. Transepithelial resistance with isotonic solutions was 60 ohm-cm² and increased significantly to 79 ohm-cm² with AVP. Osmotic water permeability (P_w) was determined by imposing an osmotic gradient and measuring H₂O flux using creatinine or ¹⁴C-inulin as a volume marker. Creatinine and inulin permeabilities were low (8.1×10^{-6} and 8.6×10^{-7} cm/sec). Mean P_w (n=16) was 14.2 μm/sec and was not affected by the direction of the osmotic gradient, the solute (mannitol or NaCl) responsible for the gradient, or 100 nM AVP. We conclude: 1) The PSE has a moderate Cl permeability which is decreased by AVP. 2) The increase in resistance with AVP is compatible with an effect on a conductive pathway. 3) The osmotic water permeability of the PSE is low and is unaffected by AVP.

RENAL DENERVATION INHIBITS PROSTAGLANDIN E₂ (PGE₂)-INDUCED NATRIURESIS. Danuta Pawlowska*, Carla Long*, Franklyn G. Knox, Mayo Medical School, Rochester, MN

The renal effect of exogenous PGE₂ were studied in male Sprague-Dawley rats on normal sodium diet during Inactin anesthesia. Meclofenamate (5 mg/kg b.w.) was given i.v. to suppress endogenous prostaglandin production. PGE₂ (1.25 μg) was administered into the renal interstitium of the left kidney by means of a chronically implanted matrix. Clearance studies were performed in animals with innervated (n=9) and denervated (n=7) left kidneys. In the ipsilateral kidney, PGE₂ administration produced natriuresis and diuresis. Also, the contralateral kidney responded with natriuresis and diuresis. GFR and MAP were not affected by interstitial PGE₂ administration. When PGE₂ was interstitially infused in denervated kidneys, natriuresis and diuresis of ipsilateral and contralateral kidneys were abolished. Results are shown as means ± SE.

	Ipsilateral		Contralateral	
	Control	PGE ₂	Control	PGE ₂
Innervated				
FE _{Na} (%)	0.20±.05	1.43±.74	0.35±.09	1.18±.33
V (μl/min)	6±1	12±2	10±2	21±4
Denervated				
FE _{Na} (%)	1.61±.52	1.53±.47	0.60±.30	0.60±.30
V (μl/min)	30±10	34±8	12±5	12±4

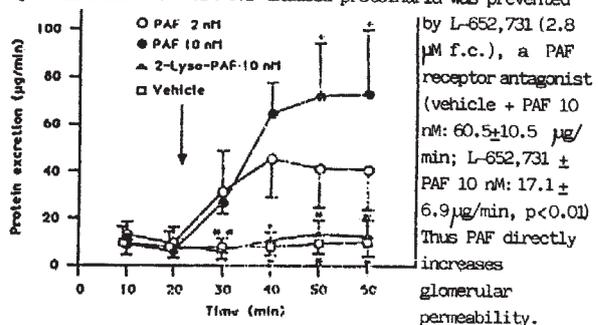
We found that the natriuretic effect of interstitially administered PGE₂ occurs in both ipsilateral and contralateral kidneys only in the presence of intact renal innervation. We conclude that neural factors contribute to the natriuretic effect of PGE₂.

DIRECT RENAL EFFECT OF OZOLINONE (OZO) IN THE DOG. J. Pedraza-Chaverri*, J.E. Benabe and M. Martínez-Maldonado, UPR School of Medicine and VA Center, San Juan, P.R.

Loop diuretics (e.g. furosemide) increase renin release (RSR). (-) OZO a loop diuretic inhibits furosemide sensitive Na⁺, K⁺, 2Cl⁻ co-transport. This should result in increased RSR if inhibition of NaCl reabsorption at the macula densa is the stimulus. We have previously proposed this mechanism to explain furosemide induced RSR. (+) OZO in contrast to its enantiomer is not diuretic, presumably because it does not bind to the transporter. The direct renal effects of these two compounds, were evaluated by infusion into one renal artery of anesthetized dogs (~ 1mg/kg). (+) OZO did not change GFR, UNaV, UClV, urine flow, urine prostaglandin (PG) excretion or RSR. In contrast, (-) OZO induced a sharp rise in UNaV, UClV without changes in GFR and a marked increase in urine PG excretion and RSR. In addition OZO did not increase RSR nor alter PG secretion from isolated glomeruli. This is the first demonstration that this specific inhibitor of the Na⁺, K⁺, 2Cl⁻ co-transport, directly stimulate PG and RSR in vivo, but not from glomeruli in vitro. These results suggest that the Na⁺, K⁺, 2Cl⁻ co-transport is critical in the regulation of PG and renin secretion, and that normal tubule-glomerular relations are required for RSR induced by OZO.

EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) AND ITS SPECIFIC RECEPTOR ANTAGONIST ON GLOMERULAR PERMEABILITY TO PROTEINS IN ISOLATED PERFUSED KIDNEY (IPK). N. Perico* F. Delaini* M. Tagliaferri* G. Remuzzi* (intr. by C.L. Pirani). Mario Negri Institute for Pharmacological Research, Via Gavazzeni 11, Bergamo - Italy.

We addressed the possibility that PAF increases glomerular permeability to proteins independently of circulating cells. Kidneys were isolated from male SD rats and perfused in closed circuit at 100 mmHg with a bicarbonate buffer containing albumin (1%), Ficoll 70 (3.5%) and amino acids. After stabilization and two 10-min control clearance periods, IPK were exposed to PAF [2 nM (n=8) or 10 nM (n=6) f.c.] or vehicle for 40 min. GFR, measured as creatinine clearance, and renal vascular resistance were unchanged when either PAF or vehicle were added to the perfusion fluid. PAF but not vehicle produced a dose-dependent progressive increase in urinary protein excretion. No changes in urinary protein excretion were found when IPK were exposed to 2-lyso-PAF, the inactive lyso-derivative of PAF. PAF-induced proteinuria was prevented



ATRIAL NATRIURETIC PEPTIDE (ANP) AND THE RENAL RESPONSE TO HYPERVOLEMIA IN NEPHROTIC HUMANS. Craig Peterson,* Berit Madsen,* Andrew Perlman,* and Bryan D. Myers. Stanford Univ., Dept. of Medicine, Stanford, California.

To elucidate the abnormality of body fluid homeostasis, we compared the atrial and renal responses to water immersion of nephrotic patients (N=10) with those of healthy controls (N=9). Nephrotics exhibited depressed baseline levels of ANP (14±3 vs 34±3 pg/ml, p<0.05) and lower rates of urine flow (\bar{V}) and sodium excretion (U_{NAV} , p<0.01). Although immersion-induced hypervolemia increased plasma ANP to equivalent levels (75±19 vs 60±6 pg/ml), the disparity in corresponding \bar{V} (5±1 vs 13±2 ml/min, p<0.01) and U_{NAV} (171±42 vs 540±65 μ eq/min, p<0.01) grew larger. In contrast, immersion caused an equivalent reduction in renal vascular resistance by 17%, (p<0.01). Despite higher renal plasma flow and lower oncotic pressure of plasma, the glomerular filtration rate and fractional clearances of dextrans of graded size remained constant during immersion in both groups. A theoretical analysis of transglomerular dextran transport revealed a decline in transmembrane hydraulic pressure difference (ΔP) by ~10% in each group. We conclude that renal vasomotor responsiveness to hypervolemia is preserved in nephrotics, but that the mediatory role of ANP in this response is uncertain. By contrast diminished responsiveness of the distal nephron to the natriuretic action of endogenous ANP could contribute to edema formation in the nephrotic syndrome.

LOW DOSE ASPIRIN IN PATIENTS WITH LUPUS NEPHRITIS.

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The intrarenal synthesis of thromboxane (TX)₂ is enhanced in pts with lupus nephritis (LN) and correlates with deteriorating renal function. In order to explore the contribution of platelet activation to these biochemical and functional changes, we randomly assigned 10 pts with LN to 4-week treatment with low-dose aspirin (20 mg bid) or placebo and monitored platelet cyclooxygenase activity (serum TXB₂), intrarenal TXA₂ and PGI₂ synthesis (urinary TXB₂ and 6-keto-PGF_{1 α}), inulin clearance (C_{IN}) and bleeding time (BT). Aspirin but not placebo administration was associated with a cumulative inhibition of platelet cyclooxygenase activity by >95% and statistically significant (p<0.01) prolongation of BT (6±0.4, 13±3.5, 12.2±1, 13.8±2.1 min at day 0, 7, 14 and 28). Neither time- nor treatment-related differences were detected in urinary TXB₂ (Aspirin: 7±1.3 vs 6.2±0.9; Placebo: 5.9±1.8 vs 5.7±2.3 ng/h, at day 0 and 28), 6-keto-PGF_{1 α} (Aspirin: 2.7±0.7 vs 2.3±0.8; Placebo: 2.9±1.2 vs 2.4±1.0 ng/h) and C_{IN} (Aspirin: 63±14, 64±16, 63±15; Placebo: 63±20, 63±21, 63±19 ml/min, at day 0, 7 and 28). We conclude that cyclooxygenase-dependent platelet activation does not contribute to the enhanced intrarenal synthesis of TXA₂ and cannot account for the functional effects of a selective TXA₂-receptor antagonist in LN.

CYTOPROTECTIVE EFFECTS OF PROSTAGLANDIN E₂ (PGE₂) ON CYCLOSPORIN A (CyA) INDUCED NEPHROTOXICITY SUPERIMPOSED ON THE ISCHEMIC INJURY FOLLOWING EXPERIMENTAL RENAL TRANSPLANTATION.

Sigmund Pomer*, Michael Rambašek*, Rüdiger Waldherr* and Lars Röhl* (intr. by P. Lundin). Univ. of Heidelberg, Depts. of Urol., Nephrol. and Pathol.

The administration of 16.16 dimethyl PGE₂ has been demonstrated to alleviate CyA induced nephrotoxicity (Makowka et al., Clin. Nephrol. 25: S89 - S95, 1986). The study was designed to evaluate whether PGE₂ can effectively protect renal transplants from CyA-induced toxicity. Inbred male Wistar rats receiving CyA following renal transplantation were divided into two groups: animals receiving orally CyA (40mg/kg/day) alone and animals treated concomitantly with PGE₂ (10ug/kg/day s.c.). The endogenous creatinine clearance was used to evaluate the functional impairment and the urinary excretion of lysosomal enzyme N-acetyl- β -D-glucosaminidase (NAG) provided the assessment of renal tubular cell damage. The CyA alone group had significant elevations of NAG activity and reductions of the creatinine clearance by the 8th treatment day. The transplant recipients group administered simultaneously with PGE₂ showed nearly normal function and significantly lower urinary NAG levels. Renal histology demonstrated severe tubulopathy (proximal tubular vacuolization, microcalcification and inclusion bodies) in the CyA alone group and nearly normal findings in the PGE₂ group. It is concluded that the CyA induced tubulopathy superimposed on ischemic injury (typical morphological features, enzymuria and functional impairment) can be markedly reduced by pretreatment and concomitant administration of PGE₂.

EFFECTS OF ILOPROST AND OKY 046 ON RENAL FUNCTION IN RENAL FAILURE INDUCED BY CAPTOPRIL IN Na DEPLETED (LNa-CEI) RATS. A. Pomerantz, M. Rathaus, E. Podjarny and J. Bernheim. Meir Hosp., Kfar-Saba and Tel-Aviv., Israel. (Intr. by J. Levi).

The development of renal failure in LNa-CEI rats is associated with a reduced synthesis of PGE₂ and 6-keto PGF_{1 α} and increased synthesis of TXB₂ by isolated glomeruli, as compared to Na depleted animals. To assess whether these changes play a role in the decrease of renal function, iloprost, a stable analogue of prostacyclin, was administered i.v. at the rate of 12.5 ng/kg/min, which does not affect systemic blood pressure.

In 5 control rats, the clearance of inulin (ml/min/100 g bw) was 0.87 ± 0.09 before and 0.85 ± 0.19 during iloprost administration. The clearance of PAH (ml/min/100 g bw) was 1.53 ± 0.24 and 1.46 ± 0.15. In 8 LNa-CEI rats, the clearance of inulin was 0.26 ± 0.03 and increased to 0.35 ± 0.03 after iloprost (p<0.05). The clearance of PAH increased but not significantly. There was no difference between 4 rats pretreated with the thromboxane synthetase inhibitor OKY 046 (100 mg/kg i.p., 16 and 2 hours before the study) and 4 untreated rats.

The results support the concept that decreased synthesis of vasodilatory prostanoids plays a role in this model of renal failure, while the physiological importance of increased glomerular thromboxane remains to be assessed. It is evident, however that other factor(s) also contribute to the decrease of renal function.

HEMODYNAMIC, HORMONAL, AND RENAL RESPONSES TO A MIXED MEAL IN NORMAL SUBJECTS: ABSENCE OF AN EFFECT OF CAPTOPRIL. DM Potter, G Glatz,* LM Demers,* JF Seaton,* and RM Hersey.* Depts. of Med., Path., Surg., & OB/GYN. Penn State Univ., Milton S. Hershey Med. Center, Hershey, Pennsylvania.

Protein loading is associated with an increase in GFR, the signal for which is unexplained. Hemodynamic and renal responses to "normal" meals is poorly characterized. We studied the effects of a 6.4 Kcal/Kg, 20% protein liquid meal in 6 normal males on a 2 mEq/Kg/day Na diet with and without acute captopril pretreatment. In the 3 hrs following the meal significant ($p < .05$ or less by 1- and 2-way ANOVA) changes in mean arterial pressure (MAP), diastolic blood pressure (DBP), heart rate (HR), atrial natriuretic peptide (ANP), norepinephrine (NOR) and serum protein concentration (PRO) were observed.

		PLACEBO		CAPTOPRIL	
		BASE	ΔMAX	BASE	ΔMAX
MAP	mmHg	84.9±2.2	-4.3	83.7±1.8	-7.5
DBP	mmHg	71.9±2.9	-7	70.2±2.1	-10.1
HR	bpm	57.9±4.4	+9.6	57.3±4.2	+10.8
ANP	pg/ml	22±0.8	+5.8	18.9±0.5	+10.9
NOR	pg/ml	211±7.6	+110	200±28	+87
PRO	gm/dl	6.6±0.1	+0.2	6.5±0.2	+0.3

GFR, FeNa, and urinary prostanoid excretion were identical with placebo and captopril. Aside from a significant increase in systolic BP and PRA with captopril, placebo and captopril responses were identical. Net fluid and sodium balances were negative.

CONCLUSIONS: A "normal" mixed meal induces a state resembling acute volume reduction due to a combination of volume loss and redistribution. The state is defended by catecholamines but not the renin system and is "paradoxically" accompanied by an increase in the vasodepressor ANP.

NEUTRALIZATION OF THE ANIONIC SITES OF CULTURED RAT MESANGIAL CELLS(MC) BY POLY-L-LYSINE(PL) EVOKES CHANGES IN EICOSANOID SYNTHESIS THROUGH A CALCIUM DEPENDENT PROCESS. Francesco Pugliese, Cristina Anania*, and Giulio A. Cinotti*. University "La Sapienza", Dept. of Medicine, Div. of Nephrology, Rome, Italy.

Changes in eicosanoid synthesis represent a feature of the biosynthetic response of rat glomerular epithelial cells to the cell surface polyanion neutralization (Pugliese et al., *Kidney Int.* 32:57-61,1987). In order to ascertain if this property was common to other glomerular cell types, we have investigated the effect of neutralizing the negatively charged sites of cultured MC by PL (MW 47,000) on prostaglandin(PG) E₂ production. Addition of PL (10 ug/ml) to MC resulted in an initial lag of 1-2 min. before PGE₂ release increased and reached maximal levels at 30-60 min. After a similar initial lag, PL slowly and progressively increased cytosolic free Calcium ([Ca²⁺]_i) in fura-2 loaded monolayers. One hour incubation of MC with varying doses of PL resulted in a dose-dependent increase of PGE₂ production: Basal=3.9±1.37; PL 1 ug/ml = 5.23±3.13; PL 2.5 = 12.9±2.8; PL 5 = 18.8±2.8; PL 10 = 31.11±11 (PGE₂ = pg/ug protein, mean±SD). PL 10 elevated [Ca²⁺]_i from basal level of 146±46 nM to 453±174 ($p < 0.001$). Addition of Heparin (5 U/ml) abolished both PL-evoked increases in PGE₂ and [Ca²⁺]_i. Removal of extracellular Ca²⁺ decreased PL-evoked PGE₂ synthesis by approximately 90%. Depletion of intracellular Ca²⁺ completely suppressed PL-induced PGE₂ synthesis in MC. We conclude that neutralization of MC fixed anionic sites by PL enhances PGE₂ production. This stimulation appears to be a Ca-dependent process.

EFFECTS OF α-hANP ON RENAL AND SYSTEMIC HEMODYNAMICS IN PATIENTS WITH DIABETES MELLITUS (DM) TYPE I. H.G. Predel*, O. Schulte-Vels*, M. Sorger*, K. Glänzer*, C. Geller*, R. Düsing, and H.J. Kramer. Univ. of Bonn, Med. Poliklinik, Bonn, W-Germany.

We evaluated the effects of i.v. α-hANP (24 ng/min/kg for 1 h) on GFR (C_{in}), RPF (C_{PAH}), renal tubular function, cardiac function (impedance cardiography) and systemic hemodynamics in 6 pts., age 25-43 yrs, with DM type I and microalbuminuria and in 6 age-matched controls (C). Basal plasma ANP of 143±19 in DM was similar to C, rose to 730±182 during i.v. ANP and was 83±14 pg/ml 1 h after cessation of ANP infusion. Maximal effects of ANP occurred between 30 and 60 min after start of the infusion. Urine flow increased approximately twofold in DM and C. GFR rose by 46% from 119 to 174 in DM and by 43% from 97 to 139 ml/min/1.73 m² in C. U_{Na}V increased by 255% in DM and by 152% in C and RPF by 19.5% from 687 to 821 in DM, but fell by 9% from 637 to 586 ml/min/1.73 m² in C. Filtration fraction rose by 40% in DM and by 55% in C. Heart rate and BP remained unaltered, whereas cardiac output initially rose by 10% from 7.5±0.7 to 8.3±0.8 l/min as compared to 16% in C, but then returned to 7.0±0.4 l/min despite continuous ANP infusion. The results demonstrate that low dose α-hANP results in a parallel rise in GFR in DM as in C but a significantly greater increase in U_{Na}V in DM than in C. Despite similar changes in systemic hemodynamics RPF markedly increased only in DM. Therefore, the renal vasculature appears to be more sensitive to circulating ANP in pts. with DM type I than in healthy subjects.

EFFECT OF BOMBESIN (B) ON VASOPRESSIN (AVP) ACTION IN THE IN-VITRO ISOLATED PERFUSED RABBIT CORTICAL COLLECTING TUBULE (CCT). K.H. Raymond, Audie L. Murphy VA Hospital and University of Texas Health Science Center at San Antonio, Texas.

Bombesin, a tetradecapeptide isolated from amphibian skin, has been shown to activate protein kinase C (PKC) and inhibit peptide binding to cellular receptors. The present study was designed to evaluate the effect of B on AVP induced hydraulic conductivity, L_p, in CCTs perfused in vitro against a 200 mOsm/kg H₂O transepithelial osmotic gradient at 37.5°C. The L_p in 4 CCTs exposed to B (20 nM) for 30 minutes was not different from the control period (9.56 ± 3.6 vs. 4.70 ± 4.3 cm/sec atm 10-7). However, the AVP (200 uU/ml) stimulated L_p in these 4 B treated CCTs was significantly reduced compared to 5 control (C) tubules (B-62.35 ± 21.3 vs. C-194 ± 38.1, $p < .03$). Subsequent exposure of the B treated CCTs to H-7 (100 uM), a relatively potent PKC inhibitor, produced a significant increase in the AVP response from 62.35 ± 21.3 to 141.46 ± 36.3, $p < .05$.

In summary, B alone has no effect on CCT hydraulic conductivity. The AVP response in CCTs pre-treated with B is significantly reduced. Although the cellular mechanisms responsible for this effect are not totally clear, the significantly increased AVP response following H-7 exposure suggests that B may act to inhibit AVP through activation of PKC.

REGULATION OF Na^+ TRANSPORT BY ALDOSTERONE AND ARGININE VASOTOCIN (AVT) IN CULTURED KIDNEY A6 CELLS. M.C. Reif, R.J.M. Bindels,* and J.A. Schafer. NRTC, Depts. of Med., and Physiol. & Biophys., UAB, Birmingham, AL.

The mechanism of synergism between aldosterone and AVT on short-circuit current (Isc, $\mu\text{A}/\text{cm}^2$) and transepithelial conductance (G, mS/cm^2) of cultured A6 cells was investigated. All experiments were performed with cells grown on Millipore filters for a period of 2 to 4 weeks in defined, serum-free medium. The cell layers were then placed in an Ussing chamber for electrical measurements. Baseline values were (n=11): Potential difference (PD), 37 ± 5 mV (apical side negative); Isc, 10 ± 1 ; G, 0.29 ± 0.03 . Omitting fetal bovine serum two days after seeding of the cells on filters did not influence the PD development. In one group of experiments, A6 cells were pretreated for 24 h with 10^{-7} M aldosterone. Isc and G were higher in these cells compared to controls (Isc: 28 ± 2 vs 16 ± 2 , G: 0.41 ± 0.04 vs 0.26 ± 0.01 , n=5) and both remained stable for at least 6 h. In control cells, 10^{-7} M AVT increased Isc within one minute after addition, reaching a maximum in 15 min and then declining slowly to baseline levels over the next 5 h. Addition of AVT to aldosterone pretreated cells resulted in a significantly greater maximal increase in Isc than in non-pretreated cells (ΔIsc : 8.1 ± 0.4 vs 4.9 ± 0.4 , n=5, $p < 0.001$). In both the aldosterone-pretreated and non-pretreated group the effect of AVT was not sustained. Reduction of the incubation time with aldosterone from 24 h to 4 h still caused a rise in Isc, but there was no detectable synergism between aldosterone and AVT. Addition of $5 \cdot 10^{-5}$ M amiloride inhibited 97% of Isc in the presence of AVT, aldosterone or both. Pretreatment with aldosterone doubled the amiloride-insensitive Isc. All the above described actions of AVT on Isc and G could be fully mimicked by $5 \cdot 10^{-4}$ M 8-Br-cAMP.

In conclusion, A6 cells grown in defined, serum-free medium exhibit hormone-dependent transepithelial Na^+ transport. There is a greater than additive synergism between aldosterone and AVT on transepithelial Na^+ transport if the cells are pre-treated for 24 h with aldosterone prior to the experiment.

EFFECTS OF ALDOSTERONE AND ADH ON INTRACELLULAR ELECTROLYTES IN THE TOAD URINARY BLADDER. Roger Rick,* Gertrud Spancken,* and Adolf Dörge.* Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama. (intr. by James A. Schafer)

Electron microprobe analysis was employed to compare the natriuretic effects of aldosterone and ADH in the toad urinary bladder. Measurements of intracellular electrolyte concentrations were performed on $0.5 \mu\text{m}$ thick, freeze-dried cryosections utilizing energy dispersive x-ray microanalysis. After aldosterone (50 nM) a statistically significant increase in the intracellular Na concentration was detectable in most bladders, together with a large stimulation of the Na transport rate as measured by short-circuit current (by 100 to 300%). On average, the Na concentration in granular cells increased by 2.4 ± 1.3 mmol/kg w.w. (n=7). A significantly larger Na increase of 19.4 ± 10.6 (n=5) was observed after a similar stimulation of transepithelial Na transport by ADH (150 mU/ml). We further noted a reduction in the intracellular Cl concentration of granular cells after aldosterone, but not after ADH. The fall in the Cl concentration was inversely related to the Na concentration increase and most pronounced in bladders demonstrating a large Na transport response. The results suggest that aldosterone, in addition to its stimulatory effect on the apical Na channel, also exerts a stimulatory effect on the Na pump. The possibility will be discussed that the stimulation of the pump is mediated by an aldosterone-induced alkalization which could explain the fall in the Cl concentration. Similar though less pronounced concentration changes were observed in basal cells, suggesting that this cell type also participates in transepithelial Na transport. Measurements in mitochondria-rich cells provided no consistent results.

EFFECT OF DOPAMINERGIC STIMULI ON VASOPRESSIN (AVP) RELEASE FROM HYPOTHALAMO-NEUROHYPOPHYSAL COMPLEXES (HNC) IN CULTURE. Noreen F. Rossi and Robert W. Schrier. Wayne State U., Detroit, Michigan and U. Colorado H.S.C., Denver, Colorado.

Dopaminergic projections exist to supraoptic and paraventricular nuclei, and dopamine (DA) is contained in neural lobe fibers. Previous studies have shown DA to be involved in AVP release, but results have been conflicting. We have studied the effects of DA, apomorphine (APM), and haloperidol (HAL) on the release of AVP from HNC in culture. AVP release rates are determined during basal and test hours. Results are expressed as percent of basal release. In the absence of ascorbate (ASC), 10^{-8} to 10^{-6} M DA results in a concentration dependent rise in AVP release with maximum release of 343 ± 42 %/HNC/h ($p < 0.001$ vs basal, N=6) in response to 10^{-6} M DA. This initial rise is followed by a decline in AVP release at higher DA concentrations to levels about twice the basal values. In the presence of 10^{-5} M ASC, maximum release of AVP to DA (379 ± 37 %/HNC/h; $p < 0.001$ vs basal, N=7) does not significantly differ from that in the absence of ASC, but the dose response curve is shifted to the right by one order of magnitude and AVP release to higher DA concentrations decreases to below basal levels. The D_2 agonist, APM produces a dose dependent rise in AVP release at concentrations at least two orders of magnitude less than DA, but no decrease in AVP is seen at higher APM doses. 10^{-6} M HAL blocks AVP release by submicromolar doses of DA. The response of AVP release to DA is biphasic, inhibited by DA antagonist, and stimulation is mimicked by D_2 agonist consistent with action on two separate DA receptor populations: one stimulating, the other inhibiting AVP.

ANGIOTENSIN CONVERTING ENZYME (ACE)-INHIBITORS ATTENUATE THE HYDROSMOTIC ACTION OF AVP IN THE RABBIT CORTICAL COLLECTING TUBULE (CCT). Diane Rouse, William Dalmeida*, and Wadi N. Suki, Baylor College of Med., Houston, TX.

We have shown that captopril (CP) inhibits AVP-stimulated osmotic water permeability (Pf) in CCT by a prostaglandin mediated mechanism. Unlike enalapril (E), a pro-drug, and its active metabolite, enalaprilat (EP), CP contains a sulfhydryl group, which may be responsible for the action of this compound. The purpose of the present study is to: 1) determine the effect of E and EP on Pf and 2) determine the effect of CP + EP on Pf. In 5 CCT segments, exposed to an osmotic gradient, lumen<bath, the addition of the pro-drug, E, had no effect on AVP-stimulated Pf. However, Pf declined from $212 \pm 58 \times 10^{-4}$ cm/sec to 139 ± 42 ($P < 0.05$) with the addition of EP, 10^{-5} M, to the bath. The subsequent addition of 10^{-4} M CP had no further significant effect ($99 \pm 20 \times 10^{-4}$ cm/sec). In 5 additional CCT, Pf declined from $137 \pm 20 \times 10^{-4}$ cm/sec to 96 ± 18 ($P < 0.005$) with the addition of CP, and the subsequent addition of EP further reduced PF to $74 \pm 10 \times 10^{-4}$ cm/sec ($P < 0.05$, vs CP).

We conclude that the attenuation of the hydrosmotic effect of AVP by CP represents an effect of ACE-inhibitors, in general, and is not dependent on the presence of a sulfhydryl group on the compound.

ROLE OF ENDOGENOUS ATRIAL NATRIURETIC FACTOR (ANF) IN REGULATION OF URINARY SODIUM EXCRETION. M. Audrey Rudd*, Stanford R. Plavin*, Julie R. Ingelfinger, Victor J. Dzau*, Brigham and Women's Hospital, Boston, MA.

Infusion of ANF produces natriuresis, vasodilation, and suppression of renin release. To determine if endogenous ANF contributes to basal regulation of sodium excretion ($U_{Na}V$), blood pressure and renin release, we examined the effect of blockade of ANF with rabbit antiserum (Ab) which was shown to be specific for ANF. Pentobarbital anesthetized aurolemic Sprague-Dawley rats (12-16wk) were surgically prepared for urine collection and blood pressure measurement. Blood samples were drawn after a 45 min control period and at 45 min after injection. Urinary sodium excretion was significantly reduced by ANF Ab ($n=7$) (0.52 ± 0.10 vs. 0.28 ± 0.10 uEq/min, $p < 0.025$). However, Ab did not alter the plasma renin concentration (PRC) (15.85 ± 3.88 vs. 13.24 ± 2.42 ng AI/ml hr) nor mean arterial blood pressure (MAP) (140 ± 5 vs. 144 ± 4 mmHg). Pre-immune serum (PRE) ($n=10$) did not influence $U_{Na}V$, PRC, or MAP. ANF levels fell from 237 ± 40 pg/ml to undetectable levels in the Ab group but were unchanged in the PRE group (356 ± 31 vs. 417 ± 89 pg/ml).

We conclude that endogenous ANF plays a role in basal regulation of $U_{Na}V$ independent of any action on blood pressure or the renin-angiotensin system. Interestingly, our data suggest that endogenous ANF does not influence PRC.

EFFECT OF ATRIOPEPTIN III ON RENAL HEMODYNAMICS AND NATRIURESIS IN DOCA-SALT HYPERTENSIVE RATS. B. Rutkowski*, E.J. Johns* (intr. by A. Cohen). Dept Physiology, The Medical School, Birmingham U.K.

The effect of atriopeptin III (APIII) on renal hemodynamics and the relationship with the natriuretic effect were investigated in normotensive and DOCA-salt hypertensive rats to elucidate action of atrial peptides. Sprague-Dawley rats were subjected to right nephrectomy and given either tap water or saline to drink plus bi-weekly injections of DOCA (15 mg/kg, s.c.) for 4-6 weeks. Two 15 min clearance periods were used before and two following APIII administration i.v. at a dose of 125, 250 or 500 ng/kg. In normotensive rats, systemic blood pressure (BP), renal blood flow (Q) and GFR remained unchanged over the course of experiment at 140 ± 2 mmHg, 23 ± 0.3 ml/min/kg and 3.4 ± 0.1 ml/min/kg, respectively. In DOCA-salt animals, BP was 169 ± 5 mmHg, Q was 23.2 ± 1.4 ml/min/kg and GFR was 4.2 ± 0.1 ml/min/kg. These variables were not altered by any dose of APIII. The urine flow (V) and sodium excretion ($U_{Na}V$) were as follow:

	Basal	APIII 125	250	500ng/kg
Normotensive				
V, ml/min/kg	42	59*	64*	69*
$U_{Na}V$, μ mol/min/kg	6.4	9.5*	10.9*	12.5*
DOCA-salt hypertensive				
V, ml/min/kg	72	105*	134*	202*
$U_{Na}V$, μ mol/min/kg	11.2	16.6*	23.4*	34.8*

*) $p < 0.05$ vs basal

These data demonstrate that acute administration of APIII had no significant effect on renal hemodynamics and a marked natriuretic effect in both normotensive and hypertensive rats. The tubular action of APIII was substantially enhanced in DOCA-salt hypertensive rats in comparison to control.

HUMAN PRO-ATRIAL NATRIURETIC FACTORS (ANF) 1-30 AND 31-67 CIRCULATE IN NORMAL HUMAN SUBJECTS. Alan L. Sallman, Chris Winters*, Jane Meadows* and David L. Vesely*. Univ. of Arkansas Med. Ctr., J. L. McClellan VA Hosp, Little Rock, Arkansas.

Recently, we have found that human Pro-ANF Factors 1-30, 31-67, & 79-98 as well as ANF 99-126, stimulate the renal particulate guanylate cyclase cyclic-GMP system (Vesely, Bayliss and Sallman, Biochem and Biophys Res. Comm. 143: 186-193, 1987). To determine if these prohormone peptide fragments circulate we have developed specific and sensitive radioimmunoassays for Pro-ANF Factors 1-30 and 31-67 in addition to ANF 99-126. Human plasma was collected in EDTA with and without the presence of protease inhibitors. The plasma was extracted then centrifuged with recovery of the supernatant, which was evaporated to dryness. This material was then reconstituted and assayed with specific antibodies (Peninsula Labs, Belmont, Ca) against either Pro-ANF 1-30, Pro-ANF 31-67 or ANF 99-126. These antibodies crossreact less than 10% with the other Pro-ANF Factor and 0% with ANF 99-126. Normal human volunteers ranging in age between 22 and 56 years (mean age=31 years) and consisting of 21 males & 13 females were studied. Levels of ANF 99-126 were similar to what others have reported (30-60pg/ml). Pro-ANF 1-30 averaged 7 fold higher than ANF, while Pro-ANF 31-67 averaged 30 fold higher than ANF. Intra and interassay variations for Pro-ANF 1-30 were 9.2% and 5.9% respectively and Pro-ANF 31-67 were 3.5% and 5.1% respectively. We conclude that Pro-ANF 1-30 and Pro-ANF 31-67, in addition to ANF 99-126, circulate in normal human beings. The physiological significance of these peptides is unknown at present.

THE EFFECT OF HIGH EICOSAPENTAENOIC ACID DIET (EPA) ON IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN) IN THE RAT. D. Sauter,* R. Young* and M. Rahman,* (intr. by T. Ing). VA Hospital and Loyola University, Hines and Maywood; IL.

We examined the effect of high EPA diet on ICGN induced by administration of cationic bovine gamma globulin (cBGG). This is a complement- and leukocyte-independent model of membranous nephropathy characterized by enhanced glomerular eicosanoid production. Rats were placed on an EPA diet (20% menhaden oil) or a diet containing 20% beef tallow (BT). After 6 weeks on the diet, rats were pre-immunized and injected with cBGG. Proteinuria, glomerular morphologic changes and glomerular eicosanoid production were examined. Proteinuria was significantly reduced in the EPA group, 160 ± 40 mg/24 hr, N=15, vs 280 ± 36 mg/24 hr, N=17, for BT group, $P < 0.02$. Glomerular PGE₂ and TxB₂ were also reduced in rats fed EPA diet (PGE₂ 0.67 ± 0.11 and TxB₂ 1.76 ± 0.33 ng/mg glomerular protein) compared to rats fed BT diet (PGE₂ 2.14 ± 0.44 and TxB₂ 6.04 ± 1.5 ng/mg glom. protein, N=8, $P < 0.01$). There were no differences in the amount or distribution of glomerular immune deposits between the two groups. As expected, in rats fed EPA diet, the T cell mitogenic response was reduced by more than 70% vs BT-fed rats. However, there was no decrease in the B cell mitogenic response. The specific immune response, assessed by measuring anti-BGG antibody levels, showed no difference between the two groups. We conclude that high EPA diet reduces proteinuria in cBGG-induced ICGN. This beneficial effect is probably due to alterations in eicosanoid synthesis.

ARGININE VASOPRESSIN (AVP) GENE REGULATION IN THE HOMOZYGOUS BRATTLEBORO RAT (HBR). R.W. Schrier, J.K. Kim, J.-B. Michel,* F. Soubrier,* L. Bankir, and P. Corvol. INSERM, U36, Paris, France; and Univ. Colorado Sch. Med., Denver, CO.

AVP messenger RNA (mRNA) for the AVP pre-hormone has been found to be present in the HBR with central diabetes insipidus. A guanosine residue deletion has been identified in the second exon of the AVP gene in the HBR. HBR also has been proposed to exhibit an abnormality in the regulation of AVP gene expression. These latter studies have been conducted in untreated HBR, thus raising the possibility that the chronic disorder in water balance may have accounted for the abnormality in AVP gene regulation. The present study was undertaken in 3 groups of HBR to examine this possibility: Group 1(G1), sham-operated; Group 2(G2), receiving 840ng AVP/day by osmotic minipump for 2wks; and Group 3(G3), receiving 840ng AVP/day for 2wks and then 24hr without AVP or water. Urinary osmolality (Uosm, mOsm/kg H₂O) in G1 before and after 2wks was not different (465 ± 26 vs 380 ± 69 , NS), in G2 was increased (368 ± 33 vs 1714 ± 177 , $P < .01$) and in G3 was increased similarly prior to AVP removal (283 ± 30 vs 1808 ± 496 , $P < .001$). AVP-mRNA was determined by solution hybridization using antisense AVP-RNA as the probe and hypothalamic (HT) pure sense AVP-mRNA as the standard (both produced by in vitro transcription from rat AVP-cDNA). AVP-mRNA of G3 was significant higher than AVP-mRNA of G2 ($9.31 \pm .59$ vs $4.31 \pm .66$ moles/HT, $P < .001$) and G1 ($9.31 \pm .59$ vs $3.38 \pm .53$, $P < .001$). Results indicate that AVP gene expression is regulated in HBR in response to 24hr of fluid deprivation after AVP treatment for 2wks prior to the study.

ATRIAL NATRIURETIC PEPTIDE (ANP), SODIUM NITRO-PRUSSIDE (NaNPr) and cGMP INDUCED RELAXATION OF CULTURED MESANGIAL CELLS (MC) ON SILICONE RUBBER SURFACE (SRS). P. Singhal, D. Schlondorff and R. Hays. Dept. of Medicine, Long Is. Jewish Medical Center & Albert Einstein Coll. of Medicine, New York.

MC are considered modified smooth muscle cells. Earlier, we reported a method for determination of relaxation on MC grown on a SRS (Kid Int 30:862, 1986). Relaxation of MC was characterized by a decrease in folds or wrinkles on a flexible substrate (SRS), while the cell was still attached to the substrate. We now report the effects of ANP & NaNPr on MC grown on SRS, observed at 36°C and photographed sequentially. Four sets of experiments were done for each group. The percentage of cells showing a decrease in number and/or magnitude of wrinkles during a control period followed by a treatment period was:

	# Cell	Control			Treatment		
		10 min.	5 min.	10 min.	5 min.	10 min.	
NaNPr (10 ⁻⁵ M)	71	21	77	93			
ANP (10 ⁻⁹ M)	25	4	8	44			
DBcGMP (10 ⁻⁴ M)	50	8	44	66			

NaNPr and ANP caused a reduction of wrinkles in more than 90% and 40% of cells respectively, indicating a relaxation response. Both ANP & NaNPr are known to stimulate cGMP production by MC. To find out whether cGMP has also contributed to this response, MC were treated with DBcGMP. The latter also led to a decrease of wrinkles in more than 60% of cells. We therefore suggest that ANP and NaNPr induce relaxation of MC and that this effect may be initiated through the release of cGMP.

POSTPRANDIAL AND FASTING PLASMA ATRIAL NATRIURETIC PEPTIDE DURING VARYING CHRONIC SODIUM INTAKES IN NORMAL HUMANS. Michael J. Solhaug,* Joey P. Granger and Janet Scott.* Depts of Peds & Physiol, Eastern Virginia Medical School, Norfolk, Virginia.

The effect of chronic dietary Na manipulation on fasting and postprandial plasma atrial natriuretic peptide (ANP) was examined in 2 studies of normal humans. In Study I, 3 separate groups of normals received diets of either low(L), normal(N) or high(H) daily Na intake for 7 days. Twenty-four hr urines for Na and K were obtained on days 6 and 7. UNaV mEq/24 hr for each group was (L)13.1±3.7, (N)150.1±19.4, (H)271.3±33.6. On the completion of day 7, a fasting plasma ANP, and serial postprandial levels were obtained:

	Fasting ANP pg/ml	Postprandial ANP pg/ml		
		30min	60min	90min
L(n=8)	31.7±2.4	30.8±3.2	32.8±2.0	33.2±2.2
N(n=8)	31.7±1.0	30.1±0.9	31.3±0.4	30.8±0.8
H(n=8)	32.1±1.5	37.6±3.4	39.6±5.6	39.2±6.5

Fasting ANP levels showed no change with alteration in Na intake. Postprandial ANP increased only in H, but not significantly. In Study II, a continuous group of normals (n=8) received the 3 Na controlled diets for 7 days sequentially L/N/H. Urine collections confirmed appropriate Na balance and fasting ANP levels were drawn on Day 7. Again fasting plasma ANP remained unchanged. Summary: 1. Fasting plasma ANP does not change significantly during conditions of chronic low, normal or high Na intake. 2. Plasma ANP increases postprandially only during chronic high Na intake. In conclusion: the maintenance of Sodium balance during chronic changes in sodium intake can occur despite no significant increase in plasma ANP under normal steady state conditions.

IMMUNE MEDIATED MESANGIAL CELL INJURY : THE ROLE OF EICOSANOIDS. R.A.K. Stahl, S. Kudelka, W. Schoeppe, U. Helmchen Dpts. of Medicine and Pathology, Universities of Frankfurt and Göttingen, F.R.G. Rabbit anti-rat-thymocyte serum (RATS) induces a complement dependent mesangial cell (MC) injury and stimulates prostaglandin E₂ (PGE₂) formation in cultured MC. We evaluated the role of eicosanoids on glomerular hemodynamics in a rat model of MC injury. I.V. application of RATS resulted in a selective mesangiolysis (ML) at 2h (demonstrated by light and electron microscopy). ML was associated with a decrease in glomerular filtration rate (GFR) (456±24 ul/min/100grbw) compared to controls (C) (837±63, P<0.001). Renal blood flow (RBF) was not different (C:2101±335; ML:1710±203 ul/min/100grbw). Pretreatment with indomethacin (INDO 5mg/kgbw) prevented the fall in GFR in rats with MC injury (800±67) and increased RBF significantly (3797±675). Glomerular PGE₂ formation in rats with ML was not different from C. Thromboxane B₂ production in glomeruli of rats with MC injury (665±213 pg/mg/min) was significantly higher compared to C (206±69 pg/mg/min). The data demonstrate that a selective MC injury results in a decrease in GFR, an effect which is prevented by INDO. This suggests that a vasoconstrictor cyclooxygenase metabolite modulates GFR in this model independently of contractile effects on MC. Vasodilatory PGs do not play a role on the modulation of glomerular hemodynamics in this model.

CALCIUM AND CYCLIC AMP AS SECOND MESSENGERS FOR VASOPRESSIN IN RAT INNER MEDULLARY COLLECTING DUCT. R.A. Star, H. Nonoguchi*, R.B. Balaban*, and M.A. Knepper, NIH, NHLBI, Bethesda, MD.

Arginine vasopressin (AVP) increases the urea permeability in the terminal part of the rat inner medullary collecting duct (IMCD). To study possible second messengers involved, we measured AVP-mediated changes in cAMP accumulation, intracellular calcium ([Ca]_i), and urea permeability (Pu). [Ca]_i was measured in isolated perfused IMCDs using the trapped fluorescent probe INDO-1. Pu was determined by measuring the urea flux resulting from a 5mM bath-to-lumen concentration gradient. Cyclic AMP accumulation was measured by radioimmunoassay following incubation of microdissected non-perfused tubules. TIME COURSE: 10 nM AVP transiently increased [Ca]_i from 131±10 to 263±14 nM (n=20). Peak response was observed an average of 87 seconds after AVP addition with return to near baseline within 3 minutes. An increase in cAMP accumulation was detectable within 1 minute of AVP (0.5 nM) exposure. Pu increased within 2 minutes of exposure to 10 nM AVP and was 50% of maximal within 5 minutes. DOSE RESPONSE: Increases in cAMP accumulation and Pu were seen with 0.01 nM AVP or greater. However, the threshold for an AVP-mediated increase in [Ca]_i was much higher (between 0.1 and 10 nM AVP). EFFECT OF EXOGENOUS CYCLIC AMP: 1 mM 8-Br-cAMP caused a large increase in Pu (from 19±4 to 50±6 x10⁻⁵ cm/s) without affecting [Ca]_i. CONCLUSIONS: 1) Cyclic AMP is the second messenger for AVP stimulation of urea permeability. 2) Vasopressin increases [Ca]_i, possibly by activation of the phosphoinositide pathway, but the physiological role of this response is not yet known.

INTRARENAL RENIN-ANGIOTENSIN SYSTEM REGULATES RENAL ACTIONS OF ATRIAL NATRIURETIC PEPTIDE. T.H. Steele and L. Challoner-Hue, University of Wisconsin, Madison, WI.

Using 2 angiotensin II receptor antagonists and 2 converting enzyme inhibitors, we assessed the effects of inhibition of the intrarenal renin-angiotensin system (IRRAS) on functional responses to atriopeptin II (APII) by isolated perfused rat kidneys devoid of angiotensinogen in their perfusate.

In kidneys from rats on a high NaCl diet, APII increased the GFR from 550 to 738 μl/min and decreased renal vascular resistance (RVR) from 2.0 to 1.9 mmHg/ml·min⁻¹ (P<0.005 for both). Urine flow (V) and fractional sodium excretion (FE_{Na}) increased (P<0.005 for both). Superimposition of IRRAS inhibitors upon APII partially reversed the increment in GFR to 613 μl/min and restored RVR to 2.0 mmHg/ml·min⁻¹ (P<0.005 for both). IRRAS inhibitors additionally increased V and FE_{Na} (P<0.01 for both). In kidneys from rats on a low NaCl diet, APII did not affect GFR, V, or FE_{Na} (P>0.05 for both), but decreased RVR (P<0.05). Superimposition of IRRAS inhibitors increased V (P<0.05), did not affect GFR or FE_{Na} (P>0.05), and reversed the decrement in RVR (P<0.02). The 4 IRRAS inhibitors acted similarly and, without APII, produced no changes in renal function.

Conclusions: The IRRAS: (1) Amplifies the action of APII to increase GFR. (2) Opposes APII actions to increase V and FE_{Na}. (3) Is not responsible for diminished renal reactivity to APII following NaCl restriction.

MESANGIAL CELL REPLICATION IS SPECIFICALLY STIMULATED BY ARGININE VASOPRESSIN (AVP). R.B. Sterzel & M.B. Ganz*, VAMC-Yale U Sch Med, New Haven, CT.

AVP and angiotensin II (Ang II) bind specifically to vascular smooth muscle-like mesangial cells (MCs) and affect contraction. We tested whether these peptides also modulate growth behavior of rat MCs in early subculture (#2-5). Subconfluent, serum-starved MCs were exposed to AVP (10⁻¹⁰ to 10⁻⁷M), Ang II (10⁻¹⁰ to 10⁻⁷M), the MC mitogen PDGF (1.5ng/ml to 5.0ng/ml) or vehicle in the presence of .5% fetal calf serum and insulin. To assess DNA replication, MC uptake of ³H-thymidine (24h pulse) was determined on days 1, 2 and 4. The cpm results in replicate wells were (mean±SEM):

	d1	d2	d4
AVP (10 ⁻⁸)	3105±470	5713±784	8219±955
Ang II (10 ⁻⁸)	697±109	839±66	959±175
PDGF (2.5ng/ml)	5813±735	1836±414	1004±259
Vehicle Control	556±177	689±164	790±143

Comparable changes of MC replication were shown by the counting of detached MCs. The V₁ AVP-antagonist, PMP inhibited only AVP-induced promotion of MC growth (by up to 80.4%). The activator of protein kinase C, TPA failed to synergize with AVP to stimulate DNA synthesis but by itself was mitogenic (d2 and 4) when added to vehicle. Addition of the possible contaminant, endotoxin did not affect the above findings. Conclusions: 1) Similar to the cytokine, PDGF, the contraction-inducing peptide AVP is strongly mitogenic for rat MCs. 2) This effect is specifically inhibitable and may be mediated by TPA-like activation of protein kinase C. 3) The lack of similar effects by Ang II is presently unclear but could be due to loss of Ang II receptors on rat MCs in culture.

DOWNREGULATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) RECEPTORS IN SUPERFUSED RABBIT GLOMERULI. Thomas J. Stokes and Erika Tetens*.

Washington University Medical School, St. Louis, MO.

Clinical states associated with persistent volume expansion and elevated ANP levels may be associated with reduced natriuresis in response to ANP. Experimental models have indicated that persistently elevated ANP concentrations result in receptor down regulation. To characterize the time course and characteristics of altered responsiveness to ANP in freshly isolated, normal rabbit glomeruli we examined a model of glomerular superfusion. Following 1 hour of equilibration perfusion with RPMI 1640 at 37°C, the glomeruli were perfused with 0.1 μ M ANP for 21 minutes. Cyclic GMP released into the perfusate fell rapidly (50% of maximum at 12.3 \pm 0.8 minutes, n=7) and was virtually eliminated by 21 minutes. Following a 30 minute ANP-free washout period, glomeruli were again perfused with 0.1 μ M ANP. The cGMP released upon reexposure to ANP was significantly less than upon initial exposure (34.7 \pm 2.6% reexposure vs initial exposure, p<0.01). Perfusion of the glomeruli with 0.1 mM dibutyl- α -cGMP or 8-bromo-cGMP for 21 minutes did not produce desensitization of the subsequent cGMP response to ANP.

Competitive binding studies following perfusion with 0.1 μ M ANP for 21 minutes and 15 minutes washout indicated reduced glomerular ANP receptor density compared to glomeruli perfused with medium alone (76.0 \pm 10.5 vs 130.0 \pm 20.0 fmoles/mg protein, n=3).

We conclude: 1) perfusion of rabbit glomeruli with ANP rapidly results in desensitization of the cGMP response to ANP 2) this effect is not mimicked by dibutyl- α -cGMP or 8-bromo-cGMP 3) desensitization is associated with reduction in ANP receptor density.

RESPONSE TO ALPHA HUMAN NATRIURETIC PEPTIDE (hANP) INFUSION IN SEVERE CHRONIC RENAL DISEASE. CP Swainson, Royal Infirmary, Edinburgh, UK.

In patients with renal failure, plasma immunoreactive ANP concentrations (Ir-ANP) are raised. hANP infusion causes a diuresis and natriuresis, accompanied by increases in GFR and ERPF, and a fall in MAP and PRA in normal subjects. To examine the effects of hANP in severe renal disease, patients with chronic glomerulonephritis (GFR <30 ml/min) were studied before and after infusion of both hANP (5 pmol/kg/min) for 1 hour and placebo, in random order, after stabilisation on a 100 mmol Na⁺, 80 mmol K⁺ and 1 g protein/kg body wt diet for 3 days. Inulin and PAH clearances, urinary electrolytes, plasma hormones and BP were measured every 15-30 min, and Ir-ANP every 2 min after the end of the hANP infusion. Baseline Ir-ANP were elevated (34.4 SD9.0 pmol/l, normals 6-14 pmol/l). Compared with placebo, hANP infusion caused significant increases in urine flow and U_{Na}V (200% and 192% respectively), ERPF and GFR (136% and 55% respectively), and PRA fell by 40%. Peak Ir-ANP at the end of the infusion was 278 SD142 pmol/l and the t_{1/2} was 4.8 SD2.7 min. MAP fell by 7 SD4 mmHg.

In a low dose, hANP causes significant changes in electrolyte excretion, renal haemodynamics and systemic BP and suppression of PRA in subjects with severe renal disease, without a marked increase in t_{1/2}, and these effects are probably mediated by an increase in receptor numbers as described in the uraemic rat, and comparable to the response of normal subjects.

INHIBITORY GTP-BINDING PROTEIN, Ni, MEDIATES INHIBITION OF VASOPRESSIN (AVP)-DEPENDENT cAMP PRODUCTION BY EPINEPHRINE, PGE₂, AND HIGH AMBIENT Ca²⁺ IN MEDULLARY COLLECTING TUBULES (MCT) AND THICK ASCENDING LIMBS OF HENLE (MAL) OF MOUSE KIDNEY. K. Takaichi* and K. Kurokawa. IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo, Japan

AVP via cAMP increases water permeability in MCT and stimulates NaCl reabsorption in MAL. α 2-adrenergic agonist in MCT and PGE₂ and high ambient Ca²⁺ in MAL inhibit AVP-dependent cAMP production thereby modulate urine concentration. The present study was aimed to clarify mechanisms underlying the inhibition of AVP-dependent cAMP production by these agents using MCT and MAL isolated from outer medulla of mouse kidney. Preincubation of MCT and MAL with 1 μ g/ml pertussis toxin (PT) for 3 and 6 hrs, respectively, resulted in ADP-ribosylation of an about 40K dalton protein, presumably α subunit of Ni. Epinephrine, 10⁻⁶M, via α 2-adrenergic stimulation, inhibited AVP-dependent cAMP production in MCT. Preincubation of MCT for 3 hrs with PT abolished inhibition of AVP-dependent cAMP production by epinephrine. PGE₂ and high (5 mM) ambient Ca²⁺ both inhibited AVP-dependent cAMP production in MAL. Preincubation of MAL for 6 hrs with PT abolished the inhibition by PGE₂ and 5 mM Ca²⁺. Preincubation of MCT or MAL with PT for 1 hr was ineffective in ADP-ribosylation and did not reverse the inhibition of AVP-dependent cAMP by epinephrine, PGE₂ or 5 mM Ca²⁺. Results indicate that the inhibition of AVP-dependent cAMP production by α 2-adrenergic agonist in MCT and by PGE₂ and high Ca²⁺ in MAL may be mediated via activation of inhibitory GTP-binding protein Ni of adenylate cyclase.

ATRIAL NATRIURETIC PEPTIDE (ANP) IN 70 PATIENTS WITH END STAGE RENAL FAILURE (RF). CLINICAL SIGNIFICANCE OF PERIODIC DETERMINATIONS.

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Since elevated concentrations of ANP are associated with volume overload (VO) or congestive heart failure (CHF) 70 patients with RF undergoing hemodialysis (HD) or hemofiltration (HF) were investigated. To elucidate the influence of the dialysis procedure clearance-studies and isovolemic HD/HF were performed.

10 patients with pulmonary congestion showed extremely elevated ANP plasma concentrations before (m:923 fmol/ml) and after (m:376 fmol/ml) HD or HF. In 42 patients without clinical signs of VO or CHF ANP levels were elevated twice the normal range of healthy controls (17-36 fmol/ml; n=50; 3 SD). In 18 patients with moderately elevated ANP concentrations (178 \pm 58 fmol/ml) 11 (A) decreased to normal levels after intensive ultrafiltration whereas 7 (B) remained unchanged; this group (B) demonstrated pathologic left ventricular ejection fraction (39,8 \pm 11%), atrial and ventricular enddiastolic diameters compared to normal values of A.

According to the molecular weight of ANP (3205D) ANP was detected in hemofiltrate and clearance-rates were calculated C ANP: 50 ml/min.

During isovolemic HD or HF ANP concentrations remained unchanged suggesting that the intravascular volume is the main factor for ANP secretion. Persistent high ANP concentrations in HD/HF patients after intensive ultrafiltration indicate CHF and normal values suggest adequate rehabilitation.

PLASMA RENIN ACTIVITY IN HUMANS IS DIRECTLY RELATED TO DIETARY POTASSIUM. E. Toffelmire,* R. Hernandez, A. Carneiro,* A. Sebastian, and M. Schambelan. Dept. of Med., Univ. of Calif., San Francisco, CA

The concept that renin secretion is inversely related to dietary K^+ intake has emerged largely from studies in rats and dogs. However in humans, we found that restriction of dietary K^+ suppresses renin secretion (KI 25:336, 1984). We have now evaluated the renin secretory response in normal volunteers to increased dietary K^+ . The increase in K^+ intake (0.7→2.1 meq/kg/day for 7-10 days) was effected by administration of KCL (n=9) or $KHCO_3$ (n=6), or by equimolar substitution of K^+ for Na^+ without change in anion intake (Na^+K , n=7). Plasma renin activity (PRA, ng/ml/h) was measured after overnight recumbency (OR) and after 2 hrs of upright activity (UP) during control (CONT), K^+ loading (KLOAD), and recovery (REC) periods. PRA increased significantly (* $p < 0.02$, ** $p < 0.001$) with KCL and Na^+K and slightly with $KHCO_3$ and decreased during the subsequent REC period:

	PRA-OR			PRA-UP		
	CONT	KLOAD	REC	CONT	KLOAD	REC
KCL	1.8 +0.4	2.8* +0.5	1.4 +0.3	3.1 +0.5	4.7* +0.8	3.2 +0.5
$KHCO_3$	1.7 +0.6	2.4 +0.6	2.1 +0.6	3.8 +1.0	4.0 +1.2	3.9 +1.1
Na^+K	2.6 +0.4	7.5** +1.2	1.7 +0.4	6.6 +1.1	11.0* +1.4	3.8 +0.6

KLOAD was associated with transient natriuresis during KCL (9/9) and $KHCO_3$ (4/6), and chloruresis during Na^+K (7/7); plasma K^+ did not increase significantly. Taken together with our previously reported studies, the present results indicate that in humans, in contrast to rats and dogs, renin secretion is directly related to dietary K^+ intake.

EFFECTS OF MINERALOCORTICOID ON KININASES IN RAT MEDULLARY COLLECTING DUCTS. Kimio Tomita, Kazutomo Ujii*, Yoshitaka Maeda*, Yasuhiko Iino Naoki Yoshiyama*, and Tatsuo Shiigai*. Tokyo Med. & Dent. Univ. Tokyo, and Toride-kyodo Hosp. Ibaraki, JAPAN

There are several reports that indicate discrepancy between urinary kallikrein excretion and urinary kinin excretion. Kininases may have critical role in the regulation of kinin concentration. Recent report showed the presence of kininases in all along the rabbit nephron segments. To elucidate the regulatory mechanism of the kallikrein system, we investigated the kininase activity following the treatment with deoxycorticosterone pivalate (DOC).

Five mg of DOC was intramuscularly injected in the back muscle of male SD rats (about 150g) 1-7 days before collection of tubules. Cortical collecting ducts (CCD) and outer medullary collecting ducts (OMCD) were collected under microscope after collagenase treatment. Kininase activity was measured using enzyme-immunoassay method (Dainippon Pharm, Osaka).

In OMCD, total kininase activity was lower in 3-days (2.84±0.09 pg/mln/mm, n=8, m±SE) and 7-days treatment of DOC (2.77±0.11, n=7) when compared to age-matched control (3.86±0.07, n=10). Those decrease in activity were mainly due to decrease in kininase II activity. On the other hand, kininase activity of CCD did not change after treatment of DOC.

Our data showed that kininase activity in rat OMCD decreased by DOC treatment. This suggests that mineralocorticoid stimulates kinin destruction in one way, besides well-known stimulative effects of mineralocorticoid on kallikrein activity.

DOWN-REGULATION OF ANGIOTENSIN II (AII) RECEPTORS IN VASCULAR SMOOTH MUSCLE CELLS (VSMC) IN CULTURE. ME Ullian,* SL Linas. U. Colorado Health Sciences Center, Dept. of Medicine, Denver, Colorado.

The effect of AII on regulation of binding to AII vascular receptors in vivo is complex. Increases and decreases in receptor number (B_M) and increases in binding affinity (K_D) have been reported. To determine the specific effects of AII on AII receptor binding, we performed binding studies in VSMC from rat mesenteric arteries. Binding of ^{125}I -AII was time, temperature, dose and protein-dependent. Bound ligand was rapidly translocated from the cell surface into an acid-resistant intracellular compartment at 22°C, but not at 4°C. Exposure to unlabeled AII at 22°C caused time and dose-dependent decreases in cell surface binding. Scatchard analysis revealed that B_M was reduced by 31% after 30 min exposure to 6×10^{-8} M AII while K_D was unchanged. Comparable decreases in total binding were observed after exposure to comparable doses of putative AII receptor antagonists Sar¹ Ala⁸-AII and Sar¹ Leu⁸-AII, but not after exposure to norepinephrine or aldosterone. Decreases in cell surface binding after exposure to AII were partially prevented by incubating cells at low temperatures (30% loss at 22°C vs 10% loss at 4°C). Recovery from maximum down-regulation was complete by 60 min at 22°C and was prevented by the lysomorph agent chloroquine, but not by the protein synthesis inhibitor cycloheximide. Conclusions: AII causes down-regulation by translocation of AII receptors from the cell surface to the cell interior where they can be recycled to the cell surface via a lysosome-dependent process.

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) AND CHOLECYSTOKININ OCTAPEPTIDE (CCK8) REDUCE SODIUM AND WATER REABSORPTION IN THE LOOP OF HENLE. Robert J. Unwin*, Amabel C. Shih* and Gerhard Giebisch. Yale Univ. Med. Sch., Dept. of Physiol., New Haven, Connecticut.

To test whether the reported renal actions of the vasoactive neuropeptides VIP and CCK8 are direct or indirect, we used free-flow renal micropuncture to study their effects on proximal and distal tubule function, in male Sprague-Dawley rats. At calculated doses of 0.4-0.7 (VIP; n=5) and 10-15 (sulphated CCK8; n=7) nmol/h, neither peptide altered mean arterial blood pressure, whole kidney g.f.r., or U_{Na} and U_{K} , compared with timed controls (n=9). Apart from a slight fall in $\%H_2O$ reabsorption during CCK8 infusion (40.6±2.5 vs. 46.3±1.6; mean±SE; n=22,34, respectively, P=0.05), there were no significant changes in proximal tubule function. Early distal tubule (<50%) delivery of Na and H_2O increased significantly; K delivery also increased with VIP, but not CCK8:-

	C	CCK8	VIP
s.n.g.f.r. (nl/min)	51.8±1.8(35)	49.6±3.2(21)	46.5±3.0(17)
$\%H_2O$ reabs.	79.3±2.2(13)	69.0±2.5(10)	68.9±2.5(5)
$\%Na$ deliv.	7.7±1.5(9)	13.3±0.8(10)	15.9±3.6(5)
$\%K$ deliv.	10.0±2.5(9)	11.9±1.5(10)	30.3±9.4(5)

Late distal delivery of Na and K was unchanged, indicating enhanced reabsorption of these ions along the distal tubule. $\%H_2O$ reabs. was still reduced by CCK8 (73.9±2.9 vs. 88.5±2.9; n=7, P<0.01), but not VIP.

We conclude that both peptides have direct inhibitory effects on ion and H_2O transport in the loop of Henle.

EFFECT OF ATRIAL NATRIURETIC PEPTIDE (ANP) ON THE SUPERFICIAL PROXIMAL TUBULE AND LOOP OF HENLE. Anja van de Stolpe*, Kristina Blouch* and Rex L. Jamison, Depts. of Med., Stanford Univ., Stanford, CA and Univ. of Rochester, Rochester, NY.

To determine if ANP inhibits reabsorption in the proximal tubule and Henle's loop, rats were anesthetized and prepared for recollection micro-puncture of late proximal (LP) and early distal (ED) tubules. Experimental (E, n=11) rats received ANP, 0.8 μ g/kg BW prime and 0.1 μ g/min per kg BW infusion; control (C, n=12) rats the vehicle only. Each group was studied before (Period I) and during (II) ANP or vehicle, respectively. Results: GFR (μ l/min), SNGFR (nl/min), tubule fluid Na concentration (TFNa, mEq/l), fractional Na delivery (TF/P (Na/inulin) \times 100, %) and Na excretion (UNaV, μ Eq/min per gKW), were (means):

	GFR	SNGFR	TFNa	ED	LP	ED	UNaV
				[TF/P (Na/in) \times 100]			
C I	1351	40	45	50	4.2	0.16	
C II	1213	43	47	57*	6.1**	0.33	
E I	1134	41	41	53	5.5	0.19	
E II	1083	45	58**	74***	10.0***†	2.01***†	

*p <0.05, **p <0.01, period II vs. I; †p <0.05, EII vs. CII.

GFR and SNGFR did not change significantly. After ANP UNaV increased 10-fold and K, Ca, Mg and H₂O excretion 3-fold. Comparing E II to C II, fractional Na delivery to the LP tubule increased significantly more after ANP. Results were similar for K, Mg, Ca and H₂O. TFNa and TF_{Mg} at the ED tubule rose after ANP. Fractional delivery of Na, Ca, Mg and H₂O to the ED tubule increased more after ANP than in control rats. These findings suggest that ANP (1) inhibits reabsorption in the superficial proximal tubule and (2) impairs normal reduction of Na and Mg concentration by Henle's loop.

CHARACTERISTICS OF THE RESPONSE OF CYTOSOLIC CALCIUM TO VASOPRESSIN IN LLC-PK₁ CELLS. JM Weinberg, JA Davis,* and JA Shayman. VA Medical Center and University of Michigan, Ann Arbor, Michigan.

LLC-PK₁ cells have long been known to have receptors for vasopressin (VP) mediating a cAMP response and have recently been shown to exhibit transient increases of cytosolic Ca in response to VP. These responses and their interrelationships have been further characterized. Basal cytosolic Ca and cAMP were 72.5 \pm 5.6 nM (N=104) and 25.9 \pm 1.9 pmol/mg protein (N=19), respectively. 100 nM arginine VP (AVP) and lysine VP (LVP) raised cytosolic Ca to 415.8 \pm 94.9 (N=11) and 469.8 \pm 163.2 (N=5) nM, respectively, and increased cAMP by 15-20 fold. dEt₂Tyr(Et)AVP and (CH₂)₅Tyr(Et)AVP, antagonists of the V₁ class of receptors at 1 μ M did not themselves affect cytosolic Ca, but completely blocked the responses to 100 nM AVP and LVP. The analogues did not affect cAMP or the response of cAMP to AVP. dVDAVP (1 μ M), a V₂ agonist, and forskolin (10 μ M) did not affect cytosolic Ca or the Ca response to AVP; however, each did increase cAMP by 15-20 fold. Neither exposure to cholera toxin (10 μ g/ml) nor pertussis toxin (1 μ g/ml) for 16 hours affected basal Ca or the response to AVP. Fluoride (5 mM) with and without Al³⁺ (50 μ M) did not consistently affect either basal Ca or the response to subsequent AVP. 50 and 100 ng/ml phorbol myristate acetate substantially diminished the Ca responses to AVP. Inositol trisphosphate, isolated by high voltage electrophoresis and measured both enzymatically and radiochemically, demonstrated time dependent changes in response to AVP.

ENDOTOXIN STIMULATION OF MESANGIAL CELL PGE₂ IS INHIBITED BY PERTUSSIS TOXIN AND MAINLY ASSOCIATED WITH ACTIVATION OF PHOSPHOLIPASE A₂. Jin Wang, Mark Kester, and Michael J. Dunn. Depts. of Medicine and Physiology, Case Western Reserve Univ. and Univ. Hospitals of Cleveland, Ohio.

We recently demonstrated a stimulatory effect of endotoxin (ET) on PGE₂ production in rat mesangial cells (MC). Arachidonic acid (AA), required for PGE₂ synthesis, can be released by activation of either phospholipase (PL) A₂ or combined PLC and 1,2-diacylglycerol (DAG) lipase. GTP-binding (G) protein(s) may couple ET "receptor"-mediated events with activation of PL(s). We thus determined whether G protein(s) and activation of PLA₂ and/or PLC mediated the ET-induced PGE₂ synthesis. Pertussis toxin, an inactivator of G protein(s), inhibited ET (60 μ g/ml)-stimulated PGE₂ synthesis in a dose-related manner, with maximum inhibition (80%) at 100 ng/ml PT (n=4). PT had no effect on A23187-stimulated PGE₂ generation. Direct or indirect PLA₂ blockers, mepacrine (10⁻⁴M) and trifluoperazine (5 \times 10⁻³M), inhibited the ET-evoked PGE₂ synthesis by 75% and 55%, respectively (n=3). Stimulation of PLA₂ by ET was supported by increments in lysophosphatidylcholine by 44% at 1 min and 65% at 5 min over controls (n=3). Additionally, ET induced a time-dependent elevation of DAG which peaked at 1 min with 95% increments and returned to control values at 15 min (n=6). However, ET did not augment cytosolic free calcium, nor was inositol trisphosphate (IP₃) elevated after ET. We conclude that ET-induced PGE₂ synthesis is mediated mainly through a PLA₂-dependent release of AA. The formation of DAG without elevations of cytosolic free calcium and IP₃ indicates ET activation of PLC independent of polyphosphoinositide turnover.

ATRIAL NATRIURETIC FACTOR (ANF) AND SALT ADAPTATION IN TELEOST FISH (GILA ATRARIA (GA)). C. Westenfelder, F.M. Birch, R.L. Baranowski, M.J. Rosenfeld*, D.K. Shiozawa*, and C. Kablitz. Dept. of Medicine & Biology, Univ. of Utah and VA Med. Ctr., Salt Lake City, Utah, and Dept. of Zoology, Brigham Young Univ., Provo, Utah.

The exact function of ANF in animal and human physiology remains to be defined. Because "euryhaline" fish are able to maintain constant serum osmolality when water salinity varies between that of fresh and ocean water, we investigated whether GA, a salt tolerant fish native to springs of glacial Lake Bonneville, produces and potentially "uses" ANF to adapt to different water salinities. In Study I, immunoreactive ANF (ir-ANF) in fish from a "fresh" water (Na: 26.2 meq/L) and from a "1% NaCl" spring (Na: 154.7 meq/L) was significantly different (146 \pm 27 and 347 \pm 21 pg/ml respectively, P < 0.01). Fish heart extract caused a significant diuresis and natriuresis in 7 rats and contained ANF-like material of 3000 dalton (by HPLC). In Study II, 36 fish were transferred from a "1% NaCl" spring to tanks. 12 fish were maintained at 1% NaCl, 12 each were gradually adapted to "fresh" water or to 2% NaCl. ir-ANF levels after 2 weeks of study were 343 \pm 55 in 1% NaCl fish, 213 \pm 20 in fresh water fish, and 691 \pm 79 in 2% NaCl fish (all pg/ml). These values differed significantly from each other (P < 0.01). Electronmicrographs of atrial and ventricular cardiocytes demonstrated large numbers of paranuclear secretory granules. We conclude that GA produces ANF-like material that resembles mammalian ANF immunologically, physiologically, and physicochemically. Because circulating ir-ANF levels rose in parallel with water salinity, we propose that ANF may be an as yet unrecognized mediator of salt tolerance in "euryhaline" fish.

ATRIAL NATRIURETIC FACTOR (ANF) "DEFICIENCY STATE" CREATED BY IMPLANTATION OF ARTIFICIAL ATRIA AND VENTRICLES IN CALVES. C. Westenfelder, F.M. Birch, R.L. Baranowski, J.B. Riebman*, D.B. Olsen*, G.L. Burns*, and C. Kablitz. Dept. of Medicine & Surgery, Univ. of Utah and VA Med. Ctr., Salt Lake City, Utah.

We created in 3 calves an "ANF-deficiency" state by removing all cardiac sources of ANF (replacement of native heart with artificial atria and ventricles). Animals were observed for up to 26 days postoperatively in order to determine the physiological effects of "ANF-deficiency". Whenever possible, postoperative measurements were compared to those obtained in the "ANF-replete", i.e. preoperative state. Immunoreactive ANF (ir-ANF) levels prior to implantation were 66 and rose to 313 pg/ml after 6 L N.S. i.v./ 20 min. This elicited a significant diuresis and natriuresis. Postoperatively, ir-ANF levels fell to 16-26 pg/ml, and salt retention occurred (edema, elevated blood pressure). Infusion of 6 L N.S. i.v./ 20 min resulted only in a significant diuresis and minimal rise in Na excretion. Reduction of cardiac output (CO) from 8 to 4 L/min led to further salt retention without an increase in ir-ANF, and to elevated atrial pressures, and a fall in GFR. Subsequent increase of CO to 8 and then 13 L/min produced a significant rise in urine flow, while Na excretion and ir-ANF remained depressed. Angiotensin II infusion raised blood pressure, but had no effect on renal function and peripheral ir-ANF. Jugular vein ir-ANF rose, however, to 61 pg/ml. Morphine (365 mg i.v.) had no effect on peripheral and jugular vein, and spinal fluid ir-ANF. Conclusion: 1. following total heart replacement, ir-ANF levels remained > 0; 2. this relative "ANF-deficiency" caused selective Na retention and prevented acute Na (not water) excretion in response to 6 L N.S. i.v. or high CO; 3. angiotensin II but not morphine may cause ANF release from the Central Nervous System.

NATRIURETIC AND DIURETIC EFFECTS OF ATRIAL PEPTIDE (SC-44900) IN MAN WITH CHRONIC RENAL FAILURE. DW Windus, TJ Stokes, R Dean*, J Morgan*, T Wright*, S Klahr. Washington Univ. School Med. and GD Searle & Co. St. Louis, MO and Skokie, IL.

Atrial peptide produces a natriuresis and diuresis in normal man and in rats with experimentally-induced chronic renal failure (CRF). The purpose of this study is to determine whether humans with CRF will respond in a similar fashion. Eight adults with GFR's ranging from 9 - 61 ml/min underwent a 4-hour intravenous infusion of either 0.05 (n=4) or 0.1 (n=4) $\mu\text{g}/\text{kg}/\text{min}$ of the 24-amino acid hormone (SC-44900). Hourly measurements of urine flow rate, inulin and PAH clearances and electrolyte excretions were performed before and during hormone infusion. No differences in response were seen comparing the two doses and the data are grouped. Urine flow rate increased from 3.6 ± 0.6 to 6.0 ± 0.9 ml/min ($P < 0.01$) and sodium excretion rate increased from 118 ± 15 to 238 ± 55 $\mu\text{Eq}/\text{min}$ ($P < 0.05$) comparing the baseline to the mean of the four-hour infusion. The fractional excretion of sodium during the infusion was $168 \pm 18\%$ of baseline. There was no significant change in inulin or PAH clearances comparing baseline studies (33 ± 7 and 150 ± 27 ml/min, respectively) to the mean during drug infusion (36 ± 7 and 165 ± 33). The peak hourly urinary cGMP excretion during drug infusion was 799 ± 123 compared to 334 ± 108 pmol/min during the baseline period ($P < 0.02$). There was no correlation between baseline GFR and fractional increase of urine flow or sodium excretion ($r = 0.1$ and 0.2 , respectively). Thus, patients with CRF have a natriuretic and diuretic response to atrial peptide associated with an increased urinary cGMP excretion. This response does not appear to be dependent on alterations of GFR or renal blood flow.

MECHANISM OF DIMINISHED ADENYLATE CYCLASE (AC) RESPONSE TO ARGININE VASOPRESSIN (AVP) IN AGING HUMAN COLLECTING TUBULES (CT). Patricia D. Wilson and David Hreniuk. UMDNJ-RWJ Med. Sch., Dept. of Physiol. & Biophys, Piscataway, N.J.

An age-associated decline in renal concentrating ability occurs despite unaltered pituitary AVP release. Our previous studies showed a dramatic decrease in the ability of AVP to stimulate osmotic water flow in old isolated perfused CT (Am. Soc. Ren. Biochem. Metab. 1987). To elucidate the mechanism of this defect, AC activity and H^+ -AVP binding were studied in cultured human CT microdissected from donors of increasing age (18-62yr). Basal levels of AC did not alter with age, but in CT from "old" (>45yr) donor kidneys a dramatically diminished response of AC to AVP ($1\mu\text{M}$) stimulation was seen by comparison to levels in cultured CT from young adults. The ability of old CT to respond to the postreceptor agonists forskolin (Fo, $25\mu\text{M}$) and NaF (10mM) was also substantially diminished. AC levels in fmole cAMP/30min/mg protein were:

	Basal	AVP	Fo	NaF
Young (18-40yr)	82 ± 16	510 ± 138	2296 ± 480	891 ± 98
Old (45-62yr)	99 ± 21	182 ± 38	250 ± 114	67 ± 12

n=8
Receptor binding studies with H^+ -AVP ($5 \times 10^{-9}\text{M}$ and $5 \times 10^{-6}\text{M}$) showed no alterations with age: cpm/mg protein were 47 ± 3 and 742 ± 80 respectively for young CT; 50 ± 5 and 564 ± 75 respectively for old CT.

We conclude that renal aging is associated with a dramatic reduction in sensitivity of CT epithelial cells to the action of AVP, mediated via cAMP and is caused by reduced AC catalytic activity but no receptor loss.

EFFECT OF THYROID HORMONE STATUS IN ATRIAL NATRIURETIC PEPTIDE CONCENTRATIONS IN RAT PLASMA. N.L.M. Wong, E.F.C. Wong*, D.C.K. Hu*, and J.H. Dirks. Dept. of Medicine, University of British Columbia, Vancouver, B.C., Canada.

Recent studies in man have shown that hypothyroidism is associated with a reduction in circulating atrial natriuretic peptide (ANP) levels. The effect of hypothyroidism on circulating ANP was examined in 12 normal rats (Group I), 12 hypothyroid rats (Group III) and 12 hypothyroid rats treated with levothyroxine, 2 mg/day (Group II). Hypothyroidism was induced by adding 0.025% methimazole to the drinking water for three weeks. TSH (I, 1.25 ± 0.14 $\mu\text{U}/\text{ml}$; II, 1.24 ± 0.18 $\mu\text{U}/\text{ml}$; III, 2.29 ± 0.14 $\mu\text{U}/\text{ml}$) T_3 (I, 0.83 ± 0.04 nmol/L; II, 1.01 ± 0.08 nmol/L; III, 0.78 ± 0.03 nmol/L) and T_4 (I, 38.3 ± 7 nmol/L; II, 26.8 ± 2.5 nmol/L; III, 7.9 ± 0.8 nmol/L[†]) were measured. Renal functions were also examined in these animals. Hypothyroidism causes a reduction in GFR (I, 2.92 ± 0.19 ml/min; II, 1.99 ± 0.09 ml/min; III, 1.25 ± 0.05 ml/min). In contrast to previous reports, plasma Mg was lowered in hypothyroid rats (P Mg, I, 1.08 ± 0.02 mM; II, 1.01 ± 0.02 ; III 0.98 ± 0.01) and was coupled with a slight increase in Mg excretion (FEMg, I, $15.0 \pm 0.7\%$; II, $15.5 \pm 0.7\%$; III, $17.9 \pm 0.8\%$). Plasma ANP was 98 ± 8 pg/ml in normal rats (I) and 62 ± 12 pg/ml in the hypothyroid rats (III). Replacement therapy caused a significant rise in ANP (181 ± 21 pg/ml) in Group II. Thus, hypothyroidism is accompanied by a reduction in circulating ANP which was reversed by replacement therapy.

[†] $p < 0.01$ compared to Group I.

TWO DISTINCT VOLTAGE-GATED CALCIUM CURRENTS IN BOVINE ADRENAL GLOMERULOSA CELLS. N. Yamashita*, H. Matsunaga*, I. Kojima* and K. Kurokawa. IVth Dept Int Med, Univ Tokyo Sch Med, Tokyo, Japan

Aldosterone secretion by adrenal glomerulosa cells increases in response to angiotensin II, ACTH, and a rise in extracellular K^+ . This stimulation of aldosterone secretion continues in the presence of these agonists and depends on sustained Ca^{++} influx. We reported the presence of low-threshold and transient-type voltage-gated Ca^{++} currents in rat adrenal glomerulosa cells and suggested it may explain an initial Ca^{++} influx in response to a rise in extracellular K^+ (Pluegers Arch 408: 351, 1987). In the present study, we analyzed Ca^{++} currents of single bovine adrenal glomerulosa cells using whole-cell patch clamp techniques. Patch pipet solution included cAMP to suppress Ca^{++} channel rundown and CsCl to block K^+ channels. Two types of voltage-gated inward Ca^{++} channel currents were identified. One was a transient (T) type which decayed within 100 ms, characterized by a low threshold voltage (about -60 mV) similar to that seen in rat adrenal glomerulosa cells. Another was a long-lasting (L) type which shows a more positive threshold potential. It has been shown that a L-type Ca^{++} channel inhibitor nifedipine inhibits agonist-stimulated Ca^{++} influx and sustained aldosterone secretion while a L type Ca^{++} channel agonist BAY-K 8644 evokes sustained Ca^{++} influx and sustained aldosterone secretion. Thus, our data indicate that while T type Ca^{++} channels may explain initial Ca^{++} influx in response to an elevation in extracellular K^+ , L type Ca^{++} channels may allow sustained Ca^{++} influx which is necessary for sustained aldosterone secretion.

ATRIAL NATRIURETIC PEPTIDE (ANP) INHIBITS ^{22}Na UPTAKE IN RABBIT INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. M.L. Zeidel, D. Kikeri,* M. Burrowes,* P. Silva, and B.M. Brenner. Harvard Medical School, Boston MA.

ANP inhibits transport-dependent oxygen consumption in IMCD cells. To clarify the mechanisms of sodium handling and their regulation by ANP in the IMCD, we measured uptake of ^{22}Na , intracellular pH (pHi, using the fluorescent indicator, BCECF), and membrane potential (PD, using the cyanine dye DiS-C3-(5)) in fresh suspensions of IMCD cells. Sodium uptake was inhibited 44.4±6.2% (n=5, SE) by amiloride (A, 0.1mM) but was insensitive to the same concentration of hydrochlorothiazide (1.9±7.9%), or furosemide (1.0±7%). Resting pHi of IMCD cells was 7.41±0.03 (n=7). The rate of recovery from acid loading (NH₄Cl, 20mM) was 0.13±0.01 pH units/min in the absence of sodium, 0.19±0.01 in the presence of 20 mM extracellular Na, and 0.11±0.01 in the presence of 0.1 mM A and Na. Acidification of the cell interior stimulated ^{22}Na uptake 2.23±0.3 fold (n=7); A blocked this stimulation. Preloading IMCD cells with 130 mM KCl followed by dilution into N-methyl D glucamine buffer rendered PD of IMCD cells more negative than similar cells diluted into KCl, and stimulated ^{22}Na uptake 1.78±0.12 fold; A inhibited this PD-sensitive Na uptake. ANP inhibited Na uptake 37.5±4.3% (n=5); inhibition by A and ANP was not additive. ANP inhibited PD-stimulated Na uptake as effectively as A. Conclusions: Sodium entry in IMCD cells can occur by two amiloride-sensitive mechanisms, Na/H exchange and electrogenic Na entry. ANP inhibits Na entry in IMCD cells via the electrogenic pathway.

PRODUCTION OF ANGIOTENSINOGEN (A) BY PRIMARY CULTURED RABBIT RENAL PROXIMAL TUBULAR CELLS. N. Yanagawa, P. Eggna*, A. Friedal* and O.D. Jo*. Sepulveda VAMC, UCLA Sch. of Med., Los Angeles, CA.

In the kidney, angiotensin converting enzyme (ACE) has been shown to be present in proximal tubule (PT). The role of ACE in PT is not clear. Since increasing number of studies suggest the existence of local angiotensin generating systems and A mRNA has been detected in extra-hepatic tissues including the kidney, we have examined the possibility of local angiotensin system in PT. Purified rabbit PT was prepared and cultivated in serum-free medium with defined hormones (hydrocortisone, insulin, transferrin) as described (J. Cell Biol. 95:118, 1982). A and renin activity in culture medium were determined 2 wks after explantation by angiotensin I radioimmunoassay. Both A (2.8 ± 0.5 ng/mg/72hr, n=6) and renin activity (35.5 ± 5.0 ngAI/hr/mg) were detected. A level was found to (i) increase with time, (ii) increase by 60% with renin inhibitor (pepstatin, 10 μM), and (iii) decrease with hormone-deprivation. To further ascertain that PT is the tissue origin for A production, A was also detected in culture medium 4 wks after explantation of microdissected rabbit proximal convoluted and straight tubules (3.9 ± 1.3 and 4.2 ± 0.6 ng/mg/72hr, n=4, respectively). These results thus indicate the presence of angiotensin generating system in PT.

HYPERTENSION

INCREASED CYTOSOLIC FREE Ca CONCENTRATION ($[Ca^{2+}]_i$) IN PROXIMAL TUBULES OF SPONTANEOUSLY HYPERTENSIVE RAT (SHR): CONCURRENCE WITH THE LOW 1,25(OH)₂D LEVELS AND RESPONSES TO A HIGH Ca DIET. I. Ahmed,* S. Tafi,* M. Resigan,* B. Eby* & K. Lau. Michael Reese Hosp. & Univ. of Chicago, IL.

It is unclear whether the reduced serum 1,25(OH)₂D levels in adult SHR is due to defective synthesis or down-regulation by excess Ca. To resolve this issue, we measured $[Ca^{2+}]_i$ in age- and sex-matched SHR and normotensive Wistar Kyoto (WKY) control, using the method of ratioing the fluorescence emitted at 505 nm during dual excitation (340 & 380nm) of fura 2-loaded tubules. All rats were equilibrated with a normal 0.6% P diet. Proximal tubule suspensions were obtained by collagenase treatment of kidney cortical slices. Harvested and studied in 1 mM Ca buffers, $[Ca^{2+}]_i$ was higher in 4-weeks old male SHR (164 ± 12 + vs 121 ± 6 nM) (†, p < 0.05 vs WKY). To exclude the possible effect of in-vitro Ca flooding, the experiment was repeated with Ba (1 mM) substituting for Ca. Fluorescence was read without Ba. (§, p 0.05 vs SHR on 0.87% Ca diet).

Diet	Ca	Plasma Ca^{2+} (nM)	Cytosolic Free Ca Concentration (nM)		
			In Buffers With [Ca]= "0"	2.5 mM	3.9 mM
WKY	0.87%	1.30	173 ± 7	248 ± 17	304 ± 25
SHR	0.87%	1.23 †	207 ± 13 †	304 ± 21 †	376 ± 21 †
SHR	2.00%	1.28 §	254 ± 17 §	436 ± 68 §	536 ± 79 §

Female SHR showed similar increases in $[Ca^{2+}]_i$ (198 ± 13 + vs 159 ± 13 nM), associated with reduced 1,25(OH)₂D levels.

We conclude: (1) Resting $[Ca^{2+}]_i$ in proximal tubule is elevated in both young and adult SHR, regardless of gender. (2) Increased buffer Ca concentration accentuates and a high Ca diet further aggravates this cytosolic abnormality. (3) Coupled with the reduced serum ionized Ca, these data further implicate deranged membrane Ca regulation in the SHR. (4) Their reduced 1,25(OH)₂D levels, however, reflect appropriate physiologic down-regulation from elevated $[Ca^{2+}]_i$.

NA AND H₂O BALANCE IN EVOLVING CANINE NEONATALLY-INDUCED COARCTATION HYPERTENSION (NICH) DURING CHRONIC ENALAPRIL (CEI): RESPONSE TO LOW SODIUM DIET (LSD). S.P. Bagby & E. Fuchs. Depts. Med. & Surgery, VA Med. Ctr. & Ore. Health Sciences Univ., Portland, Oregon

During ad lib Na diet, chronic oral Enalapril (3mg/kg BID) fails to alter development of proximal systolic BP excess or impair the normal GFR in NICH as compared to CEI-treated littermates. To further test AII-dependence of SysBP and renal function, we superimposed LSD after 4 mo. in 4 groups randomized as neonates: Coarct/CEI, Coarct/Placebo (P), Control/CEI, Control/P (n=4 each). Serial measurements included forelimb SysBP, plasma creatinine (PCR), GFR (¹⁴C-Inulin disappearance), and daily Na and H₂O balances. During 12 days of LSD, Coarcted and Controls responded similarly to CEI: a) no change in SysBP; b) greater increase in PCR (p < .02) with trend (NS) toward greater fall in GFR; c) more negative sodium balance (p < .005) due to lower Na intake (NS) and higher (p < .06) UNaV; and d) lower Uosm (p < .01) with higher UV (p < .025) despite normal osmolar excretion. During LSD, Coarcted and Control dogs differed only for SysBP (p < .005) and fecal Na excretion (p < .02), both higher in Coarcted dogs. Data indicate that during LSD, the significant AII-dependence of GFR, renal Na conservation, and urinary concentrating capacity in Coarcted dogs is similar to that in littermate Controls. We conclude that during LSD, as reported for ad lib Na intake, AII does not appear to be essential to maintenance of SysBP excess and plays a normal role in regulation of renal function in canine NICH.

INCREASED FREE CYTOSOLIC CA²⁺ (iCa²⁺) IN LYMPHOCYTES FROM THE SPONTANEOUSLY HYPERTENSIVE RAT: A T CELL SELECTIVE PHENOMENON? DC Battle, G. Janss, M LaPointe, and J Llibre, Northw. Univ. and Lakeside VA Medical Center, Chicago, IL.

Ca²⁺ is reported to be increased in cells with contractile properties (e.g. platelets) from the SHR. To gain insight into the possible generalized nature of Ca²⁺-related alterations in this experimental model of genetic hypertension we measured lymphocyte iCa²⁺ in cells obtained from the spleen and the blood of SHR (n=24) with sustained hypertension (10-24 weeks of age) and in age-matched normotensive Wistar-Kyoto rats (WKY) (n=24). iCa²⁺ was estimated by monitoring Fura-2 fluorescence at two excitation wavelengths (340 and 380 nm). iCa²⁺ was significantly increased in SHR lymphocytes obtained from the blood (127±8.0 vs 91±6.0 nM, p<0.01) but not in those obtained from the spleen (74±7.1 and 76±6.9 nM). Because the population of spleen cells is heterogeneous (about 40% T cells and 60% B cells) while blood lymphocytes are predominantly T cells we reasoned that a rise in iCa²⁺ in SHR lymphocytes could be limited to T cells. To examine this possibility, iCa²⁺ was measured in SHR (n=13) and WKY (n=10) spleen cells which were subjected to selective B lymphocyte depletion using the nylon wool method. In this preparation of predominantly T cells (approx. 90%), iCa²⁺ was significantly higher in SHR than in the WKY rats (118±17 and 59±2.9 nM, respectively, p<0.01). The data suggest that T cells but not B cells have increased iCa²⁺ in the SHR. This observation may be related to existing evidence that a defect in the immune system plays a role in the pathogenesis of this model of genetic hypertension.

THERAPEUTIC ADVANTAGE OF DIETARY SODIUM RESTRICTION OVER DIURETIC THERAPY IN REDUCING GLOMERULAR INJURY IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). J.A. Benstein, H. D. Feiner, M. Parker, L. D. Dworkin. New York Univ. Med. Ctr., N.Y., N.Y.

Although chronic restriction of dietary sodium has long been a mainstay of antihypertensive therapy, its role in preventing end organ damage in hypertension has not been clearly defined. To examine the effect of sodium restriction on glomerular injury in hypertension, we studied groups of SHR, uninephrectomized (UNX) at 6 weeks of age, and given either standard, 2% Na chow (CON, n=8), 0.45% Na chow (LSC, n=8), or standard chow plus hydrochlorothiazide added to their water (HCTZ, n=8). Serial determination of awake systolic blood pressure by tail cuff technique revealed that severe hypertension developed in all 3 groups. Shown below are mean arterial pressure (AP), protein excretion rate (U_{PROT}V), inulin clearance (C_{IN}) and kidney weight (WT) measured at 42 weeks of age. Plasma renin activity (PRA) in anesthetized rats was determined by radioimmunoassay.

GROUP	AP mmHg	U _{PROT} V mg/24h	C _{IN} ml/min	WT g	PRA ng AI/h
CON	183±6	57±9	2.3±0.2	3.1±0.03	15.0±1.9
LSC	178±4	25±2 *†	2.3±0.2	2.6±0.1 *†	34.0±4.9 *
HCTZ	171±9	59±5	2.1±0.2	3.2±0.1	41.6±6.3 *

Mean±S.E.; * P<0.05 vs CON; † P<0.05 vs HCTZ

Although PRA's suggested that both salt restriction and diuretic caused a similar degree of sodium depletion, systemic hypertension was unabated in UNX SHR on either regimen. LSC rats had decreased renal size, less proteinuria and fewer sclerotic glomeruli (6±2%, P<0.05) than CON (13±4) or HCTZ (16±3). Thus, dietary sodium restriction lessens hypertensive nephrosclerosis in UNX SHR by a mechanism not dependent on reduction in systemic blood pressure or glomerular filtration rate and unrelated to PRA.

EFFECT OF CAPTOPRIL ON RENAL FUNCTION IN HYPERTENSIVE DOGS WITH UNILATERAL RENAL ARTERY STENOSIS, STUDIED WITH RADIONUCLIDE DYNAMIC SCINTIGRAPHY (RDS). Michael D. Bentley*, Stephen G. Ritter*, Manuel L. Brown*, Mary J. Fiksen-Olsen*, J. Carlos Romero and Prince K. Zachariah*. Depts. of Biophys. and Physiol., Hypertension, and Radiol., Mayo Clinic, Rochester, Minnesota.

The purpose of this study was to determine the suppression of renal function by captopril on dogs with renovascular hypertension. Five hypertensive dogs with experimental unilateral renal artery stenosis were examined by ^{99m}Tc-DTPA and ¹³¹I-Hippuran RDS, 10 minutes following the administration of intravenous bolus injections of 0.2, 0.5, and 1.0 mg/kg captopril. These doses reduced mean arterial pressure from a control value of 138 ± 12 to 105 ± 9, 106 ± 20, and 92 ± 5 mmHg, respectively, and increased plasma renin activity from 12 ± 3 to 52 ± 10, 66 ± 7, and 55 ± 12 ng/ml/hr, respectively. The time-activity curves of both ^{99m}Tc-DTPA and ¹³¹I-Hippuran RDS indicated that renal function in the stenotic kidney decreased progressively in relation to increasing doses of captopril. In addition, the function of the contralateral kidney was partially impaired with 1 mg/kg captopril. One hour after the administration of 0.5 mg/kg captopril, the function of the stenotic kidneys was partially restored and, by two hours, the RDS time-activity curves were comparable to the control scans. These data demonstrate a reversible, dose-dependent and time-limited suppression of stenotic kidney function by captopril in renovascular hypertension and provides support for the use of captopril in the diagnosis of renovascular hypertension by RDS.

EFFECTS OF ENALAPRIL (EN) AND HCTZ IN GENETICALLY HYPERTENSIVE DOGS. K.C. Bovée, M.P. Littman,* W.L. Chung,* S.G. Montor,* and M.J. Thoolen*, School of Veterinary Medicine, Univ. of Pennsylvania, Phila. PA and DuPont Co, Wilmington, DE.

We previously reported an inbred line of essential hypertensive dogs - Penn Hypertensive Dog (PHD). The purpose of this study was to determine antihypertensive and renal effects of EN, HCTZ and EN + HCTZ in 3 groups of PHD: hypertensive, borderline, and normotensive. Mean direct arterial diastolic BP in these groups was 104, 95, and 88 mm Hg, respectively. No differences in GFR, ERPF, Na⁺ reab., K⁺ reab. and PRA were found between 3 groups. Dogs were given EN (3mg/kg BID PO), HCTZ (5mg/kg BID PO), or EN + HCTZ daily for 1 week periods interrupted by control weeks. EN + HCTZ significantly reduced BP by 13-26 mm Hg in all 3 groups, while EN or HCTZ alone were ineffective. During EN and EN + HCTZ the pressor effect of AI (1ug/kg IV) was effectively blocked. EN caused increased ERPF in all dogs; no differences between groups were found. HCTZ caused decreased K⁺ reab. in all dogs with no difference between groups. EN + HCTZ caused increased ERPF, significantly decreased Na⁺ reab. and ameliorated K⁺ losing effect of HCTZ in all dogs. EN + HCTZ caused a significant increase in GFR in the hypertensive group only. Thus, elevated pressure in these dogs cannot be attributed to increased formation of AII. This hypertensive model differs from others by its resistance to converting enzyme inhibition.

Na-SENSITIVE BLOOD PRESSURE, FAMILY HISTORY OF HYPERTENSION AND RED CELL Na TRANSPORT IN YOUNG NORMOTENSIVE ADULTS. M. Canessa, C. Laski and B. Falkner. Dept. Medicine, Brigham and Women's Hospital, HMS, Boston, Ma and Dept. Pediatrics Hahneman Univ., Philadelphia, Penn.

The effect of chronic (14 days) oral Na loading was studied in a representative sample of young normotensive whites (RWS, n=24), young normotensive blacks (RBS, n=35) and black borderline hypertensives (BBH, n=22). Red cell Na-K-Cl cotransport (Km and Vmax) and Na pump (Vmax) were studied prior to Na loading. Na-sensitive (SS) blood pressure was an increase in mean arterial pressure higher than 5 mm Hg and Na insensitive lower than 5 mm Hg after Na loading. In RWS, 46% had family history of hypertension (FH+) and 16% were SS. In RBS, 69% had FH+ and 49% were SS. In the BBH, 86% had FH+ and 14% were SS.

RWS had significantly lower cell Na content, Km for Na cotransport and higher Na pump than RBS. SS subjects in RWS, RBS and BBH had lower activity of Na-K-Cl cotransport (Vmax or Km) than Na insensitive subjects. SS subjects of the BBH group also showed higher cell Na and lower activity of the Na pump than salt insensitives.

The results indicate that a reduced activity of Na cotransport is associated with SS blood pressure in the 3 groups, while a reduced activity of the Na pump and cotransport is found when the blood pressure is borderline.

GENETIC SUSCEPTIBILITY TO CYCLOSPORIN (CS)-INDUCED HYPERTENSION (HTN): RELATION TO ADRENAL RESISTANCE TO ANGIOGENSIN (AII). D.W.S. Chan*, S. Lustig*, N. Stern*, P. Eggena* and D.B.N. Lee, VA Medical Center, Sepulveda and UCLA School of Medicine, Los Angeles, California.

Relative adrenal resistance to AII has been observed in spontaneously hypertensive rats (SHR) and in some patients with essential HTN. We have demonstrated that CS induces a similar abnormality and accelerates the HTN in SHR. We now study the effect of 2 wk intragastric CS (10-20 mg/kg/d) on BP and adrenal response to AII in SHR, Wistar-Kyoto rats (WKY) and the normotensive Sprague Dawley rats (SD). Control rats (C) received vehicle (olive oil) only. CS-treatment (CSRx) caused elevation in plasma renin concentration with no parallel increment in aldosterone (ALD). In vitro AII-stimulated ALD secretion by zona glomerulosa cells from all CSRx rats was reduced.

Treatment [n]	BP-Initial (I)	BP-Final (F)
SHR-C [12]	163±4 ^{a,b}	164±3
SHR-CSRx [12]	160±3 ^{a,b}	194±5 ^{c,d}
WKY-C [8]	130±4 ^a	131±4
WKY-CSRx [8]	130±4 ^a	148±4 ^{c,d}
SD-C [12]	117±5	123±5
SD-CSRx [10]	113±4	128±5 ^c

a: vs SD BP-I, P<0.01; b: vs WKY BP-I, P<0.01; c: vs respective BP-I, P<0.01; d: vs C BP-F, P<0.01. Final BP (BP-F), vs initial BP (BP-I), was not different in all 3 C groups, but increased in all CSRx groups: markedly in SHR, moderately in WKY and least in SD. BP-F was also higher in CSRx (vs respective C) in SHR and WKY but not in SD. Thus, both adrenal resistance to AII and genetic susceptibility to HTN may be important in CS-HTN.

LEAD (Pb) INCREASES RED BLOOD CELL (RBC) Na/Li COUNTERTRANSPORT (CTT). A. Dreisbach,* E. Chun,* M. Naumoff,* V. Batuman. VA Med. Ctr., E. Orange NJ and UMDNJ-NJ Med. School, Newark, NJ.

The mechanisms of lead-induced hypertension are not well understood. RBC Na/Li countertransport is believed to be a marker of a similar transport system in the kidney and has been found increased in essential hypertension. To determine if changes in salt transport are involved in lead-induced hypertension, we studied the effect of 20, 5, 3 and 1 μM of in vitro Pb on RBC Na/Li countertransport. RBCs were obtained from 19 healthy donors on no medications (aged 19 to 41; 14 male, 5 female), and loaded with Li to 4.53 ± 0.17 mM (mean ± SEM) intracellular concentration. Na/Li countertransport is defined as the difference between Li efflux into Na containing and Mg containing media in presence of 0.1 mM Ouabain at 37°C. All measurements are made in duplicate. Results are summarized in the table. Differences are analyzed by paired t testing.

Pb (μM)	N	Na/Li CTT (mmol Li/liter RBC x hr ⁻¹)		
		No Pb	With Pb	P
1.0	7	0.250±0.03	0.261±0.03	0.06
3.0	5	0.269±0.04	0.293±0.03	<0.05
5.0	5	0.270±0.06	0.301±0.07	<0.02
20.0	2	0.336±0.14	0.579±0.02	-
All Pb	19	0.270±0.02	0.312±0.03	<0.01

Pb concentration in medium correlated positively with Na/Li countertransport (R = 0.99, P<0.001). These data reveal that Pb enhances Na/Li countertransport, previously shown only in genetically transmitted hypertension. Increased renal transport of sodium may be an important mechanism in lead-induced hypertension.

CHANGES IN CALCITRIOL STATUS AND RELATED PARAMETERS IN THE YOUNG HYPERTENSIVE RAT (SHR). T. Drücke,* PA Lucas,* P Bourgoïn,* A Pointillart,* J Merke,* M Garabédian,* M Thomasset,* B Lacour,* E Ritz,* DA McCarron,INSERM U90 and 120; CRNS UA-583; INRA; Paris, FR, Dept Int Med, Heidelberg, FRG & Div Nephrology, Portland, OR.

Reported data on calcitriol status and calcium (Ca) and phosphate (P) metabolism of the young SHR are conflicting. To address this issue, we have measured the following parameters at both 5 and 12 weeks of age in male SHR and normotensive controls (WKY): serum calcitriol (s1,25; pg/ml); specific binding of tissue calcitriol receptors (r1,25; Bmax, fmol/mg protein); Ca and P balance (bCa, bP: mg/day); intestinal vit D dependent CaBP (9K). Results as means \pm SEM, n=5-10 rats:

		s1.25	r1.25	bCa	bP
5wk	SHR	75.1 \pm 5.7	155 \pm 1.9	62.3 \pm 2.6	31.2 \pm 0.6
	WKY	32.9 \pm 5.8	130 \pm 4.6	57.7 \pm 2.4	25.7 \pm 1.1
	P	<0.001	ns	ns	<0.01
12wk	SHR	38.0 \pm 6.2	172 \pm 4.9	53.2 \pm 4.6	5.8 \pm 1.3
	WKY	29.3 \pm 6.3	123 \pm 6.6	67.9 \pm 2.7	13.2 \pm 1.7
	P	ns	<0.01	ns	<0.05

Thus, s1,25 in the SHR (vs WKY) evolved from increased to similar values whereas r1,25 changed in the opposite direction. Similarly, there was reversal of initially raised bP, and bCa tended to follow the same pattern. CaBP of 4 intestinal segments was lower in 12-wk old SHR than WKY (studies in 4-wk old rats in progress). Initially significantly lower urinary Ca and P excretion in the SHR tended to be higher at 12 wks of age. In conclusion, apparently contradictory reports as to calcium and phosphorus metabolism in the young SHR may be explained and related parameters during its early development.

INCREMENT IN EFFECTIVE RENAL PLASMA FLOW PREDICTS RESPONSE TO CAPTOPRIL IN ESSENTIAL HYPERTENSION. M. Eldadah*, Saleh H. Abu-Romeh* (intr. by Richard L. Tannen) University of Kuwait, Faculty of Medicine, Kuwait.

It has been shown that monotherapy with Captopril (C) increases effective renal plasma flow (ERPF) and lowers blood pressure (BP) in about two-thirds of patients with essential hypertension (EH). Two-week course of C, 50 mg twice daily was given to 11 male patients with EH (mean age, 40 y) of short duration (8 mo) while ingesting normal salt (157 \pm 72 mEq), to ascertain whether correlation exists between BP control and increment in ERPF after C. ERPF was determined by a single injection of 131 I O-I-hippurate utilizing a computerized program (Schlegel Method). Baseline ERPF (450 \pm 76) increased to 526 \pm 40 ml/min/1.73 m² during C while GFR did not change significantly. BP was normalized in 8 patients whose basal ERPF were low (75% of expected or lower) and which significantly increased (in every patient) during C. On contrary the other 3 patients, whose BP did not respond to C, manifested fairly normal ERPF which failed to increase any more during C therapy.

It is concluded that ERPF determination is a clinically valuable test in predicting the response of EH patients to monotherapy with C.

VASOPRESSIN AND POSTURAL BLOOD PRESSURE CHANGES IN HYPERTENSION. Fernando Elijovich,* Simon Neubort,* and Guy Valliquette,* (intr. by Robert Saffirstein). Mount Sinai School of Medicine, New York, NY and Helen Hayes Hospital, W. Haverstraw, NY.

Plasma vasopressin (VP) was measured in 45 fasting hypertensive patients after 4 weeks of therapy and 30 min sitting. VP levels were distributed bimodally without overlap. Means (\pm SD) were 10.3 \pm 2.8 (L0, n=32) and 23.2 \pm 8.3 (H1, n=13) pg/ml. Sex distribution and the presence of diabetes and retinopathy were similar in H1 and L0 groups. More Blacks and smokers were found in the L0 group, but this was not significant. Only 2 patients had LVH by EKG; both were in the H1 group.

Upright diastolic (UDBP) and mean (UMBP) blood pressures were higher in H1 than in L0 (mean \pm SE: 108 \pm 2 vs 103 \pm 1, p<0.02 and 130 \pm 3 vs 121 \pm 1, p<0.02, respectively). Upright systolic and all sitting blood pressures were not different between groups. Orthostatic rise in diastolic BP (ORTH) was greater in H1 than in L0 (6 \pm 2 vs 3 \pm 1) but this did not reach significance (p=0.11).

VP correlated with UMBP (r=0.31, p<0.02) and ORTH (r=0.31, p<0.05) but not with serum Na, K, Osm, urinary Na excretion, or PRA. A borderline correlation was found between VP and age (r=-0.26, 0.1>p>0.05).

Our data suggest that circulating VP may play a role in regulating postural blood pressure changes in hypertensives. Whether VP-mediated orthostatic rises in blood pressure bear any relationship to the development of LVH remains to be elucidated.

NIFEDIPINE PREVENTS PRESSURE-INDUCED AFFERENT ARTERIOLAR VASOCONSTRICTION IN ISOLATED PERFUSED HYDRONEPHROTIC KIDNEYS FROM HYPERTENSIVE RATS (SHR). Murray Epstein, Koichi Hayashi*, Rodger Loutzenhiser*, V.A. Med. Ctr. & Univ. of Miami, Sch. Med., Miami, FL, 33125

The renal hemodynamic response to calcium antagonists may be exaggerated in hypertension as a consequence of an increased renal vascular resistance (RVR). Many factors contribute to RVR in this setting, including a direct renal vascular response to increased renal perfusion pressure. We utilized the isolated perfused hydronephrotic rat kidney to assess directly the possible role of renal arterial pressure (RAP) as a mediator of afferent arteriolar tone in the hypertensive kidney. Chronically hydronephrotic kidneys were excised from SHR's, and perfused with artificial medium. Afferent arteriolar diameter (AAD) was measured directly using video-microscopy as RAP was increased from 80 to 180 mm Hg. The maximal decrease in AAD of 21 \pm 3% was observed at 160 mm Hg, a value that was well below the range of blood pressure in these animals (181-212, mean 194 \pm 6 mm Hg, tail cuff method). Nifedipine (NIF) (1 micromolar) completely abolished the pressure-induced afferent vasoconstriction.

RAP mm Hg	80	100	120	140	160	180
AAD (μ m)	20 \pm 2	20 \pm 1	19 \pm 1	17 \pm 1*	16 \pm 1*	16 \pm 1*
AAD-NIF	19 \pm 1	19 \pm 1	19 \pm 1	18 \pm 1	18 \pm 1	19 \pm 1

(mean \pm SE, n=5, * p less than 0.05 vs 100 mm Hg) Thus, perfusion pressure directly stimulates a contractile response in the afferent arteriole that is inhibited by NIF. We conclude that an inhibition of pressure induced afferent arteriolar vasoconstriction may contribute to the salutary renal hemodynamic effects of calcium antagonists in hypertension.

ADRENERGIC RECEPTORS (AR) AND VASCULAR RESPONSIVENESS IN CHRONIC RENAL FAILURE (CRF) George Fadda,* Shaul G. Massry, Mahmoud El-Rafai* and Vito M. Campese, Div. Nephrol. Univ. So. Calif, Los Angeles, California.

Patients and rats with CRF have reduced pressor response to norepinephrine (NE). This is related to the high levels of parathyroid hormone (PTH) which stimulates vasodilator prostaglandins production. To determine whether CRF also affects pressor response to NE through changes in α_1 AR or in plasma NE, and whether excess PTH affects these parameters, we have measured plasma NE and the number of binding sites (B_{max}) and the K_D of α_1 AR in isolated mesenteric arteries of CRF, normocalcemic CRF-PTX and control (C) rats. Plasma NE was greater ($p < 0.01$) in CRF than in C (67 ± 14 vs 32 ± 3.1 ng/dl), but it was not different from CRF-PTX rats. The B_{max} of α_1 AR was lower ($p < 0.05$) in CRF than C (80 ± 10 vs 173 ± 29 fm/mg protein), but it was similar in CRF and CRF-PTX rats. The K_D in CRF was higher than in C (1125 ± 150 vs 666 ± 110) but was similar to CRF-PTX rats. The data show that 1) number and affinity of α_1 AR are reduced in CRF and this is not related to excess PTH. 2) this abnormality in α_1 AR may contribute to the reduced pressor response to NE in CRF and 3) the effect of PTH, on the vascular response to NE is not related to changes in plasma levels of NE or in binding sites for NE.

PRESSOR EFFECTS ON GLOMERULAR BASEMENT MEMBRANE. Falkner, B, Katz, SM, Critz, C*. Hahnemann University, Dept. of Pathology, Philadelphia, PA.

The Spontaneously Hypertensive Rat (SHR) was used to examine the early morphologic development of glomerular hypertensive abnormalities. Kidneys of male SHR were compared to male Wistar-Kyoto (WKY) control rats at 8, 15 and 52 weeks of age. Blood pressure, obtained by carotid cannulation, demonstrated development of hypertension in SHR by 15 weeks. By light microscopy, WKY showed no major alterations with increasing age. At 8 weeks, SHR was histologically identical to WKY. By 15 weeks, SHR showed segmental thickening of glomerular basement membrane (GBM), trace increase in mesangial cellularity, but no vascular lesions. The vessels and glomeruli of WKY in all age groups showed no immunofluorescence, but SHR exhibited a progressive increase in glomerular staining for fibrinogen. Superficial cortical and juxta-medullary glomeruli, sampled separately, were undistinguishable ultrastructurally. By electron microscopy, SHR glomeruli at 8 weeks were unremarkable compared to WKY. At 15 weeks, SHR showed effacement of foot processes plus segments of thick, split and multilayered GBM, changes not seen in WKY at 15 or 52 weeks. The SHR GBM (subendothelial) and the expanded mesangial matrix contained loose, fibrillar to coarsely granular material. At 52 weeks, SHR exhibited increasing severity of glomerular lesions seen at 15 weeks. The data indicate that hypertensive structural changes occur in the glomerular capillaries. Since these changes, consequent to the elevated systemic pressure, precede classic arteriolar smooth muscle hypertrophy, they reflect pressure-mediated glomerular injury.

INFLUENCE OF ENALAPRIL ON PROTEINURIA IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Leonard G. Feld, James E. Springate*, Judith B. Van Liew, Depts Pediatr and Physiol., Univ at Buffalo, Children's Hospital, V.A. Med Ctr, Buffalo, N.Y.

We have previously reported that a low protein diet without or in combination with triple antihypertensive drug therapy (chlorothiazide, reserpine, hydralazine) does not prevent renal injury in the SHR. The present study examined the influence of enalapril (E) on urinary protein excretion in SHR followed to 37 weeks of age. Treated SHR (N=16) were placed on a 24% protein diet with E (50mg/L) added to the drinking water at 15 weeks of age. Untreated SHR (N=7) and Wistar-Kyoto (WKY) (N=8) served as age-matched control groups. Enalapril lowered blood pressure from 160 ± 2 to 106 ± 4 mmHg. At 37 weeks, urinary protein excretion (mg/24 hr x 100g BW) in treated SHR was 7.8 ± 0.6 compared to 12.6 ± 0.9 ($p < 0.01$) in untreated SHR and 3.3 ± 0.2 ($p < 0.01$) in WKY. Proteinuria in the E treated SHR is similar to the excretion rate reported with triple drug therapy (8.8 ± 0.2). In addition, urinary albumin excretion in E treated SHR significantly ($p < 0.01$) increased from 0.34 ± 0.04 at 15 to 0.63 ± 0.10 at 37 weeks of age.

We conclude that enalapril does not provide any advantage over triple antihypertensive drug therapy in retarding the development of proteinuria in the SHR through 37 weeks of age.

EFFECTS OF BLOOD IONIZED CALCIUM ON SYSTEMIC ARTERIAL PRESSURE. Susan K. Fellner, Roberto M. Lang*, and David A. Bushinsky, University of Chicago, Chicago, IL.

How variations in blood ionized calcium (Ca^{2+}) affect blood pressure independent of changes in extracellular fluid volume and in other biochemical variables is unknown. To address this issue, we dialyzed 10 stable hemodialysis patients with baths differing only in calcium concentration on 3 occasions chosen in random order in 1 week. Ultrafiltration was adjusted to achieve the patient's estimated dry weight. Post dialysis we measured Ca^{2+} , arterial blood gases, electrolytes, calcium, phosphorus, BUN, creatinine, albumin and hematocrit. After patients rested supine for 15 minutes, at least 10 measurements of blood pressure and pulse were recorded with a Dinamap 1946P monitor.

Post dialysis 3 different levels of Ca^{2+} were achieved, but there was no change in any other measured biochemical variable, weight or heart rate. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were different at each level of Ca^{2+} .

Bath Calcium	Ca^{2+} (mM)	SBP (mmHg)	DBP (mmHg)
Low	$1.03 \pm .03$	116 ± 5	73 ± 4
Medium	$1.37 \pm .03^*$	$134 \pm 4^*$	$80 \pm 3^*$
High	$1.67 \pm .07^{**}$	$146 \pm 4^{**}$	$88 \pm 4^{**}$

+, $p < 0.001$ vs. low calcium; **, $p < 0.001$ vs. medium calcium; values are mean \pm S.E.

We conclude that systemic arterial blood pressure varies directly with blood ionized calcium independent of changes in blood pH or electrolytes.

ANGIOTENSIN CONVERTING ENZYME INHIBITOR (CEI) SUPPRESSES ACCELERATED GROWTH OF GLOMERULAR CELLS IN VIVO AND IN VITRO. A. Fogo*, Y. Yoshida*, I. Ichikawa, R. Hoover* and T. Homma*. Depts. of Pediatrics & Pathology, Vanderbilt University, Nashville, TN.

Progressive deterioration of glomerular structure and/or function secondary to diabetes or subtotal renal ablation has been shown to be ameliorated by several antihypertensive drugs, including CEI, verapamil (VPL) and hydralazine (HLZ). Common to these conditions is the marked glomerular hypertrophy preceding sclerosis. To determine if these drugs have a similar protective effect in other conditions characterized by accelerated glomerular growth, we studied maturation in 4-5 week old rats with (n=19) or without (n=19) unilateral nephrectomy (NPX). Non-NPX rats given CEI (enalapril, 50 mg/L drinking water for 5-6 weeks) had average maximum planar area (PAm_{max}) of glomeruli determined on serial sections 14% less than age-matched non-NPX controls (p<.001). PAm_{max} was 23% less in NPX-CEI rats vs NPX controls (p<.001). VPL (50 mg/L) or HLZ (80 mg/L) did not affect PAm_{max} in NPX or non-NPX rats. Increases in dry kidney weights after NPX and NPX-CEI were 28% and 16%, respectively. There was no significant effect with VPL or HLZ. The body weight of the rats was not affected by any of the drugs. Glomerular capillary pressure (PGC) was essentially the same in NPX vs NPX-CEI (55 mmHg vs 52) and non-NPX vs non-NPX-CEI (49 mmHg vs 49). The effect of CEI, VPL, HLZ (dose ranges 10⁻⁴, 10⁻⁵ and 10⁻⁶M for all drugs, in RPMI with 15% serum) on in vitro glomerular cell growth was then examined using cultured rat mesangial cells. Initial maximum cell counts on day 1-3 for CEI, VPL and HLZ were 163%, 155% and 152% of untreated control, respectively. At day 6, all 3 drugs resulted in marked inhibition of growth (CEI 54%, VPL 64%, HLZ 54% of untreated control at 10⁻⁶M). These studies demonstrate that CEI, VPL and HLZ have variable potentials to suppress the growth of glomerular cells in vivo and in vitro through mechanisms independent of glomerular hemodynamics. This suppressive effect on glomerular hypertrophy and/or mesangial cell growth may be crucial in the drugs' ability to diminish subsequent sclerosis.

A PROSPECTIVE EVALUATION OF THE CAPTOPRIL TEST FOR THE DETECTION OF RENOVASCULAR HYPERTENSION. ED Frederickson,* M Buccì,* N Loon,* JC Peterson, R Thompson,* CS Wingo, and CS Wilcox. Division of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville, Florida.

Renovascular hypertension (RVH) is curable but rare and, therefore, requires a sensitive screening test. A potentiated increase of plasma renin activity (PRA; ng/ml/hr) following oral captopril (50 mg) has been proposed as such a test. (AJM 80:633, 1986). We evaluated this test prospectively in 100 patients, 71 with essential hypertension (EH) and 29 with RVH. We determined the maximum sensitivity (sens), specificity (spec), positive and negative predictive values (+PV, -PV) of 3 criteria, separately and combined: a stimulated PRA of 5.7, an absolute increase of 4.7, and a percent increase of 150, or 400 if the initial PRA was less than 1.4. Values in () refer to the 62 patients not taking diuretics.

	Sens (%)	Spec (%)	+PV (%)	-PV (%)
Stim PRA	100(100)	79(86)	66(75)	100(100)
Abs Δ PRA	93(94)	87(95)	71(89)	97(98)
% Δ PRA	72(78)	86(93)	68(82)	88(91)
Combined	72(78)	87(95)	70(88)	89(91)

PRA increased from 8.7±1.1 to 30±4.6 (p<0.001) in RVH, but only from 2.1±0.3 to 4.9±1.2 (p<0.01) in EH. Changes in BP were not predictive of RVH. Conclusions: 1) This prospective analysis confirms the utility of the Captopril Test for detecting RVH; 2) diuretics decrease the specificity of the test; 3) the test can be simplified using a single stimulated PRA≥5.7, which correctly identified all 29 patients with RVH and predicted improved BP control with angioplasty or surgery in 17 of 19 attempts.

GLUCOSE INTOLERANCE AND SPONTANEOUS HYPERTENSION. Cynthia Gaboury, Njeri Karanja, David McCarron. Oregon Hlth. Sci. Univ., Portland, OR.

Preliminary reports suggest that spontaneously hypertensive rats (SHR) exhibit glucose (G) intolerance. Whether the intolerance is due to impaired insulin (I) synthesis and production or impaired peripheral glucose utilization is unknown. Twenty SHR and 20 Wistar Kyoto (WKY) controls were challenged with an IV G load (IVGTT) of 0.125 mg/100g body wt. Plasma G and I were measured at 0, 15, 30, 60, 120 and 240 mins. To assess in vivo insulin stimulated peripheral glucose uptake, a pancreatic suppression test (PST) was performed by infusing exogenous G (8mg/kg/min) I (2.5-4 μg/kg/min) and somatostatin (1ng/kg/min) for 3 hours. Steady state G (SSG) and I (SSI) were determined at 0, 140, 150 and 160 min. IVGTT Results:-

	0	15	30	60	120	240
G(mg/dl)	140±12	417±78	243±73	169±35	156±27	154±20
I(ml)	41±28	146±66	78±25	46±17	42±15	40±11
WKY						
G	141±16	546±65	439±53	341±49	272±51	144±35
I	20±9	48±24	50±20	54±28	62±36	34±10

The SHR had relative fasting hyperinsulinemia and a marked insulin response at 15 and 30 min. The WKY exhibited a blunted I response to IV G as shown by higher G values at 15, 30, 60 and 120 min P<0.05. Despite a PST SSG value 30% higher (143±20 vs 109±25; P<0.001) in the SHR, SSI was 7% lower (103±14 vs 111±13; P<0.03) in SHRs, indicating insulin resistance. We conclude the SHR has reduced peripheral insulin sensitivity. The SHR may be a model of both spontaneous hypertension and type II diabetes reflecting the common clinical association between these two disorders.

REGULATION OF NA BALANCE IN NOREPINEPHRINE (NE) HYPERTENSION: ROLE OF PRESSURE NATRIURESIS.

J.E. Hall, H.L. Mizelle*, L.L. Woods, and J.-P. Montani*. Univ. Miss. Med. Ctr., Jackson, Miss.

The goal of this study was to quantitate changes in mean arterial pressure (MAP) and renal function during chronic increases in plasma NE, and the role of the pressure-natriuresis mechanism in controlling NA balance in NE hypertension. In 6 conscious dogs in which renal artery pressure (RAP) was allowed to increase during chronic NE infusion (0.2 μg/kg/min), NA excretion (U_{Na}V) rose from 71±2 to 112±14 mEq/day and MAP increased from 99±3 to 109±3 mmHg on the first day. On days 2-7, U_{Na}V returned toward control while MAP averaged 108±2 mmHg. GFR and effective renal plasma flow (ERPF) did not change, averaging 85.9±4.0 and 235±17 ml/min, respectively, during 7 days of NE, comparing to controls of 84.1±3.9 and 252±20 ml/min. When RAP was servo-controlled for 7 days during NE infusion, the transient natriuresis was abolished; U_{Na}V averaged 72±5 during control, 77±13 during the first day of NE, and 65±4 mEq/day during 7 days of NE. GFR and ERPF did not change significantly during NE infusion with RAP held constant. MAP did not plateau but continued to rise from 102±3 to 137±3 mmHg after 7 days of NE. When servo-control of RAP was stopped while NE infusion was continued for 7 days, U_{Na}V increased to 120±13 mEq/day and MAP decreased to 116-125 mmHg. These data indicate that in normal dogs, chronic increases in plasma NE produce mild hypertension associated with sodium loss due to pressure natriuresis. However, when pressure natriuresis is impaired by servo-controlling RAP, the hypertensive effects of NE are much more severe.

CEREBROSPINAL FLUID (CSF) AND CSF Ca^{2+} IN SPONTANEOUSLY HYPERTENSIVE RAT (SHR): INFLUENCE OF DIETARY CALCIUM. Daniel Hatton, Jean-Baptiste Rouillet, Chris McCormick, Chantal M. Rouillet, David A. McCarron, Oregon Health Sciences University, Portland, Oregon.

CSF Ca^{2+} is postulated to be a central regulator of blood pressure and may counteract the pressor effect of increased CSF Na^+ . CSF Ca^{2+} is regulated by the choroid plexus (CP) cells, whose electrolyte transporting properties parallel those of duodenal enterocytes, a cell line of the SHR in which decrease Ca^{2+} transport has been identified. We sought to compare a) CSF Ca^{2+} (CaCSF) and CSF volume in SHR and WKY, and b) the effect of a 2% calcium diet to a 1% calcium diet on CaCSF and volume. We fed 5-week-old male WKY and SHR either 1% or 2% calcium diet for 13 weeks. CSF was collected by cisterna magna puncture within 1 min of exsanguination. The mean \pm SEM collected volume (V_{CSF}) and CaCSF were:

	WKY _{1%} n=12	WKY _{2%} n=8	SHR _{1%} n=7	SHR _{2%} n=8
BW gm	324 \pm 7.0	317 \pm 8	299 \pm 8	283 \pm 3
V_{CSF} μ l	101.4 \pm 4.7	97.9 \pm 7.2	133.8 \pm 10.7	127.9 \pm 5.6
CaCSF mg/L	56.9 \pm 0.6	56.1 \pm 0.4	59.6 \pm 0.5	59.5 \pm 2.5

ANOVA showed that SHR had lower BW than WKY ($p < .01$), higher collected volume V_{CSF} ($p < .001$), and increased CaCSF ($p < .001$). There was no significant effect of 2% Ca^{2+} diet on these parameters. We conclude that CSF Ca^{2+} homeostasis of SHR is abnormal and that the production and/or reabsorption of CSF appears to be altered as well. These findings suggest another cellular site of abnormal electrolyte and fluid transport in the SHR.

RACIAL DIFFERENCES IN FIBROBLAST Na/H ANTIPORT. Norio Hatori,* Hauro Tomonari,* Burton P. Fine,* Abraham Aviv, NJ Medical School, Newark, NJ

To explore the predisposition of blacks to essential hypertension we characterized the Na/H antiport in serially passed skin fibroblasts from 15 normotensive blacks and 15 normotensive whites. The antiport activity was measured in quiescent cells acidified with nigericin to intracellular pH (pHi) of 6.2-6.6. Using BCECF, change (Δ) in the pHi was monitored for 30 sec after activation of the antiport, while the Δ pHi was linear. The antiport activity was higher in blacks than whites at all levels of acidification ($p < 0.05$ for pHi of 6.5 and 6.4 and $p < 0.01$ for 6.3 and 6.2). Kinetics of the antiport activation by extracellular Na (at pHi 6.3) showed that the V_{max} values (mean \pm SEM) were 0.330 ± 0.025 and 0.233 ± 0.011 pH units/30 sec for blacks vs whites, respectively ($p < 0.02$). There were no significant differences in the basal pHi (7.33 ± 0.04 and 7.29 ± 0.03), K_m (47.6 ± 6.81 and 33.5 ± 2.74 mM), and cellular buffer capacity (13.5 ± 1.6 and 15.0 ± 1.8 $\text{mmol} \cdot \text{l}^{-1} \cdot \text{pH}^{-1}$) for blacks and whites, respectively. The higher activity of the antiport in blacks was also confirmed without cellular acidification by its activation with human serum ($p < 0.01$). Assuming that findings in skin fibroblasts reflect a generalized phenomenon, higher activity of the Na/H antiport in the proximal tubule may explain the predisposition of blacks to salt sensitive essential hypertension. Inasmuch as our observations were made in serially passed cells, they indicate racial differences in the genetic makeup which controls the expression of Na/H antiport activity.

THE ANTIHYPERTENSIVE EFFECT OF β -BLOCKADE AFTER RENAL TRANSPLANTATION. F. Huysmans* F.v.Heusden*, A. Hoitsma*, J. Wetzels* and R. Koene. Univ. Hospital, Dept. of Nephrol. Nijmegen, The Netherlands.

We previously demonstrated that host kidneys (HK), especially if glomerulonephritis (GN) is the original renal disease (ORD), may contribute considerably to the prevalence of hypertension (HT) in renal transplant (TP) recipients. The role of renin in HT after TP is much more prominent in patients with than in those without HK. Since β -blockers generally lower blood pressure (BP) more in patients with a high PRA, we retrospectively studied the antihypertensive effect of β -blockade in TP recipients with and without HK. Patients with TP artery stenosis or unstable renal function were excluded. Thus, 39 patients could be studied. None of 10 hypertensive patients without HK showed a decrease of mean arterial pressure (MAP) of over 10%, BP being $165 \pm 6/108 \pm 3$ before and $165 \pm 7/105 \pm 3$ mmHg during β -blockade (NS, Δ MAP -1.7%), whereas BP decreased by $14.9 \pm 1.3\%$ from $161 \pm 3/104 \pm 1$ to $138 \pm 3/88 \pm 2$ mmHg in 19 patients with HK in situ and GN as ORD ($p < 0.001$). In 10 patients with HK and interstitial nephritis as ORD, decrease of BP on β -blockade (Δ MAP-6.8%, $p < 0.005$) differed significantly from that in the 2 other patient groups. Doses of β -blocker, pretreatment BP, endogenous creatinine clearance, and age were similar in the 3 patient groups. The data suggest that β -blockade effectively reduces BP in TP recipients only if HK are the source of HT. This is possibly related to differences in plasma renin activity or in innervation of the HK and the graft. Further investigation of this phenomenon may clarify the exact mechanism of the antihypertensive action of β -blockade.

URINARY DIVALENT CATION EXCRETION IN EXPERIMENTAL HYPERTENSION. Elizabeth Israel*, R. Ernest Sosa*, Joseph M. Gertner*, John H. Laragh, and Lawrence M. Resnick*. Cardiovascular Center, Cornell University Medical Center, New York, New York.

To investigate renal handling of calcium and magnesium in hypertension, we measured urinary sodium (UNaV), calcium (UCaV), magnesium (UMgV), creatinine (UcrV), and serum 1,25 dihydroxyvitamin D (1,25 D) levels in 2-kidney, 1-clip (2K-1C), sham-operated (S-op), DOC-saline (DS) and saline-only (UNx-NaCl) rat models ($n=10$ for each model) on both high (1.8%, HiCa) and Lo (0.2%, LoCa) calcium diets.

UCaV, but not UMgV was increased in each hypertensive rat model on both LoCa and HiCa, relative to either UNaV ($p < 0.001$ for DS vs UNx-NaCl and 2K-1C vs S-op, LoCa, $p < 0.001$ DS vs UNx-NaCl, HiCa) and/or UcrV ($p < 0.01$ DS vs UNx-NaCl, LoCa, $p < 0.005$ DS vs UNx-NaCl and 2K-1C vs S-op, HiCa). On HiCa vs LoCa, UCaV increased in each species, while UMgV reciprocally decreased ($p < 0.01$) only in normotensive rats. UCaV and UMgV were significantly correlated only in normotensive animals ($r(\text{UNx-NaCl})=0.73$, $p < 0.01$; $r(\text{S-op})=0.76$, $p < 0.01$). Similarly, the 1,25 D response to Lo vs HiCa was blunted in each hypertensive animal ($p < 0.05$).

We conclude 1) enhanced calcium excretion is characteristic of renin and sodium-volume dependent 2^o hypertension, not just of 1^o, genetic hypertension, and 2) hypertension dissociates renal calcium and magnesium excretion. We hypothesize that at least some of these changes are mediated by inadequate responsiveness of 1,25 D to alterations in dietary mineral intake.

PARATHYROID HORMONE (PTH) INHIBITS Na-H EXCHANGE IN CULTURED VASCULAR SMOOTH MUSCLE CELLS. A.M. Kahn, R.A. Zimmer*, and S.S. Navran*. Univ. of Texas Medical School and Baylor College of Med., Houston, Texas.

Intravenous PTH acutely lowers blood pressure, and PTH relaxes vascular smooth muscle in-vitro. The mechanisms of these effects are unknown. Since PTH inhibits Na-H exchange in proximal tubular cells, and agonist-stimulated Na-H exchange activity in vascular smooth muscle has been linked to contraction, we wished to determine whether PTH inhibits Na-H exchange in vascular smooth muscle. Primary cultures of vascular smooth muscle cells from canine femoral artery were grown to confluence. The 1 min uptake of 10 mM ^{22}Na was 2.5 pmol/ug protein. When the cells were acidified inside by preincubating them with the K-H exchanger, nigericin, ^{22}Na uptake increased to 5.2 pmol/ug protein. The nigericin-stimulated component of Na uptake was inhibited 90% by 1 mM amiloride, an inhibitor of Na-H exchange. When cells were preincubated for 1 hour with $5 \times 10^{-8}\text{M}$ PTH, the nigericin-stimulated component Na uptake was inhibited 50%. 10^{-6}M dibutyryl cyclic AMP also inhibited Na uptake. It is concluded that cultured vascular smooth muscle cells from canine femoral artery contain a Na-H exchanger, and that PTH, and its second messenger, cyclic AMP, inhibit Na-H exchange in these cells. Inhibition of Na-H exchange may account for the vasorelaxant effects of PTH.

EFFECTS OF CENTRAL ALPHA-2 RECEPTOR STIMULATION ON HEMODYNAMIC RESPONSES TO AIR-JET STRESS IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). S. Knardahl*, D.R. Kapusta*, J. Koepke, G.F. DiBona, and A.K. Johnson*. Depts of Internal Med. and Psychology, Univ. of Iowa & VAMC. Iowa City, IA.

Intracerebroventricular (ICV) administration of the α -2 agonist guanabenz (G) abolishes the increase (\uparrow) in renal sympathetic nerve activity that results from air-jet stress (AJS) in conscious SHR, but does not alter the \uparrow in mean arterial pressure (MAP) or heart rate. Central α -2 receptor stimulation may exert selective control over regional sympathetic nerve activity. To investigate this, changes in vascular resistance (R) were measured using doppler flow probes (flow velocity) on the abdominal aorta, renal and superior mesenteric arteries in SHR receiving either ICV saline or ICV G (0, 5, 25, and 75 μg). G produced a dose dependent \downarrow in MAP. Similarly, 5 and 25 μg G produced graded reductions in baseline (B) renal R, but this returned to pretreatment level after 75 μg . B hindquarter R \uparrow after 5 μg G but was not reduced further with 25 or 75 μg . G produced a dose dependent \uparrow in B mesenteric R. Although G \downarrow MAP, the response to AJS was unchanged. After 5 and 25 μg G, renal R \uparrow during AJS; however a \downarrow in R occurred after 75 μg . Similarly, mesenteric R \uparrow during AJS after 5 μg G but was \downarrow after 75 μg . Hindquarter R was not affected by 5 μg G and was converted to an \uparrow in R after 75 μg . These data indicate that central α -2 receptor stimulation by G exerts selective, dose dependent changes in sympathetic outflow to the kidneys and skeletal muscle vasculature in SHR during rest and AJS.

RELATIONSHIP BETWEEN HYPERTENSION AND INSULIN SENSITIVITY IN HUMANS. Nieri Karania, Cynthia Morris, Cynthia Gaboury, David McCarron, Oregon Health Sciences University, Portland, Oregon

An association between hypertension and hyperinsulinemic glucose (G) intolerance independent of age, weight, sex and antihypertensive medication has been reported. Disorders of Ca^{2+} metabolism exist in both hypertension and diabetic patients. In a study designed to assess the effects of Ca^{2+} metabolism on BP, fasting G and insulin (I) were determined on 71 hypertensives (HTN) and 39 normotensives (NL) before and after 12 wks of placebo, Ca^{2+} supplementation with CaCO_3 or dietary Ca^{2+} . At baseline, fasting I was significantly higher in HTN than NL (9.8 ± 6.0 vs 6.0 ± 3.3 $P < 0.002$) despite comparable fasting G values. The correlation between wt and I was significant ($r = 0.48$; $P < 0.00001$) and explained 35% and 15% of the variation in I for females and males respectively. After adjustment for wt, the correlation between BP and I was not significant and explained an additional 3% of the variation in I. After Ca^{2+} supplementation, I and G did not differ between NL or HTN. The change (Δ) in plasma I and the Δ in SBP were positively, significantly correlated after 12 wks of observation ($r = 0.23$; $p < 0.008$) as was Δ I with Δ DBP ($r = 0.24$, $p < 0.006$). These changes were independent of treatment and Δ body wt, indicating that a fall in BP is associated with a reduction in I.

In conclusion, 1) hypertension is characterized by reduced I sensitivity, 2) Ca^{2+} effect on G utilization cannot be estimated with fasting parameters of carbohydrate metabolism and 3) BP reduction is associated with I reduction.

CALCIUM (Ca^{++}) CHANNEL BLOCKADE VS. VARIATION IN EXTRACELLULAR Ca^{++} : HEMODYNAMIC AND NATRIURETIC DIFFERENCES IN THE ISOLATED RAT KIDNEY. DE Khani*, RM O'Donovan*, JD Scandling, DB Ornt, JL Izzo Jr. Dept. of Medicine, U. of Rochester, NY.

Ca^{++} -related renal function changes were studied in an improved stabilized in vitro constant-flow isolated rat kidney perfusion model (GFR ~ 1 ml/min/g for > 2 hrs). In 1 set of studies, perfusate Ca^{++} was varied. At normal Ca^{++} (2.3 mEq/L), addition of norepinephrine (NE, 4 ng/ml) increased perfusion pressure (43 ± 3 mmHg), glomerular filtration rate (GFR, $.12 \pm .02$ ml/min/g) and fractional Na excretion (FE Na, $1.6 \pm 0.6\%$) over baseline. Similar effects were caused by angiotensin II (AII, 35 pg/ml). High perfusate Ca^{++} (3.3 mEq/L) enhanced NE-induced pressor responses and GFR and FE Na increases. Low perfusate Ca^{++} (1.4 mEq/L) blunted these responses to NE. In other studies at normal Ca^{++} , lanthanum (1 mM) blunted the NE-induced pressor, GFR and natriuretic responses in a pattern similar to low Ca^{++} . However, while verapamil (1 μM) blunted NE-induced pressor effects (11 ± 2 mmHg, $p .05$), NE-induced increases in GFR ($.19 \pm .04$ ml/min/g) and FE Na ($0.6 \pm 0.3\%$) were maintained; a similar pattern was seen with AII. Thus, verapamil increased the ratio of GFR/perfusion pressure after both AII and NE ($p < .05$).

Conclusions: 1. At constant flow, NE and AII increase GFR by greater constriction of efferent (eff) arterioles. 2. Low Ca^{++} blunts and high Ca^{++} enhances the pressor, GFR, and natriuretic effects of NE and AII, suggesting both afferent (aff) and eff arteriolar effects. 3. Verapamil maintains GFR at lower perfusion pressure, suggesting selective aff arteriolar dilatation.

EFFECTS OF ENALAPRIL (En) ON BLOOD PRESSURE (BP) CONTROL AND WEIGHT CHANGE IN HEMODIALYSIS (HD) PATIENTS. JS Kilpatrick*, G DeVault, K Abreo, ST Brown, L Bairnsfather*, L Stevens*, LSU Med Ctr., Shreveport, LA.

En is an antihypertensive agent that inhibits the formation of angiotensin II which is a potent vasoconstrictor and known stimulator of the thirst mechanism. As such, En should improve BP control and decrease interdialytic weight gain (IDWG) especially in volume resistant, hypertensive HD patients. To test this hypothesis a prospective study was undertaken to evaluate the effects of En on overall BP control, IDWG, and deviation of post-dialysis weight from dry weight (PD-DW) in 8 hypertensive (BP>140/90) chronic HD patients while both on and off En. All patients were on at least 1 additional BP medication of another class-type. Hypertensive events were arbitrarily defined as systolic BP (SBP) or diastolic BP (DBP) >20 mmHg above the initial BP measurement on HD, while hypotensive events, as SBP or DBP >20 mmHg below the initial BP measurement.

There were no significant differences in hyper- or hypotensive events, IDWG, or PD-DW when patients on En were compared to those off En. During the second half of dialysis all patients experienced a significant increase in both systolic ($p<.003$) and diastolic ($p<.001$) hypotensive events whether or not on En.

We conclude that (1) En does not alter hyper- or hypotensive events on HD (2) En does not decrease IDWG and PD-DW (3) En does not alter the tendency of mean SBP and DBP to fall in the latter half of HD (4) any beneficial effects of En could have been masked by concomitant administration of other antihypertensive agents.

IMPAIRMENT OF RENORENAL REFLEXES IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) DEPENDENT ON ELEVATED BLOOD PRESSURE. U.C. Kopp & L.A. Smith*. Univ. of Iowa Col. of Med. & VA Med. Ctr. Iowa City, IA.

Stimulation of renal mechanoreceptors (MR) by increasing ureteral pressure (tUP) or renal chemoreceptors by renal pelvic perfusion with 0.9 M NaCl increases ipsilateral afferent renal nerve activity (ARNA), decreases contralateral efferent RNA and increases contralateral urinary sodium excretion (UNaV) in normotensive Wistar Kyoto rats (WKY) but not in 14-15 wks old SHR with established hypertension. Impaired renorenal reflexes may contribute to the enhanced RNA and sodium retention in SHR and potentially contribute to hypertension. We examined if this impairment was dependent on the elevated blood pressure (BP) by studying renorenal reflex responses to renal MR and CR stimulation in prehypertensive 5-6 wk SHR, and in 14-15 wk SHR treated with captopril to prevent development of hypertension. Age matched WKY were used as controls. BP was 110 ± 6 and 103 ± 6 mmHg in 5-6 wk SHR and WKY, and 112 ± 3 and 97 ± 3 mmHg in captopril treated 14-15 wk SHR and WKY. In 5-6 wk SHR, $n=7$, tUP 30 mmHg increased ipsilateral ARNA $120\pm 38\%$ and contralateral UNaV from 0.4 ± 0.1 to 0.5 ± 0.1 $\mu\text{mol}/\text{min}/\text{g}$. In captopril treated 14-15 wk SHR, $n=10$, tUP increased ipsilateral ARNA $65\pm 21\%$ and contralateral UNaV from 1.3 ± 0.2 to 1.5 ± 0.3 $\mu\text{mol}/\text{min}/\text{g}$, (all $p<0.05$). tUP caused similar effects in WKY. In addition, renal CR stimulation caused similar renorenal reflex responses in 5-6 wk SHR and captopril treated 14-15 wk SHR as in WKY. Thus the impaired renorenal reflexes in adult SHR are dependent on the elevated blood pressure which may alter the sensitivity of renal MR and CR.

VOLUME AND NEURAL ALTERATIONS IN THE PREHYPERTENSIVE DAHL-S RAT. Theodore A. Kotchen, Claude Genain,* Sreenivas Reddy,* Cobern Ott. Univ. of Kentucky School of Medicine, Depts. of Medicine & Physiology, Lexington, Kentucky.

Peripheral sympathetic nervous system tone may be estimated by the rate of tissue norepinephrine (NE) efflux after in vivo tyrosine hydroxylase inhibition (alpha methyltyrosine). To evaluate neural mechanisms in the pathogenesis of salt sensitive hypertension, NE content of heart and interscapular brown fat was measured in separate groups of Dahl salt sensitive (S) and salt resistant (R) rats either without or 6, 11, and 16 hours after alpha methyltyrosine. Animals were maintained on either 7% or 1% NaCl for 5 days. Extracellular fluid volume (ECF-inulin space) and plasma volume (RISA) were measured in additional animals on these NaCl intakes. Blood pressure was higher ($p<0.01$) in S (136 mmHg \pm 2SE) than in R (129 ± 2) and was not affected by diet. ECF did not differ in S (19.3 ± 1.5) and R (17.2 ± 1.2), however, plasma volume was higher ($p<0.05$) in S (3.6 ml/100 gm \pm 0.1 vs 3.0 ± 0.2) In fat, in S, the rate of tissue NE efflux (k) was similar on 1% NaCl (0.081 hr $^{-1}$ \pm 0.012) and 7% NaCl (0.073 ± 0.013); in contrast, in R, k was less steep ($p<0.006$) on 7% NaCl (0.001 ± 0.012) than on 1% NaCl (0.047 ± 0.012). Similarly, in heart, k in R was less ($p<0.001$) on 7% NaCl (0.011 ± 0.011) than on 1% NaCl (0.66 ± 0.008); in S, k did not differ on the two NaCl intakes. We conclude that the subsequent development of hypertension in S is related both to an increased plasma volume and also to the failure to decrease peripheral sympathetic tone in response to high dietary NaCl.

MILD POTASSIUM (K) DEPLETION CAUSES SODIUM (Na) RETENTION AND INCREASES BLOOD PRESSURE (BP). G.G. Krishna, E. Miller*, S. Kapoor*. Temple Univ. Sch. of Med., Philadelphia, PA.

Potassium depletion increases BP by unknown mechanisms. The role of altered Na balance was studied in 8 healthy normotensive males ingesting an isocaloric diet supplemented with either 90 (K₉₀) or 10 (K₁₀) mEq of K daily. Each subject was studied in a metabolic ward on both diets separated by 4 weeks. Na intake, 120-190 mEq/d, was kept constant for each subject.

		STUDY DAYS			
		3	5	7	9
UNaV	K ₉₀	156+22*	142+22*	136+14*	153+12*
	(mEq/d)	101+15	98+16	96+14	105+15
U _K V	K ₉₀	86+7*	70+3*	63+5*	67+5*
	(mEq/d)	33+3	20+1	18+2	13+1

The systolic (S), diastolic (D), and mean (M) BP and plasma (P), aldosterone (A), and K were obtained on day 10:

	SBP	DBP	MBP	PA	PK
	----- (mmHg) -----			(ng/dL)	(mEq/L)
K ₉₀	117+3	77+3*	90+2*	7.4+1.4*	4.0+0.1*
K ₁₀	125+5	83+3	97+3	2.4+0.5	3.4+0.1

* indicates $p<0.05$ K₉₀ vs. K₁₀

On day 10 subjects on K₉₀ excreted 21+4% of a 2L acute saline load over 6 hours but on K₁₀ they excreted only 13+3% ($p<0.05$). Changes in GFR, RBF, urinary dopamine, norepinephrine (NE), PA, P epinephrine, P NE, plasma renin activity (PRA) and AVP were similar on K₁₀ and K₉₀.

Short term potassium depletion impairs renal Na excretion and increases BP. These changes occur independent of GFR, RBF, PA, PRA, AVP and catecholamines.

ATTENUATION OF HYPERTENSION (HTN) BY PARENTERAL ADMINISTRATION OF CALCIUM. T.W. Kurtz* and R.C. Morris. Univ. of California, San Francisco.

Interpretation of the antihypertensive effect of supplemental oral calcium is complicated by the fact that large amounts of calcium so administered can induce Na^+ diuresis and phosphorus (Pi) depletion (by binding Pi in the gut), both of which can decrease blood pressure. To determine whether chronic parenteral administration of supplemental Ca^{++} in small amount can attenuate HTN, we measured mean arterial pressure (MAP), sodium balance (Na Bal), plasma phosphorus (Pi), and blood ionized calcium (ICa) in pair-fed uninephrectomized rats given deoxycorticosterone (DOC) intramuscularly, a 4% NaCl - 0.5% Ca^{++} diet, and continuous intraperitoneal infusions of 5% dextrose or CaCl_2 (7.8 - 15.6 micromoles/hr) by osmotic pump. After 3 weeks of infusion (*p = .01):

Infusion	MAP mm Hg	PI mg/dl	Na Bal mmol/100g	ICa mg/dl
dextrose	149 ± 2	6.8 ± 0.2	5.8 ± 2.2	4.73 ± 0.03
CaCl_2	136 ± 3*	7.3 ± 0.2	7.8 ± 2.1	4.81 ± 0.10

These findings demonstrate that chronic, continuous parenteral administration of calcium in small amount can attenuate DOC hypertension without inducing a decrease in either plasma Pi concentration or retention of sodium. The findings are consistent with the hypothesis that this form of salt-sensitive HTN depends on a disorder in calcium metabolism that is susceptible to modulation by supplemental calcium.

DO TRUE SUBLINES OF THE WISTAR-KYOTO RAT (WKY) EXIST IN THE UNITED STATES? T.W. Kurtz* and R.C. Morris. Univ. of California, San Francisco.

We recently reported evidence of substantial biologic variability in "WKY" rats from different commercial sources in the U.S. (Hypertension 10:127, 1987). We now report information which suggests that "WKY" rats commercially available in the United States do not even constitute sublines of the official "WKY" strain, i.e., the strain established in Japan and formally recognized by the International Society of Hypertension. Most, if not all, commercially available "WKY" rats in the United States descend from an NIH strain of "WKY". NIH records indicate that the NIH strain of "WKY" originated from "noninbred" normotensive Wistar rats sent from Kyoto to NIH in 1971 (NIH rodent catalogue, NIH publication no. 81-606). Dr. Yukio Yamori states, however, that he donated "the first WKY strain" to NIH in 1977 (Hypertension 10:131, 1987). Thus, it would appear that "WKY" breeders distributed by the NIH do not even constitute a subline of the official WKY strain. If so, then the "WKY" rats sold by various commercial suppliers also do not constitute sublines of the official WKY strain. Given these considerations, and the recommendations of the Nomenclature Committee of the International Society of Hypertension, it might be that most of the "WKY" rats bred in the United States should not be carrying the "WKY" strain name.

IMPAIRED CALCIUM EFFLUX IN ENTEROCYTES OF SPONTANEOUSLY HYPERTENSIVE RAT (SHR). Bernard Lacour, Chantal M. Rouillet*, Phillip A. Lucas*, David A. McCarron, Tilman Drücke*, Hôpital Necker, Paris, FR; Ore. Hlth. Sci. Univ., Portland, Oregon, USA.

Decreased active Ca^{2+} absorption and impaired Ca^{2+} influx in proximal duodenal isolated enterocytes of 12-14 week old SHR vs. normotensive WKYs have been reported by our laboratories.

As Ca^{2+} efflux and $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism might affect these disturbances, we evaluated the Ca^{2+} efflux rates in both SHR and WKY (12-14 weeks old) and the effect of ouabain (4mM) or absence of sodium (replaced by choline) on this efflux. The enterocytes were loaded with ^{45}Ca for 30 min. The fraction of initial ^{45}Ca remaining in the cell was determined over a 60 min period and the efflux rate constant calculated (mean ± SEM):

	Quabain	Efflux %/hr	Na	Efflux %/hr
WKY(7)	-	51.5 ± 6.0	WKY(18)	+ 58.9 ± 3.5
WKY(7)	+	33.4 ± 5.7	WKY(15)	- 40.3 ± 2.8
SHR(7)	-	41.1 ± 3.6	SHR(19)	+ 38.4 ± 3.4
SHR(7)	+	34.2 ± 4.3	SHR(16)	- 42.7 ± 4.0

The Ca^{2+} efflux rate constant from 5 to 60 min was lower in the SHR (34.3 ± 1.4 %/hr) than in the WKY (61.9 ± 1.4 %/hr), $p < 0.01$, n=11 pairs. Ouabain and Na absence reduced Ca^{2+} efflux rate constant in the WKY ($p < 0.05$ and $p < 0.001$, respectively). No significant reductions were observed in the SHR. We conclude Ca^{2+} efflux is impaired and baseline activity of $\text{Na}^+/\text{Ca}^{2+}$ exchange is reduced in SHRs, possibly from an intrinsic abnormality of the SHR membrane.

FREE CYTOSOLIC CALCIUM IN PLATELETS: A COMPARISON OF FURA 2 VERSUS QUIN 2. BS Levine, CJ Kert*, CF Sico*, KC Norris and S Muallem.* Med & Resch Svcs, VA Med Ctr, West LA and UCLA Sch Med, LA, CA

Abnormal platelet (Plt) cytosolic Ca ($\{\text{Ca}\}_i$) metabolism in hypertension (EH) has been attributed solely to abnormal pump-leak across the Plt plasma membrane based on data using the fluorescent dye Quin-2 (Q2). A second generation fluorescent dye Fura 2 (F2) is available which is superior to Q2 in measuring changes in $\{\text{Ca}\}_i$ in other cell types. We therefore compared basal and thrombin (T) stimulated $\{\text{Ca}\}_i$ in Plt of healthy subjects using both Q2 and F2. $\{\text{Ca}\}_i$ was measured by the method of Tsien using 2 μM F2 or 10 μM Q2. Basal $\{\text{Ca}\}_i$ was 138 ± 15 nM with Q2 and 120 ± 4 with F2, $p > 0.05$, n=5. In the absence of extracellular Ca $\{\text{Ca}\}$, adding T, 0.5 U/ml, produced a small rise in $\{\text{Ca}\}_i$ with Q2 but induced dramatic changes in $\{\text{Ca}\}_i$ with F2. With F2 $\{\text{Ca}\}_i$ displayed a multiphasic pattern: an initial rapid rise; a slight decline; a second rise; a return to baseline. In the presence of Ca, 1.3 mM, $\{\text{Ca}\}_i$ increased rapidly after T-stimulation when measured with each dye but was multiphasic only with F2. In the absence of $\{\text{Ca}\}$, addition of the ionophore ionomycin (5 μM), before or after T stimulation produced a rapid increase in $\{\text{Ca}\}_i$ with Q2 and an additional rise in $\{\text{Ca}\}_i$ with F2. Conclusions: F2 is superior to Q2 in evaluating changes in $\{\text{Ca}\}_i$ produced by T; Q2 measures predominantly influx of $\{\text{Ca}\}$; Q2 does not deplete Ca stores but may interfere with its release after T-stimulation; with F2 the Ca signal may reflect release from internal stores and influx across the plasma membrane; conclusions regarding abnormal Ca metabolism in EH based on data using Q2 require evaluation with F2.

NATRIURESIS AFTER CAROTID ARTERY TRACTION (CAT) IS MEDIATED BY A γ -MSH-LIKE PEPTIDE. S.-Y. Lin*, S.A. Mazbar*, E. Wiedemann*, and M.H. Humphreys, Divisions of Nephrology and Endocrinology, San Francisco General Hospital, San Francisco, CA.

Acute unilateral nephrectomy stimulates sodium excretion ($U_{Na}V$) from the remaining kidney through reflex pathways involving carotid sinus baroreceptors (CSB). We have recently shown that the efferent limb of the reflex is an increase in plasma immunoreactive (IR) γ -MSH concentration, an anterior pituitary peptide with natriuretic properties. Since activation of CSB by CAT also causes natriuresis (Keeler, AJP 226:507, 1974), we determined if this maneuver could by itself lead to an increase in plasma IR- γ -MSH. In 10 anesthetized rats, sham CAT caused no change in $U_{Na}V$, and plasma IR- γ -MSH was 30 ± 17 (SD) fmol/ml 60 min after the sham procedure. In 17 rats undergoing CAT (25 gm weight to R carotid artery via a ligature draped over a pulley) $U_{Na}V$ increased from 760 ± 364 to 1494 ± 1153 neq/min ($p < .02$) and IR- γ -MSH was 59 ± 18 fmol/ml ($p < .01$ vs sham). A significant correlation existed between the increase in $U_{Na}V$ and plasma IR- γ -MSH concentration ($r = 0.38$, $p < .05$). In 5 additional rats treated with control rabbit serum at the time of the experiment, CAT caused $U_{Na}V$ to rise from 954 ± 543 to 2238 ± 1523 neq/min ($p < .05$). In 6 rats receiving anti- γ -MSH antiserum, CAT failed to increase $U_{Na}V$ (863 ± 495 vs 1011 ± 610 neq/min, $p = NS$). These results indicate that CAT results in an increased plasma concentration of IR- γ -MSH as well as natriuresis. These two consequences of CAT are related, since anti- γ -MSH antiserum blocked the natriuresis. γ -MSH may regulate $U_{Na}V$ in response to CSB activation.

EFFECT OF ORAL CALCIUM, POTASSIUM, NIFEDIPINE, AND DIGOXIN ON NATRIURESIS IN NORMAL MAN. Friedrich C. Luft, George R. Aronoff, and Myron H. Weinberger, Dept. of Med., Ind. Univ., Indpls. IN

Many factors influence renal sodium (Na) excretion and blood pressure. We tested the effect of dietary calcium (Ca; 500 mg bid), potassium (KCl; 60 mEq), nifedipine (N; 10 mg tid), digoxin (D; 0.25 mg/d), or no treatment, (CTR) on acute natriuresis in 14 subjects who received 150 mEq Na diets and 2L normal saline iv over 4 hr. Na excretion ($U_{Na}V$), inulin and PAH clearance (Cl I, Cl PAH), renin (PRA), and aldosterone (PA) were measured. All regimens were given in random order. Data (mean) are below:

regimen	CTR	Ca	K	N	D
$U_{Na}V$ (mEq/d)	352	335	369	387*	343
Cl In (ml/min)	112	92	100	118	107
Cl PAH (ml/min)	573	487	514	544	487
PRA (ng AI/h)	5.7	5.5	5.8	8.7*	5.8
PA (ng/dl)	9.6	7.8	12.1*	8.7	6.0

* (differs from other groups $p < 0.05$)
N caused a relative natriuresis compared to the other groups. N increased PRA, while K increased PA. Ca, K, and D did not influence natriuresis. No regimen altered GFR, blood flow, or blood pressure. No regimen influenced the response of blood pressure to saline. N promoted natriuresis in spite of increased PRA and dissociated PRA from PA. The natriuretic effects of N may augment its efficacy in hypertension.

NON-INVASIVE MEASUREMENT OF HEMODYNAMIC EFFECTS OF ENALAPRIL IN HYPERTENSION. Armando Lindner and Alice J. Meacham,* Veterans Administration Med. Ctr. and Univ. of Washington, Seattle, Washington.

Systemic hemodynamic effects of Enalapril (CEI) were measured in patients with essential hypertension, to evaluate the drug mechanism of action and the usefulness of the non-invasive Doppler-ultrasound (Ultracom) method. This measures ascending aortic diameter (AD) and blood velocity to derive cardiac output (CO) and peripheral vascular resistance (PVR). CEI was given as single drug therapy ($n = 11$) or in combination with thiazides (HCTZ, $n = 8$). Hemodynamic findings during the baseline (B) and at the end of 2 months of treatment (T) were as follows:

CEI SINGLE DRUG THERAPY (n=11)				
PERIOD	MEAN BP	AD	CO	PVR
	(Torr)	(mm)	(L/min)	(Arbit. Units)
B	120 \pm 3	30.4 \pm 0.4	5.0 \pm 0.3	24.6 \pm 1.7
T	105 \pm 2	29.3 \pm 0.7	5.8 \pm 0.3	18.5 \pm 0.9
P	0.001	NS	0.05	0.005
COMBINED CEI AND HCTZ (n=8)				
B	127 \pm 4	31.0 \pm 0.6	5.0 \pm 0.3	26.2 \pm 1.8
T	102 \pm 6	29.3 \pm 0.7	5.9 \pm 0.4	16.3 \pm 2.5
P	0.001	NS	0.005	0.001

The BP fall with Enalapril in both groups was due to a marked fall in PVR (26 and 30%, respectively). Heart rate was unchanged. In contrast to earlier invasive methods, Ultracom demonstrated a significant improvement in CO in both groups, and established its validity for repeated hemodynamic testing in hypertension.

PRONOUNCED NON-CORONARY INTERSTITIAL MYOCARDIAL FIBROSIS IN UREMIC PATIENTS

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We have previously demonstrated selective activation of pericytes, deposition of collagen and enlargement of interstitial space in myocardium of uremic rats (+ ACE inhibitors). To further examine whether interstitial fibrosis of myocardium (IFM) is found in humans, we examined heart tissue (post-mortem) of uremic patients (no stenosing coronary lesions or diabetes): 30 pat. terminal renal failure, no dialysis; 102 pat. hemodialysis for variable periods (0.5-12 y.), 11 pat. CAPD, 16 pat. renal transpl.; 41 pat. with hypertension but no renal failure as controls. No IFM was found in hearts of 500 non-hypertensive, non-uremic, non-diabetic pat. Sect. examined by 2 observers without knowledge of diagnosis. IFM was clearly different from perivascular fibrosis or vascular scars and consisted of fine spun collagen fibers surrounding cardiomyocytes.

Fibrosis score:	0	1	2	3
hypertension (n=41)	5 (12%)	30 (73%)	6 (15%)	0 (0%)
uremia (n=30)	0 (0%)	3 (10%)	25 (83%)	2 (7%)
dialysis (n=102)	9 (9%)	30 (29%)	51 (50%)	12 (12%)

IFM has recently been recognized as risk factor for reentry arrhythmias and diastolic left ventricular malfunction in essential hypertension; therefore IMF may also have obvious clinical implications in uremic patients.

DISORDERED ANGIOTENSIN II (ANGII) RECEPTOR REGULATION IN TYPE I DIABETES MELLITUS. Johannes F.E. Mann,* Hannalore Mürtz,* Jeanne Sis,* Klaus Usadel,* Eberhard Ritz* (intr. by F. Luft) Univ. of Heidelberg, Heidelberg, West Germany.

We investigated ANGI and ANF plasma levels and binding site characteristics in normotensive Typ I diabetics (age: 21-55 y, duration of diabetes: 0.2-18 y). Patients had no proliferative retinopathy or microalbuminuria, and were compared with normotensive, age- & sex-matched healthy volunteers (n=10 each). Blood was taken at 9 am after 30 min. rest and processed for determination of ANGI and ANF blood levels (RIA after extraction) and for ANGI and ANF binding site characteristics on platelets (Mann et al, J Hypertension 1985, 3:131-7). Urinary sodium excretion varied between 150-290 mmol/d. Plasma ANGI levels were higher in diabetics than in controls (16±1 vs 8±1 fmol/ml) as was B_{max} of platelet ANGI binding sites (13±2 vs 6±1 sites/cell) with no detectable change of apparent dissociation constant (K_D 150±8 vs 148±19 pM). Plasma levels of ANF were not different between diabetics and controls, but B_{max} of ANF binding sites was lower in diabetics than in controls (9±1 vs 17±3 sites/cell) with no change of K_D (14±2 vs 17±4 pM). Increased ANGI plasma levels in conjunction with increased number of ANGI, and decreased number of ANF binding sites in diabetics may contribute to the known tendency for sodium retention and for the development of hypertension in these patients.

RENAL EFFECTS OF NICARDIPINE. A. Montanari A. Novarini, P. Coruzzi. Inst. Semeiotica Med., Parma, Italy. (Intr. by J.P. Knochel)

Mean arterial pressure (MAP), renal plasma flow (RPF), glomerular filtration rate (GFR) and Na excretion (UNaV) were measured during infusion of PAH and inulin before and after acute i.v. nicardipine (NC) 5 mg in 8 untreated essential hypertensives at 200 mM Na diet. 3-5 days after the protocol was repeated under dopamine (DA) blockade with metoclopramide (MCP) 10 mg i.v. Table summarizes our results (MAP, mmHg, GFR and RPF, ml/min, UNaV, μmol/min, a=.05, b=.01)

	Control I	NC	Control II	NC+MCP
MAP	122±14	104±19 ^b	114±13	97±14
RPF	602±121	639±136 ^g	613±127	649±36
GFR	98±18	104±19 ^b	103±20	102±16
UNaV	149±28	402±146 ^b	143±50	423±102 ^b

NC did not change RPF, although renal vascular resistance fell about 20%. GFR was slightly but significantly increased. This increase was prevented by DA blockade. Marked natriuresis occurred in the presence

of no changes in RPF and with or without DA blockade thus showing that NC-induced natriuresis was independent of both direct DA tubular effects and MCP-inhibited increase in GFR. Our data suggest that NC exerts its natriuretic effect mainly through direct inhibition of tubular Na reabsorption.

DIETARY VS SUPPLEMENTAL CALCIUM TO REDUCE BLOOD PRESSURE. Cynthia D. Morris, Njeri Karanja, David A. McCarron, Oregon Health Sciences University, Portland, Oregon.

Calcium supplementation (SCa) reduces blood pressure (BP) in some hypertensives (HTN) and normotensives (NL), but the effect of increased dietary Ca (DCa) is unknown. We sought to determine if DCa could reduce BP similar to SCa. 82 subjects with mild HTN (MAP≥105) on no medication and 45 NL (MAP<105) were separately randomized to receive either 1500 mg DCa through a programmed intervention, 1 g SCa as CaCO₃ or placebo (Pl) for 12 wks. BP was measured for a 4 wk period at baseline and every 2 wks thereafter.

Mean age was 45.0±9.6 yrs with 72 ♂ and 55 ♀; baseline BP was 139±8/90±6 in HTN and 118±11/74±8 in NL. BP response to treatment was averaged after 8-12 on therapy and compared to a baseline average. In all males, supine SBP decreased significantly with CaCO₃ compared to placebo (-6.2 mmHg, p=.03) as did supine DBP (-3.1 mmHg, p=.07). The following supine BP changes were seen in males:

	DCa	SCa	Pl
HTN	-2.9/-0.7	-6.4/-3.3	-0.7/-0.1
NL	-4.1/0	-5.7/-2.8	-1.4/-1.2

Comparing Pl to SCa in HTN, SBP was reduced (p=.07) as was DBP (p=.10); only SBP was reduced in NL (p=.10). In HTN females, there was a non-significant trend for reduction of standing BP with DCa but not SCa compared to Pl (DCa -8.0/-6.4; SCa -4.4/-1.0; Pl -3.5/-3.6 mmHg). No BP changes were seen in NL females. We conclude: 1. SCa reduces BP in males; 2. BP response to Ca delivery (DCa vs SCa) may differ between sexes.

DIETARY Ca AND Fe INTERACTION ON HEMATOCRIT IN THE SHR. Martin Muntzel, Daniel C. Hatton, Jeff Absalon, David A. McCarron, Oregon Health Sciences University, Portland, Oregon.

Manipulation of dietary Ca in the spontaneously hypertensive rat (SHR) between the ages of 21 and 28 days of age produces a significant decrease in BP and hematocrit (Hct) in animals on high Ca (2.0%) diets relative to animals on low Ca (0.1%) diets. Normal diet (1.0%) animals have intermediate values. The simultaneous fall of Hct and BP suggests that the changes in BP may be a consequence, in part, of the decrease in Hct and change in viscosity. The difference in hematocrit across diets may have been due to competitive inhibition of Fe uptake by Ca.

To examine the possibility that the BP change may have been 2° to alteration in Hct, 21-day-old SHRs were placed on one of 6 Ca/Fe diets. Three of the diets were those used previously, and three were replications of the Ca/Fe ratios of those diets using constant Ca (%) and varying Fe (ppm). The results are as follows:

Diet	1	2	3	4	5	6
Ca/Fe	0.1/45	1.0/445	1.0/105	1.0/60	1.0/38	2.0/75
N	8	8	8	8	9	8
Hct	35.5	37.11	36.17	32.4	28.49	27.45
BP	105.3	100.5	99.1	96.6	97.8	96.9

ANOVA indicated that on constant Ca diets, variations in Fe produced differences (p<.001) in Hct but no difference in BP. In contrast there were significant differences in both Hct and BP on the three original diets (1,4,6). We conclude that variations in Hct alone are not sufficient to alter BP in SHR. However, dietary Ca may interact with Hct and/or viscosity to produce variations in BP.

RACIAL DIFFERENCES IN FIBROBLAST ^{45}Ca WASHOUT.
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Abraham Aviv, NJ Medical School, Newark, NJ.

We recently showed that the Na/H antiport is hyperactive in cultured skin fibroblasts from blacks. Since this system plays an important role in cellular Ca mobilization, we examined ^{45}Ca transport in quiescent fibroblasts from blacks and whites. ^{45}Ca washout was described by a three exponential function with coefficients a_{1-3} (representing, in fraction, hypothetical cellular pools) and factors k_{1-3} (representing washout rate constants). There were no racial differences in ^{45}Ca washouts under basal conditions. Serum (10%) produced a prompt and dramatic increase in the overall ^{45}Ca washout associated with rise in k_1 and a_1 . The washout rate from serum-stimulated cells in the presence of 2mM Ca was more rapid in blacks than in whites, as shown by a higher k_1 (mean \pm SEM) (0.874 ± 0.030 and $0.742 \pm 0.026 \text{ min}^{-1}$, for blacks and whites, respectively; $p > 0.01$, $n=12$) and a greater a_1 (0.691 ± 0.009 and 0.629 ± 0.015 for blacks and whites, respectively; $p < 0.02$). The k_1 of serum stimulated cells in Ca-deficient medium was also higher for blacks than whites (1.16 ± 0.037 vs $1.02 \pm 0.032 \text{ min}^{-1}$; $p < 0.05$). No differences were observed with respect to the source of serum (from blacks vs whites). No racial differences were observed in ^{45}Ca uptake with or without serum. Our findings indicate a greater Ca mobilization by serum in blacks, which may relate to a higher Na/H antiport activity. If this phenomenon is generalized, it can predispose to essential hypertension by increasing contractility of vascular smooth muscle.

PLATELET CYTOSOLIC CALCIUM IN ESSENTIAL HYPERTENSION: QUIN 2 VERSUS FURA 2. KC Norris, CJ Kert,* S Muallem*, BS Levine. Med & Rsch Svcs VA Med Ctr WLA and UCLA Sch Med LA, Ca

It was reported that in essential hypertension (EH) basal platelet (Plt) free cytosolic $[\text{Ca}]_i$ is high, but increases normally after thrombin (T) stimulation. These data, obtained with the fluorescent dye Quin 2 (Q2), were interpreted to suggest that plasma membrane fluxes are abnormal in EH but release of internal Ca stores is intact. Studies from this laboratory (Levine, ASN 87) show that Q2 inhibits Ca release from internal stores following T-stimulation. Therefore we reassessed Plt $[\text{Ca}]_i$ using the fluorescent dyes Fura 2 (F2) or Q2 in 11 subjects, 5 control (Con) and 6 with EH. Mean basal $[\text{Ca}]_i$ with Q2 in Con was $138 \pm 15 \text{ nM}$ vs $114 \pm 11 \text{ nM}$ in EH, (NS). In contrast, in the same Plt preparation $[\text{Ca}]_i$ with F2 was higher in EH than Con, $217 \pm 27 \text{ nM}$ vs $120 \pm 4 \text{ nM}$, $p < 0.05$. Further studies using F2 showed a correlation between blood pressure and $[\text{Ca}]_i$, $R = 0.55$. T, 0.5 U/ml added to Plts in Ca-free media caused a marked rise in $[\text{Ca}]_i$ with F2 which was multiphasic; although the absolute rise in $[\text{Ca}]_i$ in Con ($592 \pm 104 \text{ nM}$) vs EH ($512 \pm 60 \text{ nM}$) did not differ, the % rise was less in EH. Unlike prior studies using Q2, when F2-loaded Con Plts were incubated with Ouabain, 0.1 mM , for 1 hr, basal $[\text{Ca}]_i$ rose to 343 nM vs vehicle 235 nM , $p < 0.05$. In summary: 1) In the same population a higher resting $[\text{Ca}]_i$ was detected in Plts from EH with F2, but not with Q2; 2) Ca release from internal stores is abnormal in EH; 3) Ouabain raises $[\text{Ca}]_i$. These studies suggest that both internal Ca release, as well as plasma membrane Ca fluxes are abnormal in EH; the exact mechanism remains undefined.

MECHANISM OF VASCULAR SMOOTH MUSCLE CELLS (VSMC) CONTRACTION BY PHORBOL ESTERS. K. Okada,* C. Caramelo,* P.H. Tsai,* and R.W. Schrier. Univ. Colorado Sch. Med., Denver, CO.

In the present study, the effect of phorbol-12-acetate-13-myristate (PMA) was examined on VSMC in primary culture. A time-related (up to 60 min), dose-dependent contraction of VSMC, as assessed by both digital computerized imaging and photoplannimetry, was observed at different doses of PMA (10^{-7} , $41.6 \pm 1.8\%$; 10^{-6} M, $27.8 \pm 2.1\%$; and 10^{-5} M, $13.4 \pm 1.0\%$, $p < 0.01$ between doses). The effect of PMA (10^{-6} M) on cell contraction was enhanced by the Ca^{2+} ionophore, ionomycin (10^{-7} M, 27.8 ± 2.1 vs $36.1 \pm 1.5\%$, $p < 0.05$) and decreased (PMA 10^{-7} M) by Ca^{2+} -free medium (41.6 ± 1.8 vs $21.1 \pm 3.4\%$, $p < 0.005$). Atrial natriuretic factor (ANF, 2×10^{-7} M) did not alter this effect of PMA (10^{-6} M, 41.6 ± 1.8 vs $36.6 \pm 6.0\%$, NS, or 10^{-5} M, 27.8 ± 2.1 vs $28.3 \pm 3.4\%$, NS). The calmodulin antagonist, calmidazolium (5×10^{-6} M) also did not alter the effect of PMA (41.6 ± 1.8 vs $41.0 \pm 6.0\%$, NS). While neither ANF nor PMA altered cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$), as measured by fura 2, ionomycin (10^{-7} M) increased $[\text{Ca}^{2+}]_i$ by $84 \pm 5 \mu\text{M}$ and $218 \pm 14 \text{ nM}$ ($p < 0.005$) in the absence and presence of Ca^{2+} in medium. In conclusion, the present results are compatible with an important role of protein kinase C-mediated phosphorylation in vascular smooth muscle contraction by a mechanism independent of Ca^{2+} -calmodulin activation and of any action of ANF. This effect can nevertheless, however, be modulated by an increase or decrease in $[\text{Ca}^{2+}]_i$ and by the influx of Ca^{2+} from the extracellular medium.

REVERSIBLE DEPRESSION OF Na-K-ATPase AND LYSOSOMAL PROTEASES BY ANGIOTENSIN BLOCKADE IN ISOLATED PROXIMAL TUBULES OF CLIPPED RAT KIDNEY. C.J. Olbricht, H.-J. Groene*, E. Gutjahr*, E. Warnecke*, U. Helmchen*, and K.M. Koch*. Hannover Med. School and University of Göttingen, Fed. Republic of Germany

Angiotensin-converting-enzyme (ACE) inhibition in 2 kidney 1 clip (2K1C) rats leads to a dramatic decrease of GFR and to tubular atrophy in the clipped kidney (Lab. Invest. 54:645 1986). To elucidate biochemical aspects of this form of atrophy Na-K-ATPase and lysosomal proteases Cathepsin B+L were measured in the microdissected proximal tubule segments S1, S2, S3 of the clipped kidney by ultramicroassays. Three groups of male rats were studied, including rats with 2K1C hypertension (C); rats with 2K1C treated by the ACE inhibitor MK 421 for 14 days (MK); and MK-rats 7 days after stop of MK 421 and removal of clip and contralateral kidney (R). Enzyme activities are given as pmol/mm tubule length/min incubation.

Group	n	Na-K-ATPase		Cathepsin B+L		KW
		S1	S3	S1	S2	
C	14	123±28	26±5	12±2	9±1	845±43
MK	15	147±6*	97±3*	57±1*	47±1*	464±52*
R	10	119±11§	26±4§	12±2§	14±2§	1162±76§

(mean±SEM); * $p < 0.05$ (C vs MK); § $p < 0.05$ (R vs MK) Kidney weight (KW), Na-K-ATPase, and Cathepsin B+L in the clipped kidney decreased after prolonged ACE inhibition (MK vs C). Following stop of ACE inhibition, removal of clip, and contralateral nephrectomy (R), KW increased and the enzyme activities returned to control values. Thus, the atrophy of the clipped kidney following ACE inhibition is a state of tubular inactivity that is potentially reversible. This seems to be relevant for the management of renovascular hypertension.

EXTRACELLULAR CALCIUM $[Ca^{2+}]_o$ MODULATES RAT AORTIC SMOOTH MUSCLE CELL MEMBRANE POTENTIAL (V_{mc}).
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Although $[Ca^{2+}]_o$ homeostasis and balance participate in regulation of blood pressure the cellular mechanism behind this effect is unclear. The possibility exists that electrophysiological properties of vascular smooth muscle are affected by $[Ca^{2+}]_o$. Modulation optics assisted intracellular glass microelectrode recordings of V_{mc} were obtained in cultured, dispersed rat aortic smooth muscle (RASM) cells. Control V_{mc} was -44 ± 1 mV (n=59). Neither 8-Br-cAMP nor Angiotensin had an effect on V_{mc} . K^+ for Na^+ substitution depolarized V_{mc} from -50 ± 3 mV (n=12) to -11 ± 1 mV (n=4) indicating that RASM is primarily K^+ conductive. $[Ca^{2+}]_o$ reduction also depolarized V_{mc} :

pCa^{2+}	a_pCa^{2+}	b_pCa^{2+}	Recovery
-4.0 ± 1 mV	-28.6 ± 4 mV	-13 ± 1.8 mV	-56 ± 2 mV
(n=19)	(n=4)	(n=6)	(n=4)

$pCa^{2+} = -\log[Ca^{2+}]_o$; Mean \pm SE, $a_p < 0.02$; $b_p < 0.01$.
Verapamil (Vp) mimicked $[Ca^{2+}]_o$ reduction:

Control	$a_{10\mu M Vp}$	$b_{10\mu M Vp}$	$c_{100\mu M Vp}$
-49.5 ± 2 mV	-39 ± 3.5 mV	-27 ± 4 mV	-16 ± 2 mV
(n=16)	(n=12)	(n=9)	(n=4)

$a_p < 0.02$; b and c $p < 0.01$

We conclude that the Ca^{2+} activated K^+ channel of RASM cell membrane is highly sensitive to $[Ca^{2+}]_o$. It is likely that extracellular Ca^{2+} plays a critical role in the regulation of the resting V_{mc} and excitability of vascular smooth muscle. Modulation optics facilitate intracellular microelectrode studies in single non confluent cells.

ASYMMETRIC REDUCTION OF GLOMERULAR ANGIOTENSIN II RECEPTOR SITES IN EARLY 2K1C GOLDBLATT RATS: ROLE OF THE INTRARENAL RENIN-ANGIOTENSIN SYSTEM (RAS). *Ira Pion**, Barry Wilkes and Sabrina Silverman*, Div. of Nephrol./Hypertension, North Shore Univ. Hospital & Cornell Univ. Med. College, Manhasset, New York.

Renal functional impairment occurs prior to the development of hypertension in the 2K1C Goldblatt model. We studied the properties of glomerular angiotensin (AII) receptors during the early normotensive phase of the Goldblatt model. Experiments were performed 7 days after the application of a 0.20 mm clip to the left renal artery in male SD rats (289 ± 6 g BW). There were no changes in mean arterial pressure (control, 111 ± 3 vs 2K1C, 112 ± 7 Torr) or plasma renin activity (control, 2.75 ± 48 vs 2K1C, 2.21 ± 57 ng/ml/min). Glomerular AII receptor density was decreased in clipped compared to contralateral kidneys (341 ± 170 vs 1232 ± 105 fmol/mg, $p < 0.02$) suggesting homologous down-regulation of the receptors by tissue AII. Enalapril (10mg/kg) restored AII receptor number to control levels (clipped, 1691 ± 185 vs contralateral, 1841 ± 328 fmol/mg, $p = NS$) suggesting that elevated local concentrations of AII down-regulated glomerular AII receptor sites in clipped kidneys. Renal renin content (RRC) was elevated by 44% in clipped compared to contralateral kidneys ($p < 0.002$). There was no evidence of suppression of RRC in contralateral kidneys compared to normal controls. We conclude that the pre-hypertensive phase of the 2K1C model is characterized by activation of the intrarenal RAS which precedes changes in the plasma RAS.

CALCITRIOL SYNTHESIS IS DECREASED IN SPONTANEOUSLY HYPERTENSIVE RATS. *S Patel** and C Hsu. Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

To investigate the pathogenesis of abnormal Ca metabolism, such as hypocalcemia, decreased intestinal Ca absorption and hypercalciuria, in spontaneously hypertensive rats (SHR), we have measured metabolic clearance rate (MCR) and production rate (PR) of calcitriol by the constant isotope infusion method in 5, 8, 12, 16 and 20-week-old normotensive Wistar Kyoto rats (WKY) and SHR. PR of calcitriol were progressively decreased in both WKY and SHR as they grew older, but more so in SHR (SHR, 38.4 ng/kg/day, 26.3, 17.7, 19.7, and 11.9 vs. WKY, 42.3, 33.1, 28.2, 28.1, and 20.3, all $p < .01$). Thus the values were significantly lower in SHR after 12 wks of age. MCR of calcitriol, however, were not different between WKY and SHR. Therefore, decreased syntheses of calcitriol account for the lower plasma levels of calcitriol in SHR after 12 wks of age (SHR, 12 wks, 50.1 pg/ml; 16 wks, 58.3; 20 wks, 41.7 vs. WKY, 12 wks, 78.4, 16 wks, 83.2; 20 wks, 67.3, all $p < .01$). Acidosis or decreased renal function could not account for the decreased synthesis of calcitriol, since the blood pH and pCO_2 and creatinine clearances were similar between WKY and SHR. Plasma concentrations of ionized Ca were also lower in SHR after 12 weeks of age. Plasma concentrations of calcitonin were significantly higher in 16-week-old SHR (41.6 ± 1.5 pg/ml) than in age-matched WKY (30.5 ± 1.7 , $p < 0.001$). The values, however, were not different between 8- and 12-week-old WKY and SHR. We believe that the decreased synthesis of calcitriol could be the pathogenetic factor for the development of abnormal Ca metabolism in SHR. Age of animals should be considered when studying the Ca metabolism in SHR.

INFLUENCE OF STRESS ON PLASMA AND TISSUE LEVELS OF AN ENDOGENOUS LIGAND TO THE DIGITALIS RECEPTOR. *Albert L. Rauch*, Michael F. Callahan*, Vardaman M. Buckalew, Jr. Section of Nephrology, Bowman Gray School of Medicine, Winston-Salem, NC.

We have previously reported the presence of an endogenous ligand to the digitalis receptor in delipidated and desalted extracts of plasma and tissue. To examine the role of this ligand in the regulation of Na,K ATPase activity, its plasma and tissue levels were examined in rats after 5 min of intermittent electrical shock. The ligand was extracted by homogenization in ethanol, delipidated with organic solvents and desalted with a C18 column. Levels were quantitated with a radioreceptor assay using 3H -ouabain and erythrocyte ghosts and expressed as pmoles of digitalis-like activity/g of tissue. Extracts of plasma and tissue produced a displacement of 3H -ouabain that was parallel to that produced by cold ouabain. Activity in tissue was: adrenal (309 ± 48.1), striatum (262 ± 58.1), hippocampus (210 ± 50.5), hypothalamus (198.3 ± 35.7), cortex (143 ± 26.0), spleen (122 ± 42.7), brainstem (92.2 ± 21.2), liver (90.3 ± 17.2), cerebellum (83.7 ± 21.9), kidney (50.3 ± 8.0); the activity in plasma was 8.0 ± 1.2 ml. Stress increased the activity in the hypothalamus by 66.2% to 329.0 ± 49.0 ($p < 0.05$). Stress decreased the activity in the liver by 67.9% to 29.0 ± 8.8 ($p < 0.005$) and in the brain cortex by 33.6% to 95.0 ± 12.2 ($p < 0.05$). Stress did not affect the ligand levels in the other tissues or plasma. These results suggest the ligand in tissue may regulate Na,K ATPase activity in an autocoid-like manner.

EFFECT OF 1,25(OH)₂D₃ ON Ca²⁺ HANDLING IN PROXIMAL DUODENAL ENTEROCYTES FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR). C.Rouillet*, E. Young, T.Drücke*, D.McCarron. Hôpital Necker, Paris FR and Ore Hlth Sci Univ, Portland, USA.

We previously reported that both plasma 1,25(OH)₂D (1,25-D) levels and cytosolic Ca²⁺ influx (J_c) in isolated enterocytes were decreased in the SHR and that a Ca²⁺ supplemented diet (DCa²⁺) normalized J_c in the SHR. To determine the effect of 1,25-D₃ on J_c, 50 ng/day of the hormone (D) or vehicle alone (V) were injected intraperitoneally for four days in 18 male SHRs and 18 male WKYs (12-13 WOA). Plasma 1,25-D levels were measured and a two phase analysis of ⁴⁵Ca influx (0.25 to 45 min) was performed ($\bar{x} \pm \text{SEM}$):

Tx	Plasma 1,25-D	Flux J _c	Pool P _c
WKY V	48.8 ± 4.1	0.74 ± 0.05	10.7 ± 1.4
SHR V	39.0 ± 3.9	0.41 ± 0.04	9.5 ± 1.2
WKY D	162.4 ± 19.9	0.98 ± 0.09	16.6 ± 1.8
SHR D	189.6 ± 25.3	0.64 ± 0.05	13.6 ± 1.3

1,25-D levels (pg/ml) were reduced in control (V) SHR (p < 0.02) but were similar to WKY in D treated animals. J_c (nmol Ca/mg protein) increased significantly with D treatment in both SHR and WKY although, unlike our previous findings with DCa²⁺, J_c remained lower in SHR than WKY (p < 0.001). D treatment increased the cytoplasmic pool (P_c) in both SHR and WKY, an effect not observed with DCa²⁺. DCa²⁺ and 1,25-D₃ have different effects on enterocyte Ca²⁺ handling. These findings suggest that abnormal J_c in SHR represents a defect in cell Ca²⁺ metabolism and not just reduced plasma 1,25-D.

RELATIONSHIPS BETWEEN VASCULAR REACTIVITY AND WALL/LUMEN-RATIOS OF THE UNTOUCHED KIDNEY IN CHRONIC HYPERTENSIVE RATS.

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To examine renal vascular reactivity (VR) in the presence of structural changes, we infused different agonists (angiotensin II (A II), noradrenaline (NE), phenylephrine (PHE), vasopressin (AVP)) directly into the unclamped kidney of chronic (6 mo) two-kidney, one-clip hypertensive rats before and after removal (4 we) of the clamped kidney. Dose-response curves were obtained in 4 groups: (I)=normal controls (n=12), (II)=controls after unilat.NX (n=7), (III)=hypertensive rats before (n=12) and (IV)=rats with persistent hypertension after NX of the clamped kidney (n=9). Individual wall/lumen-ratios (WLR) were calculated after planimetric examinations of cross-sectioned arteries. Relationship between inner radius (R) and mediathickness (D) was determined for each kidney in order to achieve D-values at a constant R=50 μm. VR to A II was decreased (P<0.01) in group III only despite an increase of WLR. VR to NE and PHE was increased (P<0.01) in group III but not in group IV despite a similar increase of WLR in both groups. VR to AVP did not differ between groups. Captopril did not influence VR to NE or PHE. In all groups, VR was directly correlated (P<0.01) with WLR for NE and PHE only. Slopes of the D/R-relationship were increased (P<0.01) in group III and IV, indicating a more pronounced pressure load of the proximal vascular bed.

Conclusion: The study demonstrates specific alterations of VR in kidneys with nephrosclerosis, which cannot be solely regarded as a consequence of structural changes. The decrease of VR to A II might reflect changes in receptor-occupancy, -number or post-receptor events in chronic renovascular hypertension with nephrosclerosis.

HYPERTENSION IN JORDANIAN CHILDREN:

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42 children with Hypertension were seen at Jordan University Hospital during the last 20 months. Twenty six were females and sixteen were males. Their ages ranged between 1-20 years. Five patients presented with Hypertensive encephalopathy. Systolic B.P. ranged from 170-220mmHg, and the diastolic B.P from 90-130mmHg. Primary hypertension was found in 5 patients, while 37 patients and secondary Hypertension:- with 31 patients had Renal Parynchymal disease, 5 had renal artery stenosis, and 1 patient had 11 hydroxylase deficiency. The Etiology of renal parynchymal diseases were as follows:- 8 had acute glomerulonephritis, 7 had end stage kidney disease requiring dialysis support, 6 had nephrotic syndrome equally distributed between focal glomerulosclerosis and membranoproliferative GN, 4 patients had chronic renal functional impairment (Serum creatinine 1.8-3.5mg/dl), 3 had pre-eclampsia, and 3 had polycystic kidney disease (Adult type). Normal B.P was achieved in 3 patients with renal artery stenosis following surgical correction. Most of the patients with acute G.N. normalized their B.P. once the kidney function is back to normal. Patients with other renal parynchymal disease continued to have mildly elevated B.P. with treatment or dialysis. In conclusion, nearly 90% of our Hypertensive children had secondary Hypertension, an incidence is comparable in what has been observed in Western Children.

INFLUENCE OF CAPTOPRIL ON THE CONTROL OF GLOMERULAR FILTRATION RATE (GFR) DURING ACUTE HYPOTENSION IN NORMAL MAN.

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Recent experimental data and studies in patients with renal artery stenosis suggested that the control of GFR during reduction of arterial pressure (MAP) may be dependent on an intact renin-angiotensin system (RAS). However, in normal man direct evidence for the role of the RAS in regulating GFR during hypotension is lacking. Therefore, we studied GFR and renal plasma flow (RPF) changes in 9 male volunteers during acute nitroprusside-induced reduction of MAP on a low (LSD, 11 mEq/d) and normal (NSD, 200 mEq/d) sodium diet before and after 50mg captopril (C). Basal-MAP averaged 81 (LSD) and 82 mmHg (NSD) resp.(NS). NP reduced MAP to 68 (LSD) and to 67 mmHg (NSD) resp.(NS). After C, MAP was reduced by NP to 64 (LSD) and to 61 mmHg (NSD) resp. Basal-GFR averaged 122 (LSD) and 131 ml/min (NSD) resp.(NS) and was reduced to 99 (LSD) and to 108 ml/min (NSD) by NP. After C, GFR was reduced by NP to 88 (LSD) and to 100 ml/min (NSD) resp. Basal-RPF averaged 750 (LSD) and 894 ml/min (NSD) resp.(P<0.05). During MAP-reduction, RPF decreased to 528 (LSD) and to 661 ml/min (NSD) before C, but increased to 808 (LSD) and to 942 ml/min (NSD) after C. Therefore, filtration fraction (FF) increased from 17% to 19% (LSD, P<0.05) and from 15% to 17% (NSD) before C, but decreased to 11% (LSD, P<0.01) and to 11% (NSD, P<0.01) after ACE-inhibition. LSD increased absolute values of plasma renin activity (PRA) at any MAP, but the percentage increase of PRA during MAP-reduction was not different between both sodium states.

Conclusion: In normal man, the control of the pre/postglomerular resistance-ratio and, therefore FF, during acute pharmacologically-induced hypotension is dependent on an intact RAS, probably acting on efferent resistance.

RENAL DOPAMINE-1 DEFECT IN SPONTANEOUS HYPERTENSION. Mouin G. Seikaly, Robin A. Felder, Gilbert M. Efsner, and Pedro A. Jose. Georgetown Univ. Med. Ctr., Wash., D.C. and Univ. Virginia Med. Ctr. Charlottesville, VA

Renal dopamine (DA) receptors decrease sodium transport. However, the spontaneously hypertensive rat (SHR) of the Aoki-Okamoto strain retains sodium despite increased renal DA. We tested the hypothesis that the abnormal sodium handling in SHR is related to decreased dopaminergic activity, by studying the effects of the intrarenal infusion of the DA-1 agonist, SKF 38393, in SHR and normotensive Wistar Kyoto rats (WKY) 11-16 wk with renal nerves intact or after acute unilateral renal denervation (DNX). Specificity of DA-1 effects of SKF 38393 was verified since its natriuretic effect could be blocked by the DA-1 antagonist SCH 23390 (n=6). In WKY, occupation of renal DA-1 receptors by SKF 38393, (10^{-9} - 10^{-7} M) resulted in a natriuresis (n=6) that was enhanced after DNX (n=6). In addition, DA-1 agonist increased glomerular filtration rate (GFR) in WKY only after DNX. In SHR, DA-1 agonist increased sodium excretion only after DNX (n=6) and then to a lesser extent than normotensive controls; GFR was not affected (n=6). (DNX alone increased sodium excretion to a greater extent in SHR than WKY). The decreased natriuretic response to DA agonist in SHR was not due to differences in renal DA-1 receptor density (1.3 ± 0.3 pmol/mg protein, for SHR n=4; 1 ± 0.2 for WKY, n=4), affinity or distribution determined by radioligand binding and autoradiography with the DA-1 antagonist 125 I-SCH 23982. We suggest that the abnormal renal sodium handling in SHR is due both to increased renal nerve activity and decreased post DA-1 receptor activity.

SERIAL PLASMA RENIN ACTIVITY IN NORMAL AND HYPERTENSIVE PREGNANCY. Phyllis A. Taufield*, Maurice L. Druzin*, Jean E. Sealey, John H. Laragh. Cornell University Medical College, New York, New York.

Plasma renin activity (PRA) is reported to be lower than normal in hypertensive pregnancy but there is a considerable overlap. We measured serially, PRA, creatinine clearance (Ccr), and urine aldosterone (Ualdo) in 22 pregnant women with chronic hypertension (CHT) and 10 normals (NP) to determine whether a change in PRA reflected clinical events. Superimposed preeclampsia (PE) developed in 9 CHT and in 7, PRA decreased prior to or at the time of diagnosis. In contrast PRA rose or was unchanged in 11 of 13 with uncomplicated CHT and 9 of 10 normals in the 3rd trimester. Ualdo followed a similar pattern in that there was no rise at the end of pregnancy in CHT-PE as there was in NP or CHT.

	NP	CHT	CHT-PE
PRA (ng/ml/hr)			
2nd trimester	13.1±5.8	7.8±4.7**	12.1±5.1
3rd trimester	25.0±25.4	10.1±5.7	6.6±5.5*
Ualdo (μg/day)			
2nd trimester	133±66	89±51	74±53
3rd trimester	229±70	112±50**	67±54**

*p<.05 compared to 2nd trimester and NP;

**p<.05 compared to NP

We conclude that PRA, Ccr, and Ualdo fall in most women with CHT when PE develops. A drop in these measurements may be an early indication of the development of PE, and early serial measurement of PRA may aid in the diagnosis and treatment of patients with chronic hypertension during pregnancy.

EFFECTS OF OUBAIN ON A SIMPLE BIOLUMINESCENT ASSAY FOR THE DETECTION OF Na^+ , K^+ -ATPase INHIBITORS (CIRCULATING NATRIURETIC FACTORS). B. Todd Sitzman*, and Roberta M. O'Dell-Smith. University of New Orleans, Department of Biological Sciences, New Orleans, Louisiana.

There is considerable evidence supporting the existence of endogenous Na^+ , K^+ -ATPase inhibitors in essential hypertension and chronic renal failure. A bioluminescent assay providing a simple and highly sensitive method for the detection of these circulating natriuretic factors has been proposed (Weisberg, 1983). This proposed assay involves the incubation of Na^+ , K^+ -ATPase inhibiting factor with Na^+ , K^+ -ATPase and ATP. Residual ATP is then determined by adding luciferin/luciferase solution, resulting in a quantitatively measurable instantaneous and stable level of light (luminescence). Oubain, a known Na^+ , K^+ -ATPase inhibitor, has been used as a functional analogue of circulating natriuretic factor and the proposed assay has been modified for use in liquid scintillation systems (H^3 channel). Ideally, luminescence should have been directly proportional to Na^+ , K^+ -ATPase inhibitor present in the incubation medium; however, it has been shown that luminescence is "inversely" proportional to the level of ouabain present. This inverse relationship is linear over the range 10^{-4} to 10^{-11} M ouabain. It has been shown that ouabain does not directly inhibit the luciferin-luciferase reaction, supporting the possibility of a ouabain-ATPase complex (luciferase inhibiting) which might explain the inverse relationship between luminescence and ouabain concentration. Although the use of ouabain does not conform to the ideal assay conditions, the effects of other known Na^+ , K^+ -ATPase inhibitors (circulating natriuretic factors) on this system are being tested to determine the applicability of this simple bioluminescent assay for the detection of Na^+ , K^+ -ATPase inhibitors.

STRUCTURAL AND FUNCTIONAL CHANGES BEYOND ARTERIAL STENOSIS DURING CHRONIC ACE INHIBITION (ACEI) IN CONSCIOUS AORTIC COARCT RATS. SC Textor, L. Smith-Powell*, Renal Laboratory, City of Hope Nat. Med. Ctr., Duarte, CA

To study the effects of renal artery pressure (RAP) reduction beyond vascular stenosis during angiotensin inhibition, we studied conscious interrenal aortic coarct (Co: 0.330 mm) rats during enalapril Rx (ACEI: 6 mg/kg/d + low Na diet) or similar RAP by more severe coarct. Post-stenotic RAP was measured via the iliac artery. Plasma flow (ERPF), GFR, filtration fraction (FF), sodium excretion (FENa) and urinary n-acetylglucosaminidase (NAGA) were measured serially over four weeks. Glomerular tuft volume (Vg) after in-vivo perfusion fixation at 120 mm Hg was determined by morphometric analysis. (Mean ± sem, * p<.01 vs no Rx)

	No Rx (6)	ACEI (10)	Severe Co. (6)
RAP (mm Hg)	88±5	51±6*	62±5*
FF (%)	27±5	19±3*	-
NAGA (mU/osm)	14±5	9±2	275±40*
Vg ($\mu\text{m}^3 \times 10^+6$)	2.30±.13	1.17±.14*	0.6±.08*

GFR fell 53% during ACEI, although ERPF was preserved ($9.1 \pm .7$ vs $7.5 \pm .5$ ml/min, NS). Low FENa ($0.10 \pm .02\%$) and stable NAGA indicated normal tubular function, in contrast to severe coarct which induced tubular necrosis and glomerular atrophy. Hence, glomerular function and tuft volume in stenotic kidneys fell during ACEI over four weeks. These data demonstrate that hypoperfusion beyond vascular stenosis during ACE inhibition induces glomerular alterations not reversed by perfusion at physiologic pressures.

HIGH K DIETS PROTECT AGAINST HYPERTENSIVE INJURY TO ARTERIAL ENDOTHELIAL CELLS. Louis Tobian and Tokuichiro Sugimoto*, Univ. of Minnesota, Mpls, Mn

Two lines of evidence indicate that high K diets protect arterial endothelial cells from hypertensive damage: I. Acetylcholine (ACh) relaxes arteries by causing release of a relaxing factor from endothelial cells (EDRF). Nitroprusside (NP) relaxes arteries by direct action on smooth muscle. We obtained aortas from 9 SHRsp on 2.1% K diet & from 11 SHRsp on .75% K diet, with approx equal BPs (155 vs 156). In a bath, rings of aorta were pre-contracted with norepinephrine. In the high (2.1%) K rats, 10^{-7} ACh relaxation averaged 85% of 10^{-7} NP relaxation. In the "normal" (.75%) K rats, ACh relaxation averaged only 42% of NP relaxation, a 50% decrease ($p < .001$), indicating greatly reduced EDRF from damaged endothelial cells. In both groups average NP relaxations were almost identical. Thus, "normal" K diets permitted extensive hypertensive damage to endothelial cells, whereas high K diets prevented this damage. II. In 2 other 25-rat groups of SHRsp (BPs 209 vs 207), intimal lesions & thickening were assessed "blindly" in aorta & mesenteric arteries. This thickening is primarily the result of hypertensive injury to endothelial cells. In the high (2.1%) K group, intimal lesions & thickening averaged 54% less in the aorta & 45% less in mesenteric arteries, compared to that in the "normal" (.75%) K group ($p < .001$), indicating much less injury to endothelial cells, with intimal thickening scores equalling those of normotensive WKY rats. Thus a high K diet greatly reduces hypertensive injury to endothelial cells even when BP is not lowered. Moreover, severe renal intimal thickening is the key lesion in the malignant hypertension of blacks.

HYPERTENSION EXACERBATES THE SEVERITY OF CHRONIC HYPERNATREMIC DEHYDRATION(CHD). H.Trachtman* (Intro: B.Gauthier). Dept.Peds., SUNY-Stony Brook, Schneider Child.Hosp.of LIJMC, New Hyde Park, NY.

The blood brain barrier(BBB)is an important anatomic feature of the brain that affords protection against sudden changes in the composition of the extracellular fluid. Since acute hypertension disrupts BBB integrity, the effect of sustained hypertension on cerebral adaption to CHD was studied. Models of hypertension included adult(20 wk) SHR's and Sprague-Dawley rats with uninephrectomy and DOCA-NaCl induced blood pressure elevation. Control animals included untreated or sham-injected Sprague-Dawley rats, respectively. CHD was induced by 24hr water deprivation followed by loading with 1M NaCl for 72 hr. Brain water compartment sizes (ml/100 g dry wt) were determined at the time of death, using chloride space to indicate extracellular water.

	SHR Study(n=12)		DOCA-NaCl Study(n=12)	
	Control	Exp.	Control	Exp.
BP(mmHg)Pre-	122±5	173±4**	127±3	168±2**
S.Na ⁺ ,Final	191±3	189±2	198±3	198±2
Wt.loss(%)	21±1	19±2	21±1	21±2
Brain TTW*	363±4	327±7**	373±8	337±10**
Brain ICW*	234±7	194±6**	233±6	189±8**
Brain ECW*	127±3	134±3	141±4	148±2

*TTW-total tissue water; ICW-intracellular water; ECW-extracellular water. Results as mean ± SEM.

** $p < 0.025$, Exp. vs Control.

These results indicate that hypertension exacerbates cerebral shrinkage during CHD. This increased severity of CHD is a consequence of hypertensive injury to the BBB that permits more rapid osmotic equilibration of the ICW and ECW compartments during sustained hypertonic stress.

ENDOGENOUS DIGOXIN-LIKE MATERIAL (EDLM) LEVELS DURING THE ONTOGENY OF HYPERTENSION IN THE SHR RAT. H.Trachtman*, F.Chasalow* (Intro: B.Gauthier). Dept.of Peds., SUNY-Stony Brook, Schneider Child. Hosp. of LIJMC, New Hyde Park, NY.

A primary renal Na⁺ retaining defect with consequent release of an inhibitor of the Na⁺-K⁺-ATPase is considered important in the genesis of essential hypertension(EH). This natriuretic factor is measurable in serum since it is cross-reactive with antidigoxin antibodies in standard RIAs. To determine the role of the EDLM in EH, we measured this material at various ages in the SHR rat, an experimental model of this disease.

SHR rats (Taconic Farms) were maintained on a standard rat chow diet. Blood pressure (BP) was measured using a tail-cuff apparatus in awake, conscious animals. Sera were obtained for measurement of EDLM using a commercially-available digoxin RIA kit(Dupont)(normal:undetectable).

	Wt(g)	BP(mmHg)	Hct(%)	EDLM(ng/ml)
3 wk (n=12)	48±1	114±2	44±1	1.00±0.39
10 wk (n=9)	150±2	158±3	44±1	0.087±0.006
20 wk (n=14)	358±6	204±4	54±1*	<0.05±0.00*

Results: Mean±SEM; * $p < 0.001$, 20 wk vs 3 & 10 wk

These results indicate that EDLM is present in high levels in pre-hypertensive(3 wk) and juvenile (10 wk) SHR rats but not adult animals with sustained hypertension. While this material may be important in the development of elevated BP, it is unnecessary for maintenance of the abnormality. These findings may have relevance to attempts to detect EDLM in the sera of patients with EH.

RED CELL SODIUM-LITHIUM COUNTER-TRANSPORT AND PROXIMAL SODIUM REABSORPTION IN NORMAL AND HYPERTENSIVE MAN. Myron H. Weinberger, Jean B. Smith*, Friedrich C. Luft, Dept. Med. Ind. Univ. Indpls, IN

To examine the relationship of red cell sodium-lithium counter-transport (CTX) to renal fractional lithium and sodium excretion (FELi, FENa), we studied 13 normal (N) and 25 hypertensive (H) persons who did not differ in age. N and H were subjected to 150 mEq Na diet and 2L volume expansion with i.v. saline over 4 hr on one study day. On the next day, they underwent contraction with furosemide 40 mg for three doses and a 10 mEq Na diet over 24 hr. Urine was collected during the 4 hr saline infusion and throughout the study. Data before and after saline (mean) are below:

variable	before	after	P
MABP (N)	83	84	NS
(mmHg) (H)	106	113*	0.05
FENa (N)	0.67	1.36	0.05
(%) (H)	0.66	1.93*	0.05
FELi (N)	18.4	23.2	0.05
(%) (H)	20.0	28.1*	0.05

* (H>N, $p < 0.05$)

CTX in N (0.22) and in H (0.28) was not different ($p = 0.14$). H had exaggerated natriuresis but no inverse relationships were observed between FELi or FENa and CTX, nor were there differences between H and N before saline. The data do not suggest that H have increased proximal Na reabsorption, or that CTX reflects it.

EFFECT OF BETA-CARBOLINE AND SALT ON BLOOD PRESSURE IN RATS: A NEW "STRESS-SALT" PARADIGM. James H. Wible*, Jr., Joseph A. DiMicco*, Jana Erner*, Friedrich C. Luft, Depts. Pharmacology-Toxicology and Medicine, Ind. Univ. Indpls. IN.

Within the CNS, anxiolytic benzodiazepines bind to specific receptor sites associated with the post-synaptic GABA receptor complex. Beta-carbolines bind to the benzodiazepine receptor site but cause the opposite effect; they are anxiogenic. Administration of noreleagine (N), an anxiogenic beta-carboline, allowed us to test the hypothesis that pharmacologically induced stress coupled with a high salt intake (HS), elevates blood pressure in rats. We gave either N (2 mg/kg) or vehicle (VE) twice daily i.p. to rats (n=12) receiving either HS (8% NaCl) or low salt (LS, 0.9% NaCl) diets for 4 weeks. Mean +/- SE data (mmHg) are below:

regimen	control	after 2wks	after 4wks
N/HS	120+/-4	139+/-3	146+/-4
N/LS	121+/-4	132+/-4	134+/-4
VE/HS	122+/-3	123+/-5	122+/-5
VE/LS	122+/-3	114+/-3	121+/-4

Pressures were measured in conscious rats by tail cuff. ANOVA revealed that N/HS was different (p<0.05) from the other groups. The data suggest a synergistic effect of BC and HS (stress/salt) on blood pressure in rats. This model may have particular utility in the study of stress interactions. The mechanisms are under investigation.

ROLE OF THROMBOXANE IN SYSTEMIC AND RENAL RESPONSES TO ANGIOTENSIN. C.S. Wilcox and W.J. Welch. Div. of Nephrology and Htn., Univ. of Florida, Gainesville, FL.

Angiotensin II (AII) releases vasodilator prostaglandins (PG), which offset its vasoconstrictor action. We assessed the role of a vascular and mesangial cell constricting PG, thromboxane (Tx), in modulation of response to AII in anesthetized rats. Intravenous AII (500 ng/kg/min) increased (p<0.01) MAP by 44±5 mmHg and increased (p<0.05) renal excretion of TxB₂ by 20%. Pretreatment with a Tx receptor antagonist (SQ-29,548; SQ, 8 mg/kg; n=8) attenuated (p<0.001) the rise in MAP to 12±2 mmHg. GFR was reduced by AII in vehicle-treated rats (1.8±0.3 to 1.3±0.3 ml/min; p<0.05) but did not change consistently after SQ (2.3±0.2 to 2.1±0.1; ns). Since RPF was reduced similarly by AII in both groups, the AII-induced increases in filtration fraction (FF) were greater (p<0.01) in rats receiving SQ (27±2 to 41±4%) compared to vehicle (26±4 to 28±3%). In another protocol, the pressor responses to injections of AII (1 to 300 ng/kg) or phenylephrine (1 to 300 µg/kg) were contrasted in rats pretreated with vehicle or SQ. Whereas the BP-log dose curve for AII was shifted to the right by SQ (p<0.01), which blocked 40% of the pressor responses, there was no effect of SQ on the pressor responses to phenylephrine. Conclusions: 1) AII releases Tx from the kidney which counteracts the rise in FF; this is consistent with a mesangial action for Tx; 2) Tx mediates 40-70% of the pressor response to infused AII; and 3) Tx mediation of AII pressor response is specific and independent of the increase in BP.

SERUM PARATHYROID HORMONE (PTH) LEVEL AND BLOOD PRESSURE IN HUMANS. E.W. Young, R. Brown, A.M. Dolney, D.A. McCarron, C.D. Morris, Oregon Health Sciences University, Portland, Oregon.

Elevated serum PTH levels have been reported in essential hypertension. PTH has vasodilator properties and is essential to the regulation of calcium (Ca) homeostasis; thus the increased PTH levels may be related to BP control. Using a two-site immunochemiluminometric assay capable of detecting changes in PTH within the physiologic range, we sought to define the relation of serum PTH to BP before and during interventions designed to lower BP by augmenting Ca²⁺ intake. The effects of dietary Ca (Dca 1500 mg/d), supplemental Ca (SCa 1000 mg/d), and placebo (P) were evaluated for 12 weeks in hypertensive (137±8/94±5, n=82) and normotensive (120±10/76±9, n=45) subjects. Baseline serum PTH level was positively correlated with supine systolic (r=.2; p=.016), supine diastolic (r=.16; p=.05), standing systolic (r=.19; p=.02), and standing diastolic (r=.18; p=.027) pressures. Baseline urinary Ca excretion (normalized for creatinine excretion) was inversely correlated with baseline serum PTH levels for all subjects (r=-.22; p=.02), suggesting an appropriate renal response. SCa and Dca had significantly different effects on serum PTH concentration (p<.001), as PTH was suppressed by SCa but not Dca. The BP response to SCa was significantly worse (p=.005) in subjects with higher baseline PTH values. In contrast, BP response to Dca was unrelated to levels of PTH. We conclude that PTH levels within the physiologic range correlated directly with BP, and the type of Ca intervention had different effects on PTH that may predict BP response.

AUTOMATIC INDIRECT AMBULATORY BLOOD PRESSURE MONITORING IN THE DIAGNOSIS OF HYPERTENSION. Prince K. Zachariah*, Sheldon G. Sheps*, Duane M. Ilstrup*, Cynthia R. Long*, Christopher A. Carlson*, and Daniel J. Wilson. Mayo Clinic, Div. of Hypertension and Dept. of Statistics, Rochester, Minnesota.

We analyzed results of 24-hr. ambulatory indirect blood pressure determinations (ABPM) obtained in 128 untreated hypertensive patients (pts) in an effort to develop criteria for diagnosing borderline (BH) or sustained (SH) hypertension. Pts were classified as having BH or SH on the basis of the mean of 12 office diastolic blood pressures (DBP). A total of 103 pts had SH with DBP>90 mmHg, while 25 pts had BH with DBP<90 mmHg. ABPMs were obtained with a Del Mar P3 unit. Results are shown [systolic (S) and diastolic (D)] below:

	SH (n=103)		BH (n=25)	
	S	D	S	D
Office	149+15	99+6	135+13	87+2
Awake	144+16*	96+7*	132+14	88+4
24-Hr.	140+16*	93+7*	127+14*	84+4**

*p<0.0001 and **p<0.0005 compared with office BP

The mean ADBP was >90 mmHg in 91 pts during awake hrs. and 68 pts over 24 hrs. Awake and 24-hr. DBP loads, defined as the % of ABPM DBP >90 mmHg, were higher in pts with SH (69±22%, 58±21%, respectively) vs. BH (42±16%, 33±13%, respectively). Observed DBP loads were higher than those reported for normals (<15%). Thus, mean awake and 24-hr. ABPM and DBP loads are higher in pts with SH vs. BH; and ABPM and DBP load may aid in differentiating between BH and SH.

EFFECT OF MACRONUTRIENTS ON BP IN SHR. M. Zein, J. Areas, C. Garcia, J. Knapka, H. Preuss, Georgetown Univ. Med. Ctr., Dept. Med., Washington, DC.

For one month, we placed 30 SHR on 5 diets each containing different proportion of macronutrients and followed BP. The basic control diet (I) derived energy 1/3 from fat, 1/3 from protein and 1/3 from CHO (sucrose). The next 2 diets (II, III) were relatively high in CHO (+50%) with the calories balanced by alterations in proteins and fats respectively. The last 2 diets (IV, V) were low in CHO (+11%) and high in proteins and fats respectively. To keep mineral intake constant, the minerals added were kept constant for caloric equivalents, because rats tend to be isocaloric. We found the increase in body weight gain to be fairly constant over the 4 wks of study in all groups; however, the average BP rose significantly in groups II, III compared to Group I by the second week of diet. (218 and 214 mm Hg vs 195 mm Hg at 4 wks, $p < .01$). The differences were not significant in groups IV and V (199 and 202 mm Hg). In 6 additional SHR, replacement of sucrose by glucose in diet II also caused a significant increase in BP (222 mm Hg after 4 wks, $p < .01$). Na, K, Mg, Ca and P urinary and fecal excretion were essentially similar among all groups. We conclude that the proportion of macronutrients in the diet can influence the BP of SHR. High CHO ingestion augments BP and this effect is independent of whether the balance in calories is derived from protein or fat exclusion. Glucose raises BP as effectively as sucrose. Accordingly, the ingestion of a high proportion of CHO in the diet may play a role in hypertension.

INDOMETHACIN (IND) STIMULATES THE PROLIFERATION OF RAT CULTURED MESANGIAL CELLS (MD). Masaaki Arakawa†, Kazukiyo Yoshida†, Kazuei Narita†, Shoji Kagami†, Takashi Oite† and Fujio Shimizu* (intr. by F. Marumo). Niigata Univ. Med. School, Dept. of Med. (II) and Immunol. of Nephrol. Inst., Niigata, Japan.

It is well known that IND has a favorable effect on proteinuria. One explanation is that IND controls glomerular eicosanoid synthesis, leading to alteration in response of intrinsic glomerular cells. This work was undertaken to elucidate the regulatory effect of humoral factors such as prostaglandins (PGs) and interleukin-1 (IL-1) on MC in vitro system using IND.

Glomeruli were obtained from the kidneys of Wistar rats by sieving method. Primary MC were obtained as outgrowths from isolated glomeruli cultured for more than 4 weeks. Assay for cell proliferation was measured using ^3H -thymidine. IND had an activity of enhancing the DNA synthesis of MC. Then, we compared the effect of IND with that of purified human IL-1. IND could enhance the proliferation of MC at any concentration of fetal bovine serum (FBS) (20, 5, 1.25%), whereas IL-1 could stimulate it only in low FBS concentration (1.25%). PGE₂ concentration of culture supernatant was higher according to serum concentration without IND, whereas remarkable reduction of the PGE₂ synthesis was observed in the presence of IND regardless of FBS concentration.

We concluded that the effect of IND was mediated by the suppression of endogenous PGE₂ production. Local release by MC of growth factors and PGs may be an important determinant associated with glomerular damage.

IMMUNOLOGY/PATHOLOGY— BASIC OR EXPERIMENTAL

INHIBITION OF RAT GLOMERULAR VISCERAL EPITHELIAL CELL (GEC) PROLIFERATION BY HEPARIN (H). S. Adler, New York Medical College, Valhalla, NY.

Proliferation of GEC is an important feature of several types of human and experimental glomerulonephritis but little is known about the factors controlling their growth. To determine whether glycosaminoglycans (GAG's) present in the intercellular matrix might influence GEC growth we exposed proliferating GEC in culture to several GAG's. Cloned lines of rat GEC were established and studied during their 10-15th passage. H inhibited growth of six clones studied in a dose dependent manner with an IC₅₀ < 20 μg/ml in four. Cytotoxic effects of H were not detected by examination of morphology or trypan blue exclusion and cells re-grew normally following removal of H. Other GAG's (chondroitin SO₄, dermatan SO₄, heparan SO₄ and hyaluronic acid) had no effect in this system. De-N-sulfated H, lacking anticoagulant activity, was not inhibitory while a low molecular weight H possessing anticoagulant activity was. Comparison of high (HAC) and low (LAC) anticoagulant activity H revealed a loss of inhibitory activity with LACH (IC₅₀ = 10 μg/ml for HACH; > 200 μg/ml for LACH). Increasing the amount of serum in the growth medium (up to 20%) resulted in a linear increase in H inhibition of GEC growth ($r = 0.56$; $p < 0.001$).

These studies demonstrate that H inhibits GEC growth by its anticoagulant effect and suggest that it does so by interacting with a serum factor. This is in contrast to prior reports that H inhibition of mesangial and vascular smooth muscle cell growth is independent of anticoagulant activity. A better understanding of the control of GEC proliferation may yield new therapeutic stra-

ANTI-LAMININ AUTOANTIBODIES AND MERCURY-INDUCED MEMBRANOUS GLOMERULOPATHY. Jan Aten*, Jan A. Bruijn*, Aletta Veninga*, Emile de Heer*, and Jan J. Weening* (intr. by Ph. J. Hoedemaeker). Dept. Pathology, Univ. of Leiden, The Netherlands.

HgCl₂ can induce a transient autoimmune syndrome in rats, with development of glomerulonephritis (GN) and proteinuria (P). The pathogenesis of GN was studied in DZB rats, given HgCl₂ s.c. at days 1, 3, 5, 7 and 9. Transient nephrotic range P occurred in 53% of the animals, reaching maxima at day 17. Direct immunofluorescence (IF) showed IgG_{2a} in a granular pattern along GBM at day 15 in all rats and C9 only in rats with P. Electron microscopy (EM) revealed subepithelial aggregates. Eluates from day 15 glomeruli bound in a linear pattern along GBM and TEM in normal kidney, as shown by indirect IF; at indirect immuno EM, binding was found to be confined to the cell membranes of glomerular and tubular epithelium adjacent to the GBM and TEM, respectively. Eluates and sera from day 15 were reactive against collagenase-digested rat GBM, and, more specifically, against laminin, but did not bind to collagen IV, fibronectin or renal tubular epithelial extract (RTE), as determined by ELISA. I.v. transfer of eluates induced variable degrees of P, up to 100 mg/24h after 5 days; IF showed a fine granular distribution of IgG_{2a}. Eluates could inhibit attachment of glomerular epithelial cells in vitro and induce cytotoxicity, as shown by increased release of carboxyfluorescein.

A pathogenetic role is hypothesized in this model for autoantibodies with reactivity against glomerular epithelium, involving specificity for a cell-binding epitope on the laminin molecule.

DEGRADATION OF GLOMERULAR BASEMENT MEMBRANE (GBM) BY PURIFIED MAMMALIAN NEUTRAL METALLOPROTEINASES. William H. Baricos, Gillian Murphy,* Youwen Zhou,* Hung H. Nguyen,* and Sudhir V. Shah, Depts. of Biochem., Med., and Cell Physiol., Tulane Medical School, New Orleans, LA and Strangeways Research Laboratory, Cambridge, U.K.

Neutral metalloproteinases (NMP) degrade components of the extracellular matrix and may play an important role in proteinuria by degrading GBM. In the present study we have examined the effect of the three major NMP, collagenase, gelatinase and stromolysin, on GBM degradation, *in vitro*. ³H-labelled GBM was incubated at pH 7.5 with the purified enzymes for 24 hours at 37°C and GBM degradation expressed as percent radioactivity released into the supernatant. Our data (mean ± SEM) show:

ADDITION	GBM DEGRADATION % release
None	2 ± 0.15(7)
Collagenase (2U)	6 ± 0.59(3)
Gelatinase (2U)	46 ± 2.2 (4)
Stromolysin (2U)	59 ± 5.8 (4)

GBM degradation by gelatinase and stromolysin was: dose dependent (0.02-2.0 units); exhibited a broad pH optimum (pH 6-8.5); and was completely inhibited (>95%) by the metal chelator 1,10 phenanthroline (2mM). Collagenase (2U) did not significantly enhance the GBM degradation by either gelatinase (0.2U) or stromolysin (0.2U) alone. The presence of NMP in neutrophils and glomeruli is well documented. Our results indicate that gelatinase and/or stromolysin may be the major NMP involved in GBM degradation.

AMELIORATION OF HABU SNAKE VENOM (HSV)-INDUCED GLOMERULAR INJURY AND CELL PROLIFERATION BY SULINDAC: POTENTIAL ROLE FOR PLATELET CATIONIC PROTEINS (PCP). J.L. Barnes, Depts. of Pathol., Brown Univ. & Rhode Island Hosp., Providence, R.I.

Platelets have been implicated as mediators of mesangial cell proliferation. Of interest is a potential role for secreted platelet cationic proteins (PCP), some of which are growth factors. This study examines the effect of Sulindac, an inhibitor of platelet thromboxane A₂ generation, on the development of glomerular cystic and proliferative lesions and localization of PCP following HSV. Uninephrectomized rats received Sulindac (60 mg/kg/d) or vehicle, prior to and following HSV (2 mg/kg, i.v.). Glomerular cysts, proliferative nodules and mixed (cystic plus proliferative) lesions were quantitated and PCP localization was examined 48h following HSV. Sulindac substantially reduced the total number of glomerular lesions and preferentially reduced proliferative lesions when compared to controls:

Lesion	Vehicle(N=14)	Sulindac(N=14)	p value
cysts	7.9±2.0%*	5.5±1.4%	NS
nodules	5.8±1.1%	2.0±0.8%	<0.01
mixed	17.3±3.6%	8.6±3.0%	<0.01
total	31.0±6.5%	16.1±5.0%	<0.02

* Percent of all glomeruli examined.

PCP localized in glomerular lesions in both groups and paralleled the severity of disease. Overall intensity of PCP staining was less in Sulindac treated rats. Sulindac unaltered RBF and GFR prior to HSV ruling out hemodynamic factors. The concomitant localization of PCP and glomerular lesions and amelioration by antiplatelet therapy supports a role for PCP in proliferative glomerular disease.

SURFACE CHARGE DISTRIBUTION IS AN IMPORTANT DETERMINANT OF ANTIGEN BINDING IN THE GLOMERULAR CAPILLARY WALL. Stephen Batsford* Michael Mihatsch*, Arnold Vogt*(intr. by Bernd Sterzel). Dept. of Immunology, Freiburg, F.R.G., Institute of Pathology, Basel, Switzerland

The concept of charge dependent deposition of antigens/immune complexes(IC) is extended by the demonstration that moieties of net anionic charge, but with discrete positive regions exhibit affinity for the glomerular basement membrane(GBM). A charge hybrid(polar) molecule was constructed by covalently coupling human serum albumin(HSA) to small(17 and 20 residue) poly-L-lysine(PLL)chains. Immunofluorescence revealed binding of HSA-PLL to glomerular structures after i.v. injection(1mg/100g body weight), HSA-PLL(20) was intenser than HSA-PLL(17). Radioisotopic quantitation(on isolated glomeruli) showed that HSA-PLL(20) was effectively eliminated by 72h. Uptake of HSA-PLL was a function of the number of lysine residues, binding of HSA-PLL(20) was 2.5 times higher than HSA-PLL(17) (P<0.01). Prior injection of a small polycation(PEI 1200) reduced uptake of HSA-PLL(20) to 25%, demonstrating the charge based nature of the interaction. Administration of HSA-PLL followed(15 min) by anti-HSA antibody produced IC formation in the capillary wall, giving rise to a granular immunofluorescence pattern and discrete subendothelial and subepithelial deposits by electronmicroscopy. These immune complexes persisted for several weeks. Molecules with polar structure do occur naturally and may be able to initiate IC formation, in addition to highly cationic antigens.

INVESTIGATION OF THE ROLE OF GP 330 IN THE PATHOGENESIS OF HEYMANN NEPHRITIS (HN). M. Behar, A. Katz and M. Silverman, Membrane Biology Group, Univ. of Toronto, Toronto, Ont.

There is evidence that a brush border membrane (BBM) constituent gp 330 is nephritogenic (J. Exp. Med. 157: 667, 1983). But controversy exists as to whether gp 330 is the sole pathogenic antigen in HN. We have studied the immunopathology of HN in Sprague-Dawley (SD) and Brown Norway (BN) rats. HN is not inducible in the BN strain (J. Immunol. 155: 875, 1975). Following a single injection of emulsified Fx1A circulating antibodies (Ab) against BBM were detected at time intervals from 0 - 12 weeks using the western blot technique. At sacrifice glomerular deposits were assessed by indirect immunofluorescence (IIF) and electron microscopy (EM). Within 3 weeks both SD and BN rats show a repertoire of circulating Ab to BBM including anti gp 330. By 12 weeks all SD but none of the BN rats showed proteinuria. But small subepithelial deposits were detected in glomeruli of both species. Passive HN was studied by implanting in a nude rat a solid tumor secreting a rat monoclonal Ab obtained through the hybridoma technique from the spleen of a rat with active HN. Large amounts of circulating anti gp 330 were detected in the serum. EM and immuno EM showed small subepithelial deposits and IIF showed segmental punctuate staining in the glomeruli. The nude rats never developed proteinuria. Our results imply that anti gp 330 Ab may play a role in the pathogenesis of HN but its presence does not appear to be sufficient to cause proteinuria.

TUMOR NECROSIS FACTOR (TNF) AS A NEW MEDIATOR OF GLOMERULAR INJURY. Tullio Bertani*, Mauro Abbate*, Carla Zoja*, Pietro Ghezzi* and Giuseppe Remuzzi* (intr. by M.J. Dunn). Mario Negri Institute for Pharmacological Research, Bergamo and Milan - Italy.

TNF is a polypeptide hormone produced by activated macrophages, which has been implicated in neutrophil and cultured endothelial cell activation, tumor cell lysis and endotoxin shock. Because macrophages participate in immune glomerulonephritis we assessed whether TNF causes glomerular damage. 18 rabbits were given human recombinant TNF (kind gift of Dr. L.S. Lin, Cetus Co., Emeryville, CA) (0.08, 0.8, 8 µg/kg/h) as a continuous 5 hour i.v. infusion, and killed at hour 5 and 24 from the beginning. All rabbits given 0.8 and 8 µg/kg/h TNF developed anemia (Ht values - % decrease - at hour 5: 0.8 µg/kg/h, 15%; 8 µg/kg/h, 17%), leukopenia (leucocyte count - % decrease - at hour 5: 0.8 µg/kg/h, 47%; 8 µg/kg/h, 63%) and thrombocytopenia (platelet count - % decrease - at hour 5: 0.8 µg/kg/h, 45%; 8 µg/kg/h, 55%). Rabbits given 8 µg/kg/h also had renal failure (serum creatinine from 0.95±0.13 to 1.72±0.55 mg/dl). By light microscopy (LM) polymorphonuclear cell infiltration was the prominent finding at hour 5 in animals given 0.8 and 8 µg/kg/h. By electron microscopy (EM) in the same animals a dose-dependent glomerular endothelial cell damage was detected at hour 5 with mild mesangial and epithelial changes. Leukocyte and platelet infiltrates were also documented at hour 5. At hour 24 LM and EM findings were unremarkable. Thus TNF causes glomerular injury and may be regarded as a new mediator of macrophage-dependent damage in glomerulonephritis.

IMMUNE COMPLEXES (IC) FORMED IN VIVO IN PRIMATES ARE CLEARED BY THE ERYTHROCYTE-IC CLEARING MECHANISM. D Birmingham*, FG Cosio, LA Hebert, Ohio State Univ, Dept of Medicine, Columbus, OH.

Erythrocyte (E) of man and other primates bear complement receptors type 1 (CR1) which permit opsonized IC to bind to E. Infusion of preformed IC into nonhuman primates has revealed the existence of an E-IC clearing mechanism in which IC bind to E-CR1 (J Clin Invest 77:82,1986). The present studies determined if IC assembled in vivo are also handled by the E-IC clearing mechanism. Studies were carried out in 6 cynomolgus monkeys. The E-CR1 levels in these monkeys ranged from 35 to 3200 CR1/erythrocyte. ¹²⁵I-BGG was infused intravenously before (N=4) and after (N=6) immunization with BGG. Prior to immunization, IV infusion of ¹²⁵I-BGG resulted in negligible binding of ¹²⁵I-BGG to E (mean 8% ± 1 SE). However, after immunization with BGG, infusion of ¹²⁵I-BGG resulted in IC formation (> 40S) and binding of IC to E, within 2 min of the ¹²⁵I-BGG infusion. Peak binding of IC to E was 11 to 32% and was proportional to log CR1/E. In 3 immunized animals E-CR1 were enumerated before and 4 hr after infusion of a large antigen load. E-CR1 decreased in each animal (mean 40% ± 22 SE). In 2 animals, catheters were placed in the hepatic and renal veins to assess for uptake of IC bound vs nonbound to E. These experiments showed that IC bound to E did not deposit in kidney but were removed from E by liver, just as we found in the studies using preformed IC. Conclusion: These studies provide further evidence that, in primates, the E-IC clearing mechanism could be an important defense against IC-mediated disease.

CATIONIC STREPTOCOCCAL PROTEINASE AND HUMAN RENAL BASEMENT MEMBRANE SHARE EPITOPES. Martin Bohus*, Stephen Batsford*, and Arnold Vogt*(Intr. by Helmut Rennke). Dept. Immunology, Institute of Medical Microbiology; D-7800 Freiburg, F.R.G.

Group A Streptococci produce an extracellular cationic protein (proteinase), which is a putative nephritogenic antigen in acute poststreptococcal glomerulonephritis (APSGN) (Vogt et al., Clin. Nephrol. 20:271-279, 1983). Proteinase specific polyclonal and monoclonal antibodies were prepared in rabbits and mice respectively. These antibodies recognized the active proteinase as well as its precursor (zymogen) by immunoblotting (30 and 43 Kd respectively). In addition, bands were seen with soluble preparations (collagenase digest, acetic acid, guanidine-HCl, and 0.5M NaCl extracts) of human and rat GBM as well as with the EHS mouse sarcoma. Sera from patients convalescing from APSGN produced the same blotting pattern with GBM and EHS antigens as the anti-proteinase-antiserum. When tested against anti-proteinase-antiserum in an Ouchterlony plate, proteinase and EHS antigen revealed common determinants. The glomeruli of rabbits immunized with proteinase showed deposits of IgG in a granular pattern by immunofluorescence. The eluted antibody reacted with GBM and EHS antigen, as well as with the proteinase. When Wistar rats were given the rabbit anti-proteinase antiserum i.v. (1ml/100g body weight), immunofluorescence revealed deposition of rat antibody along the capillary wall in a granular/linear pattern. These results indicate that streptococcal proteinase and GBM tissue components share epitopes. This could be an additional mechanism in APSGN causing tissue injury.

RENAL INVOLVEMENT IN MURINE CHRONIC CRAFT-VERSUS-HOST DISEASE (GVHD). J.A. Bruijn*, P.C.W. Hogendoorn*, W.E. Corver*, E.H. van Elven*, Ph.J. Hoedemaeker, G.J. Fleuren.* University Institute of Pathology, Leiden and S.S.D.Z. Delft, The Netherlands.

We studied renal pathology in murine chronic GVHD, a model for human systemic lupus erythematosus. GVHD was induced by 4 i.v. injections of lymphocytes from DBA/2 donor mice into (C57Bl.10xDBA/2)F1 hybrids at 3-4 day intervals. Severe renal disease had developed in all recipients after 12 to 14 weeks. Significant albuminuria, hypoalbuminemia and oedema as well as decreased creatinin clearance and uremia were observed. In the kidneys of diseased animals membranous alterations with subepithelial electron-dense deposits and spike formation, as well as glomerular mesangial, segmental and diffuse proliferations, and in the most severe cases global glomerular sclerosis developed. These lesions are also typical of human lupus nephritis and can be divided according to the WHO classification. From the second week on, a linear deposition of IgG and IgM along the glomerular capillary wall was seen. Around week 5, this changed to a more granular pattern especially for IgG. Using indirect immunofluorescence and ELISA techniques, autoantibodies were found in sera and kidney eluates directed against renal tubular epithelial antigens (RTE), as well as against basement membrane components laminin and type IV collagen. We conclude, that GVHD offers a suitable model for studies on lupus nephritis. The pathogenetic role of anti-RTE and anti-basement membrane autoantibodies demands further study.

STREPTOZOTOCIN INDUCES MHC EXPRESSION IN KIDNEY BY INDUCING T CELLS TO RELEASE GAMMA INTERFERON. S. Cockfield, J. Urmson, P.F. Halloran, University of Alberta, Edmonton, Alberta, Canada.

The administration of multiple low doses of streptozotocin (STZ) produces diabetes mellitus in susceptible mouse strains. We investigated the effects of STZ on MHC expression in kidney and other tissues using histologic techniques, RIA on tissue homogenates, and specific mRNA levels. STZ in doses of 40 mg./kg./d ip for 5 days produced increased levels of both Class I and Class II MHC products in kidney with a peak effect at 14-21 days followed by a gradual return toward baseline values. Kidney tissue sections revealed parallel increases in tubular staining of Class I and II antigens. Male and female mice were affected equally and the induction occurred independently of the hyperglycemic effect. In general, changes in liver, heart and pancreas MHC expression paralleled those seen in kidney. Renal mRNA levels correlated closely with MHC product expression as measured by RIA (Class I $r=.87$, $p<.001$; Class II $r=.93$, $p<.001$) suggesting that control is likely at the transcriptional level. The mechanism of the diabetogenic effect of multiple low doses of STZ is not known, but the MHC changes appear to be due to T cell stimulation: nude mice failed to develop a significant increase in MHC products. The increased expression was blocked in a dose dependent fashion by neutralizing mAb against murine interferon gamma or by cyclosporine. We conclude that STZ stimulates T cells to release lymphokines, including interferon gamma, which massively increases MHC expression in many tissues at the transcriptional level.

THE INFLUENCE OF NON H-2 GENES ON MURINE APOFERRITIN INDUCED IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN) IN H-2^d MICE. BJ Connor*, PA Frymoyer, AH Tatum*, J Gavalchin*. SUNY-Health Sci. Ctr., Dept. of Med. and Pathol., Syracuse, NY.

Previous work with apoferritin ICGN in H-2 congenic mice demonstrated that H-2^d mice were the most susceptible (KI 31:319). The role of non H-2 genes in ICGN was studied in 4 strains of H-2^d mice with different non H-2 genes. B10.D2, Balb/c, NZB, and DBA/2J mice were injected with 4 mg apoferritin IP qd for 28 days. B10.D2 and Balb/c mice developed proliferative and crescentic ICGN. NZB mice developed proliferative and crescentic ICGN with wire loop lesions suggestive of lupus. DBA/2J mice developed only minimal mesangial proliferation without crescents or necrosis. Electron microscopy showed subepithelial and mesangial deposits in B10.D2; moderate mesangial deposits in Balb/c; marked mesangial, subendothelial and subepithelial deposits in NZB. Immunofluorescence demonstrated the presence of IgG, IgM, C3 and apoferritin in these deposits. The DBA/2J mice had only minimal mesangial deposits. Control mice had no renal lesions. The NZB mice developed significantly elevated anti-double stranded DNA antibodies. Anti-apoferritin antibody levels correlated with severity of disease. These experiments demonstrate that non H-2 genes alter the H-2^d determined disease susceptibility seen in H-2 congenic mice. NZB genes can alter the disease so that it resembles lupus and DBA/2J genes can substantially ameliorate the disease.

GLOMERULAR IMMUNE COMPLEXES (IC) FORMATION: THE ROLE OF ANTIBODY (Ab) PRECIPITATING CHARACTERISTICS. FG Cosio, AP Bakaletz*, JD Mahan. Ohio State University, Departments of Medicine and Pediatrics, Columbus, Ohio.

It has been previously suggested that precipitating (P) Ab are more nephritogenic than nonprecipitating (NP) Ab but this hypothesis has not been tested critically. Herein we evaluated the formation of glomerular IC in rats injected IV with an antigen (DNP-Gelatin, DNP-GL) which binds to glomerular mesangium. 2 hr later, we infused either P or NP mouse monoclonal anti-DNP Ab. Controls received either DNP-GL or Ab alone. We demonstrated previously that P anti-DNP Ab (an IgG1) and NP anti-DNP Ab (an IgG2b) have the same isoelectric point and affinity (J Imm 138:2587,1987). Kidney biopsies were done 2, 4, 24 and 48 hr after injection of Ab. By light microscopy there were no significant abnormalities. By immunoperoxidase, P Ab was present in the glomerular mesangium up to 48 hr after injection. By contrast, NP Ab was present after 6 hr but absent after 24 hr. By electron microscopy, glomeruli in both groups of rats demonstrated subendothelial deposits and increased GBM density. Utilizing ¹²⁵I DNP-GL we demonstrated that the failure of NP Ab to persist in mesangium was not related to removal of antigen from the mesangium. Conclusion: Both P and NP Ab can form electron dense deposits in glomeruli. However, IC formed with NP Ab may be less nephritogenic because NP Ab is more rapidly removed from the mesangium.

NEUTROPHIL (PMN)-DERIVED ELASTASE (E) MEDIATES GLOMERULAR INJURY IN VIVO. W.G. Couser, M. Vissers*, S. Klebanoff*, C.E. Alpers, and R.J. Johnson. Univ. of WA, Seattle, WA, and Univ. of MI, Ann Arbor, MI.

Based on the ability to digest GBM in vitro proteases are presumed to participate in PMN-mediated glomerular injury. Most potent in digesting GBM is E which is present in PMNs, monocytes and platelets and degrades collagen, laminin and fibronectin. However, the effects of proteases in physiologic concentrations on glomerular function and morphology have never been established in vivo. We studied purified human PMN-derived E (MW 30,000, PI > 8.5) perfused ex vivo into the renal artery of rats in concentrations of 10-50 µg. Controls received E inactivated by MSAAPV-CK but of equivalent PI (E-I). Both E and E-I bound diffusely to the glomerular capillary wall in equivalent amounts as detected by antibody to E. However, on day 1 rats perfused with E > 25 µg had an immediate and persistent increase in urine protein excretion (192 ± 13 mg/day, n=13; E-I 19 ± 2, n=6, $p < .001$) but had no increase in urinary OH-proline excretion (E: .14 ± .06 mg/mg creatinine, E-I: .16 ± 0.1, normal .15 ± 0.9, $p > .2$). E perfused rats had no significant glomerular histologic changes, and exhibited only focal podocyte effacement without detectable alterations in the GBM by EM.

We conclude that E is a potent mediator of glomerular injury in vivo, its effect is charge-independent and may not involve degradation of GBM collagen. Thus proteases as well as oxidants can contribute independently to inflammatory cell-mediated glomerular injury.

IN VIVO TURNOVER OF [³⁵S]-LABELLED PROTEOGLYCANS IN NORMAL RAT GLOMERULI. M. Davies, L.A. Beavan, R. Mason, J. Couchman and M. Williams (Intr. by R.B. Sterzel). Univ. of Wales College of Med., Dept. of Renal Med., Cardiff, Wales and Charing Cross Hospital, London, U.K.

The turnover rate of glomerular proteoglycans (PG) and glycosaminoglycans (GAG) in the normal rat was investigated. Animals were injected I/P with 2 doses of 1 mCi Na₂³⁵SO₄/100 g body weight at t=-7 h and t=-3.5 h. At t=0 the animals were pulse chased and labelled kidneys removed at different times up to t=163 h. Prior to removal the kidneys were perfused with cetylpyridinium chloride, a procedure which enhanced the recovery of glomerular PG 3-fold. Glomeruli were isolated and analysed by a) papain/NaOH to release total GAG, portions of which were treated with either chondroitinase ABC or nitrous acid to remove chondroitin sulphate (CS) and heparan sulphate (HS) respectively, b) extraction under dissociative conditions followed by immuno-precipitation with an antiserum which reacts specifically with glomerular basement membrane (GBM) HSPG and c) light microscopy autoradiography. The turnover of total GAG was biphasic with t_{1/2} of 19 h and 60 h respectively. The HSPG (estimated chemically) also turned over at a similar rate but the CSPG was slower. The turnover of GBM HSPG was also biphasic and even more rapid than the general pool of glomerular HSPG (t_{1/2} of 5 h and 20 h). The disappearance of grains within the glomerulus was consistent with the above data.

These results imply that the turnover of newly synthesized glomerular PG in normal rats is rapid.

EFFICACY OF PROTEASES IN REDUCING PROTEINURIA AND DEPOSITS IN GLOMERULONEPHRITIS INDUCED BY CATIONIC ANTIGEN IS IMPROVED BY TARGETING WITH AVIDIN A. S. N. Emancipator, R. B. White* and M. E. Lamm. Case Western Reserve University, Cleveland, OH.

Administration of proteases to rats and mice can ameliorate experimental glomerulonephritis. To determine if avidin A (Av), a naturally cationic protein which binds to glomeruli in a distribution similar to cationic bovine gamma globulin (cBGG), can target biotinylated proteases (bP) to the site of immune deposits, we induced a membranous glomerulopathy in rats with cBGG. Before treatment, there were no differences in proteinuria among the groups in each experiment. On d 13-17, some rats received 1 mg/d Av iv; rats later received a mixture of 2 mg chymopapain and 1 mg subtilisin ip bid (nP), biotinylated proteases at the same doses (bP), or saline. In the first experiment, 7 rats given Av+bP had 17±13 mg proteinuria/d after treatment, significantly less than the 39±9 or 38±13 mg/d in 7 rats given Av+nP or 4 rats given bP protease alone, respectively (p<0.01); all treatment groups had less proteinuria than the 51±10 mg/d in the 4 rats given saline. In a second experiment with more severe nephritis, 11 rats given Av+bP excreted 55±6 mg proteinuria/d versus 76±11 mg/d in 11 rats given bP alone and 62±7 mg/d in 10 rats given Av+nP; 9 rats given saline had 104±15 mg/d and 5 rats given Av+saline had 84±18 mg protein/d (p<0.01). Immunofluorescent deposits were reduced in rats given Av+bP, but the difference was significant only in the first experiment. We conclude that Av targets bP to glomerular capillaries, improving the efficacy of protease therapy of membranous nephropathy. No toxicity was observed in any treated rat.

INTERSTITIAL NEPHRITIS INDUCED BY PROTEIN OVERLOAD PROTEINURIA. Allison Eddy, Lori McCulloch*, and Jennifer Adams*. The Hospital for Sick Children, Toronto, Ontario.

Proteinuria induced in rats by several immunologic and chemical mechanisms is associated with the development of tubulointerstitial nephritis (TIN). To investigate the possibility that proteinuria participates in the pathogenesis of TIN, we studied the renal interstitium in a model of protein overload proteinuria. Following uninephrectomy, rats received daily i.p. injections of 1.0 gm of bovine serum albumin (BSA) or saline (controls) until sacrifice at 1, 2, 4, or 7 wks. Sections of frozen renal tissue were stained with a panel of anti-rat monoclonal antibodies [OX19-T-cells; W3/25-T-helper cells and macrophages (Mo); OX8-T-cytotoxic cells; OX42-Mo; Ia-MHC class II antigens; RCLA-all marrow cells] and positive TI cells were quantitated by epifluorescence microscopy.

BSA rats developed proteinuria with mean rat urinary albumin levels at 1, 2, 3, and 6 wks of 35.6±21.8, 97.2±46.1, 63.6±40.8, and 58.6±24.4 mg/24 hr (controls = 0.17±0.16 mg). BSA was detectable in the plasma with mean values of 26.8±3.8, 27.8±2.7, 20.3±6.2, and 7.0±1.1 mg/ml (controls = 0.3±0.04 mg) at 1, 2, 4, and 7 wks. Plasma creatinine was not elevated. A significant mononuclear cell infiltrate was present in the interstitium of BSA rats at all periods.

Antibody	Tubulointerstitial Cells (+cells/1000 TI cells)**				
	Controls	Wk 1	Wk 2	Wk 4	Wk 7
OX19	9.8	14.5	54.0*	37.0*	38.8*
W3/25	86.8	128.3*	197.5*	142.3*	124.8*
OX8	7.1	9.0	37.8*	32.3*	30.5*
OX42	15.9	75.0*	92.5*	92.0*	91.5*
Ia	23.0	57.8*	98.0*	68.0*	72.8*
RCLA	67.4	120.3*	164.3*	105.8*	160.0*

*p<.01,**Results expressed as group mean (n=4 BSA, n=8 control). In BSA rats, C3 and C5b-9 neoantigens but not IgG were present along the luminal border of many tubular epithelial cells. These results suggest that proteinuria may play a role in the pathogenesis of TI injury.

ANTI-GRANULOCYTE CYTOPLASMIC AUTOANTIBODY (AGCA) SPECIFIC FOR MYELOPEROXIDASE(MPO) IN VASCULITIS RELATED AND IDIOPATHIC NECROTIZING AND CRESCENTIC GLOMERULONEPHRITIS (NCGN).

RJ Falk, R Terrell, JC Jennette, Depts. of Med. and Path. Univ. of North Carolina, Chapel Hill, NC.

We sought to identify the antigenic specificity of AGCA in patients with Wegner's (WG), Polyarteritis (PAN), Lupus, and idiopathic NCGN (INCGN). Purified human leukocytes were subject to nitrogen cavitation and centrifugation. The supernatant was tested by ELISA for AGCA. Positive reactions (>2 standard deviations (sd) above normal mean (n=41)) were seen in 10/12 WG/PAN, 16/20 INCGN, 8/12 Lupus, and 5/62 other renal lesions (ORL). Analyzed by groups, WG/PAN and INCGN sera were significantly different from normal (P<0.01). Subcellular leukocyte constituents were then isolated by percoll sedimentation. AGCA positivity was found in fractions enriched for azurophilic granules (AG) (P<0.01). Specificity for molecular constituents of AG was investigated by ELISA. Positive reactions with MPO (>2 sd of normal mean (n=10)) were found in 7/12 WG/PAN, 5/9 INCGN, 1/4 Lupus, and 1/6 ORL. MPO values in WG/PAN and INCGN were equivalent, but different from normal values (P<0.01). Only one Lupus serum and no others reacted with alkaline phosphatase or lysozyme. These results were confirmed by dot blot ELISA using entirely different reagents.

AGCA in WG/PAN and INCGN react with myeloperoxidase. A common serological marker in diverse clinical conditions associated with NCGN suggests a similar pathogenesis.

AMPLIFICATION BY ENDOTHELIAL CELLS OF THE MONOCYTE PROCOAGULANT SIGNAL INDUCED BY GBM/ANTI-GBM IMMUNE COMPLEXES.

Katherine Fitting, Bryan Wharram*, Margaret Vissers*, Roger Wiggins. Univ. of Michigan, Ann Arbor, MI.

Fibrin formation may play an important role in glomerular injury. We therefore examined the procoagulant signal produced by human monocytes (M) incubated with GBM alone and GBM that had been preincubated with anti-GBM antibody, in the presence and absence of cultured human umbilical vein endothelial cells (E). All experiments were done in the absence of serum, and reagents contained < 0.1 ng/ml LPS as assessed by limulus assay. Procoagulant activity (PCA) was measured by one stage coagulation assay and was tissue factor-like as assessed by deficient plasmas. M incubated alone at high concentrations (> 2x10⁶/ml) produced PCA. At lower concentrations the presence of GBM in the absence of antibody induced some PCA synthesis, but the presence of anti-GBM antibody markedly enhanced PCA production. PCA was seen after 2 hours of incubation, peaked at 10-20 hrs and declined thereafter, and was inhibited by cyclohexamide. The presence of E enhanced PCA 10-fold at a ratio of 10 E:1M. In the absence of M the E did not produce PCA. These findings emphasize the importance of the collaboration between E and M for PCA production (possibly due to the action of IL-1 and TNF α on E as described by Bevilacqua and colleagues, PNAS 83,4533, 1986). They are compatible with the concept that activation of relatively few M within glomeruli may cause fibrin formation by inducing tissue factor synthesis by endothelial cells.

T-LYMPHOCYTES IN RABBIT SERUM SICKNESS NEPHRITIS. S.J. Foster,* TE Brelje,* LG Hunsicker. VA Med. Center and Univ. of Iowa, Iowa City, Iowa.

The basis for glomerular monocytic infiltration in acute serum sickness nephritis (ASSN) is uncertain. To evaluate the role of T-cells in ASSN, monoclonal antibodies against rabbit T-cells were prepared (XIID₂₃, VIIIE₁₀) or obtained from NIH (L11/135) and were used to stain isolated whole glomeruli in an indirect immunofluorescence assay. In normal rabbits each clone stained a small number cells per glomerulus (mean \pm sd; XIID₂₃ 11.4 \pm 4.0; L11/135 14.1 \pm 5.5; VIIIE₁₀ 18.0 \pm 9.5). When normal rabbits received 800 rads total body irradiation with right kidney shielded, there was equal depletion of stained cells from shielded and irradiated kidneys in parallel with a decrease in WBC. Scatter radiation to the shielded kidney was 200 rads; however, direct irradiation with 200 rads resulted in no depletion of stained cells nor significant decrease in WBC. These findings are consistent with origin of these cells from the circulation. In rabbits with bovine albumin induced ASSN, there was a marked increase in glomerular T-cells during disease. Sequential daily renal biopsies showed a rapid increase in glomerular T-cells at immune clearance, of 24 hour duration and paralleling the peak infiltration of monocytes as estimated by non-specific esterase stain. There was no apparent correlation between the extent of T-cell infiltration and the degree of proteinuria in different rabbits. The correlation between the T-cell and monocytic infiltration suggests a role for T-cells in glomerular inflammation; however, the lack of correlation with proteinuria leaves their role in the pathogenesis of glomerular injury unclear.

SUSCEPTIBILITY TO APOFERRITIN INDUCED MURINE IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN) IN H-2 CONGENIC MICE IS ASSOCIATED WITH ISOTYPE SWITCHING. PA Frymoyer and AH Tatum*. Dept. of Med. and Pathol., SUNY-HSC, Syracuse, NY.

Susceptibility to apoferritin ICGN in congenic B10 mice is dependent upon genes in the H-2 region (KI 31:319) with the degree of susceptibility being B10 (H-2^b) < B10.BR (H-2^k) < B10.D2 (H-2^d). In these experiments the role of isotype switching was studied. ICGN was induced utilizing 4mg of horse spleen apoferritin (HAF) i.p. q.d. for 28 days. IgG and IgM anti-HAF responses were measured using ELISA assays. The data at weeks 2 and 4 were similar and at week 4 were (mean \pm SE):

Strain	IgM (%pos. cont.)	IgG (mcg/ml)	IgG/IgM Ratio $\times 10^{-2}$
B10	133 \pm 13	1.4 \pm 0.18	1.2 \pm 0.2
B10.BR	182 \pm 17	8.6 \pm 2.4	5.1 \pm 1.4
B10.D2	165 \pm 27	23.7 \pm 6.7	15.0 \pm 4.0
p<	0.05	0.002	0.001

All strains were able to develop an anti-HAF IgM response of similar magnitude at each time point although there were small significant differences that do not correlate with disease susceptibility. The B10 mice which are not susceptible failed to switch from IgM to IgG anti-HAF whereas the B10.BR mice with intermediate susceptibility were able to switch to a modest degree and the B10.D2 mice with marked susceptibility had a vigorous switch to IgG. This data suggests that isotype switching is important in disease pathogenesis. H-2 may be influencing disease susceptibility because isotype switching is dependent upon helper T cells and genes in the MHC region.

INTERACTION OF ANTIBODIES WITH SURFACE ANTIGENS OF HUMAN AND MONKEY GLOMERULAR EPITHELIAL CELLS (GEC). A. Fukatsu, M. Milgrom, J. Miller, L. Olson, F. Milgrom, J. Brentjens and G. Andres. Dept. Micro. and Path., SUNY at Buffalo, Buffalo, NY, and Dept. Surgery, Univ. of Miami, Miami, FL.

Heymann glomerulonephritis, a rat model for human membranous glomerulonephritis (GN), is due to antibodies (Ab) reactive with surface antigens (Ag) of GEC. We tested the hypothesis that similar mechanisms may induce subepithelial immune deposits (ID) in monkey and in human glomeruli. Ag of brush border (BB) of human proximal tubules, and of plasma membranes of human GEC, rabbit Ab to BB and GEC, and monoclonal Ab to GEC (GEC M Ab) were prepared. BB Ab induced weak, and GEC Ab and GEC M Ab strong Ag redistribution on cultured GEC at 37°C. Monkeys immunized with BB Ag developed tubular disease, but not GN. BB and GEC Ab injected into monkeys bound diffusely to glomerular cells and induced fine granular ID during autologous phase. GEC M Ab induced fine granular ID on day 3 and on day 14 without deposit of monkey IgG. Normal human kidneys perfused for 4 hrs at 37°C with BB Ab or GEC Ab had diffuse binding of rabbit IgG to glomerular endothelial and epithelial cells, but not granular ID. GEC M Ab bound diffusely to human GEC at 30 min, and formed fine granular ID at 4 hrs. Controls had no ID. These results show that some Ab to human GEC induce Ag redistribution and formation of small ID. Failure to induce more severe lesions may be due to lack of relevant Ag on GEC, comparable to rat gp 330, lack of critical concentration of relevant Ab, insufficient time of kidney perfusion, or to a combination of these factors.

NONCATIONIZED ANTIBODIES IN IMMUNE COMPLEXES ARE DEPOSITED IN GLOMERULI BY THE PRESENCE OF A SMALL FRACTION OF CATIONIZED ANTIBODIES. V. Joyce Gauthier* and Mart Mannik* (Intro. WG Couser). Univ. of Washington, Dept. Medicine, Seattle, WA.

The ability of preformed immune complexes (ICs) to deposit when composed entirely of cationic antibodies has been previously demonstrated (J Exp Med 156:766, 1982). In order to determine if a small population of cationic antibody molecules would be capable of mediating deposition of immune complexes composed of naturally occurring anionic and neutral antibodies, mixtures containing 10 and 25% unlabelled native or chemically cationized rabbit antibodies to human serum albumin (anti-HSA) and radiolabelled native rabbit anti-HSA were used to prepare preformed immune complexes at 5 times antigen excess. C57BL/6 mice in triplicate received 0.5 mg doses IV and were sacrificed at 5 minutes. Glomeruli were isolated and the quantity of deposited native radiolabelled antibodies determined:

	ng native rabbit Ab per kidney	
	10% mix	25% mix
Mixes of ICs with no cationized antibody	11.21±5.40	4.43±1.70

Mixes of ICs with cationized antibody	30.95±5.29 p<0.02	140.25±23.46 p<0.01
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Control experiments utilizing nonspecific cationized rabbit IgG showed no enhanced deposition of complexes containing only native antibodies. Thus small populations of cationic antibodies are sufficient to mediate the deposition of anionic or neutral antigens and antibodies.

THE RATE OF CLEARANCE OF PATHOGENIC IMMUNE COMPLEXES (IC) IS NOT IMPAIRED IN LUPUS ERYTHEMATOSUS. Norman A. Granholm*, Rodney R. Nunley*, and Tito Cavallo. Department of Pathology, Rhode Island Hospital and Brown University, Providence, Rhode Island.

Published data indicate that the clearance of IgG coated RBCs is impaired in lupus patients; whether they have, also, impaired clearance of soluble IC is unknown. We investigated the rate of clearance of pathogenic IC (greater than Ag₂Ab₂) in three strains of murine lupus [(NZBxNZW)F₁ females (B/W), MRL-lpr/lpr males, (MRL), BXSB males] before (2 months of age), during (3.5 months of age), and after (5.0 months of age) development of nephritis. C57BL/6 mice (C57) served as normal controls. The curves of disappearance of IC (BSA-antiBSA; 0.1mg/g body weight) were resolved into three components: transient, intermediate (clearance of pathogenic complexes greater than Ag₂Ab₂), and persistent. The clearance rates of pathogenic IC expressed by their weighted average (WA) values (WA = 0.5 x mg IC x component percent of total + t_{1/2}) are shown in the Table.

Groups	2.0 months	3.5 months	5.0 months
C57	0.54 ± 0.18*	0.34 ± 0.06 _s	0.75 ± 0.21
B/W	0.64 ± 0.20	0.79 ± 0.12	0.89 ± 0.12
BXSB	0.42 ± 0.04	0.54 ± 0.23	0.61 ± 0.10
MRL	0.49 ± 0.07	0.25 ± 0.05	0.38 ± 0.06

* Values are Mean mg/hr ± SEM. § Kruskal-Wallis test, p ≤ 0.05 compared to age matched C57.

Because the clearance rate of pathogenic IC is not decreased in murine lupus compared to control mice, defective clearance of IC does not appear to be a contributory factor to the pathogenesis of lupus erythematosus.

EFFECTS OF A SPECIFIC THROMBOXANE A₂ SYNTHETASE INHIBITOR (TxA₂SI) ON DEVELOPMENT OF IMMUNE COMPLEX GLOMERULONEPHRITIS. G.F. Grauer,* C.A. Culham,* R.R. Dubielzig,* and R.B. Grieve.* (intr. by D.P. Simpson) School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin.

Previous studies assessing the effect of TxA₂SI on the development of glomerulonephritis (GN) have had variable results. We studied the effect of a specific TxA₂SI, 1-benzylimidazole (1-BIM), on the development of GN induced by *in situ* formation of immune complexes. Twelve beagles were immunized with an aqueous-soluble *Dirofilaria immitis* antigen and subsequent to at least 5-fold increases in serum antibody titer, 6 mg of homologous antigen was infused into the left renal artery. Six dogs were treated once daily starting the day of infusion with 0.75 mg/kg 1-BIM in saline and 6 dogs were given saline only. Light, electron, and immunofluorescent microscopy 10 days after antigen infusion, revealed a mononuclear cell (MON) proliferative GN in the left kidney with polymorphonuclear leukocyte (PMN) infiltration and fibrin deposition (FD). MON proliferation and FD were graded using a 0 to 3+ scale and PMNs/20 random glomeruli were counted. IgG deposits were observed in a segmental fine granular pattern and the GBM was routinely thickened. Anti-*Dirofilaria immitis* antibodies were demonstrated in all kidney eluates. 1-BIM had no effect on IgG deposition, GBM thickening, or concentration of antibody in kidney eluates, however, 1-BIM lessened MON proliferation, PMN infiltration, and FD (Table). These data suggest that TxA₂ is an important mediator in the development of immune complex GN.

	1-BIM Treated	Controls	p value
MON proliferation	1.0±0.89	2.17±0.41	< 0.02
PMN infiltration	9.75±5.5	17.6±3.5	< 0.02
Fibrin deposition	0.67±0.52	1.67±0.82	< 0.05

EFFECT OF DIETARY PROTEIN COMPONENTS ON ACQUIRED POLYCYSTIC KIDNEY DISEASE (APKD) IN THE RAT.

H.-J. Groene, E. Groene, M.H. Weber, U. Helmchen. University of Goettingen, West Germany (intr. by M.J. Dunn).

The pathogenesis of renal cysts and the abnormal proliferation of the cyst epithelium in APKD are not fully understood. As renal growth in young rats can be modulated by dietary protein composition we tested if different dietary proteins can influence cyst development. Rats with daunomycin (D) nephrosis that develop renal cysts served as an experimental model. Six week (wk) old male rats, unilaterally nephrectomized, were injected with D (5mg i.v./kg BW) or vehicle (C) and paired 2 diets: P and N, differing only in composition but not in content (18% of protein; P: vegetable and animal protein; N: casein as only protein. Diets P and N were free of cystogenic drugs. Urinary protein excretion rose 1wk after D and did not vary between the 2 D-groups. Proximal tubular cell mitosis increased in both D-groups compared to C (4-fold in PD and 8-fold in ND at 2wk after D; autoradiography). 6wk after D kidney weight was 2-fold larger in ND than in PD, with more cysts in ND: PD (n=10): 10±1; ND (n=15): 28±4 % of proximal tubules. RBF and GFR - comparable in both D-groups during cyst genesis - were decreased (GFR at 6wk: PD: 0.28±0.1; ND: 0.14±0.05; C: 1.78±0.24 ml/min). Rats with aminonucleoside nephrosis showed similar cyst formation with N-diet. Control rats did not develop cysts. Thus, an experimental model of pronounced APKD with decreased renal function was observed in a short period of 6wk. Time sequence analysis indicated that glomerular proteinuria initiated proximal tubular cell proliferation. Dietary casein had a permissive effect on cyst genesis. It may be therapeutically relevant that dietary proteins can influence tubular cyst development.

ROLE OF THE TERMINAL COMPLEMENT PATHWAY (TCP) IN IMMUNE DEPOSIT CLEARANCE. G.C. Groggel, A.O. Haakenstad*. Univ. of Utah School of Medicine and VA Medical Center, Salt Lake City, Utah.

Rabbits deficient in the 6th component of complement (C6D) have a more severe form of accelerated autologous anti-GBM nephritis (AAGBM) than normal rabbits (N) (KI 31:321, 1987). A possible role for C6 and the TCP in the clearance of immune complexes (IC) and solubilization of immune deposits (ID) was investigated. HSA - 125 I anti-HSA IC (containing 10 mg anti-HSA/ml at 5X antigen excess) were infused (2mg anti-HSA/kg) into C6D and N rabbits. IC clearance and tissue distribution were measured. In vitro solubilization of glomerular 125 I sheep anti-GBM ID formed in AAGBM was attempted. 2000 isolated glomeruli were incubated at 37°C for 48h with N, C6D or heat-inactivated (HI) rabbit serum and % of ID solubilized calculated.

There were no significant differences in serum IC levels at 5, 10, 15, 30 mins, 1, 2, 4, 8, 16, 24, 48, 72, and 96 hrs. 45% of IC were cleared by 1h and 86% by 24h. At 1h, N kidneys contained 0.56%±0.20 of injected IC and C6D had 0.11%±0.09, while at 24h N had 0.00% and C6D had 0.13%±0.12. At 1h N livers contained 34.2%±5.54 and C6D had 25.9%±2.11 and by 24h these were reduced to 0.25%±0.05 and 0.22%±0.06. Spleen and lung localization was <1% of injected IC. Solubilization of glomerular ID from AAGBM was 12.1%±3.66% with N, 10.1%±2.34 with C6D and 9.3%±1.64% with HI serum.

Further studies are needed to determine the significance of these observations and to define whether the TCP has a significant role in the solubilization of glomerular ID.

INTERFERON- γ (IFN γ) ANTAGONIZES THE BENEFIT OF DIETARY FISH OIL (FO) ON MURINE LUPUS. M. Guo*, S. Tateno*, A.H. Williams*, D.R. Robinson*, and R.B. Colvin, Mass. Gen. Hosp., Boston, MA 02114.

These studies test whether the beneficial effect of FO is related to decreased expression of major histocompatibility (MHC) antigens or response to IFN γ . 60 MRL/lpr mice (H-2^k) were placed on diets with 15% FO or beef tallow (BT). 15 mice in each group were given recombinant IFN γ (20,000 U qod ip, for 4-8 weeks); controls received saline. Proteinuria-free (<3+) survival at 8 weeks was increased by FO (p<.03), but this effect was overcome by IFN γ (p<.01). Control non-autoimmune mice (B10.BR) have little renal MHC expression, aside from IA⁺ dendritic cells. In contrast, MRL mice had increased renal class I and II MHC antigens, which progressed over 4 weeks, as judged by immunoperoxidase, using monoclonal antibodies to K^k (3-83P) and IA^k (10-3.6.2). K^k was increased in glomeruli, proximal tubules and arterial endothelium. IA^k was increased in glomeruli and tubules. Periarthral mononuclear cells were positive for both. FO had little effect on this progression, with the possible exception of inhibiting glomerular K^k. IFN γ exaggerated the abnormal vascular K^k and tubular IA^k in both groups similarly. However, FO inhibited the K^k response of glomeruli and tubules, and the increase in intraglomerular IA^k cells (all p<.04). IFN γ increased the IgG deposition in glomeruli, but had no effect on the vasculitis, which was worse on FO (p<.05). The dramatic MHC changes that occur *in situ* in murine lupus apparently precede glomerular damage. The observed antagonism of certain effects of IFN γ may related to the beneficial effects of FO.

EFFECT OF ADMINISTERING LARGE DOSES OF DNA ON GLOMERULAR DEPOSITS AND RENAL DISEASE IN NZB/W MICE. A.O. Haakenstad, A Lanker-Wilson* (intr. by G. Groggel). VA Medical Center and University of Utah, Salt Lake City, Utah.

Large intravenous (iv) doses of sonicated DNA used previously reduced glomerular deposits in NZB/W mice (Kid Int 31:322, 1987). Due to difficulties with iv injections of concentrated, viscous DNA solutions, subsequent studies utilized intraperitoneal (ip) DNA injections.

NZB/W females (4-5 mo) received 15 mg sonicated, calf thymus DNA ip, 5 days weekly for 8 wks. Prior to DNA or PBS (control mice) injections the left kidney was removed. The right kidney was obtained at 8 wks, and both scored (0-4+) by immunofluorescence microscopy.

Mice(n)	Admin	Kidney	IgG	IgM	C3
8	DNA	day 0	1.4±0.9	0.9±0.6	0.8±0.9
		8 weeks	1.8±0.9	1.1±0.8	2.1±0.6
		p value	n.s.	n.s.	0.005
8	PBS	day 0	1.7±1.2	0.7±0.7	1.4±1.3
		8 weeks	3.0±0.9	1.7±1.2	3.0±0.9
		p value	0.038	n.s.	0.01

IgG and IgM deposits did not change in DNA injected mice but C3 deposits increased significantly. IgG, IgM and C3 deposits increased in control mice, however, the IgM increase was not significant.

BUN and urine protein did not significantly change in DNA injected mice, while significant increases in BUN (P=0.001) and proteinuria (p=0.011) occurred in control mice. AntiDNA antibody titers (ELISA) were lower in DNA injected mice than in control mice, but the differences were not significant.

BACTERIAL LIPOPOLYSACCHARIDE (LPS) INCREASES RENAL MHC EXPRESSION BY TRIGGERING NON T CELLS TO RELEASE GAMMA INTERFERON. Philip F. Halloran, Div. of Nephrology & Immunology, University of Alberta, Edmonton, Alberta, Canada.

We studied the effect of LPS (S. Minnesota) on the expression of major histocompatibility complex (MHC) genes in mouse kidney, using histology and radioimmune assay (RIA) to assess products and hybridization to assess specific mRNA. LPS 5-25 ug i.p. x 2 induced Class I in glomeruli, tubules and arteries, and Class II only in tubules. RIA revealed large increases in total Class I (5 fold) and Class II (2-3 fold). Northern and slot blots revealed parallel increases in specific mRNA for Class I, Class II, and B₂ microglobulin. Similar changes were observed in other tissues, e.g. heart and pancreas. These changes could be induced in normal mice; T deficient nude mice; and mice with severe combined immunodeficiency (SCID) but were severely reduced in LPS resistant C3H/HeJ. The effects of LPS were inhibited by cyclosporine and by a monoclonal against interferon gamma. The changes in Class II could be simulated by administration of rIFN gamma but not rIFN alpha or poly I /poly C, an IFN alpha/beta inducer. We conclude that LPS induced transcription of Class I and II and B₂ microglobulin genes in mouse kidney and other tissues, by inducing a population of non T cells to release gamma interferon; and that this pathway is inhibited by cyclosporine. This mechanism may be relevant to the reactions of the kidney to local and systemic bacterial sepsis.

MODULATION OF COLLAGEN SYNTHESIS IN GLOMERULAR EPITHELIAL CELLS (GEC) BY INTERLEUKIN 1 (IL-1) AND SUPERNATANTS OF MESANGIAL CELLS (GMC). Gertrud M. Hänsch, Ingo Torbohm, Johannes v. Kempis and Klaus Rother. Institut für Immunologie der Universität Heidelberg, FRG.

We studied factors modulating the collagen synthesis of cultured human GEC. Recently, we reported that the terminal complement components C5b-9 enhanced the collagen synthesis. We now report on the effect of IL-1 on human GEC. Highly purified IL-1 as well as IL-1 containing supernatants of activated macrophages increased the collagen synthesis, measured as uptake of ^3H -proline into collagenase digestible proteins. The newly synthesized material consisted mainly in collagen type IV. Collagen production was also enhanced, when GEC were incubated with supernatants of cultured GMC, known to produce an IL-1-like factor. Analysis of the radioactive material by SDS-PAGE and autoradiography revealed, that in addition to the 180 kD band, seen following stimulation with purified IL-1, also collagen split products appeared, most probably generated by a GMC-derived collagenase. Thus, the interaction of GEC and GMC appears to be twofold: GMC-products enhance the collagen synthesis of GEC, but also promote the degradation of collagen. In vivo, this interaction could modulate the turnover of the glomerular basement membrane.

INVOLVEMENT OF ANTIBODIES (ab) DIRECTED AGAINST LAMININ, TYPE IV COLLAGEN AND FIBRONECTIN IN AUTOLOGOUS (AICN) AND HETEROLOGOUS (HICN) IMMUNE COMPLEX GLOMERULONEPHRITIS. P.C.W. Hogendoorn, J.A. Bruijn, E. de Heer, J.M. Foidart, L.J.C.M. van den Broek, Ph.J. Hoedemaeker, G.J. Fleuren. University of Leiden, Holland and Liège, Belgium.

AICN and HICN are induced by active or passive immunisation against renal tubular brush border antigens (RTE). Within RTE gp330 and gp90 are recognized to be of pathogenetic importance. We investigated whether ab directed against other antigens within RTE could be operative within the autologous or heterologous antisera and eluted ab from kidneys of diseased rats. Both in AICN and HICN ab directed against laminin, type IV collagen and fibronectin were present as shown by ELISA and Western blotting. Ab with these specificities were separated by affinity chromatography and tested for their activity in vivo by injection into naive rats. Direct immuno-EM demonstrated linear binding to glomerular epithelial and endothelial cell surfaces and the laminae rarae. Moreover, these ab were able to activate complement and to induce a transient proteinuria (max 62.5 mg/24hr, controls 15 mg/24hr). These ab might be important in AICN or HICN either by a direct nephritogenic potential, or by synergistic or complementary action with other ab specificities (e.g. anti-gp330 and/or anti-gp90). These findings could explain differences in pathogenicity between monospecific anti-gp330 ab and polyclonal anti-RTE ab observed earlier.

RECOGNITION OF ANTIGEN (Ag)-SECRETING RENAL EPITHELIAL CELLS BY Ag-SPECIFIC HELPER T CELLS. W.H. Hines*, C.J. Kelly, T.P. Haverty*, and E.G. Nettson, Renal Section, U. of Pa., Phila., PA.

We developed a murine proximal tubular cell line (MCT) in order to study the interaction between epithelial cells and T lymphocytes in experimental interstitial nephritis. MCT cells constitutively produce the target Ag (3M-1; 30,000 Mr) of this disease as shown by immunoprecipitation from biosynthetically labelled cells. Supernatants from cultured MCT cells also contain IL-1-like activity (13 units/ml), and MCT cells express MHC class II Ag's on their cell surface. We employed a cloned L3T4⁺ helper T cell line (M-30) specific for 3M-1 to determine if tubular epithelium could act as an Ag-presenting cell. The Ag specificity of M-30 was demonstrated by incubating helper cells with irradiated splenic feeders and various Ag's in a ^3H TdR uptake assay: purified 3M-1 = 20,411 ± 1046 cpm, stimulation index (SI) = 29.4 (p < .001); 3M-1 depleted soluble renal Ag = 1437 ± 394 cpm, SI = 2.1 (p > .05). M-30 also proliferates in a dose-response fashion when incubated on increasing numbers of irradiated MCT cells. M-30 incubated alone was 816 ± 15 cpm, with 1X10⁴ irradiated MCT cells was 1465 ± 119 cpm (SI = 1.8), with 5X10⁴ MCT was 2836 ± 34 cpm (SI = 3.5), and with 2X10⁵ MCT was 4562 ± 118 cpm (SI = 5.6) (av. p < .001). The proliferation of M-30 on irradiated MCT was inhibited more than 50% by αL3T4^+ antibody, but was not inhibited by equal amounts of αL2 control (p < .005). Thus, MCT cells produce IL-1, elaborate class II MHC determinants on their surface, and secrete 3M-1. MCT cells also support the growth of 3M-1 specific helper cells in a dose-response fashion, and this growth requires cell interaction molecules. Our work suggests that tubular epithelium can provide an antigen-presenting function for T lymphocytes in autoimmune interstitial nephritis.

POTENTIAL PATHOGENIC ROLE OF LUPUS ASSOCIATED MEMBRANE PROTEIN (LAMP), IN SYSTEMIC LUPUS ERYTHEMATOSUS. L. Jacob*, M.A. Léty*, D. Kerjaschki*, J.F. Bach*, D. Louvard* (int. by Dr Anagnostopoulos). INSERM U 25, Hôpital Necker, Département de biologie moléculaire, Institut Pasteur, Paris, France. Institut für Pathologische Anatomie, University of Vienna, Austria.

We recently demonstrated that several monoclonal anti-DNA antibodies react with a cell-surface protein expressed at the surface of various cell types involved in lupus pathogenesis, including normal human glomeruli, T and B cells, erythrocytes, platelets and neuronal tissue. Eluted IgG from kidneys of MRL/lpr/lpr lupus mice reacted specifically with this protein, called LAMP. Importantly, LAMP is located in clathrin coated pits on the surface of kidney glomerular epithelial cells suggesting that LAMP is a receptor. Anti-LAMP antibodies were detected in lupus mice sera as well as in 25 human active systemic lupus erythematosus (SLE) sera (two without anti-DNA antibodies), but not in the serum of 10 rheumatoid arthritis, 6 scleroderma, 4 primary sicca syndrome patients, and 10 normal human subjects. Anti-LAMP antibodies were found in the serum of 5 other active SLE patients but not in these patients in inactive state. LAMP is modified at the surface of spleen and glomerular cells from MRL/lpr and B/W lupus mice in contrast to spleen and glomerular cells from BALB/c and CBA/ca normal mice. This change appears between 1 and 3 weeks during the life of MRL/lpr/lpr lupus mice. Such modifications might result in the appearance of a non self antigen and therefore elicit an immune response. These data indicate that LAMP probably plays a pathogenic role in SLE.

REGULATION OF DNA SYNTHESIS IN HUMAN MESANGIAL CELLS BY PEPTIDE MITOGENS. E. Jaffer,* C. Saunders,* P. Shultz* and H.E. Abboud. Department of Medicine, VA Medical Center, and Case Western Reserve University, Cleveland, Ohio.

We have recently reported that purified platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) are potent mitogens for cultured human mesangial cells. In the present studies, we examined the effect of recombinant PDGF, purified EGF and transforming growth factors-beta and alpha (TGF-beta, TGF-alpha) on DNA synthesis in the same cells, using ^3H -thymidine incorporation into acid insoluble precipitate. Recombinant PDGF increased DNA synthesis in quiescent cells with half maximal effect occurring at a concentration of 2.5 ng/ml (nine fold). EGF and TGF-alpha also stimulated DNA synthesis in a similar manner with half maximal increase occurring at a concentration of 2.5 ng/ml (two and a half fold). Low concentration (0.01 and 0.05 ng/ml) of TGF-beta enhanced DNA synthesis induced by 10 ng/ml EGF but not PDGF. In contrast higher concentration of TGF-beta inhibited EGF and PDGF-induced DNA synthesis. Inhibition was observed at a concentration of 0.25 ng/ml and was maximal at 1 ng/ml (50% inhibition). At high concentration, TGF-beta did not interfere with ^{125}I EGF binding to mesangial cells, suggesting that its inhibitory effect was not caused by competition at cell surface receptors. These data suggest that mesangial cell DNA synthesis can be differentially modulated by peptide mitogens. Since all four peptides have been shown to be released by platelets or activated macrophages and lymphocytes, their interaction with mesangial cells may provide a mechanism for the regulation of cell proliferation in glomerular diseases.

PLATELETS (Plts) MEDIATE NEUTROPHIL (PMN)-DEPENDENT GLOMERULONEPHRITIS (GN) INDUCED BY SUBENDOTHELIAL IMMUNE COMPLEX (IC) DEPOSITS. R.J. Johnson, C.E. Alpers, W.G. Couser. Univ. of Washington, Seattle, WA.

GN associated with subendothelial IC deposits (SLE, MPGN) is often characterized by PMN infiltrates and platelet activation. In a model of subendothelial IC GN induced by renal artery perfusion with Concanavalin A (Con A) followed by rabbit anti-Con A IgG, GN was characterized by extensive subendothelial IC deposits, glomerular PMN and Plt infiltrates, capillary loop thrombosis and proteinuria (113 ± 35 mg/day). Selective PMN depletion abolished glomerular PMN infiltrates and reduced proteinuria to 35 ± 17 mg/day ($p < .01$). To study the role of Plts in this PMN-dependent model, 14 rats were selectively depleted of Plts to $< 20,000/\text{mm}^3$ with anti-Plt serum prior to induction of Con A GN and compared at 24 hrs to 14 control GN rats treated with normal IgG that maintained Plt counts of $> 600,000/\text{mm}^3$. Plt depletion had no effect on PMN levels or function or on glomerular deposition of ^{125}I labeled anti-Con-A antibody, Con A, C3 or glomerular PMN infiltrates. However, Plt depleted rats had a marked reduction compared to controls in urine albumin excretion (7 ± 6 mg/day vs. 55 ± 36 , $p < .01$) and fractional albumin clearance ($.05 \pm .01$ vs. $.41 \pm .09$, $p < .01$) and a reduction in glomerular capillary thrombosis from $> 75\%$ of loops to $< 25\%$.

We conclude that Plts play an essential role in the mediation of the acute phase of PMN-induced glomerular injury in experimental subendothelial IC GN.

IMMUNOGLOBULIN PRODUCTION STIMULATED BY 1-OLEOYL-2-ACETYLGlycerol IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS. S. Jordan, R. Sakai*, and D. Yamaguchi. Cedars-Sinai Medical Center VAMC West Los Angeles, Los Angeles, Ca.

The stimulation of protein kinase C (PKC) is thought to be an early event in B-cell activation. Phorbol-12-myristate-13-acetate (PMA) and 1-oleoyl-2-acetylglycerol (OAG) both activate PKC. The effects of PMA and OAG on immunoglobulin (Ig) production and proliferation were examined in human peripheral blood mononuclear cells (PBM). IgG and IgM were determined by an enzyme-linked immunosorbent assay (ELISA) and proliferation by ^3H -thymidine incorporation (TdI). OAG stimulated IgG and IgM production in a dose-dependent manner from 1 to 100 ug/ml. However, maximal Ig production stimulated by OAG was only 50% of maximal Ig production stimulated by pokeweed mitogen (PWM). PMA at doses from 1 to 100 nM did not stimulate Ig production. At doses that stimulated Ig production, OAG did not cause an increase in TdI. PMA caused a small but statistically insignificant increase in TdI. OAG did not stimulate an increase in IL-2 generation nor an increase in IL-2 receptor expression. H-7, a PKC blocker, did not alter spontaneous Ig generation. Conclusions: 1) OAG stimulates Ig production independent of PBM proliferation; 2) The mechanism of Ig stimulation of OAG may be via PKC activation; 3) PBMs respond differently to OAG and PMA although both are PKC activators; 4) The OAG effect is not through an initial generalized activation of T-cells but possibly through stimulation of B-cell specific T-cell factors or a direct effect on B-cells.

FIBRONECTIN (FN) PRODUCTION IN HUMAN GLOMERULAR CELLS. Debra Kees-Folts*, John D. Mahan, Cynthia McAllister*, Barry Shannon*, Fernando G. Cosio. The Ohio State University, Depts of Pediatrics and Medicine, Children's Hospital, Columbus, Ohio.

Increased glomerular FN is seen in many forms of glomerulonephritis (GN) and may participate in acceleration of the inflammatory process. Human endothelial cells treated with activated neutrophil release products (ANRP) increase FN secretion and FN is associated with chemoattraction and adherence of neutrophils and macrophages in vitro.

Human mesangial (MC) and human glomerular capillary endothelial cells (GCEC) were grown separately in culture. Cell supernatant FN was measured by ELISA from untreated cells, cells exposed to ANRP and cells exposed to ANRP and catalase (ANRP-C).

Supernatant Fibronectin (picograms/cell \pm SD)			
MC	Untreated	ANRP	ANRP-C
5 min	$0.56 \pm .39$	$0.39 \pm .27$	
4 hr	$3.68 \pm .88$	$1.22 \pm 1.35^*$	
24 hr	5.55 ± 2.15	$2.66 \pm .81^*$	$2.31 \pm .53^*$
GCEC			
5 min	0.10	$0.78 \pm .5^*$	
4 hr	$1.25 \pm .32$	$3.10 \pm .98^*$	
24 hr	10.51 ± 3.62	$1.26 \pm 1.39^*$	$2.20 \pm 1.05^*$

(* = $p < 0.01$ vrs untreated)

Conclusions: Treatment of human MC with ANRP decreased the release of free FN. GCEC exposed to ANRP increased FN release at 4 hr but had decreased supernatant FN by 24 hrs. The GCEC results at the early time periods support a role for FN in the inflammatory process in GN. These effects of ANRP on MC and GCEC are independent of neutrophil oxygen free radical release.

SUSCEPTIBILITY TO MURINE ANTI-TUBULAR BASEMENT MEMBRANE (α TBM) DISEASE IS FUNCTIONALLY LINKED TO CONTRASUPPRESSION. C.J. Kelly, H. Mok*, and E.G. Neilson, U. of Pa., Philadelphia, PA.

The functional process underlying linkage of autoimmune diseases to the MHC is enigmatic. In murine α TBM disease, susceptibility maps to a class I locus, H-2K. Susceptible (SJL) and non-susceptible (B10.S(8R)) strains differ in their T cell responses to renal tubular antigen (RTA). The critical difference is selection of a Lyt-2⁺ nephritogenic effector cell (T_{DTH}) in SJL and [SJLxB10.S(8R)]F₁ mice, and an L3T4⁺ T_{DTH} cell in B10.S(8R) mice which doesn't typically transfer disease. Selection is regulated by I-J⁺ T cells. Using *in vitro* induction of T_{DTH}, we have examined the relative role of suppressor (Ts) and contrasuppressor (Tcs) cells in SJL, B10.S(8R), and F₁ mice. Removal of Vicia Villosa lectin adherent (VV⁺) Tcs cells from T_{DTH} induction cultures resulted in selection of L3T4⁺ T_{DTH} from all three strains (Avg. DTH to RTA = $17 \pm 0.7 \times 10^{-3}$ in. after α Lyt-2 + C vs. 3.7 ± 0.5 after α L3T4 + C, $P < 0.001$). Further depletion of Ts cells from the VV⁺ cells with α I-J⁵ + C' resulted in differentiation of both T_{DTH} phenotypes in all three strains. Removal of Tcs cells thus affected T_{DTH} phenotype only in susceptible mice. The Tcs is Lyt-2⁺ and I-J⁺ (T_{DTH} is L3T4⁺ after removal of these cells vs. Lyt-2⁺ with depletion of L3T4⁺ VV⁺ cells, $p < 0.001$). Tcs function is mediated by a soluble factor (TcsF) which is RTA binding, I-J⁺, H-2K and Igh-restricted. Co-culture of F₁ TcsF with B10.S(8R) spleen cells results in nephritogenic Lyt-2⁺ T_{DTH} selection (histologic disease severity 2.0 (scale 0-2) after cell transfer vs. 0.0 after transfer of B10.S(8R) cells alone). The functional correlate of H-2K linked susceptibility to disease is a regulatory process, contrasuppression, which uniquely distinguishes susceptible from non-susceptible mice.

LOCALIZATION AND TRANSPORT OF C5b-9 BY THE GLOMERULAR EPITHELIAL CELL (GEC) IN EXPERIMENTAL MEMBRANOUS NEPHROPATHY (MN). D. Kerjaschki*, M. Schulze*, S. Binder*, and W.G. Couser. University of Vienna, Vienna, Austria and the University of Washington, Seattle, WA.

In the passive Heymann nephritis (PHN) model of MN in rats, subepithelial immune deposits result from antibody binding to GP330 on the GEC membrane, and proteinuria requires assembly of complement C5b-9. Urinary C5b-9 excretion is detectable only when deposits form on the GEC membrane and not in other models with equivalent glomerular C5b-9 deposits. (Kidney Int. 31:329, 1987) To better understand the processing of C5b-9 by the GEC resulting in urinary excretion, we utilized a monoclonal antibody to rat C5b-9 neoantigens in sequential immunohistochemical studies of PHN. On day 1, C5b-9 was detected only in immune deposits at the GBM-podocyte interface. By day 3, C5b-9 was present (without IgG or GP330) in numerous multivesicular bodies and was visible being exocytosed into the urinary space coincident with increased urinary C5b-9 excretion. By immunoblotting C5b-9 was present in degraded form in membrane vesicles in the urine. In contrast, no significant C5b-9 was found intracellularly or in the urinary space of MN induced with cationic IgG despite equivalent subepithelial C5b-9 deposits or in C5b-9 independent nephrotoxic nephritis.

We conclude that in the PHN model of MN C5b-9 is assembled on the GEC membrane at sites of immune deposits, is selectively transported intracellularly and then extruded into the urinary space. This process may contribute to GEC dysfunction and proteinuria.

CHIMERIC INTERLEUKIN-2-TOXIN SELECTIVELY TARGETS TO ITS RECEPTOR AND BLOCKS DELAYED TYPE HYPERSENSITIVITY. V.E. Kelley, O. Pankewycz*, J. Murphy*, and T.B. Strom. Brigham & Women's Hosp., Beth Israel Hosp., Boston Univ. Med. Sch., Boston, MA

Expression of interleukin-2 receptor (IL-2R) marks a critical and pivotal event in initiation of an immune response. Targeting the IL-2R with antibody effectively suppresses T cell mediated events including transplant rejection and autoimmunity. Since IL-2 binds more avidly to its receptor than the IL-2R Mab and because Mab therapy is complicated by the induction of anti-idiotypic antibodies, we examined the *in vivo* effects of a chimeric IL-2 toxin fusion protein upon suppression of a T cell mediated response. The chimeric molecule is the product of a gene in which IL-2 sequences replace the receptor binding domain of diphtheria toxin (dt). BALB/c mice were immunized with trinitrobenzene sulfonic acid, challenged 6 days later and DTH units (u) measured after 24 hrs. IL-2 toxin μ g/mouse/d suppressed DTH from 47 ± 4.1^u in the CRM45 (a truncated dt lacking any receptor binding domain) control to 20 ± 1.7^u ($p < .005$). IL-2 toxin suppressed DTH at doses as low as 0.05 μ g/d. Compared with IL-2R Mab therapy the toxin therapy was 2 fold more potent. The IL-2 toxin selectively eliminated IL-2R bearing T cells in draining lymph nodes. In immunized mice expression of IL-2R+ T helper and T suppressor cells was 14% and 18%, respectively. Injection of IL-2 toxin reduced these IL-2R+ cells to the amount detected in unimmunized mice 5% and 3%, respectively. Thus, IL-2 toxin selectively eliminates IL-2R+ T cells and blocks the T cell mediated DTH. Targeting of IL-2R+ activated T cells with IL-2 toxin is a precise and selective immunotherapeutic.

MECHANISM OF INTERLEUKIN 1 (IL1) STIMULATION OF PROXIMAL TUBULAR (PT) GLUCOSE TRANSPORT. Donald E. Kohan*, and George F. Schreiner. Washington Univ., Renal Division, St. Louis, Missouri.

Previous studies from our laboratory have demonstrated IL1 stimulation of Na-dependent glucose and aspartate transport in PT cells. We have now investigated the mechanism of this stimulatory effect of IL1. PT cells obtained by enzymatic digestion of mouse renal cortex were grown to confluent monolayers on Petri dishes or collagen membranes. Preincubation with IL1 for at least 12 hrs. caused a two fold increase in apical to basal transepithelial ¹⁴C-glucose movement. Preliminary data indicate a similar increase in transepithelial ²²Na transport. The IL1 enhanced glucose and Na transport coincided with a 15% increase in Na/K ATPase activity. IL1 did not change the number of Na/glucose cotransporters as assessed by ³H-phlorizin binding. No effect of IL1 on total protein synthesis, cell number, cell ultrastructure, or brush border alkaline phosphatase activity was detected. These data indicate that IL1 stimulates transepithelial glucose transport in cultured PT cells. Preliminary evidence indicates that transepithelial Na transport is also increased by IL1. This stimulation appears to be specific for Na-dependent transport and may be related to changes in the activity of Na/K ATPase.

GENETIC INFLUENCE ON THE NEPHRITOGENICITY OF ANTI-HEPARAN SULFATE-PROTEOGLYCAN (HS-PG) ANTIBODIES. B. Lelongt*, H. Makino*, and Y. Kanwar. Dept. Path Northwestern University, Chicago, Illinois.

Genetic influence on the nephritogenic potential of anti-HS-PG antibodies was investigated in various strains of rats, i.e., Fischer (Fi), Long Evans (Le), Brown Norway (Bn), and Lewis (Li). The rats were injected with rabbit anti-HS-PG and sacrificed 2, 4, 6, and 8 weeks later. Kidney tissues were utilized for light & electron microscopy and immunofluorescence. Urinary proteins & plasma anti-rat IgG levels were determined. No significant proteinuria and no detectable plasma anti-rat IgG were observed in Fi & Le strains. The other two strains revealed a dramatic proteinuric response, i.e., 20-40 for Li and 50-60 mg/24hr/100 g B.W. for Bn rats. The Li had 5-10 fold higher plasma anti-rat IgG than Bn rats. The kidney sections were equally reactive with anti-rabbit IgG in all the strains. Fi were negative for anti-rat IgG, and remaining three strains were strongly positive. Rat C3 was negative in Fi and Le, mildly positive in Li, and strongly reactive in Bn rats. All the strains exhibited knob like thickening of the glomerular basement membrane, and hypercellularity of the glomeruli. The monocyte count was highest in Bn rats. The electron-dense immune deposits were not observed in Fi rats. The Le strain had moderate number of subepithelial deposits. The concentration of these deposits were highest in Bn and Li strains, even after 2 weeks.

In conclusion, these data indicate that there are wide differences in the nephritogenic response to proteoglycan antibodies among various strains of rats.

RAT GLOMERULAR MESANGIAL CELLS RELEASE CYTOTOXINS/TUMOR-NECROSIS FACTOR-ALPHA. Robin P. Lowry and Dominique Blais*, Royal Victoria Hospital, McGill University, Montreal, Canada.

Glomeruli isolated from rats with an autologous form of Nephrotic Serum Nephritis release tumor-necrosis factor-alpha (TNF-alpha) and a serine protease with cytotoxic activity in greater quantities than glomeruli of normal rats (R. Lowry Clin Res 35:552A, 1987). While we have accrued evidence to suggest that cells of the monocyte/macrophage lineage contribute significantly to cytotoxin/TNF-alpha release in nephritic rats, the cellular source of such factors is unclear. Since mesangial cells have been shown to release the monokine IL 1, we assessed release of cytotoxins/TNF-alpha by cultured rat glomerular mesangial cells in long term culture. Glomeruli were isolated by sieving, collagenase treated and mesangial cell outgrowths cultured in 3T3 conditioned media. Supernatants (N=11) of subconfluent mesangial cell cultures, apparently devoid of macrophage contamination (nonspecific esterase -), were cytotoxic to the L929 cell line (cpm release 4-10 x background). Antiserum to murine recombinant TNF-alpha (rMuTNF-alpha, Genentech Inc.; 40 U/ml) inhibited the cytotoxic activity of mesangial cell supernatants by 40 +/- 20% (M. +/- S.D.). In the same experiments anti-TNF antiserum (40U/ml) inhibited cytotoxicity by rMuTNF-alpha, 250-0.1 pg/ml, by 17-100%. We conclude that rat glomerular mesangial cell release cytotoxins, including TNF-alpha, on in-vitro culture. Since TNF-alpha is now recognized to be one of the most potent proinflammatory mediators additional studies are clearly required to define the role of mesangial TNF-alpha in glomerular physiopathology.

A NEW MODEL OF EXPERIMENTAL GLOMERULONEPHRITIS (EXGN) INDUCED IN RAT BY HELIX POMATIA AGGLUTININ (HPA) AND ITS ANTIBODIES. Seichi Matsuo, N. Aoi,* F. Yoshida,* Y. Watanabe,* and N. Sukamoto.*

3rd Dept. Int. Med., Nagoya Univ., Nagoya, Japan. HPA recognizes terminal N-acetyl-D-galactosamine residues, which are masked by sialic acid in normal rat glomeruli. Partial digestion with neuraminidase (NRD) and subsequent HPA administration in an ex vivo kidney perfusion system resulted in the binding of HPA on the surface of glomerular endothelial cells (GEN). Further perfusion of rabbit anti-HPA antibodies (RbAHPA) caused the formation of granular immune deposits on the GEN surface. EXGN was induced by i.v. injection of RbAHPA before and after perfusion, ex vivo perfusion of the left kidney with NRD and HPA (eluate recovered from renal vein), and reestablishment of blood circulation. Non perfused right kidney was removed. 15 minutes after revascularization, immune complexes (ICs) containing rabbit IgG, HPA and rat C3 were seen in the subendothelial space. In a time course, there was decrease of subendothelial ICs with concomitant appearance of subepithelial ICs. By the 7th day, ICs localized mainly in the subepithelial space with mild GBM thickening. No autologous reaction was seen by this time. Mild to moderate proteinuria was detected. Control rats, in which experiments were done by omitting either one of NRD, HPA or RbAHPA, showed no subepithelial deposits nor proteinuria. It was considered that this model was characterized by (1) in situ IC formation on the GEN surface induced by planted non-glomerular antigen (HPA) and antibodies in the initial stage, and (2) subsequent movement of ICs from luminal side to subepithelial area resulting in membranous transformation.

THE CLEARANCE OF GLOMERULAR IMMUNE COMPLEXES (IC) WITH EXCESS ANTIGEN (AG) IS NOT VIA THE PERIPHERAL CIRCULATION. K. Melton*, G.B. Melton*, Brooke Army Med. Ctr., San Antonio, Tx. and L.Y.C. Agozola, Walter Reed Army Inst. of Res., Wash., D.C.

The final pathway of egress of IC from the mesangium (mes) of mice following excess AG administration has never been determined. We hypothesized that a possible route of mes IC clearance is via the renal lymphatics. Soluble IC of human serum albumin (HSA) and anti-HSA prepared with I - 125 labeled, reduced and alkylated antibody (AB) at 5 - fold AG excess were injected into mice. Fifty - fold AG excess was injected 12 hrs after IC administration. Kidney tissues were examined by direct IF before and after excess AG injection. Complete removal of IC was demonstrated. Whole blood disappearance of the IC was plotted as the fraction of I - 125 remaining (\pm SD) vs. time after IC injection :

1 min	15 min	1 hr	2 hr	4 hr	8 hr			
0.88	0.76	0.68	0.57	0.48	0.43			
After excess AG injection :								
1 min	15 min	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	72 hr
0.23	0.23	0.21	0.22	0.21	0.15	0.11	0.11	0.11
\pm .01	\pm .27	\pm .06	\pm .14	\pm .14	\pm .03	-	-	\pm .06

We concluded that mes IC can be completely removed by excess AG administration. Analysis of peripheral blood after injection of excess AG did not reveal an increase in radioactivity. Failure to detect an increase in radioactivity may be secondary to hemodilution or non - vascular routes of clearance. These non - vascular routes of IC clearance from the mes of mice remain to be investigated.

ANTI-INTERLEUKIN-2 RECEPTOR ANTIBODY SUPPRESSES INSULINITIS IN NOD MICE AND INDUCES ANTI-IDIOTYPIC ANTIBODIES. O. Pankewycz*, R. Hassarjian*, T.B. Strom* and V.E. Kelley. Brigham and Women's Hosp., & Beth Israel Hosp., Boston, MA

Anti-T cell monoclonal antibody (Mab) therapy is an important immunosuppressive agent for transplantation and autoimmunity. Treatment of non-obese diabetic (NOD) mice with 5ug/day of the anti-interleukin-2 Receptor (IL-2R) Mab, M7/20, from 5 wks of age for 5 wks prevents autoimmune insulinitis (2.1±0.5 in controls to 0.7±0.4 in M7/20 treated p<0.5). A possible complicating feature of Mab therapy is the appearance of host anti-allotypic and anti-idiotypic antibodies to the therapeutic agent. We have evaluated the antibody (Ab) response of NOD mice treated with 10ug/day of M7/20 (rat IgM, k) in a direct binding ELISA assay. Pretreatment and serum from mice treated for 1 wk did not have detectable anti-M7/20 IgG; while all mice produced Ab by 2 wks (1:800 titer) with increasing amounts at 3 wks (=/>1:3,200). The 3 wk serum contained anti-idiotypic anti-M7/20 Ab. This serum reduced the binding of M7/20 to Con A stimulated spleen cells by 49.2±11.3% and yet did not affect the binding of 7D4, another rat IgM, k anti-IL-2R Mab. Following absorption on a polyclonal Rat IgM Sepharose column, the immune serum bound to M7/20 but did not bind 7D4. Simultaneous anti-idiotypic Ab but not control Ig, was more effective in neutralizing the immunosuppressive effects of M7/20 on *in vivo* expression of DTH. Thus, anti-IL-2R Mab therapy causes paradoxical clinical effects: it suppresses insulinitis but engenders the formation of neutralizing antibodies that may limit the duration of effective treatment.

REVERSAL OF PROTEINURIA AND REDUCTION OF MORTALITY IN NZB/W F₁ LUPUS MICE BY PROSTAGLANDIN E₁ (PGE₁), ILOPROST AND A THROMBOXANE SYNTHASE INHIBITOR (TSI). A. Parbtani,* W.F. Clark and J.W.D. McDonald*. Univ. of Western Ontario, Dept. of Medicine, London, Ontario, Canada.

An over production of thromboxane (TX) by the glomeruli and an increased urinary excretion of TXB₂ have been associated with abnormal glomerular function and histology in lupus nephritis. These observations suggest an imbalance between TX and prostacyclin, and provide a rationale for using therapeutic agents which could restore this balance. The present study was undertaken to assess the effects of PG-analogues and TSI in NZB/W F₁ lupus mice. To provide a clinically relevant assessment of these agents, mice were treated after the onset of proteinuria with either TSI (100 mg/kg/bid; n=20) PGE₁ (200 µg/mouse; n=20), iloprost (10 µg/mouse/tid; n=18) or saline (control group; n=30). Mortality and proteinuria were assessed at weekly intervals. Urinary TXB₂ and 6 keto PGF_{1α} levels were measured prior to the onset of proteinuria (18 wk) and at the end of the study (40 wk). Mortality was significantly reduced in the treated groups (PGE₁ = 35%, p<0.01; iloprost = 38%, p<0.01; TSI = 65%, p<0.05) compared to the controls (73%). A significant proportion of mice in the treatment groups showed a reversal of proteinuria. All except the iloprost treated group showed a significant age-related increase of the urinary TXB₂ levels. All groups showed an age-related increase of 6 keto PGF_{1α} excretion. PGE₁, iloprost and TSI reduce mortality and reverse proteinuria in NZB/W F₁ mice. These beneficial effects did not correlate with urinary TXB₂ or 6 keto PGF_{1α} levels.

INDUCTION OF RENAL FIBROSIS BY NEPHROTOXIC NEPHRITIS. Sem H. Phan*, Greg Downer, Roger Wiggins, Univ. Michigan Med. Sch., Dept. Pathol., Ann Arbor, Michigan.

We have used a rabbit model of nephrotoxic nephritis to examine the induction of renal fibrosis. New Zealand white rabbits were treated with guinea pig (GP) anti-glomerular basement membrane IgG following prior sensitization to GP IgG. At selected time points, renal cortical tissue (RC) and glomeruli (G) were analyzed for hydroxyproline (HP) content and rates of collagen synthesis. HP content of both G and RC was significantly elevated (up to 2-3 fold of controls) at 14-16 days, and remained elevated up to 55 days in the cortex. Collagen synthesis in RC was significantly elevated as early as 3-4 days, peaking at 7-15 days (>200% of controls) and returning to control levels by day 21. Staining of cortical sections with anti types I & III, or IV collagen revealed both increased interstitial and basement membrane collagen in the fibrotic glomeruli and interstitium. Slot blot hybridization studies of cortical mRNA using cDNA probes to human procollagen I&III revealed increased types I&III procollagen mRNA (as great as 5-fold for type I) relative to controls, with the increase being much greater for type I vs. III procollagen message. These increases are associated with an increase in growth factor activity for isolated mesangial cells in media conditioned by glomeruli isolated for 7-day animals. These data suggest that induction of fibrosis occurs earlier than can be assessed morphologically and that any intervention designed to arrest or reverse this end-stage process must occur as early as possible to maximize success.

ANTI-FX1A INHIBITS DECAY OF C3/C5 CONVERTASES (CON) ON THE SURFACE OF GLOMERULAR EPITHELIAL CELLS (GEC). R.J. Quigg, A.V. Cybulsky, J. Badalamenti,* D.J. Salant. Boston University Medical Center, Boston, MA.

Activation of complement (C) on the cell surface is regulated by membrane proteins that inhibit CON of both the classical (CP) and alternative pathways (AP). In addition to activating the CP, heterologous anti-Fx1A activates the AP on the surface of GEC (ISN Proc., p. 352, 1987), suggesting that the antiserum contains antibodies that inhibit normal regulatory proteins. This might increase GEC injury in passive Heymann nephritis. We investigated the effect of anti-Fx1A on the decay of CON formed on the surface of cultured rat GEC. Cells were exposed to a C-fixing rabbit antibody to GEC membrane antigens and then to C5-deficient human serum (C5DS) to form CON. In some experiments, C5DS contained Mg-EGTA to permit only AP CON formation. Cells were then incubated for 30 min at 22°C with sheep anti-Fx1A IgG, anti-Fx1A Fab', or nonimmune sheep IgG. Residual CON activity was evaluated by adding C3-C9 (15% normal human serum in 10 mM EDTA), and cytotoxicity was determined by release of cell-incorporated bis-carboxyethyl carboxyfluorescein (BCECF). Data are corrected for background release in EDTA alone (4.2±0.7%).

CON	Specific BCECF Release (% of maximum)*		
	anti-Fx1A IgG	anti-Fx1A Fab'	Sheep IgG
CP + AP	33.1±4.2%†	16.0±5.4%†	6.5±2.8%
AP	14.4±4.4%†	-	0.2±1.3%

*meant±SD (n=5); † p < 0.001, ‡ p < 0.01 vs. sheep IgG. If C5DS was omitted, there was no release of BCECF above background. Thus, anti-Fx1A inhibits decay of CON on the GEC surface. Inhibition may occur through binding of anti-Fx1A to a regulatory membrane protein, and is, at least in part, independent of cross-linking. This phenomenon could enhance C activation on GEC in passive Heymann nephritis.

A FISH OIL DIET IS PROTECTIVE AGAINST ACCELERATED NEPHROTOXIC SERUM NEPHRITIS (ANSN). M. Miller*, H. Holthofer*, A. Sinha*, N. Gibbons*, A. Santiago*, and L. Scharfshmidt. A. Einstein Coll. of Med., Bronx, N.Y.

Fish oil diets (FOD) preserve renal function in murine lupus, but we have found that FOD accelerates renal disease in renoprival nephropathy. In this study we examined the effect of FOD in ANSN. Female rats fed either (20%w/w) beef tallow diets (BTD) or FOD (n=7) for one month were preimmunized with 1mg rabbit IgG. Five days later nephrotoxic serum (NTS) was injected. (14C) inulin clearance was measured chronically via surgically implanted osmotic minipumps in awake animals before and after NTS (*p<0.05 FOD vs BTD here and below).

	GFR (ml/min)		PROTEINURIA(mg/24 hr)
	pre-NTS	24hr post-NTS	24 hr post-NTS
BTD	2.1±.3	.6±.1	13±3
FOD	2.1±.2	1.3±.2*	3±.3*

FOD markedly attenuated the decline in GFR and rise in proteinuria. Renal histology 48-72 hr after injection of NTS was similar between BTD and FOD rats; 4/7 rats in each dietary group had entered the autologous phase. FOD reduced glomerular eicosanoids both in normals (n=3) and in NTS (n=7).

	PGE2 (ng/mgprot/10 min)		TXB2	
	Normal	Post-NTS	Normal	Post-NTS
BTD	5.1±.6	16±2	1.1±.2	4.0±.5
FOD	.4±.1*	6±1*	.2±.1*	1.5±.3*

FOD also induced an immune defect: Five days after preimmunization, FOD rats had more rabbit IgG remaining in their serum (22±5 ug/ml) than did BTD rats (6±3) (p<0.05) suggesting that FOD impaired removal of the foreign antigen. Thus the salutary effects of FOD may result from a combination of decreased glomerular TXA2 production and defective immune surveillance.

INDUCIBLE EXPRESSION OF THE Fc RECEPTOR FOR IgG IN RAT MESANGIAL CELLS. N. Njoku*, H.I. Werber*, P.M. Hogarth and J.R. Sedor. Case Western Reserve Univ., Cleveland, OH and The Univ. of Melbourne, Australia.

Contractile mesangial cells (MC) possess a number of macrophage-like characteristics and may serve as immune effector cells in glomerulonephritis. We have recently provided evidence that immune complexes alter MC biology in a manner dependent on the Fc region of antibody. However, the presence of Fc receptors (FcR) on MC is controversial. To confirm the presence of FcR on MC, Northern blot analyses of rat MC RNA were performed using specific probes for the murine FcR IgG receptor. Nonsynchronized MC contain a 1.9 kb mRNA transcript which hybridized to a cDNA that identifies both currently recognized FcR IgG species. A lower molecular weight mRNA species was detected in the FcR⁺ P388D₁ macrophage, and no transcripts were identified in FcR⁻ CHO cells. Preliminary studies using cDNA probes specific for each gene product demonstrated that MC express the β but not the α FcR gene product. Cycling MC had enhanced FcR expression 24 hrs after serum stimulation. Southern blot analysis of DNA obtained from MC and P388D₁ macrophages demonstrated restriction fragment length polymorphisms which may explain the different molecular weights of the FcR mRNA species demonstrated above. These results provide further evidence that MC in culture can be induced to express an FcR-related gene. The binding of immune complexes to FcR present on MC activated within an inflamed glomerulus may be an important mechanism of glomerular immune injury.

EFFECTS OF CYCLOSPORINE (CSA) IN EXPERIMENTAL FOCAL GLOMERULOSCLEROSIS (FGS). NS Narman, Jr* and FG Cosio, Ohio State University, Dept of Medicine, Columbus, OH.

CSA decreases proteinuria in patients with FGS but the mechanism of action and effects of CSA on histology are unknown. In this study, FGS was produced in 11 male Sprague-Dawley rats subjected to unilateral nephrectomy (UNX) and i.p. injections of puromycin (PAN) (4.2 mg/100 g bw, 0 wk x 3). CSA (25 mg/kg) (N=6), or vehicle (OIL) (N=5) were administered, via gavage, for 8 weeks. Tail cuff systolic BP, serum creatinine, and 24 hr urine protein were measured at weeks 0, 1, 4 and 8. At week 8, rats were sacrificed and renal histology was evaluated semi-quantitatively. PAN produced an average 65-fold (peak) increase in proteinuria at week 4 (455 mg/d vs baseline of 7 mg/d). CSA administration had no significant effects on proteinuria, BP or creatinine. Renal histology demonstrated FGS in 24±6 and 21±2% of glomeruli in CSA and OIL treated rats, respectively. A second group of UNX/PAN treated rats received CSA (N=5) or OIL (N=4) starting at week 8 when proteinuria was still 20-fold above baseline (week 8 = 157 mg/d, baseline = 7 mg/d). In this group of rats CSA, but not OIL, resulted in an acute and significant decline in proteinuria (week 9: CSA 49±14, OIL 150±43 mg/d; p<.02) which persisted for 3 weeks. OIL rats demonstrated progressive rise in BP from weeks 8 to 15 but CSA rats did not (week 8 = 130 mmHg for both and week 15 = 122 mmHg and 142 mmHg for CSA and OIL-treated rats, respectively). Renal histology demonstrated FGS in 22±7.2 and 29 ± 12% of glomeruli in CSA and OIL-treated rats, respectively. CONCLUSION: CSA reduces proteinuria and hypertension in experimental FGS, however, CSA therapy does not prevent the development of FGS.

IMMUNOSUPPRESSIVE EFFECT OF 15-DEOXYSPERGUALIN ON MURINE LUPUS NEPHROPATHY. Michihito Okubo, Keiichi Inoue,* Naoki Umetani,* Naoyuki Sato,* and Kouju Kamata. Kitasato Univ. Sch. Med., Dept. of Med., Sagami-hara, Japan.

We recently reported that 15-deoxyspergualin (dsp, J. Antibiotics 35: 1665, 1982) significantly prolonged the life span of New Zealand Black/White F1 mice (B/WF1) without reduction of body wt (Xth Intern. Congr. Nephrol., 1987). To analyze the mode of therapeutic action of dsp on nephropathy, B/WF1 were treated with dsp, 6 mg/kg, every other day, or with the carrier only, starting at 14 wks of age. At various ages, spleen, blood, urine and kidneys were obtained. Splenic T cell subsets were flowcytometrically analyzed. Serum IgG anti-DNA antibody levels were determined using solid phase radioimmunoassay, and proteinuria was semi-quantitated. Renal histological lesions were studied with both light microscopy and immunofluorescent antibody method. The results were evaluated as reported previously (Clin. Immunol. Immunopathol. 33: 31, 1984). Total spleen cells as well as L3T4+ T cells in the dsp-treated mice were significantly lower through 24 to 32 wks of age compared with the control mice. Dsp suppressed the age-dependent elevation in IgG anti-DNA titers and the development of proteinuria. Renal histological score, which represented the sum of intra-capillary and extra-capillary cellular proliferation and glomerular tuft necrosis, and the intensity of anti-IgG immunofluorescence both remained low through 14 to 32 wks of age. Dsp prolonged the life span and suppressed the development of lupus glomerulonephritis, which may be due to the exaggerated antibody production, by modulating the abnormal T cell function inherent to these lupus-prone mice.

EFFECT OF COMPLEMENT (C) DEPLETION ON GLOMERULAR EICOSANOID SYNTHESIS IN PASSIVE IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN). M. Rahman*, D. Sauter*, J. Broestl* and S. Emancipator. Hines VA Hospital and Loyola Univ., Hines and Maywood, IL. and Case Western Reserve Univ., Cleveland, OH.

We examined the effect of C depletion on glomerular synthesis of PGE₂ and TxB₂ in passive ICGN model in the rat. The model was induced by infusion of cationic bovine gamma globulin (cBGG) in the left renal artery, followed by infusion of rabbit anti-BGG antibody. The right kidney served as a control (CT). C depleted rats received a daily i.p. injection of cobra venom factor (CVF), starting one day before induction of passive ICGN and continued until rats were sacrificed on day 7. Proteinuria, glomerular morphologic changes and glomerular PGE₂ and TxB₂ were examined. Administration of CVF resulted in elimination of glomerular staining for C3 and prevented the development of proteinuria (8.4±1.8 mg/24 hour in CVF treated rats, N=10, vs 42.7±11.6 for control, N=10; P<0.005). PGE₂ and TxB₂ results, expressed in ng/mg glomerular protein, were as follows:

	PGE ₂		TxB ₂	
	ICGN	CT	ICGN	CT
Control	0.98±0.08	0.99±0.05	5.31±1.6*	2.7±0.4
CVF	1.0±50.29	1.0±90.45	3.7±1.3	2.2±0.42

*p<0.05 vs CT, N=7.

Glomerular TxB₂ was significantly increased after induction of passive ICGN, there were no changes in PGE₂ production. Administration of CVF inhibited the increment in TxB₂. We conclude that in passive ICGN glomerular C deposition is probably the stimulus for enhanced thromboxane production.

MONOCLONAL ANTI-DNA ANTIBODIES INDUCE KIDNEY DYSFUNCTION BY DIRECT BINDING TO RENAL ANTIGENS. E. Raz,* M. Brezis, E. Rosenmann,* Z. Neeman* and D. Eilat.* Hadassah University Hospital, Jerusalem, Israel.

While anti-DNA antibodies (Ab) may bind directly to renal antigens their role in alteration of kidney function remains unknown. We tested the binding ability of mouse monoclonal (MC) anti-DNA Ab and its influence upon renal function. Isolated rat kidneys were perfused with albumin Krebs solution alone (I), with MC mouse anti-DNA Ab (II) and with purified IgG of the same subclass (γ2b)(III), obtained from mouse myeloma. Results were as follows:

Group (n)	†GFR		ΔTRNa	
	(100 min)	(80 min)	(80 min)	(160 min)
I (5)	0.52 ± 0.11	96.7 ± 1.9	93.7 ± 3.5	
II (6)	0.37 ± 0.02	95.4 ± 1.2	76.3 ± 5.8*	
III (4)	0.42 ± 0.13	96.5 ± 0.8	90.4 ± 3.7	
Group (n)	φProteinuria			
	(80 min)	(160 min)		
I (5)	111 ± 31	125 ± 39		
II (6)	279 ± 92	1345 ± 410*		
III (4)	84 ± 35	85 ± 21		

† - ml/min; Δ - %; φ - μg/min; Mean ± SE; * p<0.01
Immunofluorescence for IgG showed diffuse granular staining of renal interstitium in group II only. Glomeruli remained unstained. Preincubation of the MC anti-DNA Ab with DNA prior to its addition to the perfusate, prevented the deterioration in renal function (n=4), suggesting lack of pathogenicity of these DNA-anti-DNA Ab complexes in this model. These data indicate that anti-DNA Ab may directly bind to kidney antigens and induce renal dysfunction.

MACROPHAGE (Mφ) CHEMOTAXIS IN THE ESSENTIAL FATTY ACID DEFICIENT (EFAD) RAT. B. H. Rovin*, J. B. Lefkowitz*, and G. Schreiner, Washington University School of Medicine, St. Louis, Mo. 63110

Previous work in our laboratory has demonstrated that in rats, a diet deficient in essential fatty acids results in an 80% depletion of resident glomerular Mφ and prevents accumulation of glomerular Mφ in response to inflammatory stimuli such as nephrotoxic serum or aggregated ferritin. We undertook the current investigation to determine if glomerular Mφ depletion in EFAD rats was secondary to a chemotactic defect. Rats were fed a normal or EFAD diet for at least 8 weeks. Peritoneal Mφ were harvested 4 days after i.p. injection of 0.5% glycogen. The chemotactic response of the Mφ was studied in a multiwell micro-chemotaxis chamber. The yield of EFAD peritoneal Mφ was 35% lower than that of control animals. There was however, no difference between control and EFAD Mφ migration in response to zymosan activated control rat serum, activated EFAD serum, or platelet activating factor. Additionally, there was no difference in the chemotactic response of normal Mφ to activated control or EFAD sera. Incubation of non-activated EFAD serum with normal rat serum during a chemotaxis assay failed to demonstrate the presence of a circulating inhibitor of Mφ migration. Preliminary experiments suggest the presence of chemotactic activity in the supernatants of cultured normal glomeruli. These results indicate that glomerular Mφ depletion in rats fed an EFAD diet is not due to an intrinsic defect of Mφ chemotaxis or serum chemotactic activity. The possibility of a defect in glomerular chemoattractant generation must be entertained.

COMPLEMENT (C) ACTIVATION ON CULTURED RAT GLOMERULAR EPITHELIAL CELLS (GEC) IS REGULATED BY MEMBRANE PROTEINS. D.J. Salant, R.J. Quigg, A.V. Cybulsky, J. Badalamenti*. Boston University Medical Ctr., Boston, MA.

The nephritogenic antibody of passive Heymann nephritis, anti-Fx1A, binds to cultured GEC membrane antigens and induces C-dependent cytotoxicity (KI, 31:328, 1987). We investigated if C activation on GEC is regulated by membrane proteins. Because the action of these proteins is reported to be species specific, we first compared cytotoxicity of heterologous and homologous C. Antibody-sensitized GEC were incubated with fresh rat (FRS), rabbit, or guinea pig sera, and cytotoxicity was assessed by release of cell-incorporated bis-carboxyethyl carboxyfluorescein (BCECF). Data are expressed as mean±SD (n=3) and are corrected for background release of BCECF. At all doses of C, cytotoxicity was greater with heterologous than homologous C (e.g., BCECF release was 21.8±0.7%, 54.3±4.3%, and 69.5±0.2% with 5% rat, guinea pig, and rabbit sera, respectively). In further studies, GEC were treated prior to BCECF incorporation with trypsin or pronase, which are known to inactivate membrane regulatory proteins on human erythrocytes, such as C3b receptor and decay accelerating factor.

anti-Fx1A	C	Specific BCECF Release (% of maximum)		
		Untreated	Trypsin	Pronase
+	10% FRS	24.3±1.3%	38.6±1.2%	71.6±0.9%
-	"	0	0	9.7±3.2%

Enzyme treatment did not affect binding of ¹²⁵I anti-Fx1A to GEC (no enzyme: 23.3±4.6; trypsin: 18.3±2.9; pronase: 19.3±4.1 ng anti-Fx1A specifically bound/mg cell protein; n=4). Thus, homologous C was less effective than heterologous C in producing GEC injury, and proteolytic enzyme treatment increased cytotoxicity with homologous C. This indicates that C activation on rat GEC is controlled by membrane protein(s).

TREATMENT OF HEYMANN NEPHRITIS (HN) BY 15-DEOXY-SPERGUALIN (SP). Naoyuki Sato,* Kouju Kamata and Michihito Okubo.* Kitasato Univ. Sch. of Med., Dept. of Int. Med., Sagami-hara, Kanagawa, Japan.

Treatment of HN was designed using SP, an immunosuppressant newly developed in Japan (J. Antibiotics 35, 1665, 1982). Thirty-one female Lewis rats were immunized with 120 µg Fx1A and B. pertussis vaccine for initial immunization and 60 µg Fx1A for booster immunization at 4 wks. Effect of SP on weight and survival of rats, development of proteinuria, antibody (ab) titers against Fx1A in the sera and antigens recognized by the sera was studied by giving 0, 0.25, 0.5, 1.0 or 2.0 mg/kg of SP, 6 times a week until 8 wks, 3 times a week until 10 wks, intraperitoneally. At 14 wks after immunization, they were sacrificed and the glomerular lesion was identified by light microscopy and graded 0-4 by immunofluorescence (IF). One of 6 rats in 0.5 or 1.0 mg SP group and 6 of 7 in 2 mg SP group were dead by 14 wks. Every rat in 0, 0.25 or 0.5 mg group showed positive proteinuria (>20mg/day); while none of 1.0- or 2.0 mg-SP rats showed positive proteinuria by 14 wks. Control group (0 mg) showed the peak ab titer of 33506±5024(SD) cpm at 6 wks; while the 1.0 mg group showed the peak of 7390±3614 cpm at 8 wks. The 330 kD, 440 kD and 700 kD glycoproteins were precipitated by serum IgG obtained from every group of rats at 8 wks. Glomerular deposits were suppressed by SP (Table).

	Dose of SP (mg/kg)			
	0	0.25	0.5	1.0
IgG	4.0±0.0(6)	3.5±0.6*(4)	3.2±0.4*(5)	1.8±1.1*(6)
C3	2.3±0.5(6)	2.0±0.8*(4)	1.8±0.8*(5)	0.0±0.0*(6)

*: p<0.01 vs 0 mg group by Mann-Whitney's U test.
SP suppressed the development of proteinuria and glomerular lesions of HN by inhibiting the ab production against Fx1A.

HISTONES CAN PROVOKE IMMUNECOMPLEX FORMATION IN THE GLOMERULAR CAPILLARY WALL.

Thomas Schmiedeke*, Frank Stoeckl*, Stephen Batsford* and Arnold Vogt* (intr. by Helmut Renke). Dept. of Immunology, Institute of Medical Microbiology, D-7800 Freiburg, F.R.G.

Histones are small (<22Kd), highly cationic (pI>11) entities which can aggregate spontaneously. We asked: 1) do histones have affinity for glomerular polyanion and can they act as planted antigen, 2) can histones neutralise fixed negative charges permitting deposition of anionic antigens(e.g. DNA) in the glomerulus. Following perfusion of 500µg of a polydisperse histone preparation(11-150Kd) via the rat aorta, intense staining for histone was seen in a capillary pattern by immunofluorescence. The histone molecules were accessible for subsequently injected (i.v.) rabbit anti-histone antibody, resulting in immune complex(IC) formation. When perfusion of histone was followed by i.v. injection of native ferritin(3mg/100g body weight) this tracer readily entered the capillary wall, in contrast to ferritin given alone. These results establish an experimental basis for the participation of histones in glomerular immune complex disease, for example in Lupus nephritis. Antibodies to histones can be observed in glomerulonephritis in man (e.g. SLE) and in mice (NZB/W F₁ and Graft versus Host models) lending support to the proposed hypothesis.

LOCALIZATION OF DECAY ACCELERATING FACTOR (DAF) IN NORMAL AND DISEASED KIDNEYS. DD Sedmak*, FG Cosio. Ohio State University, Departments of Medicine and Pathology, Columbus, Ohio.

DAF is a membrane glycoprotein (MW 70k) which inhibits complement (C') activation on cell surfaces, protecting cells from C' mediated damage. DAF inhibits both classic and alternative pathway activation of C3. DAF is present in circulating blood cells and vascular endothelium. Clinically, decreased erythrocyte DAF is associated with complement-mediated hemolytic anemia. The presence of DAF in the kidney is of interest since DAF is present in normal urine and in disease states, the kidney is frequently exposed to C' activation products. Frozen/acetone fixed normal kidney specimens (N=16) and pathologic kidney specimens (N=28) were stained with monoclonal anti-DAF antibodies (kindly provided by Dr. V. Nussenzweig) and Peroxidase labeled anti-mouse antibodies. In normal kidneys DAF was present only in the juxtaglomerular apparatus (JGA) of all superficial and deep glomeruli. DAF was not detected in other normal kidney structures. JGA DAF was decreased in 11 of 28 pathologic kidney specimens (4 of 6 SLE, 3 of 7 MN, 1 of 3 MPGN, and 1 case each of Goodpasture's syndrome, scleroderma, and interstitial nephritis). In diseased kidneys DAF was present in mesangium (N=8), interstitium (N=11), vessels (N=8) and crescents (N=1). Conclusions: In health, DAF is contained only in the JGA. In disease states, DAF tends to be decreased in JGA but is present in glomerular, vascular, and interstitial sites. DAF may play a role in containing complement injury in kidney.

A NOVEL SIGNAL TRANSDUCTION PATHWAY IN INTERLEUKIN-1 STIMULATED RAT MESANGIAL CELLS. J.R. Sedor, P. Mene*, M.S. Simonson*, and M. Kester*. Department of Medicine, Case Western Reserve Univ., Cleveland, Ohio.

Proliferation of mesangial cells and local release of mitogens may be important pathogenic determinants of glomerular immune injury. Thus, we explored recombinant interleukin-1α (IL-1)-induced phospholipase C activation and DNA synthesis in cultured rat mesangial cells (MC). IL-1 induced a dose- and time-dependent elevation of [³H]-arachidonate labeled 1,2-diacylglycerol (DAG) in MC. DAG formation was observed as early as 15 sec after IL-1 (3.6 ng/ml), peaked at 1 min with a 74.6±5.2% increment (n=5) and decreased at 5 min to a new steady level. Surprisingly, concomitant experiments demonstrated no IL-1-stimulated changes in the concentration of inositol phosphates (IP₁, IP₂, IP₃) measured in MC labeled with [³H]-myoinositol. However, arginine vasopressin (AVP) induced rapid hydrolysis of polyphosphoinositides in MC from the same culture. In corroborating experiments, IL-1 did not induce any changes in cytosolic free calcium ([Ca²⁺]_i) concentration as measured in fura-2-loaded MC whereas AVP increased [Ca²⁺]_i. Alternative sources for DAG formation were investigated and elevated concentrations of [³²P]-phosphocholine (631±63 vs. 412±29 dpm/ug protein, 3.6 ng/ml IL-1 vs. control, respectively, n=3) were noted at 1 min. In contrast to other mitogens, IL-1 did not change the cytosolic pH of BCEFC-labeled MC whereas monensin and AVP induced cytosolic alkalization. Yet, IL-1 but not denatured IL-1 stimulated proliferation as measured by [³H]-thymidine incorporation. In MC, IL-1 induces sustained DAG formation that appears to be derived from phosphatidylcholine and is independent of polyphosphoinositide turnover. IL-1-induced MC mitogenesis may, in part, be mediated by this novel mechanism of signal transduction.

COMPARISON OF MORPHOMETRIC LIGHT MICROSCOPY (MLM) AND QUANTITATIVE SCANNING ELECTRON MICROSCOPY (SEM) IN DETERMINING GLOMERULAR SIZE. L. Smith-Powell*, SC Textor, J Hardy*, M Miller*, Renal Lab. and Res. Resources, Beckman Res. Inst. and City of Hope National Medical Center, Duarte, CA

Determination of glomerular size using morphometric techniques usually assumes a homogeneous population of spherical glomeruli. SEM allows direct measurement of whole glomeruli instead of sections. We compared measurements by MLM and SEM of perfusion fixed (at 120 mm Hg) left kidneys from 18 Sprague-Dawley rats after unilateral nephrectomy (NX), sham nephrectomy (SNX) or interrenal aortic ligation (LIG). Mean glomerular diameter (dia.) in H & E sections was determined by analysis of 50-100 glomeruli. Longest and perpendicular diameters from 10-46 whole glomeruli were determined in SEM samples.

	MLM	SEM long.	SEM perp.
NX (8)	140±5	93±4 *	80±3 *#
SNX (6)	134±2	90±3 *	77±3 *#
LIG (4)	109±2	73±2 *	63±1 *#

(Microns; *, p<.01 vs morph.; #, p<.02 vs SEM long)

Tissue shrinkage for SEM averaged 33%; range: 22-45%. MLM and SEM correlated (R=0.80, P<.01) but MLM was more sensitive in detecting small changes, e.g. hypertrophy. Inter-glomerular variability by SEM was 10.0±9% per kidney. Longest and perp. SEM dia. differed by 13.4±4% (p<.01). These data demonstrate that glomeruli are sufficiently homogeneous after perfusion to use MLM, but that accurate volume calculations must consider non-spherical characteristics of glomeruli.

COMMON GERMLINE V GENE UTILIZATION IN C3NeF AND ANTI-DNA: EVIDENCE FOR IDIOTYPIC IDENTITY AS A MEANS OF REGULATING PRODUCTION OF AUTOANTIBODIES. Roger E. Spitzer and Ann E. Stitzel. SUNY Health Science Center at Syracuse, Dept. of Pediatrics, Syracuse, NY.

C3NeF is an autoantibody found predominantly in patients with membranoproliferative glomerulonephritis (MPGN). The relationship of C3NeF to other autoantibodies was studied by determining the idiotypic commonality between C3NeF and anti-DNA. Specific C3NeF was isolated and purified from 5 unrelated patients with MPGN, EBV established B cell lines from 2 additional patients with MPGN, and 1 patient with partial lipodystrophy. Anti ds DNA was isolated and purified from 3 different patients with systemic lupus erythematosus. Auto anti-idiotypic antibody to C3NeF isolated from 3 of the patients with MPGN blocked the binding of each C3NeF preparation to EC3bBb and each anti-DNA to ds DNA. Thus, all 11 autoantibodies appear to contain the same idiotype in or near the V region or antigen combining site. These data indicate that the same unmutated germline V gene is used in the production of C3NeF and anti-DNA in these patients. It is likely that this restricted idiotype represents a highly conserved and efficient mechanism for the network control of the synthesis of these and other auto antibodies.

SCARRING POTENTIAL, FIMBRIAL TYPE α -HAEMOLYSIN PRODUCTION AND HUMAN NEUTROPHIL (PMN) ACTIVATION BY UROPATHOGENIC STRAINS OF ESCHERICHIA COLI. R. Steadman, N. Topley, R. Mackenzie, M. Davies and J.D. Williams (Intr. by R.B. Sterzel). Univ. of Wales College of Medicine, Dept. of Renal Medicine, Cardiff, Wales, U.K.

The renal scarring associated with chronic pyelonephritis (CP) is directly related to the initiation of an inflammatory response. However, the bacterial characteristics required to elicit this response are poorly understood. In a rat model of CP, the degree of scarring 6 weeks after intrarenal injection of defined uropathogenic strains of Escherichia coli (E.coli) correlated with the expression of type 1 mannose sensitive (MS) fimbriae (p<0.01) but not with that of mannose resistant (MR) fimbriae or α -haemolysin. E.coli strains expressing MS fimbriae promoted in vitro release (p<0.0005) of 1st, 2nd and 3rd granule markers (including neutral protease (NP) activity, >90% of which was due to 1st granule elastase) in a dose and time dependent manner. Strains expressing only MR fimbriae did not release significant NP activity. Also there was no correlation between α -haemolysin and release of PMN neutral proteases. Neither the expression of α -haemolysin nor MS-fimbriae correlated with activation of the PMN 5-lipoxygenase pathway (LTB₄) which was independent of 1st granule NP release. Our results suggest that α -haemolysin production is not related to the ability of E.coli strains to activate PMN in vitro or to cause renal scarring in vivo. However, there was a specific interaction between MS fimbriate strains and PMN resulting in NP release which was directly related to their ability to cause renal scarring.

PROTEINURIA IN LONG AND SHORT LOOPED NEPHRONS IN AGING RATS. Coleman H. Terrell, Margaret M. Delaney, John R. Hoyer*. The Children's Hospital Philadelphia, PA.

The heterogeneity of proteinuria among nephrons in 17-month-old male Sprague-Dawley rats was assessed by a new method for study of individual nephrons. This method allows large numbers of nephrons otherwise inaccessible to direct study of proteinuria to be separated into long and short looped populations and is based on previous studies showing that antibodies to Tamm-Horsfall protein (TH) enter the glomerular filtrate of proteinuric rats and form immune complexes with TH present on luminal surfaces of the thick ascending limbs of Henle (TAL). Each TAL functions as an immunoabsorbent column since the distance along the TAL is proportional to the magnitude of proteinuria. Proteinuric nephrons are identified by using these immune deposits as a marker. 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 474±83 (mean±SEM) TAL/rat were evaluated. TALa (38.8±1.4% of all TALs) were twice as likely as TALd to contain luminal rabbit IgG deposits: 41.3±6.1% vs. 24.8±4.2%, n=9, (p=.002) and had deposits with greater intensity than TALd, (p=.027). The incidence of glomerulosclerosis was greater in inner cortical than outer cortical glomeruli: 8.1±2.0% vs. 3.2±1.3%, (p=.038). These results show that long looped nephrons make a greater contribution to proteinuria than short looped nephrons in old male SD rats and may be responsible for the greater incidence of sclerosis in inner cortical glomeruli.

PROTEOLYTIC ACTIVITY IN RAT GLOMERULI AFTER INDUCTION OF IN SITU IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN). F. Thaisz*, C. Wanner*, W. Hori*, University of Frankfurt and Freiburg, FRG (introduced by R.A.K. Stahl).

A pathogenetic role of proteases in experimental glomerulonephritis has recently been suggested. Proteases prevent development of passive Heymann nephritis. Additionally urinary excretion of neutral metalloproteases is increased in nephrotoxic serum nephritis.

To further evaluate a pathogenetic role of proteases we examined protease activity in supernatants of isolated glomeruli from rats with ICGN. GN was induced by selective perfusion of the left kidney with cationized human-IgG followed by an intravenous application of rabbit anti-human-IgG. Glomeruli were isolated by differential sieving at 24 and 48 hours, 7 and 14 days after induction of nephritis. Glomeruli were superfused in Krebs-Ringer-bicarbonate buffer. Protease activity in supernatants were determined by the azocasein test and further characterized by various inhibitors of tissue proteinases. The metalloprotease activity was significantly increased 24 hours after induction of ICGN when compared to controls, it was, however, unchanged 48 hours, seven and 14 days after induction of disease (table).

proteolytic activity (U/ μ g/min)			
C	24h	48h	7d
2.4	4.4	2.5	2.1
± 1.4	± 1.3	± 1.4	± 1.5
			1.3

We conclude that proteolytic enzymes are released from isolated rat glomeruli. Their formation is increased in rats with in situ ICGN. The proteases might contribute to development of glomerular injury and proteinuria in this experimental model. The cellular origin of proteolytic activity might be resident glomerular or blood derived inflammatory cells.

HIGH DIETARY CARBOHYDRATE ACCENTUATES PROTEINURIA AND DIABETIC NEPHROPATHY IN THE SHR/N-CP RAT WITH OBESITY AND TYPE II DIABETES MELLITUS.

MT Velasquez, PL Kimmel, OE Michaelis IV,* N Carswell,* A Abraham,* and JP Bosch, George Washington Univ., Dept. of Med. and Pathology, and Beltsville Human Nutr. Res. Ctr., ARS, USDA, Washington, D.C. and Beltsville, Maryland.

The SHR/N-cp rat is a new genetically obese rat with metabolic abnormalities of type II diabetes mellitus which worsen on high carbohydrate(CHO) diet. In the present study we evaluated the effects of high CHO diet on renal function and morphology in this model. Five week old male obese rats(n=11) and lean litter mates(n=8) were fed diets containing 54% CHO and 20% protein. Body weight, creatinine clearance(Clcr), urinary glucose(UglucV) and urinary protein(UprotV) excretion were measured at 4 and 12 weeks. Then rats were sacrificed for histopathological examination of kidneys. Results (Mean \pm SEM; *p<.01 compared to 4wks; \dagger p<.01, compared to lean rats):

	4 WEEKS		12 WEEKS	
	Lean	Obese	Lean	Obese
Body Weight, g	325 \pm 1	469 \pm 20 \dagger	406 \pm 7	609 \pm 11 \dagger
Clcr, ml/min/kg	3.5 \pm 0.3	4.0 \pm 0.7	2.7 \pm 0.3	2.6 \pm 0.3*
UglucV, mg/17h	1.4 \pm 0.2	99 \pm 13 \dagger	1.5 \pm 0.1	95 \pm 21 \dagger
UprotV, mg/17h	20 \pm 1	69 \pm 15 \dagger	19 \pm 3	90 \pm 16 \dagger

Kidneys from obese rats weighed more than kidneys from lean rats(1.5 \pm 0.06g vs 1.2 \pm 0.06g, p<.05) and showed diffuse mesangial expansion and glomerular basement membrane thickening on electron microscopy consistent with diabetic renal disease. These data demonstrate that high dietary CHO worsens proteinuria and diabetic nephropathy in the obese SHR/N-cp rat.

MIXED IgG-IgA AGGREGATES AS A MODEL OF IMMUNE COMPLEXES (IC) IN IgA NEPHROPATHY(IgAN). F.B. Waldo, J. Mestecky,* and E.C. Kohaut. Univ of Alabama at Birmingham, Depts. of Pediatrics and Microbiology, Birmingham, Al.

Patients with IgAN have IC which contain IgA, IgG and C3. Erythrocytes (RBCs) promote clearance of IC containing C3b. IgA fixes C3 poorly and IgA-IC do not bind to RBCs. To investigate these IC properties *in vitro*, we have mixed human IgG and IgA1 and heated them for 63°C for 2 hrs to form mixed aggregates(agg). On sucrose density gradients IgG agg were 11 to 19S while IgA agg were either 11S or >19S. Addition of IgA to IgG increased the agg size towards 19S. The pI of IgG agg was 7-9 and of IgA agg 4.5-5.5. The pI of mixed agg was decreased as the percent IgA was increased. IgG agg mixed with NHS caused 20% C3 activation(20 min, 37°C) while IgA agg causes no activation. There was a linear decrease in C3 activation by agg as the percent IgA increased. Mixed aggregates which contained either radio-labeled IgG or IgA were mixed with NHS (1hr, 37°C) and then solubilized, reduced and separated by 10% SDS-PAGE. Heavy molecular weight bands, consistent with covalent bonding of C3b and C3bi to Ig heavy chain were only seen in lanes with labeled IgG. Binding of agg (+C3) to RBCs was tested. IgG-C3 agg bound but IgA agg (+C3) did not. Addition of >10% IgA to an IgG-C3 agg inhibited RBC binding. We conclude that IgG in the mixed agg is responsible for C3 fixation. In contrast, IgA in mixed agg does not fix C3 but instead lowers the pI, increases the size and inhibits binding to RBCs. These properties in an IC would inhibit RE clearance, promote mesangial deposition and local complement activation.

THE EFFECT OF AEROBIC TRAINING ON GLUCOSE CONTROL, KIDNEY FUNCTION AND STRUCTURE IN THE OBESE (DIABETIC) ZUCKER RAT (OZR). Kory M. Ward*, John D. Mahan, Julia M. Lash* and William M. Sherman*. The Ohio State University & Children's Hospital, Columbus, Ohio.

The obese Zucker rat is a genetic model of Type II diabetes where hyperglycemia develops at 12-18 wks. Renal disease characterized by mesangial expansion and focal sclerosis develops by 26 wks. To assess the effects of exercise on the prevention of this form of diabetic nephropathy (DN), 8 OZR underwent treadmill exercise (OR) 1 hr/d for 5 d/wk starting at 6 wks of age. Fasting blood glucose (FBG), glycated hemoglobin (GHb), proteinuria (P) and % mesangial volume (MV) by quantitative EM were assessed at 18 wks. Lean littermates (LZ) and sedentary OZR (OS) served as controls.

	LZ	OS	OR
	(Group means \pm SEM)		
FBG mg/dl	123 (3.7)	193 (10.3)*	163 (5.7)*#
GHb %	4.5 (0.5)	6.4 (1.3)	5.5 (1.9)
P mg/d	7.5 (0.9)	41.6 (26.2)*	9.3 (2.3)#
MV %	8.8 (3.6)	21.4 (3.1)*	15.9 (3.6)*#

* = sig. vrs LZ by one-way ANOVA at p 0.05

= sig. vrs OS by one-way ANOVA at p 0.05

Conclusions: Exercise training improves glucose control and P early in the OZR. Mesangial expansion is present early in OZR and is partially prevented by aerobic exercise. This data suggests that exercise training may be beneficial in the prevention of DN in Type II diabetes. The benefits of continued exercise in this condition and the effects of aerobic exercise on more advanced DN remain to be determined.

INDUCTION OF TISSUE FACTOR SYNTHESIS BY CULTURED RAT MESANGIAL CELLS BY LPS AND BY TNF- α BUT NOT BY INTERLEUKIN-1. Roger Wiggins, Nnennaya Njoku*, John Sedor. University of Michigan, Ann Arbor, Michigan and Case Western Reserve, Cleveland, Ohio.

Both TNF- α and IL-1 have been shown to induce endothelial cell tissue factor synthesis. We therefore examined cultured rat mesangial cells to determine whether they might potentially contribute to the procoagulant signal during inflammation in response to similar signals. Rat mesangial cells were grown from isolated glomeruli in 20 percent fetal cell serum by standard methods. Confluent monolayers were incubated in serum-free medium for 96 hours to allow the cell procoagulant activity (PCA) to fall to baseline levels. Various agents were added to wells and after 6 hours of incubation the PCA was measured in each well by one stage coagulation time and confirmed to be tissue factor-like using deficient plasmas. The following results were obtained: control 2.8 \pm 0.6; PAF (10⁻⁶M) 5.4 \pm 2.2 NS; LPS (10⁻⁶g/ml) 12.8 \pm 1.8 p < 0.01; IL-1 (10 U/ml) 3.7 \pm 0.7 NS; TNF- α (100 ng/ml) 7.2 \pm 1.2 p < 0.04. In a similar experiment in the presence of a defined growth medium the values obtained were as follows control 8.4 \pm 1.3 U/mg protein, PAF (10⁻⁶M) 8.8 \pm 1.9 NS, LPS (10⁻⁶g/ml) 14.4 \pm 2; p < 0.01; IL-1 (10 U/ml) 9.3 \pm 1.5 NS; TNF- α (100 ng/ml) 14.4 \pm 5, p < 0.01. These results show that resting rat mesangial cells in culture can be induced to synthesize tissue factor in response to TNF- α and LPS but not IL-1. Induction of procoagulant activity by these signals may be relevant to intraglomerular fibrin formation under some circumstances.

GLOMERULAR HYPERTROPHY HAS A GREATER IMPACT ON GLOMERULAR SCLEROSIS THAN THE ADAPTIVE HYPERFUNCTION IN REMNANT NEPHRONS. Y. Yoshida*, A. Fogo* & I. Ichikawa. Departments of Pediatrics & Pathology, Vanderbilt University, Nashville, Tennessee.

Experimental animal models of subtotal nephrectomy (sNPX), diabetes and high protein diet feeding, which predispose to glomerular sclerosis (GS), are characterized by hypertrophy of glomeruli which precedes sclerosis, raising the possibility that the hypertrophic process may have a direct causal link to the GS. An experimental finding consistent with this notion was obtained when sclerosis index (SI: 0-4 scale) and maximum planar area (PAm_{ax}) were simultaneously determined after sNPX at the single glomerular level by serial thin-section histological analysis: Four-six weeks after sNPX (n=7), 90% of glomeruli had mild GS (SI < 1.5) with a strong positive correlation between PAm_{ax} vs SI (P < 0.005). In contrast, 12 weeks after sNPX (n=5) more than 50% of glomeruli had advanced GS (SI \geq 1.5), and the glomeruli with more severe GS tended to have decreased PAm_{ax}. In the study, a further attempt was made to identify early functional indices which may predict the degree of subsequent hypertrophy in the same glomerulus: Within a given kidney, however, values for PAm_{ax} or SI determined 4-6 weeks after sNPX had no tendency to correlate with maximum levels of SNGFR or glomerular capillary pressure assessed in the same glomerulus by serial micropuncture measurements in earlier stages. In separate groups of sNPX rats at 12 weeks, administrations of enalapril (50 mg/L drinking water)(n=5) or hydralazine (200 mg/L) + reserpine (12.5 mg/L) + hydrochlorothiazide (62.5 mg/L)(n=5) largely ameliorated GS (SI: 0.17 vs 0.20). Again, there was a positive correlation (p < 0.05) between mild degrees of GS vs PAm_{ax} at single glomerular level.

Thus, unlike adaptive hyperfunction, the hypertrophic process in the remnant glomeruli appears to impose a direct impact on the severity of glomerular sclerosis. The observations also support the view that the hyperfunction of the remnant glomeruli plays little causal role in the development of this hypertrophy.

ANTIBODY INDUCED MESANGIAL CELL (MC) LYSIS AND PROLIFERATION: GLOMERULAR HEMODYNAMIC CONSEQUENCES. T. Yamamoto,* C. Mundy,* C.B. Wilson, and R.C. Blantz. Res. Inst. of Scripps Clinic and VAMC and UCSD, La Jolla, CA.

Anti-thymocyte antibody (ATS) causes complement dependent MC lysis at one day (1d) and mesangial proliferation at six days (6d) (J. Immunol. 138: 3758, 1987). Nephron filtration rate (SNGFR), plasma flow (SNPF), glomerular capillary hydrostatic pressure (P_G) and pressure gradient (Δ P), and glomerular ultrafiltration coefficient (LpA) were measured at 1d and 6 d after ATS and in normal serum controls (C). \dagger p < 0.05 vs. C, \S p < 0.05 vs. 1d

	SNGFR (nl/min)	SNPF (nl/min)	P _G (mmHg)	Δ P (mmHg)	LpA (nl/sec/mmHg)
C	44 \pm 2	149 \pm 12	55 \pm 2	36 \pm 2	0.06 \pm .006
ATS-1d	36 \pm 2 \dagger	191 \pm 13 \dagger	64 \pm 2 \dagger	40 \pm 2	0.035 \pm .003 \dagger
ATS-6d	28 \pm 2 \dagger	116 \pm 13 \S	58 \pm 2 \S	35 \pm 1 \S	0.034 \pm .006 \dagger

MC loss was extensive in ATS-1d and capillary lumina were focally enlarged with plasma transiting the mesangial region. Mesangial hypercellularity in ATS-6d rats appeared to compress capillary lumina. LpA decreases at 1d and 6d were related to differing mechanisms: At 1d SNPF increased with MC loss distorting tertiary structure of the capillary loop. This leads to capillary flow separated from the filtering surface and the possibility of concentration polarization of proteins. At 6 d mesangial expansion 1) was associated with SNPF decreased from 1d values and 2) contributed to decreases in LpA. Selective destruction and proliferation of MC leads to architectural alterations that reduce SNGFR by different mechanisms.

IMMUNOLOGY PATHOLOGY— CLINICAL

BENEFICIAL EFFECT OF OKT3 IN STEROID RESISTANT, PREDOMINANTLY VASCULAR, REJECTION. V.B. Delaney, W.G. Campbell, J. Whelchel. Emory University, Atlanta, GA.

Three patients became oligoanuric and uremic with a return to dialysis in the early (2-5 days) post-transplant period. No clinical improvement followed pulse methylprednisolone (lg i.v. for 3-5 days). Renal biopsies, performed in all patients 2 to 5 days following pulse steroids, showed severe, predominantly vascular, rejection with almost total occlusion of the lumens of the medium and small arteries by an admixture of lymphocytes and endothelial-like cells. Lymphocytes were dense perivascularly but otherwise sparse. T-cells predominated, Helper (OKT4) or suppressor (OKT8), with minimal or no B-cells. HLA-DR (anti-Ia) expression was marked in patients. Treatment with OKT3 monoclonal antibody was associated with an immediate diuresis and improvement in serum creatinine. Repeat renal biopsies, within 4 weeks of cessation of OKT3, showed healing of the vascular lesions by focal scar formation. All 3 patients maintain normal renal function 8, 12, and 17 months later.

Summary: OKT3 was effective therapy in steroid resistant, mainly vascular rejection, not usually considered amenable to treatment. T-cell predominance may indicate a subgroup with this form of rejection who will respond favorably to OKT3.

MONOCLONAL ANTIBODIES (mAb) TO TAMB-HORSFALL PROTEIN (THP) AS PROBES OF TUBULAR OBSTRUCTION IN MYELOMA CAST NEPHROPATHY (MCN). M. De Meyer,* P. Ronco,* B. Mougenot,* P. Dosquet,* V. Lemaître,* F. Mignon,* E. Rondeau,* Ph. Vanhille* and P. Verroust*. INSERM U. 64 and Services de Néphrologie, Paris, Valenciennes, France (Intr. by C. Le Grimellec).

The observation that THP, a protein synthesized by cells of the thick ascending limb of Henle's loop, can be detected in the glomerular urinary space (GUS) in obstructive nephropathies led us to analyze its distribution in kidney biopsies from 24 patients with MCN and RF (creatinine: 548 ± 430 $\mu\text{m}/\text{l}$). Using mAb specific for 3 different epitopes, THP deposits were identified in 71/153 (46%) glomeruli from 20 patients. In one patient in whom repeated analysis of renal tissue was carried out, the diffusion of glomerular THP paralleled creatinine values but in the group as a whole, there was no significant correlation with the severity and outcome of RF. Glomerular deposits of THP were associated with interstitial deposits in 11 cases, and were also detected in 10/13 Balb/c mice bearing a K light chain excreting plasmocytoma which had developed MCN like lesions. In marked contrast, scanty THP deposits were only found in 2/66 glomeruli from 13 control patients with a monoclonal component and renal lesions distinct from MCN (including amyloidosis, nodular glomerulosclerosis, proliferative glomerulonephritis). Since the presence of THP in the GUS is attributed to retrograde flow of tubular urine, these results suggest that tubular obstruction is frequent in MCN and plays a substantial role in the genesis of RF.

DECREASED C3 LEVELS ARE A MORE RELIABLE INDEX OF SLE RENAL ACTIVITY THAN ARE DECREASED C4 LEVELS. LA Hebert, FG Cosio, WH Bay, DF Middendorf, NS Nahman, Jr*, JC Neff*, Ohio State University, Depts of Medicine and Pathology, Columbus, OH.

In 20 consecutive patients (pts) with SLE and diffuse proliferative glomerulonephritis, C3, C4 and pertinent renal parameters were measured each 2 months during SLE remission and each 2 weeks during SLE relapse. SLE renal relapse was defined as a documented increase in serum creatinine of at least 0.3 mg/dl or an increase in proteinuria: if < 200 mg/24 hr, an increase to > 1.0 gm, if < 1 gm/24 hr an increase to > 2.0 gm, if > 1 gm/24 hr, a doubling of proteinuria. On presentation, all 20 pts had low C3 (mean 56 mg/dl, range 27-73); 15 of 20 pts had reduced C4 (mean 11 mg/dl, range 3-24). (Normal C3: 97-155 mg/dl; normal C4: 13-44 mg/dl.) 7 pts developed renal failure by 6 months. The remaining 13 pts were followed long term (mean 3.1 yr) and had a total of 17 renal relapses. All renal relapses were associated with low C3. Low C3 preceded relapse in 10 of 17 instances by 4 to 40 wk (mean 14 wk). In 3 instances, the first abnormal C3 was coincident with relapse. In 4 instances low C3 occurred after onset of relapse. By contrast, C4 levels remained normal or unchanged during 11 of 17 renal relapses. If C3 was persistently less than 70 mg/dl, relapse (renal or nonrenal) was always seen. In no instance did C4 levels provide information not provided by C3 levels. Conclusion: Monitoring complement status in SLE patients with GN is useful. It is sufficient to monitor only C3 levels.

PURIFIED ANTI-PHOSPHOLIPID ANTIBODIES (APA) FROM LUPUS NEPHRITIS PATIENTS ENHANCE THROMBOXANE (TxB_2) PRODUCTION BY ACTIVATED PLATELETS

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We have shown previously IgG APA in 30% of 82 immunosuppressed patients with lupus nephritis and 85% of lupus-like nephritis, and that IgG APA are associated with intraglomerular thrombi. Circulating platelets in lupus are activated and such platelets express anionic phospholipids (mainly phosphatidyl serine) with which APA preferentially react. We studied the effect of purified APA on function of activated normal platelets *in vitro*.

APA from three patients (classical lupus nephritis, lupus-like nephritis and recurrent abortion) were purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation, phospholipid micelle incubation, and butanol extraction which leaves specificity unaltered. 0-50 $\mu\text{g}/\text{ml}$ of purified APA or normal IgG (NIG), heat aggregated IgG (HAGG), or buffer alone were incubated with unstirred normal platelet-rich plasma for 5 mins at 37°C and subaggregant doses of a stimulating agent: arachidonic acid, ADP or collagen. TxA_2 production was measured by radio immunoassay.

All three APA produced a concentration-dependent increase in TxA_2 production at 5 S.D. above NIG, HAGG or buffer. (APA 1040-1100 vs 740 ± 105 (2 SD) for NIG, HAGG, or buffer, ng $\text{TxA}_2/\text{ml}/3 \times 10^6$ platelets). This enhancement was dose-dependent and did not occur with unstimulated platelets.

This suggests that *in vivo* APA might have an important pathogenetic role enhancing platelet aggregation in platelets already activated by immune complexes in the circulation of glomerular capillaries.

SEVERE PULMONARY HAEMORRHAGE AND SYSTEMIC VASCULITIS IN ASSOCIATION WITH ISOLATED CIRCULATING IgM ANTI-NEUTROPHIL ANTIBODY. D.R.W. Jayne,* S.J. Jones,* and C.M. Lockwood.* (intr. by Eric G. Nielson)

Royal Postgraduate Medical School, London, UK. Antibodies to neutrophil cytoplasmic antigens (ANCA) can be found in the sera of patients with systemic vasculitis, such as Wegener's granulomatosis and microscopic polyarteritis by indirect immunofluorescence (IIF) and solid phase radioimmunoassay (SPRIA) and are usually of IgG class (Lancet i, 1389, 1987).

We have recently identified a subgroup of three patients with systemic vasculitis which manifested as severe acute pulmonary haemorrhage requiring assisted ventilation and renal involvement but without overt disease in other organs, in whom the ANCA were restricted to the IgM class, as detected by IIF and a modified SPRIA. A class switch to IgG class ANCA occurred in all patients after immunosuppressive therapy was started. IgG and IgM class ANCA were present by SPRIA in the glomerular eluate from the kidneys of one of the index patients who died, but were not detected in the eluate from normal kidneys, a kidney with rapidly progressive nephritis without serum ANCA or a kidney with severe anti-GBM disease. This finding suggests that ANCA may have access and bind to glomerular antigens and supports the possibility that they have a direct role in the pathogenesis of renal injury.

We conclude that analysis of different classes of ANCA will enable further classification of the systemic vasculitides and aid in the management of patients with these conditions.

A CLINICOPATHOLOGIC COMPARISON BETWEEN ANTI-GRANULOCYTE CYTOPLASMIC AUTOANTIBODY (AGCA)-POSITIVE IDIOPATHIC NECROTIZING & CRESCENTIC GLOMERULONEPHRITIS (INCGN) AND ARTERITIS-ASSOCIATED GLOMERULONEPHRITIS (AAGN).

J. Charles Jennette, W. Patrick Burgess and Ronald J. Falk. U.N.C. Sch. Med., Dept. of Pathol. & Med., Chapel Hill, N.C.

AGCA are detectable in patients with Wegener's granulomatosis (WG), microscopic polyarteritis nodosa (MPAN) and INCGN. This shared serologic marker suggests that the GN in all of these diseases may be related, if not identical. To further assess this relatedness, we compared the renal dysfunction and CN pathology in 13 cases of AAGN (all having a necrotizing arteritis in the biopsy specimen) with that in 16 AGCA-positive INCGN cases. Four of the AAGN patients had evidence for WG. Data for AAGN/INCGN included mean (\bar{X}) age 58/51, \bar{X} creatinine 6.6/6.3, \bar{X} % crescents 5C/58, \bar{X} necrosis (0-4) 3.2/2.0, % with \geq 1 Ig 23/0, % with \geq 1 C3 38/29, % with EM dense deposits 1C/7, and \bar{X} AGCA by ELISA (control \bar{X} 26.1) 91.8/67.1. Additional signs and symptoms, other than those indicative of WG, were shared by the two groups.

Our data show that AAGN and AGCA-positive INCGN have no qualitative differences; however, on the average, AAGN has a higher serum AGCA level and more glomerular necrosis than INCGN. We propose that INCGN is closely related, clinically, pathologically and probably pathogenetically, to AAGN, including WG and MPAN. INCGN may be at one end of a disease spectrum that extends from a renal-limited variant (i.e. INCGN) to widespread systemic vasculitis (e.g. WG and MPAN).

A PROSPECTIVE STUDY OF THE INCIDENCE OF ANTI-GBM AND ANTI-NEUTROPHIL CYTOPLASM ANTIBODIES IN PATIENTS WITH RAPIDLY PROGRESSIVE NEPHRITIS.

C.M.Lockwood,*D.R.Jayne,*P.Marshall,*S.Jones,* and C.O.S.Savage.* (intr. by Eric G.Nielson) Royal Postgraduate Medical School, London, UK.

We have recently developed a solid phase radio-immunoassay (SPRIA) to detect circulating antibodies to neutrophil cytoplasmic antigens (ANCA), which we find to be specific for patients with systemic vasculitis, such as Wegener's granulomatosis and microscopic polyarteritis (Lancet i,1389 1987). We use this assay with a SPRIA for anti-GBM antibodies to diagnose patients whose rapidly progressive nephritis could be in association with systemic vasculitis or anti-GBM disease (J.Clin. Lab. Immunol. 17,197,1985).

To determine the relative incidence of these two autoantibodies a prospective study was initiated. From November 1986 to July 1987 890 consecutive samples referred to us for diagnosis were tested. 47 (5%) were positive for anti-GBM, 246 (28%) were positive for ANCA, 20 (2%) were positive in both. The specificity of both antibodies could be confirmed by cross inhibition experiments.

We conclude that approximately 30% of patients with anti-GBM disease also have autoantibodies typically found in systemic vasculitis and this may have an important effect on their long term prognosis.

MUCOSAL IMMUNITY: ITS ROLE IN URINARY TRACT INFECTION AND RENAL TRANSPLANTATION.

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Specific IgA-producing cells in mucosal epithelia provide humoral protection against pathogens in the environment. Secretory immunoglobulin A (SC-IgA) is the predominant immunoglobulin in external secretions and plays a major role in protection of mucosal surfaces. However, its role in local defense from urinary tract infections (UTI) remains to be established.

A fast, simple, sensitive and reproducible enzyme-linked immunosorbent assay was developed to compare the SC-IgA concentrations in serum and urine of healthy subjects (n=30), in patients with indwelling bladder catheter (n=10), and in immunocompromised renal allograft recipients (n=25) with good graft function and without UTI. In addition, SC-IgA concentration was measured in premenopausal healthy women (n=20) during various phases of the ovarian cycle.

The SC-IgA concentration in healthy controls was 22.3 ± 7.8 mg/l in serum, 0.5 ± 0.4 mg/g creatinine (creat.) in urine; in patients with indwelling bladder catheter 45.7 ± 22.6 mg/l in serum, (p < 0.01), 6.4 ± 5.2 mg/g creat. in urine (p < 0.05); in renal allograft recipients 32.8 ± 23.5 mg/l in serum (p < 0.05), 1.9 ± 1.2 mg/g creat in urine (p < 0.01). An influence of the ovarian cycle of healthy women on serum or urinary SC-IgA could not be found. There was no significant correlation between SC-IgA values in serum and urine.

The high SC-IgA levels in patients with indwelling bladder catheter may be due to a stimulation for the mucosa-associated immune system by either mechanical irritation or bacterial invasion. The elevated SC-IgA level in urine after renal transplantation may be due to postoperative anatomic derangements with chronic urinary reflux and asymptomatic bacterial invasion. We conclude, that the immunosuppressive therapy in renal allograft recipients does not influence the SC-IgA concentration secreted by the urinary tract.

CYCLOSPORINE TREATMENT OF FREQUENTLY RELAPSING MINIMAL CHANGE NEPHROTIC SYNDROME (FR-MCNS) IN CHILDREN: RESPONSE TO THERAPY AND EVIDENCE FOR A T CELL ABNORMALITY. Mark Mentser, Barry Shannon* and John D. Mahan. The Ohio State University, Depts. of Pediatrics and Pathology, Children's Hospital, Columbus, Ohio.

Since clinical and immunologic observations support the concept that MCNS is a T cell mediated disease process, six children with steroid-responsive FR-MCNS and severe steroid side effects were treated with cyclosporine (CSA), 6 mg/kg/day for 8 weeks. All had previously received cyclophosphamide or chlorambucil but subsequently developed a frequently relapsing course. All patients were in remission at the initiation of CSA. During CSA therapy no patient experienced hypertension, decreased creatinine clearance or a trough serum CSA level greater than 100 ng/ml by RIA. No patient relapsed while on treatment, however, 5 of 6 relapsed within 1 month after CSA was discontinued.

Flow cytometric analysis of lymphocyte subsets, function-associated antigens and activation antigens was performed prior to and at the completion of therapy. Ta1, an activation T cell antigen was significantly increased (23±3%) when compared to other nephrotic children (13±6%) prior to receiving any therapy. In addition, IL-2, Ia and Ia-T11 activation antigens were increased in the CSA patients.

Conclusions: (1) CSA maintains a remission in FR-MCNS, although relapses tend to occur soon after cessation of therapy. (2) Evidence for T cell activation is present in children with FR-MCNS. Persistent T cell activation may identify a sub-group of nephrotics that will develop a frequently relapsing course.

PRESENCE OF TYPE I PLASMINOGEN ACTIVATOR INHIBITOR (PAI) IN RENAL FIBRIN DEPOSITS IN HUMAN PATHOLOGICAL CONDITIONS. B. Mougenot, E. Rondeau, B. Kruithof, J.D. Sraer. INSERM U 64, Paris, France and Chuv Lausanne. (Intr. by M. Schambellan).

The persistency of fibrin deposits in the kidney during renal diseases could reflect either a defective release of plasminogen activators (PA) or a local excess of PAI. In order to investigate this problem we studied human renal biopsies by immunofluorescence technique with the specific antibodies for fibrin, tissue-type plasminogen activator (t-PA), urokinase (u-PA), type I PAI and type 2 PAI. By this technique t-PA could be detected in the glomerular flocculus and the endothelium of small arteries of the normal control kidneys. We failed to detect significant fluorescence with other antibodies in normal kidneys. Conversely in cases of arteriolar thrombosis (renal vasculitis n=3) the positive fluorescence obtained with antifibrin antibodies at the site of thrombosis was associated with a positive fluorescence with anti-PAI and to a lesser extent with anti t-PA antibodies. No u-PA nor PAI 2 were detected in these lesions. Similarly in crescentic glomerulonephritis (n=3) the extracapillary fibrin deposits detected by IF were associated with PAI. In one case u-PA was also detected. This was in agreement with our previous findings that glomerular epithelial cells release both PAI and the inactive form of u-PA (pro-u-PA). Thus, our results support the hypothesis that PAI, which is able to inhibit both t-PA and u-PA, may play a major role in the persistency of fibrin deposits in the human kidneys during pathological conditions.

HISTOLOGIC AND ULTRASTRUCTURAL SPECIFICITY OF HIV-ASSOCIATED FOCAL AND SEGMENTAL SCLEROSIS (FSS).

V. Pardo, C. Ortiz,* J. Strauss and J. J. Bourgoignie. VAMC and U of Miami, Miami, Florida.

To determine the histologic and ultrastructural specificity of ARC-AIDS related FSS, renal biopsies from 13 patients with ARC and 19 with AIDS and from 20 patients with idiopathic FSS (IFSS) and 7 IV drug users seronegative for HIV were compared. In each case, the incidence of glomerular endothelial tubuloreticular inclusions (TRI), as well as nuclear inclusion bodies (NI) and fibrillogranular nuclear changes (FG), was evaluated by counting TRI in 20 glomerular capillaries and NI and FG in 50 randomly selected nuclei.

Glomerular epithelial cells were more hypertrophic and vacuolated in HIV-related FSS than in other groups. Early on, a tubulointerstitial nephritis was frequently present, with microcystic tubular dilatations and variegated casts appearing later.

TRI were significantly more numerous in either ARC or AIDS (10.2±7.6) than in IV drug users (4.6±4.5; p<0.02) or patients with IFSS (1.8±1.8; p<0.01). Separately, numerous TRI were also seen in 2 children with ARC and minimal lesions or mild mesangial hyperplasia. In contrast, there were no significant differences in the incidence of NI and FG among the 3 groups. Moreover, NI and FG were frequently present in other renal diseases and may represent a non-specific reaction to injury or processing artifacts.

In conclusion, we did not find a specific "marker" for HIV-related FSS. Nevertheless, the quantitative differences observed may be useful to identify HIV-associated FSS

PRIMARY "IDIOPATHIC" AMYLOIDOSIS A: IMMUNOHISTOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION. Maria M. Picken*, Blas Frangione*, Kotresha Neelakantappa, and Gloria Gallo. NYU School of Medicine, NY, NY.

Primary idiopathic amyloidosis is usually related to immunoglobulin light chain (AL) associated with immunocytic dyscrasias, while secondary "reactive" amyloidosis (AA) is related to serum amyloid A protein and typically occurs with chronic inflammation, malignancy, or Familial Mediterranean Fever. We report observations in 2 patients with nephrotic syndrome due to renal amyloidosis in whom no predisposing disease could be found. Both were characterized immunohistochemically as AA. Three year follow-up in one patient reveals no clinical evidence of associated disease. The other patient, examined at autopsy, had systemic amyloidosis and no morphologic evidence of underlying disease. The purified amyloid fibril protein extracted from tissues obtained at necropsy proved by immunoblotting and amino acid sequence to be AA, homologous to reactive and familial forms of amyloid. Extracts from both frozen and formalin-fixed paraffin-embedded kidney and spleen yielded similar monomers and dimers of the AA protein. In the extract from the paraffin embedded lung we found an additional fragment (12,000 daltons) which suggests that different processing of proteins, i.e., by polymerization and/or degradation, may occur in different organs. In conclusion: 1) Idiopathic amyloid may be of the AA type and homologous to secondary and familial forms. 2) tissue specific factors contribute to the processing of amyloid protein. Whether there is a common factor in the amyloidogenesis of the 3 forms of AA remains to be elucidated.

POLYMORPHISM OF IMMUNOGLOBULIN (Ig) HEAVY CHAIN SWITCH REGION IN IGA GLOMERULONEPHRITIS OR HSP AND ITS RELATION TO RENAL OUTCOME

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We have recently described differences of Ig heavy chain (HC) gene polymorphism at S_u and S_{α1} switch region (RFLP) between pat. with IgA-GN and healthy controls. In this study we (a) further characterized IgHC by probing the D14S1 region downstream of S_{α1}(b) compared IgA-GN with HSP and (c) evaluated correlation of RFLP to renal outcome. Genomic DNA from 62 biopsy confirmed IgA-GN and 21 HSP was digested (restrict. endonuclease SstI and Hind III), transferred to nylon membranes (Southern blot) and hybridised with a probe homologous to switch region of Ig C_u gene (S_u) which detects RFLP's in both S_u and S_{α1}. In IgA-GN frequency of 7.4 kb homozygotes was significantly increased (57.4 vs 37.7% in controls) and 6.9 kb homozygotes decreased (1.6 vs 15%) with a significant decrease of gene frequency of the 6.9 kb allele. In contrast, HSP did not differ from caucasoid control population (7.4 kb homoz. 27.3% and 6.9 kb homoz. 27.3%) suggesting immunogenetic differences between IgA-GN and HSP. In contrast to the S site, no deviation of allotype frequencies was found at the hypervariable D14S1 region 3' of S_{α1} in IgA-GN (12; 10 kb heterozyg. 61% vs 56% in controls; 12 kb homozyg. 16.7 vs 21.6%; 10 kb homozyg. 22.2% vs 23.4%). Finally, IgA-GN pat. homozyg. for 6.9 and 7.4 kb alleles at S_{α1} tended to have more adverse renal prognosis (terminal renal failure in 19.4% of homozyg. and only 4% of heterozyg.).

POLYREACTIVE ANTI-DNA ANTIBODIES(AB) ARE NEPHRITOGENIC IN HUMAN LUPUS NEPHRITIS (LN). J. Sabbaga*, O.G. Pankewycz* and M.P. Madaio New England Medical Center, Boston, MA.

To define the properties of autoAb that form immune deposits in LN, we eluted Ab from the kidneys of 2 patients with active LN [one with mesangial proliferation(Mes) and another with membranous nephropathy(Memb)] and compared their ligand binding, idiotypic and charge properties to serum anti-DNA Ab derived from both patients with active lupus(LS) and normals(NS). The kidney eluate(KE) anti-DNA Ab were the most polyreactive; they cross-reacted with denatured DNA, polydGc, polydT, polydG, polydC, ZDNA and the phospholipids cardiolipin and phosphatidyl serine. LS anti-DNA Ab cross-reacted with polynucleotides but not phospholipids, whereas NS anti-DNA Ab reacted only with polydT. An anti-idiotype(anti-Id¹) produced against serum anti-DNA Ab from one patient reacted with: anti-DNA Ab in other LS; both KE Ab; and anti-DNA Ab from NS. Anti-Id¹ did not react with non-anti-DNA Ab in LS or NS. Isoelectric focusing of Ab showed that the charge of LS and KE Ab overlapped: KE Memb Ab had pI's 4.5-8.6; KE Mes Ab had pI's 8.1-9.1 and serum anti-DNA Ab had pI's 5.4-9.0.

We conclude that anti-DNA Ab from individuals with lupus and NS are related idiotypically, however their ligand binding properties differ: the more polyreactive autoAb are present in immune deposits whereas the antigen binding properties of anti-DNA Ab in both LS and NS are more restricted. These findings suggest that polyreactivity may distinguish lupus and natural autoAb, and it may influence the capacity of lupus autoAb to form glomerular immune deposits.

INCREASED PRODUCTION OF INTERLEUKIN-2(IL-2) AND EXPRESSION OF IL-2 RECEPTOR(IL-2R) BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS(PBMC) IN PRIMARY IgA NEPHROPATHY(IgAN). F.P. Schena*, G. Mastrolitti*, I. Munno, A.R. Fracasso, N. Pellegrino, E. Jirillo*. (intr. by S.N. Emancipator). Chair of Medical Therapy and Inst. of Microbiology, Univ. of Bari, Polyclinic, Bari, Italy.

Primary IgAN is characterized by increased activity of helper T cells and decreased function of suppressor T cells. This immune imbalance may lead to an increased production of polymeric IgA. On the basis of this fact we investigated the production of IL-2 by PBMC in 13 patients(pts) with IgAN and 9 pts with chronic glomerulonephritis. IL-2 was measured by an ELISA kit on supernatants taken from 24h cultures of PBMC stimulated with 5 µg/ml of phytohemagglutinin(PHA). Analysis of IL-2R on the PBMC was carried utilizing a monoclonal antibody to TAC. Our results showed a spontaneous specific production of IL-2 in cultures of PBMC from pts with IgAN ($p < 0.025$) that increased after PHA stimulation. Furthermore, IgAN pts had significantly higher expression of IL-2R on the surface of resting PBMC than did pts with chronic glomerulonephritis ($p < 0.05$).

Our data suggest that the increased production of IL-2 in pts with IgAN may be responsible for the increased activity of helper T cells. The high number of IL-2R expressed by freshly separated resting PBMC suggests that these cells are continuously stimulated and this finding concurs with the demonstrated spontaneous hyperproduction of IL-2.

URINARY COMPLEMENT C5b-9 EXCRETION IN MEMBRANOUS NEPHROPATHY (MN). M. Schulze*, P.J. Baker*, R.J. Johnson, C.J. Pruchno*, J.V. Donadio, and W.G. Couser, Univ. of Washington, Seattle, WA and Mayo Clinic, Rochester, MN.

The pathogenesis of MN is uncertain and no measures of disease activity are available. Passive Heymann nephritis (PHN) is a model of MN in rats induced by antibody to a glomerular epithelial cell (GEC) antigen in which C5b-9 assembly on the GEC membrane mediates proteinuria. We found (Kidney Int 31:329, 1987) that urinary C5b-9 excretion was elevated in PHN but not in MN induced with exogenous antigens, and paralleled ongoing glomerular antibody deposition. To determine if this could be related to human MN, we developed an ELISA for human C5b-9 with antibodies to human C6 and C9. To correct for any spontaneous C5b-9 generation in proteinuric urine, results are expressed as C5b-9 units/mg protein $\times 10^3$. No C5b-9 was detected in 40 normal urines. In 20 patients with biopsy proven idiopathic MN, urine C5b-9 was 30.1 ± 12.3 vs 6.0 ± 1.1 in 45 non-diabetic, non-MN proteinuric patients ($p < .03$). Ten MN patients had very high values (56 ± 19 units) while 10 were not high (2.3 ± 0.9 units). Patients with higher values had shorter disease duration (34 vs 57 mos) and more proteinuria (11.4 ± 2.3 mg/mg creat vs 6.5 ± 2.5 , $p < .01$).

We conclude that elevated urinary C5b-9 is present in some patients with MN and supports an autoimmune pathogenesis for this disease. Elevated C5b-9 excretion is more common in early and severe disease and may reflect active glomerular immune deposit formation.

CHRONICITY(CI) AND ACTIVITY INDICES(AI) DO NOT PREDICT OUTCOME IN SEVERE LUPUS GLOMERULONEPHRITIS (LGN). Schwartz MM, Phillips E,* and the Lupus Nephritis Collaborative Study Group. Rush Medical College, Chicago, IL and George Washington University, Washington, D.C.

It has been suggested that a semiquantitative index (CI) of glomerular and interstitial scarring is superior to the proven value of the standard histologic classification (WHO) as a predictor of renal failure outcome in LGN. To test this point, four renal pathologists independently scored the AI/CI on 85 renal biopsies from patients with LGN followed for 109 ± 8 wks ($\bar{x} \pm S.D.$), and the mean score was related to adverse outcome (death, renal failure and stop points). Receiver operator characteristic (ROC) curves were derived from a series of 2x2 tables in which outcome was one variable and AI or CI dichotomized by a cut off point was the second. The ROC curve analysis indicated that no value of the AI or the CI provides a cut off which predicts outcome. Since the AI/CI do not have sufficient sensitivity and specificity to identify the patient who will have an adverse outcome, they are not useful in individual cases. When the patients were divided by the presence or absence of adverse outcome, a comparison of the activity (11.41 ± 0.44 vs 11.27 ± 0.50 , $p = 0.71$) and chronicity (4.12 ± 0.37 vs 3.21 ± 0.24 , $p = 0.39$) indices showed that the AI/CI do not distinguish between the two populations and had no predictive value for outcome. When the patients were divided by the single binary outcome variable, renal failure/non renal failure, the CI approached but did not reach statistical significance (4.38 ± 0.42 vs 3.19 ± 0.23 , $p = 0.06$). We conclude that therapeutic innovations based upon the AI/CI as a predictor of chronic renal failure should be held in abeyance until independently confirmed.

IDENTIFICATION OF NUCLEATED CELLS IN THE URINE SEDIMENT USING MONOCLONAL ANTIBODIES. M. Segasothy*, D. Birch*, K. Fairley*, P. Kincaid-Smith. Department of Medicine, University of Melbourne, Department of Nephrology, Royal Melbourne Hospital, Melbourne, Australia.

Carefully collected mid-stream urine samples were centrifuged for 10 minutes at 2500 rpm. Sedimented cells were washed with Hank's balanced salt solution and resuspended in a medium containing Hank's balanced salt solution and fetal calf serum. 150 μ l aliquots were deposited onto microscopic slides in a cytocentrifuge, air-dried, acetone fixed and then subjected to microwave irradiation. The specimens were analysed with monoclonal antibodies to B lymphocytes, T4 (helper/inducer) lymphocytes, T8 (cytotoxic/suppressor) lymphocytes, monocytes/macrophages, polymorphs, glomerular epithelium, proximal tubule, loop of Henle, distal tubule and urothelium and an immunoperoxidase stain. Papanicolaou stained cytocentrifuge preparations were examined for comparison. Percentages of each cell type were determined by counting up to 400 cells and were then converted into absolute number of cells/ml of urine based on the total nonsquamous nucleated cell count at the initial microscopic examination. Thus the cell profile in the urine sediment was established.

Urine from patients with a range of renal conditions including transplant recipients, acute tubular necrosis, acute interstitial nephritis, glomerulonephritis, polycystic kidney disease and analgesic nephropathy were studied. Varying types of cells in varying numbers were present in the different conditions.

We conclude that examination of the urine sediment with monoclonal antibodies and immunoperoxidase stain constitutes a useful noninvasive diagnostic tool in the assessment of renal disease.

MYELOMA CAST NEPHROPATHY: IMMUNOHISTOCHEMICAL AND LECTIN STUDIES. David A. Start,* Fred G. Silva, Loraine D. Davis,* Vivette D'Agati, and Conrad L. Pirani. University of Texas Health Sciences Center, Dallas, Texas and Columbia University College of Physicians & Surgeons, New York, New York.

Renal disease is a common cause of morbidity and mortality in patients (pts) with plasma cell dyscrasia (PCD). We examined renal paraffin sections from 53 pts with PCD (24 of which, in addition to large casts, had giant cells and polys). The following studies were performed - tubular markers: *Tetragonolobus lotus*, *Arachis hypogaea*, Tamm-Horsfall protein (THP), epithelial membrane antigen (EMA), AE1-AE3; hematopoietic cell markers: leukocyte common antigen (LCA), alpha-1-antitrypsin, alpha-1-antichymotrypsin, lysozyme, and Leu-M1.

Although tubular epithelial cells continued to stain with their respective markers (whether inflamed, thinned, dehisced, or lining casts) true intratubular giant cells were never positive for these tubular markers; alternatively, some giant cells stained with some of the hematopoietic markers. In 50% of all cases, THP and other distal tubular markers (*Arachis*, EMA, AE1-AE3) were found in Bowman's space, almost always in association with interstitial THP; this suggests that there are communications between the distal and proximal nephron, possibly through breaks in the tubules and via the interstitium. In summary, there is no evidence to suggest that intratubular giant cells are of tubular epithelial origin. Connections between the distal and proximal nephron are common in myeloma cast nephropathy.

PROFILE OF NUCLEATED NONSQUAMOUS CELLS IN URINE IN ACUTE TUBULAR NECROSIS (ATN), ACUTE INTERSTITIAL NEPHRITIS (IN) AND CRESCENTIC GLOMERULONEPHRITIS (RPGN). M. Segasothy*, D. Birch* K. Fairley*, P. Kincaid-Smith. Department of Medicine, University of Melbourne, Department of Nephrology, Royal Melbourne Hospital, Australia.

Nucleated nonsquamous cells in the urine of patients with ATN (n=10), IN (n=3) and RPGN (n=8) were identified using monoclonal antibodies and immunoperoxidase stain. The diagnoses were made on clinical evidence and in all cases of IN and RPGN and in 5 cases of ATN these were confirmed by renal biopsy. Mann-Whitney rank-sum tests were performed to determine significant differences in the numbers of B lymphocytes, T4 (helper/inducer) lymphocytes, T8 (cytotoxic/suppressor) lymphocytes, monocytes, polymorphs, glomerular epithelium, proximal tubule, loop of Henle, distal tubule and urothelium.

Polymorph counts were significantly higher in IN than in ATN ($p < 0.02$) or RPGN ($p < 0.05$); monocyte counts were higher in IN than in ATN ($p < 0.05$), in RPGN than in ATN ($p < 0.005$); glomerular epithelial cell counts were higher in RPGN than in ATN ($p < 0.005$) or in IN ($p < 0.05$); the number of nucleated nonsquamous cells was higher in IN than in ATN ($p < 0.02$) and higher in RPGN than in ATN ($p < 0.05$). There were no significant differences in the numbers of other cell types assayed in the 3 conditions however high numbers of renal tubular cells (proximal tubule, loop of Henle and distal tubule) were detected in the 3 conditions.

We conclude that ATN, IN and RPGN are characterised by high numbers of nucleated nonsquamous cells and different patterns of polymorphs, monocytes and glomerular epithelial cells in urine. High numbers of renal tubular cells are also present in these conditions.

CIRCULATING FORM OF BETA-2-MICROGLOBULIN IN DIALYSIS PATIENTS. Thomson DME, Gagnon RF and Somerville P. Montreal General Hospital, Montreal, Canada.

In eight long term dialysis patients, the circulating profile of beta-2-microglobulin (β 2M) was determined using a commercial radioimmunoassay by measuring β 2M in different fractions of their serum after molecular-sieve separation. Four patients had carpal tunnel syndrome with demonstrated amyloid in excised wrist tissues of which two were positive for β 2M. In all patients despite high serum levels (35 to 42 mg/l), β 2M eluted exclusively as a single peak in the molecular weight region of about 12,000 daltons on a calibrated Sephacryl S-200 column. Recoveries from within the peak accounted for 93% or more of the applied β 2M serum concentrations. These results were confirmed by molecular-sieve separation of the enriched β 2M-containing fractions by high pressure liquid chromatography. In conclusion immunoreactive β 2M in dialysis patients circulates as an intact monomer without evidence for the formation of aggregates or fragments.

LOW MOLECULAR WEIGHT Clq BINDING IgG IN PATIENTS WITH SLE CONSISTS OF AUTOANTIBODIES TO Clq. Shu Uwatoko* and Mart Mannik*. (intr. by WG Couser) Div. of Rheumatology, Univ. of Washington, Seattle, Washington.

The majority of Clq binding IgG in sera of patients with SLE cosediments with monomeric IgG (Medicine 66, 85, 1987) and has been thought to contain antibodies to Clq (Clin Exp Immunol 69, 98, 1987). This study was undertaken to provide rigorous proof that the low molecular weight Clq binding material consists of antibodies to Clq. Monomeric Clq binding IgG was isolated from 5 SLE plasmas by Clq affinity chromatography and gel filtration. All preparations of Clq binding IgG bound to Clq and to the collagen-like region of Clq (CLR) both by a radioimmunoassay and by an ELISA. To rule out that small DNA-antiDNA immune complexes do not cause Clq binding, the Clq binding preparations were converted to F(ab')₂ fragments, then to Fab' fragments, and digested with DNase I to degrade any DNA. The Fab' fragments continued to bind to Clq and CLR after this treatment. By isoelectric focusing heterogenous IgG molecules were present, but a highly anionic band of protein was present in each preparation. Binding of the isolated Clq binding IgG to solid phase Clq was retained in 0.3 M NaCl, whereas the binding of DNA to solid phase Clq was abrogated by this salt concentration and thus dependent on charge-charge interactions.

We conclude that the low-molecular weight Clq binding IgG in the studied patients with SLE consists of autoantibodies to Clq.

ADULT (AUTOSOMAL DOMINANT) POLYCYSTIC KIDNEY DISEASE (APKD). RESULTS OF HISTOCHEMICAL STUDIES. Regina Verani, Fred Silva and Loraine D. Davis* Univ. of Texas Med. School, Houston and Dallas, TX.

We previously reported the results of histochemical studies in autosomal recessive PKD and in acquired PKD. The purpose of this study is to document the site of origin of the renal cysts (C) and the site of epithelial hyperplasia (EH) in 9 cases of APKD. The patients were 22 to 55 yrs. old, 5 male and 4 female. The material consisted of nephrectomy specimens in all cases. The kidney weights ranged from 405 to 2030gm. Histochemical techniques utilizing *Tetragonolobus lotus* (T) for proximal tubules, *Arachis hypogaea* (A) for collecting ducts, epithelial membrane antigen (EMA) for distal nephron and antibodies against Tamm-Horsfall protein (THP) of thick ascending limb of Henle were performed. In each kidney the controls for the histochemical studies were the preserved renal tubules which showed the respective positive reactions. In all cases > 20 C were examined and the C replaced > 60% of the renal parenchyma. A and EMA were positive in > 60% of the C in 5 cases, in 30-60% of the C in 3 cases and in < 30% of the C in 1 case. T was positive in < 30% of the C in 3 cases and in rare C in 6 cases. THP was negative in the C wall but was observed in the lumen of C in 5 cases. Negative cysts to all reagents were observed in all cases. EH was observed in 13 C in 6 cases and the hyperplastic epithelium was positive for A in all C. Glomerulocysts were present in most cases. Cysts surrounded by fibromuscular tissue were seen in many kidneys. We concluded that the majority of the C in APKD are of collecting duct origin and that collecting duct C are the site of EH. The possibility that the C in APKD are primarily of collecting duct origin and that proximal tubules C and glomerulocysts are a secondary change should be considered.

ASYMMETRIC IgG SUBCLASS DEPRESSION IN CHILDREN WITH NEPHROTIC SYNDROME. Barry L. Warsaw and Irene Check* Emory Univ. School of Med, Depts. of Pediatrics and Pathology, Atlanta, GA.

To determine whether the hypogammaglobulinemia of childhood nephrotic syndrome is characterized by symmetrical depression of the IgG subclasses, we compared the IgG subclass concentrations in nephrotic patients in relapse versus remission. We utilized a highly sensitive monoclonal antibody based enzyme immunoassay (sensitive to 10 ng/ml) which allows quantitation with comparable precision of all 4 subclasses. We analyzed 30 sera from 24 nephrotic patients during relapse (alb < 3.0 gm/dl; N=18) and/or remission (alb > 3.0 gm/dl; N=12). The mean ages of the 2 groups were similar (9.8 ± 1.1 vs 9.7 ± 1.3 yrs). Results in mg/dl; geometric means and SE's compared by t-tests:

	IgG1	IgG2	IgG3	IgG4	IgG Tot
Relapse	181	58	30	17	352
	x+32	x+13	x+5	x+3	x+55
Remission	372	112	34	18	746
	x+57	x+20	x+8	x+5	x+83
p	.003	.02	NS	NS	.0007

Total IgG, IgG1, and IgG2 were significantly decreased during relapse compared to remission; IgG3 and IgG4 were not different. This pattern of asymmetric depression of IgG subclasses supports a cause other than urinary losses, affecting specific B cell populations.

DEMONSTRATION AND PARTIAL CHARACTERIZATION OF A NEW ANTIBODY-ANTIGEN SYSTEM IN A PATIENT WITH ANTI-BASEMENT MEMBRANE (BM) ANTIBODIES AND PROGRESSIVE RENAL FAILURE. M. Weber*, M. Marx*, W. Thoennes*, K.H. Meyer zum Büschenfelde*, H. Köhler* (intr. by E.J. Feinstein), 1st Dept. of Int. Med. and Inst. of Pathol., University of Mainz, FRG.

It has been shown that the epitopes of antglomerular BM antibodies (anti-GBM AB) in Goodpasture's (GP)-syndrome are localized on the globular domain (NC1) of BM collagen IV. We here report of a patient with anti-BM AB disease with AB-reactivity against a BM component different from the typical GP-target antigen.

The 66 y old woman was admitted 05/86 preoperatively because of cardiac insufficiency. S-creatinine was 1.2 mg% and 2.1 mg% respectively. An ovarian cystoma was removed and the patient discharged. 03/87 she again was admitted because of an increase in s-creatinine to 9.5 mg%. Erythrocyt., leucocyturia, and an urinary protein excretion of 4.48 g/24h was found. A kidney biopsy was performed which showed mesangioproliferative GN and a linear deposition of IgG along renal BM on immunofluorescence (IF) microscopy.

Circulating anti-GBM antibodies could be demonstrated by ELISA with a collagenase digest (CD) of human GBM as antigen and by IF on human kidney slices. In addition, indirect IF showed linear staining for IgG along tubular BM (TBM) and Bowman's capsule. However, a dot-blot assay with the purified human NC1 as antigen was negative. The antibody therefore was studied by SDS-PAGE and immunoblotting against CD of human GBM and TBM as well as purified NC1 and compared to the reactivity of anti-GBM AB in GP-syndrome.

The studies showed, that the AB lacked reactivity to NC1 but identified target antigens with the mol.-weights of 22, 25, 43, 67, and a closely spaced double band at 105 KD in the CD of GBM. In TBM antigens with similar mol.weights were identified, the 25 KD and 67 KD bands were however absent. The antigens were bound to a DE 52 column and could be eluted by increasing NaCl concentrations. Considering the known components of the BM the AB may possibly directed against nidogen and its breakdown products, which corresponds well with the apparent mol.weights of the target antigens and the behaviour on column chromatography.

The presentation demonstrates, that the anti-BM AB in this patient with anti-BM disease does react with other antigens than anti-GBM AB in GPs syndrome. The report underlines, that a more comprehensive study of autoantibodies in BM disease is necessary, and offers a more rational basis for the milder variants of anti-BM AB disease.

IN VITRO B-CELL INTERLEUKIN-2 (IL-2) RECEPTOR EXPRESSION IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)
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We have previously demonstrated the spontaneous presence of increased numbers of IL-2 receptors (IL2R) in patients with active SLE compared to inactive SLE patients and normal controls. Since B cell hyperfunction and T-cell dysfunction are both characteristic of active SLE, we undertook a study to determine whether the increased IL2R expression was solely a T-cell phenomenon. Peripheral blood lymphocytes (PBL) from four patients with active SLE, diagnosed by revised ARA criteria, and four normal donors were subjected to antibody-mediated complement lysis. The resultant B-enriched cells, 95% pure by indirect immunofluorescence, were examined for the spontaneous presence of IL2R by EIA using a murine monoclonal anti-IL2R antibody (anti-Tac). In addition, whole PBL from patients and controls were also examined for spontaneous IL2R presence. As previously noted, there was higher spontaneous IL2R presence on PBL from active SLE patients compared to normal controls ($p=0.05$). In addition, there was >300% higher IL2R expression on B-cells from active SLE patients compared to normals ($p=0.05$). The functional significance of IL2R on B-cells of active SLE patients remains to be elucidated.

ANTI-ENDOTHELIAL CELL (AEC) ANTIBODIES IN IGA NEPHROPATHY (IGAN) - A POSSIBLE PATHOGENETIC FACTOR. H.K. Yap*, R.S. Sakai*, K.T. Woo*, C.H. Lim*, V. Anantharaman*, G.C. Chiang*, S.C. Jordan. Dept. of Pediatr., Pediatr. Nephrol., Cedars-Sinai-UCLA Med. Ctr., L.A., Calif., Dept. of Pediatr., Nat. Univ. of Singapore, Renal Med. Dept., SGH, Singapore.

AEC Ab has been described in several vasculitic disorders. In IgAN, IgA and/or C3 deposition has been seen in the walls of renal arterioles. This study examined the incidence and nature of AEC activity in IgAN patients. 72 IgAN patients and 27 normals were studied. AEC activity was measured by an ELISA after 7-day primary culture of human umbilical venous endothelial cells (EC) to a confluent monolayer. The cells were positive for factor VIII R Ag and negative for DR Ag. 32% of IgAN patients vs 4% of controls were positive for either IgA or IgG AEC activity ($p<0.008$). Of the positives, 10 IgAN patients and 1 control were identified to have anti-HLA class I Ab by ELISAs based on competitive inhibition of binding of mouse monoclonal anti-HLA-ABC (W6/32) to platelets and ECs. Hence 13 IgAN patients had specific AEC activity ($p<0.008$), of which 9 were of IgA class ($p<0.05$). Gamma-IFN or IL-1 stimulation of EC did not increase the AEC activity. The IgA AEC activity was correlated with IgA immune complex levels, but this AEC activity was shown to be a specific Ab since no change in EC binding occurred after removal of IC. Significant correlations were found between IgA AEC Ab and presence of crescents ($p<0.05$), C3/IgA deposition in renal arterioles ($p<0.02$), and proteinuria >1 g/day ($p<0.03$). Hence IgA AEC Abs may be an important marker of pathogenetic activity in IgAN.

MINERAL METABOLISM

RELATIVE EFFECTS OF ORAL AND PARENTERAL VITAMIN D SUPPLEMENTATION ON GROWTH IN EXPERIMENTAL UREMIA.
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Chronic renal insufficiency is associated with poor growth due to lack of endogenous vitamin D. Both uremic hypophagia and lack of 1α -hydroxylation of $25\text{-OH}\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$ seem involved. The relative effects of oral (p.o.) and parenteral (I.P.) administration of these vitamin D metabolites on growth in experimental uremia was tested. Young male Wistar rats weighing 100-120 g were rendered uremic (U) by a two stage 7/8 nephrectomy. One control group (CP) was paired to uremics and fed the quantities of feed consumed by their uremic partners. Another control group (CA) was allowed to eat ad libitum. Three subgroups of U and CP were supplemented with either $25\text{OH}\text{D}_3$ (500 pM/10g p.o.); $1,25(\text{OH})_2\text{D}_3$ (50 pM/d p.o.); or $1,25(\text{OH})_2\text{D}_3$ (50 pM/10 g I.P.). Linear growth data were assessed by analysis of variance. Linear growth (cms.) after four weeks is shown below:

No supplement		25OH ₂ D ₃		1,25(OH) ₂ D ₃	
CA	CP	U	CP	U	I.P.
4.5 ^a	0.9 ^b	0.7 ^b	2.0 ^c	1.5 ^c	2.0 ^c
				2.0 ^c	1.9 ^c
					4.4 ^a
					2.0 ^c

Values not sharing superscripts differ significantly ($p < 0.01$).

Linear growth was similarly improved in uremic animals supplemented p.o. with either one of the vitamin D analogs. Parenteral $1,25(\text{OH})_2\text{D}_3$ appeared to offer greater appetite stimulation and growth advantage. Uremic hypophagia probably contributes to endogenous deficiency of $1,25(\text{OH})_2\text{D}_3$ by relative deficiency in precursor.

ALFACALCIDOL (1α -OH₂D₃) AND CALCIUM IONES: NEW INSIGHTS INTO THE PATHOPHYSIOLOGY OF UREMIC NEUROPATHY.

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Calcium ions, participating in numerous cellular processes, are important for the function of the peripheral and central nervous system.

In order to evaluate the effects of Ca and of 1α -OH₂D₃ on the pathogenesis of uremic neuropathy we compared the results of neuroelectrophysiological studies obtained in 10 RDT pts who underwent a six months study-period during the which they were administered with 0.25-0.50 ug/die of 1α -OH₂D₃. The neuroelectrophysiological tests performed were: Electromyography (EMG), Motor (MNCV) and Sensory (SNCV) Conduction Velocities, Somatosensory Evoked Potentials (SEP).

The mean serum Ca and P levels differed but not at a statistically extent. The EMG after 1α -OH₂D₃ modified getting a lesser amplitude of the Motor Units (MU) (1635.70 ± 810.58 vs 545.60 ± 103.70 uV, $p<0.001$) and a decrease in duration of MU. The peripheral MNCV passed from 50.50 ± 7.50 to 57.75 ± 6.80 m/sec ($p<0.02$). The P₂₇ and N₃₅ waves evoked by sural SEP converted in a more synchronous and faster program.

Ca "anchorage" by 1α -OH₂D₃ is thought to be a component of the mechanism which keeps a controlled transmembrane potential between the outer and the inside of electrical membranes, it meaning a more efficient depolarization and delivery of mediator.

METABOLIC ACIDOSIS (MA) ENHANCES THE RESISTANCE OF CANINE RENAL CORTICAL ADENYLATE CYCLASE (AC) TO PARATHYROID HORMONE (PTH) DURING DIETARY PHOSPHORUS DEPRIVATION (PD). E. Bellorin-Font, C. L. Milanes,* R. Starosta,* N. Pernalet,* J. R. Weisinger and V. Paz-Martinez. Centro Nacional de Dialisis y Trasplante and Renal Division, Hospital Universitario. Caracas, Venezuela.

PD and MA induce renal resistance to the phosphaturic action of PTH. We have demonstrated that in both situations there is a decrease in PTH-stimulated AC activity. However, studies in vivo suggest that the renal resistance to PTH in PD is reversed by MA. The present studies compare the action of PTH on the AC in renal cortical membranes from dogs with PD, PD + NH₄Cl induced MA and controls. Serum phosphorus in PD and PD+MA were 0.75 ± 0.1 Vs. 0.82 ± 0.4 mg/dL (n.s.), and HCO₃⁻ 18.7 ± 1.0 Vs. 13.1 ± 1.2 mEq/L ($p < 0.01$), respectively (4.4 ± 0.2 mg/dL and 19.5 ± 1.2 mEq/L in controls). Basal AC activity was unchanged. However, PTH stimulated AC was reduced in PD (V_{max} 1379 ± 176 Vs. 2700 ± 345 pmol cAMP/mg prot x 30 min in controls, $p < 0.01$). PD+AM further decreased PTH stimulated AC as compared to PD (813 ± 150 pmol cAMP/mg prot x 30 min, $p < 0.05$). In the presence of Gpp(NH)p, PTH-dependent AC remained decreased in PD and PD+AM, suggesting a defect in the regulatory component (G) of the AC system. Indeed, cholera toxin-dependent ADP-ribosylation of G showed more than 50 % decrease in the content of the 42,000 Mr alpha subunit in PD+MA membranes compared to controls. The present studies demonstrate that in PD, MA enhances AC resistance to PTH, probably due to quantitative changes of G, suggesting that in PD the reversal of in vivo resistance to PTH by MA is not an AC-dependent phenomenon.

IMMUNOCYTOCHEMICAL LOCALIZATION OF ERYTHROCYTE Ca⁺⁺-Mg⁺⁺ ATPase AND 28K-CALCIUM BINDING PROTEIN IN THE SAME CELLS OF HUMAN KIDNEY DISTAL TUBULES. J.L. Borke,* J. Minami,* A. Verma,* J.T. Penniston,* and R. Kumar. Mayo Clinic and Foundation, Rochester, MN.

In order to determine whether vitamin D-dependent 28K-calcium binding protein (28K-CaBP) and Ca⁺⁺-Mg⁺⁺ ATPase pump are present in the same cells of the human kidney, kidney was examined for immunoreactivity with antibodies directed against these proteins. Double-label immunocytochemistry shows that a majority of the cells of the distal convoluted tubule (DCT) contain epitopes to both of these proteins. Western blot analysis of kidney homogenates shows binding of anti-Ca⁺⁺-Mg⁺⁺ ATPase monoclonal antibodies to a major band of Mr = 140,000. Western blots of kidney homogenates also demonstrate binding of an anti-28K-CaBP polyclonal antibody to a major band at Mr = 28,000. Incubation of blots with ⁴⁵CaCl₂ demonstrates that the Mr = 28,000 band binds calcium. We have previously shown the presence of an epitope of the human erythrocyte Ca⁺⁺-Mg⁺⁺ ATPase pump in the basolateral membrane of human DCT (J Clin Invest 1987, in press). Our present study demonstrates, for the first time, that epitopes of the vitamin D-inducible 28K-CaBP and the human erythrocyte Ca⁺⁺-Mg⁺⁺ ATPase pump are present in the same cells of the human kidney DCT. Previous work in our laboratory has shown that 28K-CaBP binds calcium in a manner analogous to calmodulin, a known regulator of human erythrocyte Ca⁺⁺-Mg⁺⁺ ATPase (J Biol Chem 1987). Taken together, these findings suggest, that vitamin D-dependent CaBP may regulate a Ca⁺⁺-Mg⁺⁺ ATPase pump in the human kidney DCT.

RELATIONSHIP OF ANIMAL PROTEIN-RICH DIET TO CALCIUM METABOLISM AND KIDNEY STONE FORMATION. Neil A. Breslau,* Linda Brinkley,* Kathy D. Hill,* and Charles Y.C. Pak. UTHSCD-Southwestern Medical School, Department of Medicine, Dallas, Texas.

In order to determine whether different types of dietary protein might have different effects on calcium metabolism and on stone formation, 15 young normal subjects were studied during three 12-day dietary periods, comprising vegetable without eggs, vegetable with eggs, or animal protein. While these three diets were constant in Na, K, Ca, P, Mg and quantity of protein, they had progressively higher sulfur content. As the fixed acid content of the diets increased, urinary calcium excretion increased from 103 ± 15 (SEM) mg/d on vegetarian diet to 150 ± 13 mg/d on animal protein diet ($p < .02$). There was a significant reduction of PTH, urinary cyclic AMP and 1,25-(OH)₂D consistent with acid-induced bone dissolution. There was no change in fractional intestinal ⁴⁷Ca absorption. The inability to compensate for the animal protein-induced calciuric response may be a risk factor for the development of osteoporosis.

The animal protein-rich diet was associated with the highest excretion of undissociated uric acid due to its high purine content and to the reduction in urinary pH. Moreover, citrate excretion was reduced because of the acid load. However, oxalate excretion was lower than during the vegetarian diet (26 ± 1 mg/d vs. 39 ± 2 mg/d; $p < .02$). Urinary crystallization studies revealed that the animal protein diet conferred an increased risk for uric acid stones, but because of opposing factors, not for calcium oxalate or calcium phosphate stones.

DECREASED RECEPTORS FOR 1,25-(OH)₂D IN PARATHYROID GLANDS OF UREMIC DOGS. Alex Brown,* Adriana Dusso,* Silvia Lopez-Hilker,* Patricia Grooms,* and Eduardo Slatopolsky, Dept. of Medicine, Washington Univ., St. Louis, MO.

The active form of vitamin D, 1,25-(OH)₂D, has been shown to suppress the synthesis and secretion of parathyroid hormone in vivo and in dispersed parathyroid cell cultures. Control of transcription by 1,25-(OH)₂D is believed to be mediated by interaction of this hormone with a specific receptor within target cells. Recently, Korkor (New Eng. J. Med. 316:1573, 1987) reported that the levels of this receptor are lower in parathyroid glands from patients with chronic renal failure than in glands from kidney transplant recipients. We have examined the 1,25-(OH)₂D receptor in parathyroid glands from normal dogs and dogs made uremic by 5/6 nephrectomy. The levels of receptor were four-fold lower in parathyroid extracts from uremic dogs than in those from normal dogs (109 ± 22 vs. 463 ± 196 fmol/mg protein). No differences were observed in the binding affinity for 1,25-(OH)₂D or in the sedimentation in sucrose density gradients. Since this receptor has been shown to be upregulated by 1,25-(OH)₂D, our findings of lower levels of receptor could be attributed to decreased serum concentrations of 1,25-(OH)₂D in uremic animals. However, the data accumulated to date do not show a significant correlation between serum 1,25(OH)₂D₃ and the level of its receptor in the parathyroid gland. On the other hand, a high correlation ($r = 0.76$) was noted between log [PTH] and log receptor number. It is likely that this reduced receptor number in the parathyroid glands of uremic animals renders the gland less responsive to the inhibitory action of 1,25-(OH)₂D on the synthesis and secretion of PTH, and may contribute to the hyperparathyroidism associated with chronic renal failure.

INCREASED CALCIUM ABSORPTION AND RETENTION, WITHOUT ELEVATED SERUM $1,25(\text{OH})_2\text{D}_3$, IN GENETICALLY HYPERCALCIURIC RATS. David A. Bushinsky, R. Ben Johnston*, Carol E. Nalbantian* and Murray J. Favus*. Univ. of Chicago, Chicago, IL.

Idiopathic hypercalciuria in man is a common cause of calcium (Ca) oxalate nephrolithiasis. The pathogenesis of the increased Ca excretion is not clear but may be due to a primary increase in intestinal Ca absorption, overproduction of $1,25(\text{OH})_2\text{D}_3$ ($1,25\text{D}$) or a renal Ca leak. To investigate the cause of idiopathic hypercalciuria in an animal model, genetic hypercalciuria was selected for in rats and their Ca balance and intestinal transport studied.

Male and female Sprague Dawley rats were fed a diet adequate in Ca, and those with 24 h urine Ca excretion > 2SD over the mean were termed hypercalciuric (HC). HC males (HCM) and females (HCF) and their HC offspring were in-bred. Fourth generation HCM and HCF as well as normocalciuric males (NCM) and females (NCF) were fed 15 g of a 0.6% Ca diet for 12 d and Ca intake and output measured over the last 6 d. Blood was obtained and duodenal Ca bidirectional fluxes studied under short circuit conditions in the Ussing apparatus.

Group	n	UCA	% Abs	Ret	Jnet	$1,25\text{D}$
NCM	10	3.9±0.3	57±1	263±4	67±6	75±9
HCM	16	11.5±1.0*	65±1*	291±5*	97±19	48±6*
NCF	10	4.8±0.3	18±2	79±11	9±5	28±7
HCF	14	10.9±0.6*	25±2*	104±10*	51±15*	34±5

Values mean ± SE; n = number of rats; UCA, urine Ca, mg/6d; % Abs, % Ca absorption/6d; Ret, net Ca retention, mg/6d; Jnet, net Ca flux, nmol/cm²/h; $1,25\text{D}$, pg/ml; *, p < 0.05 vs. NC, same sex.

Hypercalciuria in this genetic model is due to increased intestinal Ca absorption, not mediated through increased serum $1,25(\text{OH})_2\text{D}_3$, and is associated with increased net Ca retention.

REGULATION OF 25-HYDROXYVITAMIN D_3 -1 α -HYDROXYLASE ACTIVITY IN PRIMARY CULTURE OF RAT PROXIMAL TUBULAR CELLS. Tai C. Chen, Christine A. Leone, Ramzi N. Nassar and Jules B. Puschett. Renal-Electrolyte Division, University of Pittsburgh, School of Medicine, Pittsburgh, PA.

Primary cultures of cells derived from rat proximal tubules (PT) were initiated and maintained in serum-free medium supplemented with insulin, transferrin, epidermal growth factor, hydrocortisone and prostaglandin E_1 (PGE_1). Renal epithelial cells grow to near confluency without detectable growth of fibroblasts. The cultured cells retain an active 25-hydroxyvitamin D_3 -1 α -hydroxylase (1 α -hydroxylase) (4-7 pmol/hr/mg protein) and many other functional properties that are characteristic of the PT. Activity of 1 α -hydroxylase is stimulated by parathyroid hormone (PTH), PGE_2 or 8-Br-cyclic AMP, and inhibited by $1,25$ -dihydroxyvitamin D_3 ($1,25(\text{OH})_2\text{D}_3$), calcium and phosphate. Time course studies indicate that a minimum of 3 to 6 hours is required for the hormones to stimulate enzyme activity. No 25-hydroxyvitamin D_3 -24-hydroxylase (24-hydroxylase) activity was detected in control or $1,25(\text{OH})_2\text{D}_3$ preincubated cells, suggesting the absence of the 24-hydroxylase enzyme in this culture system. These results suggest that primary cell culture is suitable for structural and functional analyses of 1 α -hydroxylase.

IN "VIVO" EFFECT OF AL INFUSION ON PTH RELEASE
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In "vitro" studies have proved that Al could inhibit PTH release. However, although in "vivo" studies have shown also low PTH levels in Al intoxication there is no a definite answer regarding which is the main impaired mechanism. Production or release? Thus, the aim of this study was to evaluate the acute PTH response to intravenous (iv) Al infusion (inf).

We studied 10 male wistar rats (weight 350-450g) divided into two groups. Group A (N=5): received 0.1 ml of saline. Group B (N=5): received 0.5 mg of Cl3Al in 0.1 ml of saline. The inf were performed iv in 2' into the left jugular vein (JV) and blood samples for Al, Ca^{++} , PTH (rat-PTH) and PVC were obtained from the right JV at 0, 15, 45 and 120'.

In both groups PVC decreased from 54 to 39% due to blood withdrawal. In Group A, there was also a proportional decrease in Ca^{++} (0.65 to 0.46 mmol/l) and an increase in PTH levels. On the contrary, as the table shows, in Group B, in which the mean serum Al remained very high throughout all experience (from 1300 to 3015 $\mu\text{g/l}$), there was a significant lower PTH release than Group A without significant changes in Ca^{++} , despite Group B has a similar degree of haemodilution than Group A.

PTH	Basal	15'	45'	120'	
Grupo A	130±50	195±81	163±69	181±86	* p < 0.05
Grupo B	121±54	81±20*	77±42**	63±21*	** p < 0.01

These results suggest that "in vivo" Al could also inhibit PTH release as it has been described "in vitro". In addition we observed that Al interfere with serum Ca^{++} , may be "blunting" its decrease, which in turn, might avoid further PTH increments inducing even lower PTH levels.

URINARY CALCULI IN RENAL TRANSPLANT RECIPIENTS.
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Urinary calculi are uncommon in renal transplant recipients. During a 15 year period, we observed urinary calculi in 9 of 544 kidney transplant recipients (1.7%) who had functioning allografts for more than 3 months. Calculi occurred in 6 males and 3 females. Six patients received a living related transplant and 3 a cadaveric kidney. Calculi were diagnosed as early as 3 months and as late as 3.5 years post-transplant, but most were detected within the first year. Calculi were observed in the bladder (4 cases), the transplant (3) and indeterminate (2). Crystallographic analysis of retrieved stones revealed calcium oxalate and/or phosphate (4 cases), triple phosphate (2) and uric acid (1). All patients had one or more stone-predisposing factors such as obstructive uropathy and recurrent urinary tract infection (4 cases), hyperoxaluria (3) or hypercalciuria (2). Over a long term follow-up (mean 60 months), only one patient lost his graft 14.5 years after transplant due to causes unrelated to urinary calculi. One instance of stone recurrence was noted. During this period stone removal procedures were required in 7 patients and parathyroidectomy was done in 2 patients. In conclusion, 1) urinary calculi after renal transplant are relatively uncommon, 2) predisposing factors and crystallographic composition of the calculi were identical in type but not frequency to those of non-transplant patients, and 3) with proper medical and surgical management, post-transplant urolithiasis did not appear to affect graft prognosis.

CONTROL OF PARATHYROID HORMONE (PTH) SECRETION IN UREMIA: INFLUENCE OF ALUMINUM (Al) STATUS, CALCIUM AND THE DIRECTION OF CHANGE OF CALCIUM. Cunningham J*, Altmann P*, Glead J*, Marsh F*, O'Riordan J*, (intr. by E Stalopolsky). The London & The Middlesex Hospitals, London, UK.

Previous studies have shown impairment of PTH response to acute hypocalcemia in patients with severe Al loading and osteomalacia. We have examined the control of aminoterminal PTH (N-PTH) secretion in patients with mild and moderate Al loading, without osteomalacia, to see whether PTH secretion is also compromised by Al in this much larger group of patients. 6 patients with moderate Al loading (group A: serum Al 106 ± 25 $\mu\text{g/l}$) and 6 with low Al loading (group B: serum Al 21 ± 2 $\mu\text{g/l}$) underwent a 15 minute calcium infusion (0.4 mmol/kg/hr) and were followed for 3³/₄ hrs thereafter. Baseline and peak blood ionised Ca^{2+} in groups A and B did not differ. N-PTH, initially 534 ± 258 and 559 ± 201 pg/ml suppressed rapidly and similarly to 247 ± 93 and 307 ± 105 pg/ml after 15 min in groups A and B respectively. Hypocalcemic hemodialysis for 60 min reduced Ca^{2+} by 0.19 ± 0.01 mmol/l (group A) and 0.19 ± 0.02 mmol/l (group B) with N-PTH increments of 430 ± 118 and 667 ± 269 pg/ml respectively (ns). The rate of recovery of Ca^{2+} during the first 30 min after hypocalcemic dialysis was not influenced by Al status (ΔCa^{2+} of 0.07 ± 0.01 and 0.06 ± 0.03 mmol/l in groups A and B respectively, ns). During and after calcium infusion, the concentration of N-PTH, at a given level of calcium was the same whether Ca^{2+} was rising or falling. However, during and after the hypocalcemic dialysis, N-PTH was significantly greater when Ca^{2+} was falling than when Ca^{2+} was rising, at comparable levels of hypocalcemia.

These results show that, unlike severe Al intoxication, 1) a moderate degree of Al loading has no perceptible effect on parathyroid function in vivo; 2) when $[\text{Ca}^{2+}]$ is changing in the region of hypercalcemia, N-PTH depends on the blood $[\text{Ca}^{2+}]$; 3) conversely when $[\text{Ca}^{2+}]$ is below normal, the direction of change of $[\text{Ca}^{2+}]$ is an additional major determinant of the N-PTH response to hypocalcemia.

RENAL RESPONSES TO PHOSPHORUS DEPRIVATION IN RABBITS. D. DePalco*, A.L. Theisen*, C.B. Langman, R. Bouillon*, and J.E. Bourdeau. Michael Reese Hospital (University of Chicago) and Children's Memorial Hospital (Northwestern University), Chicago, Illinois and Laboratorium voor Experimentele Geneeskunde en Endocrinologie (Katholieke Universiteit Leuven), Leuven, Belgium.

To evaluate the renal adaptations to dietary P deprivation, young growing (1.4 kg) female albino rabbits were fed a P-deficient (0.15 %) diet for 10 consecutive days while they were housed in metabolism cages. Urinary Ca excretion rates increased markedly within 24 hours of P deprivation, remained high for each of the 10 days that dietary P was low, and returned to control values within 24 hours of consuming a normal-P feed. The hypercalciuria resulted from both an increased filtered load and decreased tubular reabsorption of Ca. Urinary P excretion rates decreased gradually in response to a low P diet and reached a nadir only after nine days of deprivation. Urinary P excretion rates recovered to control values within 24 hours of feeding a normal-P diet. Increased tubular reabsorption of P alone accounted for the hypophosphaturia. Plasma [P] was reduced significantly after 10 days of dietary P deprivation, and this was associated with a significant increase in plasma [calcitriol], independent of either plasma $[\text{Ca}^{2+}]$ or plasma [parathyroid hormone]. We conclude that dietary P deprivation in the rabbit effects the hypophosphaturia, hypophosphaturia, and hypercalciuria that characterize this condition in rats, dogs, and humans. Likewise, the elevation in plasma [calcitriol] that has been observed with dietary P deprivation in healthy rats and humans also occurs in the growing laboratory rabbit.

BIOCHEMICAL PARAMETERS OF BONE DISEASE IN RANDOMLY SELECTED DIALYSIS PATIENTS.

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Aluminum (AL) toxicity has received considerable attention in chronic hemodialysis (HD) pts. with a focus on those symptomatic pts. who have normal alkaline phosphatase (AP) and depressed PTH levels. To gain a better appreciation of these variables in AL bone disease in an outpatient HD facility, we randomly studied 27 HD pts. AL levels before and 48 hours after desferoxamine infusion (DFO), AP, n-terminal PTH, and ionized calcium (iCa) were measured. Mean age of the sample was 51.1 ± 3.9 with a time on HD of 69.7 ± 8 months (range 8 to 61). PTH was 76 ± 13 pg/ml (normal=8-24), iCa was 4.8 ± 0.1 mg/dl and AP was 263 ± 39 (normal=30-115 U/L). Baseline AL was 105 ± 12 $\mu\text{g/L}$ with a mean change following DFO (ΔAL) of 140 ± 24 . There was no correlation of ΔAL with time on HD. Pts. with a positive response to DFO ($\Delta\text{AL} > 200$ $\mu\text{g/L}$) were compared to pts with a negative response.

	$\Delta\text{AL} < 200, n=21$	$\Delta\text{AL} > 200, n=6$	P
ΔAL	84 ± 11	337 ± 35	.0001
AP	224 ± 31	396 ± 133	NS
PTH	60 ± 7	132 ± 52	NS
AL	94 ± 13	143 ± 27	NS
Months	63 ± 9	93 ± 18	NS

We conclude that there are no biochemical parameters that permit the selection of patients for routine surveillance of AL related bone disease.

DISSOCIATION OF CHANGES IN PTH FROM IONIZED CALCIUM (CA) DURING HEMODIALYSIS. Iamim Eche*, Connie L. Davis, U of TX HSCD and Dallas VAMC, Dallas, TX.

Levels of PTH may influence uremic symptoms and signs in patients with ESRD, but the factors which influence PTH levels during HD have not been completely elucidated. Thus, we measured PTH levels (intact molecule, RIA) and total CA in 12 patients before and at 1h of standard HD (dialysate Ca^{++} 3.5 mEq/l, 1.1 m² dialyzer). In these patients total CA increased from 9.2 ± 2 to 10 ± 0.2 mg/dl ($p < 0.001$) as PTH decreased by $50 \pm 4\%$. This marked decline in PTH lead us to study 4 patients in which serial PTH and ionized Ca^{++} values were measured. At 5 min PTH had decreased significantly by $17 \pm 6\%$ as ionized Ca^{++} increased from only 1.1 ± 0.05 to 1.13 ± 0.04 mM/l (NS). At 60 min PTH had declined by $58 \pm 8\%$ as ionized Ca^{++} increased by $7 \pm 1\%$ from 1.09 ± 0.03 to 1.18 ± 0.03 mM/l $p < 0.005$ (or 4.4 ± 1 to 4.7 ± 1 mg/dl $p < 0.001$). The sharp decline in PTH in contrast to the modest increment in ionized Ca^{++} prompted us to measure PTH in dialysate in several patients. In one patient the clearance of PTH was 12 cc/min. We conclude that PTH declines rapidly during the HD procedure and that the increment in ionized Ca^{++} does not fully account for this decline. Rather clearance of PTH across the dialyzer may play an important role in PTH levels.

IN VIVO EVIDENCE OF EXTRARENAL C₁₉-HYDROXYLASE ACTIVITY AND ITS IMMEDIATE RESPONSE TO PTH. P.Fanti, R.F.Friedler, and H.H.Malluche, Univ. of Kentucky Med. Ctr., Dept. of Nephrology, Bone and Mineral Metabolism, Lexington, KY. PTH stimulates in vivo and in vitro C₁₉ hydroxylase (1 α OHase) through a cyclic AMP (cAMP) mediated mechanism. Administration of PTH results in immediate cellular response documented by a steep increase in intracellular cAMP. The immediate effects of PTH on 1,25 D production in vivo have not been studied.

Cannulation of the left renal vein, artery and of both ureters was performed through flank incisions in anesthetized mongrel dogs (n=6). Simultaneous arterial and venous plasma samples and urine samples were collected at baseline (BSL) and at various time interval after bolus injection of 4 U/kg bw of 1-34 synthetic bovine PTH. Plasma and urine measurements included 1,25 D, calcium, phosphorus, and creatinine. Results are shown in Table I:

	BSL	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN
Arterial						
1,25 D pg/ml	21.9 \pm 4	28.4 \pm 5*	23.8 \pm 3	17.9 \pm 3	19.2 \pm 5	21.8 \pm 7
% of BSL	100	130	109	82	88	99
Venous						
1,25 D pg/ml	26.0 \pm 5	18.3 \pm 2*	20.6 \pm 2*	18.4 \pm 3*	22.8 \pm 5	21.9 \pm 6
% of BSL	100	70	79	71	88	84

* different from BSL (p<0.05).

As early as 1 min. after PTH bolus the 1,25 D levels in the arterial blood were 30% over baseline (p<0.05). Venous concentrations of 1,25 D were lower at min. 1,3, and 5 after PTH (p<0.05).

These results suggest extrarenal production of 1,25 D and, therefore, further studies were done in 10 anesthetized dogs. They were bilaterally nephrectomized and the femoral artery was cannulated; 6 dogs received the same PTH injections, 4 were given vehicle. Arterial blood sampling and biochemical measurements were done as in the first experiment. Results are given in Table II:

	BSL	1 MIN	3 MIN	5 MIN	10 MIN	15 MIN
Arterial						
1,25 D pg/ml	25.3 \pm 1	28.1 \pm 2*	25.1 \pm 2	22.1 \pm 2	25.6 \pm 5	28.9 \pm 3
% of BSL	100	111	99	87	101	114

* different from BSL (p<0.05).

In the vehicle injected animals 1,25D levels were not different from baseline.

These data demonstrate in vivo: (1) extra renal production of 1,25 Vit D; (2) immediate response of extrarenal C₁₉ OHase to acute PTH stimulation; (3) immediate renal metabolism of 1,25 D following stimulation by PTH bolus injection.

CHRONIC METABOLIC ALKALOSIS INCREASES CALCIUM RETENTION IN RATS. Murray J. Favus*, R. Ben Johnston*, Melissa D. Tanklefsky* and David A. Bushinsky. Univ. of Chicago, Chicago, IL.

Chronic metabolic alkalosis decreases urine (U) calcium (Ca) excretion. The decrease in UCa excretion would increase the serum level of Ca, subsequently increasing the filtered load of Ca. UCa should then increase to pre alkalosis levels. That UCa remains low during alkalosis implies that either intestinal Ca absorption decreases or that Ca retention, presumably by bone, increases. To determine which, we fed adult male rats 15 g per day of a 0.6% Ca diet. After 7 days some remained on the same diet (CTL), others were changed to a matched chloride deficient diet (-Cl) which produces alkalosis in the growing rodent (ALK1) and others were fed -Cl and drank 75 mM NaHCO₃ (ALK2). Ca intake and output measured over the next 7 d. Blood was then obtained and duodenal Ca bidirectional fluxes studied under short circuited conditions in the Ussing apparatus.

Group	n	UCa	% Abs	Ret	Jnet	1,25D
CTL	13	6.0 \pm 0.4	46 \pm 1	280 \pm 7	17 \pm 9	65 \pm 6
ALK1	15	4.0 \pm 0.3*	53 \pm 2*	332 \pm 11*	28 \pm 6	64 \pm 8
ALK2	14	3.0 \pm 0.2*	48 \pm 1*	302 \pm 8*	30 \pm 8	73 \pm 5

Values mean \pm SE; n = number of rats; UCa, urine Ca, mg/ 7d; % Abs, % Ca absorption/ 7d; Ret, net Ca retention, mg/ 7d; 1,25D, 1,25(OH)₂D₃, pg/ml; +, p < 0.05 vs. CTL; #, p < 0.05 vs. ALK1.

Compared to CTL, UCa excretion fell with chronic metabolic alkalosis, and intestinal Ca absorption increased (ALK1), resulting in an increase in net Ca retention, presumably by bone, in both groups of alkalotic rats. Whether alkalosis will increase calcium retention in man, as it does in the rat, is not known.

AGE-ASSOCIATED REDUCTION IN THE CYTOSOLIC Ca²⁺ RESPONSE OF RAT RENAL PROXIMAL TUBULES TO PARATHYROID HORMONE (PTH), NOREPINEPHRINE (NE), AND ANGIOTENSIN-II. Charles R. Filburn*, and Stephen Harrison* (intr. by Bertram Sacktor). GRC, NIA, NIH, Baltimore, Maryland.

The response of rat proximal tubules to hormones known to elevate cytosolic Ca²⁺ was measured in tubules from kidneys of 6 mo and 24 mo male Wistar rats. Mean basal cytosolic Ca²⁺ measured in Quin-2 loaded tubules was slightly, but not significantly higher in tubule from 24 mo compared to 6 mo rats. Peak transient stimulation of cytosolic Ca²⁺ was 25% less (p < .01) in the older group, but a small sustained increase was the same. Since the mean BUN value of 24 mo rats was twice that of 6 mo rats, PTH responsiveness was compared in subgroups with normal and elevated BUNs. Peak increase in Ca²⁺ in tubules from 24 mo rats with normal BUN values was not significantly different that of paired 6 mo rats, while the response in tubules from rats with elevated BUNs was reduced by 42%. PTH responsiveness showed a significant negative correlation with BUN. Much larger peak and plateau increases in cytosolic Ca²⁺ elicited by NE and A-II were also reduced in tubules from older rats. Since responses to all three agonists appear to be mediated by IP₃, a diminished ability to respond to a PTH induced increase in IP₃, which is small relative to NE and A-II-induced increases, probably does not explain the reduced response to PTH. Thus, reduced PTH stimulation of IP₃ production, apparently associated with increased renal disease, may be involved in the age-associated reduced responsiveness.

SEPARATE BINDING SITES FOR INTACT PTH 1-84 AND SYNTHETIC PTH 1-34 IN CANINE KIDNEY MEMBRANES. J. Garcia, C. McConkey* and K.J. Martin. Renal Division, Washington University School of Medicine, St. Louis, MO.

Although the classical actions of parathyroid hormone (PTH) can be reproduced by the amino-terminal fragment PTH 1-34, there have been observations suggesting differences in the biological effects of the two PTH preparations. Therefore, we examined the characteristics of PTH receptor binding in canine renal cortical membranes using iodinated biologically active preparations of intact PTH 1-84 and Nle^{3,18},Tyr³⁴ PTH 1-34 amide. Radioiodinated PTH 1-84 was prepared using a solid phase lactoperoxidase technique. The PTH 1-34 analog was iodinated using chloramine T. Both radioligands were purified by reverse phase HPLC. Specific binding of ¹²⁵I PTH 1-84 reached equilibrium at 3 hours whereas binding of ¹²⁵I PTH 1-34 analog reached equilibrium at 45 mins. Binding of both radioligands were stable for 5 hours. While excess intact PTH 1-84 resulted in complete displacement of ¹²⁵I PTH 1-84, 22 \pm 1.6% of the specifically bound radioligand could not be displaced by excess PTH 1-34. These data suggested the possibility of a binding site for the carboxy-terminal region of intact PTH. Therefore, additional studies were performed with carboxy-terminal PTH fragments, PTH 35-84 and PTH 53-84. In contrast to previous studies in other systems, these fragments did not result in significant displacement of ¹²⁵I PTH 1-84. Scatchard analysis of binding of ¹²⁵I PTH 1-84 was consistent with a two site model whereas binding of ¹²⁵I PTH 1-34 suggested a single site model. These data suggest that there are binding sites in canine kidney which are selective for the intact hormone.

THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF 25-HYDROXY-26,27-DIMETHYLVITAMIN D₃ AND 1,25-DIHYDROXY-26,27-DIMETHYLVITAMIN D₃: HIGHLY POTENT NOVEL ANALOGS OF VITAMIN D₃. H.S. Gill,* J.M. Londowski,* R.A. Corradino,* A.R. Zinsmeister,* and R. Kumar. Mayo Clinic/Foundation, Rochester, MN and Cornell University, Ithaca, NY.

We synthesized 25-hydroxy-26,27-dimethylvitamin D₃ (25OH-diMeD₃) and 1,25-dihydroxy-26,27-dimethylvitamin D₃ (1,25(OH)₂-diMeD₃) from cholesterol-5-ene acid-3 β -ol and tested their biological activity *in vivo* and *in vitro*. 25OHdiMeD₃ was found to be a highly potent vitamin D analog with bioactivity similar to that of 25-hydroxyvitamin D₃ (25OHD₃). It bound rat plasma vitamin D binding protein with approximately one-third the affinity of 25OHD₃. In a duodenal organ culture system and in a competitive binding assay with chick intestinal 1,25-dihydroxyvitamin D (1,25-(OH)₂D) receptor, 25OHdiMeD₃ was significantly more potent than 25OHD₃.

1,25(OH)₂-diMeD₃ was also highly active *in vivo*. At doses of 1,000-5,000 pmoles/rat, its action was more sustained than that of 1,25(OH)₂D₃. 1,25(OH)₂-diMeD₃ bound to vitamin D binding protein about 18 times less effectively than 1,25(OH)₂D₃. 1,25(OH)₂-diMeD₃ bound to the chick intestinal cytosol receptor with an affinity one-half that of 1,25(OH)₂D₃. In a duodenal organ culture system, 1,25(OH)₂-diMeD₃ was about half as active as 1,25(OH)₂D₃. Extension of the sterol side chain at C-26 and C-27 by CH₂ groups, prolongs the bioactivity of a vitamin D sterol hydroxylated at C-1 and C-25; a sterol hydroxylated only at C-25 does not show alteration of its bioactivity *in vivo*. These newly synthesized analogs may be of therapeutic use in various mineral disorders.

INTRAVENOUS CALCITRIOL: PLASMA KINETICS AND ACUTE EFFECT ON SERUM PTH IN NORMAL AND DIALYZED SUBJECTS. WG Goodman, IB Salusky, R Horst*, G Segre*, KC Norris, JW Coburn. Depts of Peds & Med, UCLA Sch of Med & VA Med Ctrs, Wadsworth & Sepulveda, LA, CA and Harvard Med Sch, Boston, MA.

Chronic calcitriol (1,25) therapy can lower serum (S) PTH levels in dialyzed patients, but the acute effects of intravenous (IV) 1,25 and its plasma kinetics have not been studied in normal or uremic patients. Thus, 6 adolescent patients with osteitis fibrosa, age 16 \pm 3 (SD) yrs, on CAPD/CCPD (PD) and 5 normal adults (N) (39 \pm 9 yrs) received single IV doses of 1,25, 4 μ g/70 kg. S PTH, 1,25 and Ca levels were measured 2, 15, and 30 min and 1, 3, 6, 12, and 24 hrs after injections. Basal S-PTH and S-1,25 levels were 674 \pm 192 pg/ml and 8 \pm 7 pg/ml, respectively, in PD and 30 \pm 24 and 17 \pm 3 in N, p<0.005. S-1,25 values at 2 min (352 \pm 92 pg/ml in PD and 365 \pm 24 in N) and 1 hr (191 \pm 49 in PD and 190 \pm 28 in N) did not differ between groups. The plasma half-life of 1,25, determined from the slope of the plasma decay curve between 3 and 24 hrs, was 16.5 \pm 7.5 hrs. in PD and 11.4 \pm 2.2 hrs in N, NS. S-PTH and S-Ca did not change after IV 1,25 in PD. In contrast, S-PTH in N fell to 58 \pm 14% of baseline values at 15 min, p<0.01; 62 \pm 9% at 30 min, p<0.01, 69.2 \pm 15% at 60 min, p<0.01, and 68 \pm 25% at 24 hrs, p<0.05. S-Ca and ionized Ca in N were unchanged at each time interval after IV 1,25. Thus, the plasma half-life of 1,25 is similar in dialyzed and normal subjects. IV 1,25 acutely lowers S-PTH without changes in S-Ca in N but not in PD with 2 hyperparathyroidism. The data show that 1,25 rapidly lowers S-PTH, possibly by a direct mechanism independent of steroid hormone action.

CHLOROTHIAZIDE (CTZ), FUROSEMIDE (FUR) AND BUMETANIDE (BUM) DISSOCIATE CA AND NA CLEARANCES IN RAT. Mary Kay Grady* and Linda S. Costanzo. Medical Coll. of Va., Richmond, Va.

Clearance experiments were performed in saline-expanded rats to compare the effects of CTZ, FUR and BUM on renal handling of Ca and Na. With control infusions, fractional electrolyte excretion and C_{Ca}/C_{Na} were stable over time. CTZ caused significant natriuresis (p < 0.001) without calciuresis, reducing C_{Ca}/C_{Na} from 1.47 \pm 0.23 to 0.05 \pm 0.01, p < 0.001. FUR also increased fractional Na excretion (p < 0.01) without altering fractional Ca excretion and dissociated Ca and Na clearances; C_{Ca}/C_{Na} fell from 2.20 \pm 0.39 to 0.53 \pm 0.22, p < 0.001. BUM produced a pattern of results similar to that for FUR and caused the Ca/Na ratio to fall from 1.73 \pm 0.16 to 0.65 \pm 0.10, p < 0.001. In summary: (1) The dissociation of Ca and Na clearances produced by CTZ is stereotypical of its distal tubule action. (2) FUR and BUM produced a CTZ-like dissociation of Ca and Na clearances. In addition to their well-known actions in thick ascending limb, these diuretics may also have significant distal activity in the rat.

EFFECT OF HYPOCALCEMIA ON RENAL HANDLING OF PHOSPHORUS (Pi) IN THE RAT: INTERACTION WITH DIET Pi AND PTH. J.Guntupalli and E.Bourke. Dept. Of Medicine, VA Medical Center and Emory University School Of Medicine, Atlanta, Georgia.

The effects of acute hypocalcemia on renal handling of Pi are unresolved. Hence, clearance experiments were performed during Na-EGTA (100 μ mols. Kg⁻¹.h⁻¹.) induced hypocalcemia in the following groups of chronically PTXed rats. Group 1: In dietary replete rats, reduction in plasma calcium (PCa) (1.78 \pm 0.03 vs 1.37 \pm 0.03 mM, P<0.05) at constant plasma Pi had no effect (85 \pm 3 vs 83 \pm 2 μ g/min, NS) on the TRPi/GFR. Group 2: In the dietary Pi deprived rats, a similar reduction in PCa also failed to alter (89 \pm 2 vs 90 \pm 2 μ g/min, NS) the TRPi/GFR. Group 3: Superimposition of Na-EGTA induced hypocalcemia (2.25 \pm 0.14 vs 1.50 \pm 0.07 mM, P<0.05) on 1-34 PTH 2.5 U. Kg⁻¹.h⁻¹. infusion in dietary Pi deprived rats, however, fully restored (81 \pm 2 vs 67 \pm 3 μ g/min, P<0.05) the phosphaturic action of PTH. Group 4: In contrast, PTH had no effect on Pi reabsorption in the dietary Pi deprived, saline infused time control experiments where PCa levels were stable. Group 5: Similarly, in the dietary Pi deprived rats infused with Ca-EGTA, where PCa was maintained constant, PTH again failed to decrease the TRPi/GFR.

In conclusion, these studies demonstrate that acute hypocalcemia per se does not alter the tubular reabsorption of Pi both in dietary Pi repletion and deprivation. Acute hypocalcemia, however, fully restores the phosphaturic action of PTH in dietary Pi deprivation, independent of plasma Pi. These results are compatible with the recent implication of a role for intracellular Ca in the regulation of tubular Pi transport.

ATRIAL NATRIURETIC PEPTIDE LEVELS IN IDIOPATHIC HYPERCALCIURIA: EFFECT OF AN ORAL CALCIUM LOAD. Aaron Halabe, Norman L.M. Wong, Eric F.C. Wong, and Roger A.L. Sutton. Dept. of Medicine, Univ. of British Columbia, Vancouver, B.C. Canada.

The effect of an acute oral Ca load on atrial natriuretic peptides (ANP) secretion in idiopathic hypercalciuria (IH) with high calcitriol levels was examined. Six controls (3 male, 3 female mean age 40±11) and 7 (IH) patients (4 male, 3 female mean age 45±10) received an 800 mg Ca diet for one week prior to the study. On the test day, Ca 25 mg/kg as Ca gluconate was given with a breakfast containing 480 mg Ca. Plasma ANP and serum Ca and iPTH were determined prior to and at 30, 60, 90, 150 and 210 min after the Ca load. Total serum Ca rose in C group (8.9±0.4 to 9.2±0.4 mg/dl $p > 0.005$) and in IH subjects (9.2±0.3 to 9.7±0.4 mg/dl $p < 0.05$). Urinary Ca/Cr rose from 0.10 to 0.35 (mean Δ 257%) in C group vs 0.19 to 0.51 (mean Δ 182%) in IH group. Baseline serum iPTH levels were similar (C, 38±11.4 vs IH 37±15.3 pg/ml) and fell by 51% after Ca load. Calcitriol levels prior the Ca load were 35±5.8 and 53±8.6 pg/ml ($p < 0.001$) in the C and IH group respectively. Baseline ANP levels were 37±18 pg/ml in C and rose to 64±36 pg/ml ($p < 0.02$) in contrast to 14.3±6.3 pg/ml in IH that rose to 26±3.7 pg/ml ($p < 0.003$). ANP levels increased significantly after a Ca test. Further studies will be required to determine whether differences in calcitriol levels are responsible for the increased ANP levels in the IH group and whether ANP is related to the pathophysiology of IH.

PURIFICATION AND CHARACTERIZATION OF NEPHROCALCIN FROM MAMMALIAN KIDNEY TISSUES. S. Hall*, Y. Nakagawa* and F. L. Coe. Nephrology Program and Dept. of Biochem. and Mol. Biology, Univ. of Chicago, Chicago, IL

We have purified nephrocalcine, a urinary glycoprotein calcium oxalate monohydrate crystal growth inhibitor, from human urine, rat urine and from rat kidney tissue, and human embryonic kidney tissue culture medium. These nephrocalcines were of mol.wt. 14,000 in the monomeric form, and the dissociation constant toward calcium oxalate monohydrate was $\times 10^{-8}$ M. Using the same methods, we have isolated nephrocalcines from the kidney tissues of cow, dog, mouse, monkey and human. All 5 nephrocalcines purified from these mammalian kidney tissues were aggregated to dimeric, trimeric, and tetrameric forms, with an apparent monomeric mol. wt of 14,000. All were glycoproteins with a high content of acidic amino acid residues (aspartic acid and glutamic acid). Dissociation constants toward calcium oxalate monohydrate crystal surface were in the range between 10^{-7} and 10^{-8} M. Native bovine nephrocalcine showed strong amphiphilicity as measured collapsing pressure at the air-water interface using a film balance, but lost its amphiphilicity upon decarboxylation of gamma-carboxyglutamic acid (GLA). This suggests that the GLA residues in nephrocalcine have an important role in maintaining its conformation.

Nephrocalcines are present in mammalian kidney tissues and act as a basic defense mechanism against calcium stone formation in mammalian kidneys.

PARATHYROID HORMONE (PTH) CAUSES HOMOLOGOUS DESENSITIZATION OF THE CYTOSOLIC Ca^{2+} RESPONSE IN RAT RENAL PROXIMAL TUBULES. Stephen Harrison* and Charles R. Filburn,* (intr. by Bertram Sacktor). GRC, NIA, NIH, Baltimore, Maryland.

PTH is known to elevate cytosolic Ca^{2+} in rat renal proximal tubules by a cAMP-independent, apparently IP_2 -dependent mechanism. Pretreatment of tubules, prepared by collagenase digestion and Percoll gradient separation, with 1-34 rat PTH results in rapid disappearance of the Ca^{2+} response to PTH. Subsequently added norepinephrine or angiotensin-II elicit normal responses, with either normal medium Ca^{2+} or excess EGTA present. Desensitization was dependent on the level of PTH present during pretreatment and, at lower levels of hormone, on the time of exposure. The analog 3-34 b PTH, dibutyrylcAMP, and forskolin failed, both individually and in combination, to induce desensitization. Norepinephrine at 10^{-7} M, which produces a Ca^{2+} response similar to PTH, also failed to desensitize to PTH, either alone or in combination with 3-34 PTH and dibutyrylcAMP. Desensitization by 40 nM 1-34rPTH was attenuated by 10^{-6} M 3-34 b PTH. Desensitized tubules only partially recovered over 3 hr. after removal of 1-34 PTH. Thus, rPTH causes a receptor-linked, slowly reversible, homologous desensitization of PTH mobilization of intracellular Ca^{2+} .

EFFECTS OF NEPHROCALCIN (NC) ON CALCIUM OXALATE MONOHYDRATE (COM) CRYSTAL AGGREGATION AND ELECTROSTATIC SURFACE CHARGE (ESC): DIFFERENCES BETWEEN NORMAL NC (nNC) AND NC FROM HUMAN KIDNEY STONES (sNC).

B.Hess,* Y.Nakagawa,* and F.L.Coe, Nephrology Program, Univ. of Chicago, Chicago, Illinois.

Although it lacks γ -carboxyglutamic acid and forms weak air-water interfacial films, sNC inhibits COM crystal growth almost as well as nNC. But because kidney stones can form by aggregation of small crystals whose growth has been retarded, sNC could predispose to stones by failing to inhibit crystal aggregation normally. We have measured COM crystal aggregation using the rate of decrease of turbidity of a COM crystal suspension, that reflects the sedimentation velocity of aggregates induced by slow stirring. Sedimentation velocity increases with particle radius, which can increase only by aggregation, because the crystals are suspended in a solution that supports no crystal growth. Monomeric (14kD) nNC inhibited aggregation in a concentration dependent manner at $\geq 1 \times 10^{-8}$ M, whereas sNC was inhibitory at only $\geq 2 \times 10^{-7}$ M. We measured ESC, which is a basic force of particle repulsion, using the electrophoretic mobility of COM crystals in solution. In the absence of NC, COM crystals had an ESC (in mV) of $+13.8 \pm 0.6$; 5×10^{-8} M nNC changed ESC to -16.5 ± 0.6 , vs -7.8 ± 0.5 for sNC, $p < 0.001$. Corresponding values for nNC vs sNC each at 10^{-7} M were -18.2 ± 0.6 vs -12.8 ± 0.8 , $p < 0.001$; and at 5×10^{-7} M were -18.2 ± 0.4 vs -13.3 ± 0.6 , $p < 0.001$.

sNC is a defective inhibitor of COM crystal aggregation, and the defect may arise from a reduced effect upon ESC when sNC binds to the crystal surface.

ACTIVATION OF THE Ca^{2+} MESSENGER SYSTEM BY PARATHYROID HORMONE (PTH) IN THE OPOSSUM KIDNEY (OK) CELL LINE. K.A. Hruska, R. Civitelli,* and I.R. Reid*. Jewish Hosp., St. Louis, Missouri

PTH stimulation of OK cells, a proximal renal tubule-like cell line, produces a transient elevation of $[Ca^{2+}]_i$, with return to levels above baseline within 1 min, and a sustained elevation. The PTH dose-response curves for cAMP production and $[Ca^{2+}]_i$ transients were similar. Activation of the adenylate cyclase system produced $[Ca^{2+}]_i$ transients of a type different from PTH. The $[Ca^{2+}]_i$ transients stimulated by PTH were derived from IP_3 stimulated release of intracellular Ca^{2+} stores. Determination of the PTH effect on inositol phosphate (IP) release revealed a rapid increase in IP_3 and a sustained increase. The latter represented $I_{(1,3,4)}P_3$ as increased levels of inositol tetrakisphosphate (IP_4) were also found, and $I_{(1,3,4)}P_3$ is the breakdown product of IP_4 . In isolated plasma membrane vesicles of proximal tubular cells, IP_3 stimulated the initial rates of Ca^{2+} efflux. In inside out vesicles Ca^{2+} efflux directionally represents Ca^{2+} entry in intact cells. These results suggest that PTH produces an increase in Ca^{2+} entry from the extracellular space which is initiated at the time of the IP_3 stimulated Ca^{2+} transient. IP_3 stimulated Ca^{2+} entry may participate in the sustained elevation of $[Ca^{2+}]_i$ observed in OK cells. Inhibition of PTH stimulated Ca^{2+} transients and IP_3 production by pertussis toxin is compatible with this suggestion. Thus, multiple cellular pathways of Ca^{2+} flux are coordinately regulated and stimulated by PTH.

SKELETAL RESPONSE TO CALCEMIC ACTION OF PTH IS NORMAL IN RENAL FAILURE. C Hsu and S Patel*, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

It is widely believed that skeletal resistance is the mechanism of impaired calcemic response to PTH in renal failure. The action of PTH not only involves skeletal mobilization of Ca, it may also stimulate intestinal absorption of Ca and renal conservation of Ca. We have examined each of these factors and studied the calcemic response to PTH in renal failure. PTH, 3u/h/100 g, was infused for 5 hours to renal failure rats 3 weeks after 5/6 nephrectomy. In non-fasted animals, the post-PTH increments of total plasma Ca ($\Delta P_{Ca} = .71 \pm .06$ mg/dl) and ionized Ca ($\Delta P_{Ca^{++}} = .37 \pm .06$ mg/dl) of control sham operated rats were significantly greater than those of renal failure rats ($\Delta P_{Ca} = .37 \pm .02$ and $\Delta P_{Ca^{++}} = .17 \pm .01$, both $p < .01$). Urinary Ca excretion rate (U_{Ca}) remained unchanged during PTH infusion despite the increase of plasma Ca. Plasma levels of calcitriol after PTH injection were higher in control (257 ± 18 pg/ml) than in renal failure rats (162 ± 5 , $p < .001$). Pretreatment of non-fasted renal failure rats with 50 ng calcitriol i.v. corrected the abnormal calcemic response to PTH. In order to exclude the PTH effect on intestinal Ca absorption, PTH infusion was carried out in animals fasted for 18 hours. The post-PTH increments of Ca were lower, but no longer different between renal failure rats ($\Delta P_{Ca} = .37 \pm .04$, $\Delta P_{Ca^{++}} = .20 \pm .01$) and control rats ($\Delta P_{Ca} = .34 \pm .03$, $\Delta P_{Ca^{++}} = .19 \pm .01$), suggesting that skeletal mobilization of Ca was similar between the two groups of animals. We conclude that the lack of intestinal response to PTH rather than the skeletal resistance is the mechanism of impaired calcemic response to PTH in renal failure.

PTH ANALOG $[Tyr^{34}] bPTH (7-34)NH_2$ ANTAGONIZES THE INHIBITORY ACTION OF PTH ON PROXIMAL HCO_3^- REABSORPTION IN THE RAT. Ph. Jaeger, M. Tellier*, N. Fowler*, J.P. Hayslett, F. Roch-Ramel*, Lausanne University, Dpts of Medicine and Pharmacology, Lausanne, Switzerland.

Previous acute studies have shown that PTH impairs proximal HCO_3^- reabsorption. To see whether this finding is relevant to chronic primary hyperparathyroidism (PHP) and may account for the acidosis encountered in PHP, we developed an animal model of PHP in the rat with normal plasma Ca and P levels and absence of nephrocalcinosis. When HCO_3^- loaded, these animals displayed exaggerated bicarbonaturia. Free-flow micropuncture experiments were carried out in proximal tubules (PT) to localize the nephron site of this effect and to ascertain the role of PTH. Tubular HCO_3^- was assayed as total CO_2 by ultramicrocalorimetry. Three groups of TPTX rats were infused for 6 days (s.c.) osmotic minipumps) with bPTH (1-34): gr I at 0.7 U/h (normal replacement = euparathyroidism); gr II at 2.1 U/h (hyperparathyroidism); or gr III at 2.1 U/h with acute (during micropuncture) i.v. infusion of $[Tyr^{34}] bPTH (7-34)NH_2$, a PTH analog with in vivo antagonist properties in both kidney and bone. Micropuncture was carried out during i.v. infusion of 80 mM $NaHCO_3$, 70 mM NaCl and 3% polyvinylpyrrolidone at 200 μ l/min.kg BW.

	Plasma Ca (mM)	Filtered load HCO_3^- (μ mol/min.kg)	HCO_3^- deliv. to late PT (%)
I	1.63 ± 0.11 (5)	102 ± 9 (4)	31 ± 3 (16)
II	1.99 ± 0.12 (4)	105 ± 13 (4)	$47 \pm 4^*$ (9)
III	1.96 ± 0.20 (3)	118 ± 20 (3)	$36 \pm 2^*$ (11)

+ $p < 0.005$ vs I; * $p < 0.02$ vs II; X \pm SEM (n)

Fractional delivery of HCO_3^- to late PT was larger in hyper- (gr II) than in euparathyroidism (gr I) despite similar diets, plasma Ca levels and filtered loads of HCO_3^- ; this inhibitory effect of PTH was blocked by the PTH analog. Conclusion: the proximal tubule's ability to reclaim HCO_3^- is blunted in chronic PHP; the effect is caused by PTH per se and is acutely abolished by a PTH antagonist. The latter observation confers a new blocking action to this recently developed PTH analog.

INTESTINAL MECHANISM FOR THE REDUCED CA ABSORPTION BY AMILORIDE. S. Kothalia, C. Martin,* P. Rodriguez,* K. Lau. Michael Reese Hosp. & Univ. of Chicago, Chicago, IL.

Amiloride is known to enhance distal nephron Ca transport, but its effects on intestinal Ca handling and mode of action are unknown. Metabolic balance and Ussing chamber studies were therefore performed to examine these issues in rats, randomized to receive nothing (as control) (C) or amiloride (Am). The following results were obtained. (1) Single dose of Am (9 mg/kg) given acutely by gavage or by intravenous infusion in vivo produced no effects on Ca fluxes. (2) Neither mucosal nor serosal addition of Am (100 μ M) directly to the Ussing chamber elicited any changes. (3) Oral administration of Am (9 mg/kg/day) x 8 days reduced net intestinal Ca absorption and Ca retention (Table). (4) These were associated with reduced mucosal-to-serosal (J_{ms}) Ca flux ($\text{mmol} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$) across the distal colon (Table), but not across the duodenum. Lumen PD tended to be more negative with Am (-12.06 ± 2.13 vs -7.88 ± 0.82 mv), but this was not significant. There was no difference in serum $1,25(OH)_2D$ levels (45.5 ± 2.1 vs 45.4 ± 6.8 pg/ml)

	Distal Colon			Net Ca Absorption		Retained Ca (mmol/day)
	J_{ms}	J_{sm}	J_{net}	(mmol/day)	(%)	
C	34 ± 3	19 ± 2	15 ± 3	1.45 ± 0.1	34 ± 1.1	1.39 ± 0.1
Am	$25 \pm 2^{\S}$	17 ± 2	$8 \pm 1^{\S}$	$1.17 \pm 0.1^{\S}$	$28 \pm 2.3^{\S}$	$1.13 \pm 0.1^{\S}$

(Values are mean \pm SEM, \S , $p < 0.05$ vs C.)

These data indicate that prolonged but not acute amiloride administration decreases net intestinal Ca absorption and Ca retention. These effects are attributable to a reduction in active mucosal-to-serosal Ca flux across the distal colon. The mechanism is presently unclear, but is independent of differences in voltage and serum $1,25(OH)_2D$ concentration.

FUNCTIONAL HETEROGENEITY OF TAMM-HORSFALL GLYCOPROTEIN (THP): COMPARISON OF SEVERE CALCIUM RENAL STONE FORMERS (SF) TO NORMAL CONTROLS (C). B.Kim,* B.Hess,* Y.Nakagawa,* and F.L.Coe, Nephrology Program, Univ.of Chicago, Chicago, IL

THP has been described either as a promoter or an inhibitor of the aggregation of calcium oxalate monohydrate (COM) crystals. We isolated THP from urines of 6 male C and 5 male SF (> 20 stones). The limiting viscosity number (η) of THP was measured at pH 7.0. Electrostatic surface charge (ESC) of COM crystal suspensions after adding THP was derived from the electrophoretic mobility of the crystals. COM crystal aggregation was induced by slow stirring of a slurry and measured as the rate of decrease of turbidity that reflects the radius-dependent sedimentation velocity of the aggregates. Solubility of THP was generally reduced in SF. Only the soluble THP fraction was used in the experiments. Measured at ionic strength (μ) 0.19, η of THP was 73.9 ± 10.5 for SF and 30.5 ± 10.5 for C ($p < 0.025$). ESC (in mV) of COM crystals at 1×10^{-4} M THP was -22.2 ± 0.8 for C and -15.0 ± 1.2 for SF ($p < 0.001$). At 5×10^{-4} M THP, pH 7.2 and μ 0.2, crystal aggregation inhibition was only reduced in 1 SF. pH 5.7 decreased the inhibition markedly in 2/6 C and 3/5 SF.

Thus the viscosity of the soluble fraction of THP is increased among SF at physiologic μ and pH 7.0. This may - by alteration of the molecular conformation - induce a less negative ESC when THP binds to the COM crystal surface. THP at pH 7.2 is an inhibitor of COM crystal aggregation in vitro. However at pH 5.7, crystal aggregation inhibition can be markedly reduced; this seems to occur more frequently among SF.

ZINC DEPLETION LIMITS THE VITAMIN D₃ RESPONSE TO LOW PHOSPHATE DIET (LPD). PL Kimmel and CB Langman, George Washington University, Washington, D.C. and Northwestern University, Chicago, IL.

To test if the physiologic increase of 1,25 dihydroxycholecalciferol(1,25) in response to LPD is limited by zinc(Z) depletion(DEP), 7 week old male Lewis rats were randomized and pair fed Z DEP (2ppm)(-Z) or Z replete (-Z plus 5mg acetate/d) (+Z) diets containing 0.5% calcium(Ca) and 0.4% phosphate(P)(NP). After 14d, half of each group were placed on LPD (0.5% Ca, 0.1% P), and continued on +Z or -Z. The others continued originally assigned diets. 7d later complete 3d external Z, P, and Ca balance and plasma(p) concentrations of Z, P, Ca, 25 hydroxycholecalciferol(25) and 1,25 were measured. Data: Mean \pm SEM, * $p < .05$, +Z vs -Z, ANOVA.

	pPmg/dl	pCa mg/dl	pZug/dl	p1,25pg/ml
+Z/NP(6)	5.6 \pm 0.5	9.4 \pm 0.4	20.3 \pm 2.0*	25.5 \pm 10.9
-Z/NP(7)	5.8 \pm 0.1	10.1 \pm 0.2	7.7 \pm 1.8	27.7 \pm 12.6
+Z/LP(5)	4.3 \pm 0.5	9.6 \pm 0.4	25.5 \pm 1.8*	63.0 \pm 46.6*
-Z/LP(7)	4.9 \pm 0.9	10.5 \pm 0.5	11.6 \pm 8.0	39.0 \pm 15.1

p1,25 was higher in +Z/LP than all other groups, but there was no significant increase in -Z/LP. There were no differences in p25 between groups. Rats on LPD had lower urinary(U)P and higher Ca excretion than NP controls, but had similar Ca and P balance, regardless of Z nutritional status. UZ was higher and fecal Z excretion lower in LPD than NP +Z rats suggesting greater Z absorption in LPD rats.

We conclude Z DEP limits the 1,25 response to LPD. Non-hormonally mediated changes in Ca and P absorption may contribute to maintenance of balance on LPD with Z DEP in spite of lower p1,25 levels.

SODIUM-PHOSPHATE CO-TRANSPORT IN INNER MEDULLARY COLLECTING DUCT CELLS. KA Knibloe*, BS Levine, DR Mishler* and JA Kraut. Med & Resch Svc VA Med Ctr West LA and UCLA Sch of Med LA, Ca

A small portion of Pi transport occurs in the distal nephron of the mammalian kidney but little is known regarding the precise mechanism. To address this issue, we studied Pi transport in primary cultures of inner medullary collecting duct (IMCD) cells. Pi transport was measured in Costar wells at 37°C in uptake media containing in mM: NaCl 137; KCl 5; CaCl₂ 2.8; MgSO₂ 1.2; Hepes 10; K₂HPO₄ 0.1; pH 7.4. In 5 separate experiments, in the presence of Na, Pi uptake was linear for 20 min and then began to plateau. Pi uptake at 10 min in the presence of Na 1.31 ± 0.11 mmol/mg protein was significantly higher than in the presence of K or choline, 0.07 ± 0.006 and 0.006 ± 0.03 , respectively $p < 0.01$, $n=4$. When a specific inhibitor of proximal tubular Na-Pi transport, phosphonoformic acid (1.0 mM) was added, Na-Pi transport was decreased by 28 \pm 5% at 5 min, $n=3$. The maximal uptake rate (V_{max}) and affinity (K_t) calculated from an Eadie-Hofstee plot of uptake when extracellular Pi was varied between 0.01-1.0 mM, were 0.49 nmol/mg protein/min and 0.02 mM, respectively. The addition of PTH 1 nM, DBc-AMP 1 mM, or ADH 1 mM (a dose which stimulates c-AMP generation in our IMCD cells) had no effect on Na-Pi uptake compared to their respective vehicles at 3 min uptake. These data demonstrate the presence of a Na-dependent Pi transport process in IMCD cells which has a high affinity and low capacity for Pi and does not appear to be regulated by c-AMP. The precise role of this transporter and the factors that may regulate it remain to be determined.

REDUCED RENAL 1,25(OH)₂D₃ SYNTHESIS (1,25D SYN) IN CHRONIC METABOLIC ACIDOSIS (CMA) IS SECONDARY TO MITOCHONDRIAL (MITO) CALCIUM (Ca) OVERLOAD. C.B.Langman, M.J.Favus*, F.L.Coe, Northwestern Univ. and Univ. of Chicago, Nephrology Divs., Chicago Illinois.

CMA in rats fed a Ca-replete diet is associated with reduced serum levels of and proximal tubule(PT)1,25D syn. Although PT Ca content does not differ between acidotic(A) and control(C) rats (1.4 \pm .2v1.5 \pm .4 nmol/mg prot), presumptive removal of PT mito Ca by either EGTA, 0 mM ECF Ca or A23187 increases A but decreases C 1,25D syn. To more directly test the hypothesis that mito Ca controls vitamin D 1-hydroxylase activity during CMA, cortical mito were prepared in standard fashion from C and A rat kidneys and incubated in a cytosol-like(cyto)medium(125 mM KCL;HEPES; succinate). 1,25D syn was measured from conversion of 25(OH)D₃ substrate using an HPLC separation system which removed 19-nor, 10-oxo, 25D from true 1,25(OH)₂D₃ and receptor protein from calf thymus cytosol. A time course of mito 1,25D syn was established. When cyto Ca was 1-5 μ M, C mito > A mito in 1,25D syn(48 \pm 5v12 \pm 3 fmol/mg prot, $p < .05$). However, in cyto with 0 mM Ca + 2mM EDTA, A mito > C mito 1,25D syn at either pH 7.2 or 7.4: (7.2:A, 62 \pm 2vC, 48 \pm 3 fmol/mg prot, $p < 0.05$; 7.4:A, 120 \pm 10vC, 52 \pm 4, $p < 0.05$). Buffering of cyto Ca by mito(M buff) measured as reduction in cyto Ca(1-10 μ M) was reduced in AvC mito alone, but similar if A mito were first exposed to EDTA. Induced mito Ca efflux by spermine(40 mM) and sodium(18 mM) was > in AvC mito. Thus, reduced 1,25D syn in CMA is associated with reduced M buff. Induced mito Ca efflux in A mito restores 1,25D syn to normal. CMA is a state of renal mito Ca overload.

EFFECTS OF PARATHYROID HORMONE (PTH) AND 8-Br-cAMP ON CYTOSOLIC FREE Ca ($[Ca^{2+}]_i$) IN ISOLATED RABBIT CONNECTING TUBULES (CNT). K. Lau and J.E. Bourdeau Michael Reese Hosp. & Univ. Chicago, Chicago, Ill.

PTH stimulates Ca reabsorption in isolated perfused rabbit CNT. The presence of PTH-sensitive adenylate cyclase and reproduction of PTH action by cAMP analogues in CNT suggest that cAMP is the mediator. Although a cellular pathway for lumen-to-bath Ca flux is implied from energetic considerations, direct evidence is lacking. To study cellular mechanisms, individual CNT were dissected and incubated with fura-2-AM at 21° C and suffused with a physiologic solution. $[Ca^{2+}]_i$ of fura-2-loaded tubules was estimated by epifluorescence microscopy with dual wavelength excitation. When temperature was increased to and maintained at 37° C, mean $[Ca^{2+}]_i$ decreased from 407 to 197 nM over 85 min (n=6). In response to b(1-34)PTH, 0.1 nM, and acetic acid (AA), 10 μ M, the PTH vehicle, $[Ca^{2+}]_i$ changed as follows (n=6/group, p<0.05§ by ANOVA):

	Basal	Experimental (2-min periods)					Recovery	
		(1st)	(2nd)	(3rd)	(4th)	(5th)	(1st)	(2nd)
AA	267	260	261	257	255	251	247	245
PTH	278	279	311§	324§	331§	329§	329§	309

The PTH effect peaked 7.2 min following exposure ($\Delta=68$ nM) and persisted 2 min after PTH removal. Acetic acid produced no changes. 8-Br-cAMP, 1 mM, mimicked the effects of PTH, raising $[Ca^{2+}]_i$ within the first 2 min ($\Delta=31$ nM), producing a maximum ($\Delta=84$ nM) in 6 min, and sustaining an effect during recovery. We conclude: (1) at a concentration in the pathophysiological range, PTH increases $[Ca^{2+}]_i$ in rabbit CNT; (2) 8-Br-cAMP mimics this action, implicating a messenger role for cAMP; and (3) these data may be interpreted as reflecting increased transcellular Ca flux.

HYPERCALCIURIC KIDNEY STONE FORMERS EXHIBIT ENHANCED INTESTINAL CALCIUM ABSORPTION (Ca_a) DESPITE ONLY SLIGHTLY ELEVATED SERUM 1,25-(OH)₂-D CONCENTRATIONS. J. Lemann, Jr. and R.W. Gray.* Depts. of Med. and Biochem. and Clin. Res. Ctr. Medical College of Wisconsin. Milwaukee, WI.

We reexamined the relationships between the components of Ca balance and serum 1,25-(OH)₂-D concentrations among 14 hypercalciuric Ca oxalate stone formers: HS; U_{Ca} 9.5 \pm 1.8 mmol/day and 22 healthy subjects: N; U_{Ca} 4.0 \pm 1.7 mmol/day; p < 0.001, during balance studies while both groups ate diets providing normal amounts of Ca averaging 21.5 \pm 2.7 mmol Ca/day. Among N, serum 1,25-(OH)₂-D ranged from 51 to 154 pM and averaged 86 \pm 22 pM. Ca_a among N averaged 4.4 \pm 2.8 mmol/day and was correlated to serum 1,25-(OH)₂-D: Ca_a mmol/day = -0.7 + 0.06 x serum 1,25-(OH)₂-D, pM; r = 0.46; p < 0.05. Among HS, serum 1,25-(OH)₂-D ranged from 58 to 182 pM and averaged 105 \pm 33 pM; p < 0.05. Ca_a among HS averaged 9.4 \pm 2.8 mmol/day; p < 0.001 but was unrelated to 1,25-(OH)₂-D; r = 0.29. Ca balances were not different averaging +0.4 \pm 2.3 mmol/day in N and -0.1 \pm 2.1 mmol/day in HS. Serum PO₄ among N averaged 1.28 \pm 0.14 mM and was lower among HS averaging 1.14 \pm 0.13 mM; p < 0.005. Serum total Ca and iPTH levels did not differ between N and HS. As first proposed by Pak and associates, Ca_a among HS appears to be increased relative to prevailing serum 1,25-(OH)₂-D levels. Enhanced Ca_a could occur via a 1,25-(OH)₂-D independent process. Alternatively, since 1,25-(OH)₂-D is not suppressed among HS and 1,25-(OH)₂-D has been shown to enhance expression of its own intestinal receptor, such a phenomenon might also be involved.

VERAPAMIL (V) IN-VITRO INHIBITS ACTIVE Ca ABSORPTION BY RAT INTESTINE. J. Levi, Y. Hirsh* and U. Gafter*, Nephrology, Hasharon Hosp., Petah-Tikva; Tel-Aviv Univ. Med. Sch, Israel,

The effect of Ca channel blockers on intestinal absorption of Ca is controversial. We studied the effect of verapamil using metabolic balance studies and everted gut sacs from duodenum, jejunum, ileum and colon. Pairs of everted sacs were prepared from each section of gut. One was incubated with 1.5mM V (+V) and one without (-V) and ⁴⁵Ca uptake was measured. For the metabolic studies, rats were divided into 4 groups: Group 1 received V in the drinking water (20 mg/kgB.wt./day). Group 2 received subcutaneous injections of V (10 μ g/100gB.wt. twice daily). Groups 3 and 4 served as controls. A balance study was performed for 10 days and the rats sacrificed. Intestines were removed and gut sac ⁴⁵Ca uptake by the 4 segments was measured (CMP/mg weight/0.5h). In-vitro, verapamil could decrease Ca uptake by duodenum (p<0.05) and colon (p<0.01) but not jejunum or ileum.

	Duodenum	Jejunum	Ileum	Colon
V-:	1025±142	485±38	670±54	648±66
V+:	654±54	423±36	596±42	390±27

Administration of V orally or parenterally had no effect on ⁴⁵Ca uptake by any of the gut segments or on the intestinal Ca absorption and Ca balance.

In summary: While in-vivo no effect on intestinal Ca absorption, could be demonstrated, in-vitro, verapamil inhibited active duodenal and colonic Ca absorption.

THE PROTECTIVE EFFECT OF HIGH LEVELS OF PARATHYROID HORMONE (PTH) ON ALUMINUM (AL) INDUCED OSTEOMALACIA (OM). F. Llach, M. Rodriguez, and A. Felsenfeld. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, Okla.

The administration of AL to rats with renal failure produces OM. In dialysis patients, PTH appears to protect against AL-induced OM. To evaluate the effect of PTH in AL toxicity, 3 groups of pair-fed rats with surgically-induced renal failure were studied: I, controls; II, 20 mg of AL administered intraperitoneally during a 2 day period; and III, the same as II except 1-34 PTH was administered at 2 units/hr via subcutaneous Alzet pump. The PTH infusion was begun 4 days before AL administration and continued until sacrifice. Rats were sacrificed 5 and 12 days after AL. PTH did not correct the AL-induced decrease in bone formation rate (BFR). The serum calcium (Ca, mg/dl), osteoblastic osteoid (OB), total osteoid surface (TOS), relative osteoid volume (ROV), and osteoclastic resorption (OR) were:

	5 Days			12 Days		
	I	II	III	I	II	III
Ca	10.2±1	10.5±1	12.9±4	10.6±1	10.7±2	12±3*
OB, %	23±2	2±1*	34±5	15±2	12±5	22±6
TOS, %	27±2	7±1	45±7*	23±3	33±9	54±7*
ROV, %	4±1	2±1	20±4*	3±1	10±2*	31±6*
OR, %	10±1	12±2	9±2	10±1	2±1	7±2

x±SE; *p<.05 vs I.

In summary, 1) PTH elevates serum Ca; 2) AL decreases OB at 5 days and OR at 12 days; 3) PTH prevents the AL-induced decrease in OB and OR; 4) PTH+AL result in an increase in TOS and ROV; and 5) PTH does not prevent the AL-induced decrease in BFR. In conclusion, PTH prevents the toxic effect of AL on osteoblasts but does not correct the BFR.

THE EFFECT OF PHOSPHONOFORMIC ACID (PFA) ON THE Na^+ -DEPENDENT UPTAKE OF PHOSPHATE (P_i) IN OPPOSSUM KIDNEY (OK) CELLS GROWN IN VITRO. Mahmoud Loghman-Adham, Hassan Molajji*, and Thomas P. Dousa. Neph. Res. Unit, Mayo Clinic, Rochester, Minnesota.

In our previous studies, we discovered that PFA (foscarnet) acts as a specific reversible inhibitor of Na^+ -gradient [$\text{Na}^+_o > \text{Na}^+_i$]-dependent P_i transport across renal and intestinal brush border membrane (BBM). Further, PFA exhibited a phosphaturic effect *in vivo*. We now explored whether PFA acts upon Na^+ -dependent uptake of P_i in OK cells, a cell line having many properties analogous to proximal tubules. In the OK cells, uptake of $^{32}\text{P}_i$ was almost completely dependent on the presence of Na^+ in the medium. In the concentration range 0.1 mM to 5 mM, PFA elicited a dose-dependent inhibition of Na^+ -dependent P_i uptake. At 1 mM PFA, uptake of P_i (pmoles/mg protein/15 min) was inhibited by 20%; at 5 mM PFA, the inhibition was -50%. PFA had no effect on uptake of ^3H -alanine either in the presence or absence of sodium. Kinetically, inhibition by PFA was strictly competitive ($K_i \approx 2$ mM). Phosphonoacetic acid was less effective (at 5 mM the inhibition was about -25%) than PFA. Other monophosphonates, phenylphosphonic acid and phosphonopropionic acid, had no appreciable inhibitory effect. **Conclusion:** PFA is a specific competitive inhibitor of Na^+/P_i cotransport in OK cell line *in vitro*, similar to inhibition of Na^+/P_i cotransport in BBM. These results suggest that Na^+/P_i cotransporter in OK cells is homologous to Na^+/P_i cotransporter in BBM of renal proximal tubules.

GTP-BINDING PROTEINS OF PARATHYROID CELLS. J. Morrissey and C. Hayes*. Renal Div., Washington University Medical School, St. Louis, MO

A pertussis toxin substrate is an important link in the normal suppression of parathyroid hormone (PTH) secretion by calcium. Pertussis toxin substrates belong to a family of GTP-binding proteins which transduce signals from the cell surface to second messenger generating enzymes of the plasma membrane. In this study we characterize the major GTP-binding proteins of the bovine parathyroid cell. Cells were prepared by collagenase-DNase dispersion, PTH measured by RIA, GTP-binding proteins by [^{35}S]GTP γS binding and pertussis substrates by [^{32}P]ADP-ribosylation. Membrane proteins were ribosylated in the presence of toxin and separated on SDS-gels. Radioautography revealed a major substrate at 41 kDa and a minor substrate at 39 kDa. Pretreatment of the cells with pertussis toxin to derivitize 50% of the substrate *in situ* shifted the setpoint for calcium to inhibit PTH secretion from 1.0 to 1.2 mM. If 90% of the toxin substrate was derivitized *in situ*, suppression of secretion is nearly abolished. The binding of [^{35}S]GTP γS to Western Blot replicas of membrane proteins separated by SDS-gels revealed several GTP-binding proteins. In addition to the pertussis substrates there is a prominent binding protein at 21 kDa. The role of the 21 kDa GTP-binding protein in PTH secretion is not known, however, the decrease in biologically active pertussis substrates by toxin pretreatment mimics PTH secretion seen with hyperplastic human parathyroid cells.

1,25(OH) $_2$ D $_3$ PREVENTS PARATHYROID CELL PROLIFERATION IN UREMIC RATS

J. Merke, A. Szabo, E. Beier, E. Ritz (intr. by B. Brenner). Dept. Int. Med., Heidelberg, Germany

Parathyroid glands have recently been recognized as an important target for 1,25(OH) $_2$ D $_3$. This D metabolite is known to affect mRNA for per-pro PTH and the set point for PTH secretion, but its effects on parathyroid growth have been less well defined. In renal failure, parathyroid (PT) hyperplasia occurs regularly, but the extent to which this is responsive to 1,25(OH) $_2$ D $_3$ has not been examined. We studied cohorts of 9 male SD rats 21 days after subtotal nephrectomy (NX) or sham op. (CO). PT were removed by microsurgery, pooled and incubated (3h; 37°C; PBS with 10 mmol glucose; pH 7.4) in the presence of ^3H thymidine (730 nM; 5 uCi). PT were washed, sonicated, treated with 5% TCA and hydrolyzed (1N NaOH for 1h). DNA after Burton. NX animals had elevated S-Crea (1.48±0.37 vs 0.57±0.03 mg/dl in CO) and diminished 1,25(OH) $_2$ D $_3$ (81.2±8.9 vs 134±6.2 pg/ml in CO). PT protein (26.3 vs 13.6 ug/gland in CO) and DNA content (4.9 vs 3 ug/gland in CO) were increased in NX animals. ^3H dTid incorporation was increased in NX (4001 cpm per gland and 816 cpm/ug DNA in NX vs 1282 cpm/gland and 427 cpm/ug DNA in CO). Pretreatment with 1,25(OH) $_2$ D $_3$ (50 pmol i.p. 24h prior to surgery) markedly suppressed ^3H dTid incorporation in CO (-50%) and NX (-87%). The finding of adequate suppression of PT cell proliferation to 1,25(OH) $_2$ D $_3$ in uremia is of interest in view of reduced 1,25(OH) $_2$ D $_3$ PT receptors (Korkor, NEJM 316, 1573, 1987). We conclude that 1,25(OH) $_2$ D $_3$ not only diminishes PTH secretion (set point) but also PT hyperplasia (basal secretion) in uremia.

ANTAGONIST TO GROWTH HORMONE RELEASING FACTOR INHIBITS GROWTH AND RENAL PHOSPHATE REABSORPTION IN IMMATURE RATS. S.E. Mulronev*, M.D. Lumpkin* and A. Haramati, Department of Physiology, Georgetown University School of Medicine, Washington, DC.

We recently reported that the tubular capacity for phosphate (P_i) reabsorption (MAX RPi/GFR) is relatively greater in immature rats compared to adults, perhaps as a consequence of the increased demand during growth. However, the factors that contribute to this increase have not been clearly defined. In this study we evaluated the possible role of growth hormone (GH) in P_i homeostasis by using an antagonist to GH releasing factor, [N-Ac-Tyr 1 ,D-Arg 2]-GRF (anti-GRF), that has been shown to inhibit GH secretion. Chronic jugular catheters were placed in 100-140g Wistar rats (4-5 wks old), and anti-GRF was injected i.v. (100ug/kg) twice daily for 4-7 days. Control and anti-GRF rats were then anesthetized and prepared for renal clearance studies to determine the MAX RPi/GFR. During the injection period growth was dramatically inhibited in the anti-GRF animals. Body weight in control animals increased 31±3% while the increase in anti-GRF rats was only 6±2% ($p<.01$). Urinary excretion of P_i increased from 0.86±0.06 to an average of 1.37±0.11 mmol/day ($p<.01$). This was associated with a significant decrease in MAX RPi/GFR in the anti-GRF rats (3.2±0.2 umol/ml vs 4.4±0.4 umol/ml in control rats, $p<.01$). This decrease was not due to a change in basal plasma P_i concentration. We conclude that anti-GRF inhibits growth and concomitantly decreases the tubular reabsorption of P_i to the level seen in an adult rat. These results support the notion that GH contributes to the enhanced tubular reabsorption of P_i in the young, and plays a major role in the interaction between growth and renal phosphate reabsorption.

PARATHYROID HORMONE (PTH) RESPONSE TO CHANGES IN SERUM CALCIUM IN PATIENTS WITH ALUMINUM (AL) BONE DISEASE BEFORE AND AFTER DEFERRIOXAMINE (DFO) THERAPY. J. Myers*, M. Rodriguez*, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, OK.

In dialysis patients (pts), AL associated osteomalacia (OM) is associated with decreased basal and stimulated PTH levels. DFO is an effective therapy for AL associated OM. In the present study, 16 pts with AL associated OM or aplastic bone disease were studied with both a calcium (Ca) free and high Ca (4 mEq/L) dialysis before and 1 year after treatment with DFO. The level of serum Ca which maximally suppressed PTH pre and post DFO was similar, but the maximally suppressed PTH value was greater post DFO, 68 ± 21 (x \pm SE) vs. 94 ± 27 pg/ml, $p < .02$. The increase in maximally suppressed PTH value after DFO correlated with the decrease in stainable bone AL ($p < .04$). The maximally stimulated PTH in response to hypocalcemia, pre and post DFO, was similar, 458 ± 99 vs. 450 ± 86 pg/ml; however, the serum Ca level at which maximal stimulation of PTH occurred was different, $7.7 \pm .09$ vs. $8.0 \pm .15$ mg/dl, $p < .02$. Also, the Δ serum Ca (between maximal suppression and stimulation of PTH) was less after DFO, $2.3 \pm .18$ vs. $1.8 \pm .18$ mg/dl, $p < .04$.

In summary, in these 16 pts, treatment with DFO resulted in: 1) a greater maximal suppression of PTH for a similar Ca level; 2) a shift in Ca at which maximal stimulation of PTH occurs; and 3) a reduction in the serum Ca between maximal suppression and stimulation of PTH. In conclusion, although treatment with DFO did not change the absolute maximal PTH, alterations in PTH suppression and stimulation were observed.

PLASMA MAGNESIUM (Mg) CONTROLS RENAL Mg CONSERVATION DURING SHORT-TERM Mg DEPRIVATION. D.B. Ornt and J.D. Scandling. Dept. of Medicine, Univ. of Rochester, Rochester, NY.

Microperfusion study of short-term Mg-deficient rats suggests that Mg excretion is in part controlled by plasma Mg.

To test this hypothesis, *in vitro* isolated perfused kidney (IPK) studies were performed in rats after 4 days of either Mg-free or control (.04 mmol/g Mg) diet at 3 levels of perfusate Mg (P_{Mg}): low (0.8 mEq/L), normal (1.3 mEq/L) and high (2.5 mEq/L). *In vivo* study of rats on Mg-free diet showed maximal urinary Mg conservation by 4 days, $.05 \pm .01$ vs. $.60 \pm .03$ mEq/day in rats on control diet, $p < .001$. Plasma Mg was not measured at 4 days, at 10 days it was $0.85 \pm .04$ vs. $1.33 \pm .05$ mEq/L, $p < .001$. In the IPKs, mean absolute Mg excretion (U_{MgV}) in the Mg-free groups increased with each level of P_{Mg} (.03 + .01 vs. .10 + .01 vs. .30 + .05 ueq/min/g); and was similar to control (.04 + .01 vs. .11 + .03 vs. .24 + .03 ueq/min/g). The changes in fractional Mg excretion mirrored U_{MgV} . U_{MgV} was correlated to P_{Mg} at each P_{Mg} level in both the Mg-free ($r = .86$) and control ($r = .89$) groups. The correlation between P_{Mg} and U_{MgV} in the Mg-free groups was not different from that in the control groups. Other functional parameters of the IPKs, GFR, urine flow rate, sodium and potassium excretion, were similar between the Mg-free and control groups at each level of P_{Mg} .

Thus there was no *in vitro* evidence of renal adaptation for Mg conservation. Urinary Mg conservation is likely due to lowered plasma Mg during short-term Mg deprivation.

URINE CALCIUM RESPONSE TO CALCITRIOL TREATMENT IN WOMEN WITH OSTEOPOROSIS. SM Ott, CH Chesnut*, U of Washington, Seattle

A 2 yr randomized, double-blind clinical trial of calcitriol (1,25D) was conducted in 85 women with osteoporosis. We measured 24-hour urine calcium (Uca), phosphate, hydroxyproline, and creatinine (cr); and bone mass. Age was 67 ± 7 yrs; baseline GFR 65 ± 20 ml/min. For 1 month dose of 1,25D or placebo was gradually increased to .75 mcg/day. Thereafter dose and Ca⁺⁺ intake were adjusted depending on Uca. At baseline Uca correlated with GFR, $r = .43$, $p < .001$, but not with dietary Ca⁺⁺ or serum Ca⁺⁺. At 1 mo Uca/100ml GFR had not changed in placebo group (.15 \pm .08 to .16 \pm .08) but increased in 1,25D group (.16 \pm .07 to .34 \pm .10, $p < .001$). Fasting Uca/cr correlated to 24hr Uca/GFR initially ($r = .67$, $p < .001$, SEE 39%), but not after 1 mo of 1,25D ($r = .04$). Urine PO₄, TRP, hydroxyproline and bone mass did not change. Over 2 yrs, GFR declined similarly in both groups. Ultrasound studies showed no nephrocalcinosis. We conclude that 1,25D treatment in age-related mild renal failure does not necessarily lead to decline in GFR. Urine Ca⁺⁺ increases with 1,25D without evidence of increased bone resorption, suggesting increased absorption of dietary Ca⁺⁺. Fasting Uca/cr correlates with 24hr Uca at baseline, but with 1,25D there is no relationship. Urine collections must be done for 24hr to assess the urine Ca⁺⁺.

PTH LEVELS ARE SUPPRESSED AND BONE ALUMINUM LEVELS ELEVATED IN HYPERCALCEMIC DIALYSIS PATIENTS. Beth Piraino, Tai Chen, Jules Puschett. Univ. of Pittsburgh, Pittsburgh, PA.

Hypercalcemic (\uparrow Ca) dialysis patients (pts.) can have either osteitis fibrosis (OF) or low turnover osteodystrophy (LTO). We measured N terminal PTH, ionized calcium (Ca²⁺) and stainable bone aluminum (B_{A1}, as % of trabecular surface) in \uparrow Ca and non-hypercalcemic (nCa) dialysis pts., with bone biopsy proven OF and LTO. None of the \uparrow Ca pts. were on oral calcium or vitamin D. B_{A1} was higher ($46 \pm 33\%$ vs 9 ± 23 , $p < .001$) and PTH levels lower (89 ± 84 pg/ml vs 211 ± 155 , $p = 0.001$) in \uparrow Ca pts. compared to nCa pts. LTO was present in 62% (13/21) of the \uparrow Ca pts vs 29% (8/28) of the nCa pts. ($p < .05$). Results in 4 groups of pts. were (means \pm SD):

	Ca ²⁺ (mg/dl)	PTH (pg/ml)	B _{A1} (%)
\uparrow Ca - OF	$5.3 \pm 0.3^*$	$149 \pm 81^{**}$	18 ± 23
nCa - OF	$4.5 \pm 0.5^*$	$278 \pm 135^{**}$	$7 \pm 22^{**}$
\uparrow Ca - LTO	$5.5 \pm 0.4^*$	52 ± 65	$63 \pm 24^{**}$
nCa - LTO	$4.5 \pm 0.4^{**}$	70 ± 68	$15 \pm 24^{**}$
Normal	$4.9 \pm 0.1^{**}$	16 ± 5	0^{**}

* $p < .001$ vs nCa groups; ** $p < .05$ vs all other groups; *** $p < .05$ vs \uparrow Ca groups.

1,25(OH)₂D₃ levels were decreased in the \uparrow Ca - LTO group (14 ± 8 pg/ml vs all others 23 ± 10 , $p < .01$). There were weak correlations between Ca and PTH ($r = -.33$, $p < .05$) and between Ca and B_{A1} ($r = 0.39$, $p < .01$). These results support the following hypotheses: (1) \uparrow Ca is associated with suppression of PTH in dialysis pts. with OF, (2) \uparrow Ca in LTO is related to high levels of B_{A1}, (3) relatively low levels of PTH appear to play a role in the pathogenesis of LTO.

REGULATION OF THE SERUM LEVELS OF PHOSPHORUS AND 1,25-(OH)₂D IN NORMAL MAN BY NORMAL DIETARY INTAKES OF PHOSPHORUS. A.A. Portale,

B.P. Halloran*, S.T. Harris*, and R.C. Morris, Jr., GCRC, Univ. of California at San Francisco.

We have reported that in normal men, severe restriction (oral Al(OH)₃) and large supplementation (>3 g/d) of dietary phosphorus (P) induces large increases and decreases, respectively, in the production rate and serum level of 1,25-(OH)₂D, and these changes vary inversely with changes in the 24-hr mean serum level of P. Dietary P within a normal range might then also regulate serum levels of both P and 1,25-(OH)₂D. In 7 healthy men in whom dietary P was first at the upper (2.3 g) and then lower (0.65 g) limit of normal, each for 9 d, we measured fasting serum levels of 1,25-(OH)₂D and levels of P in blood drawn hourly for 24 hrs. With both intakes of P, serum levels of P exhibited the normal circadian rhythm, with both 12- and 24-hr periodicities. When P was restricted, fasting serum levels of P increased, 0.3±0.1 mg/dl, p<.02, but P levels after breakfast decreased; at 1-4 pm, by 1.4±0.2 mg/dl, p<.001. Serum levels of 1,25-(OH)₂D increased by 50%, p<.005, and varied inversely with 24-hr mean serum levels of P, r=-0.78, p<.001, but not with fasting levels of P. Over an extended range of P intake from essentially zero to >3 g/d, serum levels of 1,25-(OH)₂D ranged from 20 to 90 pg/ml, and varied inversely with 24-hour mean levels of P, r=-0.88, p<.001. These data provide evidence that in healthy men, dietary P within the normal range, and under normal physiological conditions, is a major determinant of the serum level of both P and 1,25-(OH)₂D within their normal ranges.

1,25 DIHYDROXYVITAMIN D3 (1,25) CAN POTENTIATE OR INHIBIT INTERLEUKIN 2 (IL2) mRNA ACCUMULATION IN ACTIVATED T CELLS. John L. Prehn*, Rebecca S. Sakai* and Stanley C. Jordan. Div. of Pediatric Nephrology, Dept. of Pediatrics, Cedars-Sinai/UCLA Med. Ctr., Los Angeles, California.

1,25 has profound effects on specific gene expression in activated human peripheral blood mononuclear cells (PBM). These include suppressing accumulation of mRNAs for interferon gamma (IFN), GM-CSF, and as we and others have recently shown, IL2. However, 1,25 markedly enhances induction of class II MHC gene expression in mouse monocytes by IFN, and stimulates expression of its own specific cytosolic receptor in many cells. We have estimated production of IL2 mRNA in human T cell lines (JURKAT and HuT, pretreated with 0.5 nM 1,25 for 48 h to induce 1,25 receptors) by RNA blotting and probing with cDNA. IL2 mRNA is maximal after 6 h of stimulation (PHA, 1mcg/ml+PMA, 50ng/ml). 1,25 added concurrently decreases IL2 mRNA about 50%. The same result was obtained in PBM. However, if cells were incubated with 1,25 (10nM) for 14-72 h prior to stimulation, IL2 mRNA was increased several-fold, even at 3 h. Proportional potentiation occurred in the presence of cyclosporin A (10ng/ml) or dexamethasone (1nM), concentrations which otherwise would reduce IL2 mRNA by >50%. Prolonged exposure to relatively high levels of 1,25 may prime T cells to secrete a large amount of IL2 quite rapidly when activated. In sum, 1,25 regulation of IL2 gene expression in T cells varies greatly with experimental conditions tested.

FUNCTIONAL ALTERATIONS OF DISTINCTIVE SODIUM-DEPENDENT AND pH-DEPENDENT PHOSPHATE TRANSPORT IN RENAL BRUSH-BORDER MEMBRANES. Gary A. Quamme, Dept. of Medicine, University of British Columbia, Health Sciences Centre Hospital, Vancouver, B.C.

Two distinctive sodium-dependent phosphate (Pi) transport systems have been identified in early and late proximal tubules; a high capacity process (G₁) located only in outer cortical tissue, and a high affinity (G₂) in both cortical and outer medullary brush-border membranes (BBM). A third, sodium-independent, pH gradient-stimulated exchange system (V_{max} 4.9±0.6 nmol.mg⁻¹ prot. min⁻¹, Km 0.14±0.01 mM) is present in the outer medulla, but absent in cortex. BBM vesicles were prepared from outer cortical and outer medullary tissue of pigs maintained on low (0.01%) or high (2%) Pi diets. Sodium-dependent Pi uptake of G₁ increased (V_{max} 3.15 to 13.1 nmol.mg⁻¹ prot.min⁻¹) from high to low Pi diet, whereas the changes in G₂ were small (V_{max} 0.8 to 1.5 nmol.mg⁻¹ prot.min⁻¹). There were no changes in Km values of G₁ and G₂, 4.1±0.02 and 0.2±0.07 mM, respectively. The pH-dependent process also increased (V_{max} 3.1 to 7.4 nmol.mg⁻¹ prot. min⁻¹) with no change in mean Km value, 0.15±0.001 mM. Administration of 1 U PTH resulted in a decrease in G₁ and G₂ (V_{max} 2.8 and 0.55) and in pH-dependent uptake (2.9 nmol.mg⁻¹ prot.min⁻¹) with no change in the respective Km values. In conclusion, dietary Pi intake and PTH appropriately alters Pi uptake by all three systems, sodium-dependent (G₁ and G₂) and pH-dependent, although the sensitivity of each system to these influences is unknown.

ALUMINUM AND PARATHYROID HORMONE (PTH) ACTIVITY IN PARATHYROIDECTOMIZED RATS WITH TRANSPLANTED PARATHYROID GLANDS. M. Rodriguez, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, Okla.

Aluminum (AL) toxicity is associated with decreased PTH secretion both in dialysis patients with AL associated osteomalacia and in parathyroid cell cultures. To evaluate the in vivo effect of AL on PTH secretion, parathyroid glands were transplanted (tx) from donors in 2 groups of rats 6 days after parathyroidectomy. Group I was control and II received AL 5 mg intraperitoneally x 4 days after tx. Both groups were maintained on a .05% calcium diet during the study and before tx, the mean serum calcium (Ca) was 5 mg/dl. The serum Ca, phosphorus (P) and AL, the fractional excretion of P (FeP) and the urinary cyclic AMP (cAMP) obtained 6 (I₆) and 9 (I₉) days after tx were:

	Ca (mg/dl)	P (mg/dl)	FeP (%)	cAMP (pmol/min)	AL (g/L)
I ₆	7.3±1.1	10.1±1.8	39±15	.23±.03	22±5
II ₆	7.9±1.6	10.1±2.0	21±14*	.09±.07*	296±54*
I ₉	8.8±1.6	10.1±1.2	37±20	.24±.06	16±4
II ₉	9.3±1.1	8.6±1.9	45±20	.11±.05*	454±78*

x±SD; *p 0.05 I₆ vs II₆; +p 0.05 I₉ vs II₉

In Group II, the mean serum Ca is similar to that of control at both 6 and 9 days after Tx; and cAMP, a marker of PTH activity, is decreased. Thus, in AL loaded rats: a) lower PTH activity may be present, and b) less PTH may be required to increase Ca levels. These findings suggest that AL may inhibit PTH secretion and also directly effect the plasma-bone Ca equilibrium.

IN VITRO ATHEROGENIC ROLE OF 1,25 DIHYDROXY VITAMIN D₃. J-B Rouillet*, M. Haluska*, D. McCarron, Div. of Nephrology, OHSU, Portland, OR.

An *in vivo* atherogenic role of dietary vitamin D has been reported. We sought to determine the *in vitro* effects of its active metabolite, 1,25-(OH)₂D₃, on lipid metabolism in human monocyte-derived macrophages (HMM). After 6 days of culture in RPMI 20% serum with or without 10⁻⁸M 1,25 (OH)₂D₃. Lipoprotein lipase activity in medium (LPL_M, mU/10⁶ cells) and cellular triglycerides (TG_C μg/10⁶ cells) were measured. Cells were switched to a medium containing 10% lipid deficient serum, 0.1mM [³H] oleate and either buffer (B) or 50μg/ml acetylated low density lipoprotein (AcLDL). Esterification rates (ER) [nmol [³H] oleate/10⁶ cells/24 hrs] and cellular cholesteryl ester (CE_C, μg/10⁶ cells) were measured after 24 hours. The results (m±SEM) were:

	Control(-)	1,25(OH) ₂ D ₃ (+)	p	
LPL _M (n=8)	9.4±0.3	11.1±0.3	<0.05	
TG _C (n=8)	455.0±36.7	700.4±31.0	<0.001	
ER (n=6)	CE _B	0.32±0.03	0.32±0.03	NS
	TG _B	45.4±2.9	97.6±9.2	<0.001
	CE _{AcLDL}	0.90±0.03	2.36±0.18	<0.001
	TG _{AcLDL}	56.3±3.3	85.1±5.8	<0.001
CE _{AcLDL} (n=6)	0.47±0.18	2.06±0.16	<0.001	

A time course study performed on day 0,2,4,6 showed an early (day 2) increase in TG_B esterification: (-) 3.9±0.5, (+) 42.8±8.2 (p<0.001, n=6). These results show that 1,25(OH)₂D₃ induces lipid accumulation in HMM and suggest a potential role of the hormone on *in vivo* atherogenesis.

24,25(OH)₂D₃ BLUNTS THE HYPERCALCEMIC EFFECT OF 1,25(OH)₂D₃ IN CHRONIC RENAL INSUFFICIENCY: EVIDENCE FOR THE ROLE OF SECONDARY HYPERPARATHYROIDISM. D.I. Rubinger*, Y. Krause*, and M.M. Popovtzer. Hadassah University Hospital, Jerusalem, Israel.

To examine the interaction between 24,25(OH)₂D₃ and 1,25(OH)₂D₃ in chronic renal insufficiency, 3 subsets of 5/6 nephrectomized (NPX) rats were studied: a) rats with intact parathyroids (P-NPX), b) parathyroidectomized rats (PTX-NPX), c) rats after incomplete parathyroidectomy (I-PTX-NPX). Each subset was divided into 4 groups: 1) control rats, 2) rats treated with 1,25(OH)₂D₃, 3) rats treated with 24,25(OH)₂D₃, and 4) rats treated with both 1,25 and 24,25(OH)₂D₃. After 8 days of treatment there were no changes in plasma calcium (P_{Ca}) in the control or in the animals treated with 24,25(OH)₂D₃ in the 3 subsets, while hypercalcemia was seen in all rats treated with 1,25(OH)₂D₃. After combined administration of 1,25 and 24,25(OH)₂D₃, the calcemic effect of 1,25(OH)₂D₃ was blunted in P-NPX and in I-PTX-NPX and was augmented in PTX-NPX as shown below.

Subset	P _{Ca} (mEq/L)		
	1,25(OH) ₂ D ₃	1,25(OH) ₂ D ₃ + 24,25(OH) ₂ D ₃	D
P-NPX	6.86±0.22	6.19±0.18	<0.025
PTX-NPX	7.04±0.2	7.62±0.24	<0.05
I-PTX-NPX	7.67±0.09	7.29±0.16	<0.05

These results show, 1) 24,25(OH)₂D₃ blunts the hypercalcemic effect of 1,25(OH)₂D₃ in rats with chronic renal insufficiency only in the presence of secondary hyperparathyroidism, and 2) after PTX 24,25(OH)₂D₃ augments the hypercalcemic effect of 1,25(OH)₂D₃ as shown before in rats with intact renal mass.

24,25(OH)₂D₃ BLUNTS THE HYPERCALCEMIC EFFECT OF 1,25(OH)₂D₃ BY A MECHANISM INDEPENDENT OF INTESTINAL CALCIUM (CA) ABSORPTION. D. Rubinger*, I. Krause*, and M.M. Popovtzer. Hadassah University Hospital, Jerusalem, Israel.

We have recently shown that 24,25(OH)₂D₃ blunts the hypercalcemic effect of 1,25(OH)₂D₃ in rats with reduced renal mass. To examine whether this effect is due to interference of 24,25(OH)₂D₃ with 1,25(OH)₂D₃-induced enhancement of intestinal CA absorption, the following groups of 5/6 nephrectomized rats (NPX) were examined: 1) control rats, 2) rats treated with 1,25(OH)₂D₃, 54 ng/day, 3) rats treated with 24,25(OH)₂D₃, 54 ng/day, and 4) rats treated with both 1,25 and 24,25(OH)₂D₃, 54 ng/day each. The 2 h fractional intestinal absorption of Ca⁴⁵, expressed as "area under the curve" (F_{Ca}⁴⁵) and plasma calcium (P_{Ca}) were determined after 8 days of vit. D administration, as shown below:

Group	F _{Ca} ⁴⁵	P _{Ca} (mEq/L)
Control	1086±283	5.60±0.14
1,25(OH) ₂ D ₃	2847±364 ^a	6.86±0.22 ^a
24,25(OH) ₂ D ₃	1336±272	5.51±0.09
1,25+24,25(OH) ₂ D ₃	2800±272 ^a	6.19±0.18 ^{a,b}

a = p<0.001 vs. control and vs. 24,25(OH)₂D₃
b = p<0.025 vs. 1,25(OH)₂D₃

These results show, 1) 1,25(OH)₂D₃ increases P_{Ca} and F_{Ca}⁴⁵, 2) 24,25(OH)₂D₃ alone does not alter P_{Ca} or F_{Ca}⁴⁵, 3) attenuation of hypercalcemia of 1,25(OH)₂D₃ after combined 1,25(OH)₂D₃ and 24,25(OH)₂D₃ administration is not associated with further alterations in F_{Ca}⁴⁵. Therefore, in rats with reduced renal mass, 24,25(OH)₂D₃ blunts the hypercalcemic effect of 1,25(OH)₂D₃ by a mechanism independent of intestinal calcium absorption.

Ca ABSORPTION IN TURTLE BLADDER IS MEDIATED BY A Ca-ATPase IN MITOCHONDRIAL RICH CELLS. S. Sabatini and N.A. Kurtzman, Texas Tech Univ. Health Sciences Center, Lubbock, TX.

The turtle bladder is a membrane which actively transports sodium and secretes protons. We recently demonstrated that it is also capable of bidirectional calcium transport. We postulated that the absorptive calcium flux is mediated by a Ca-ATPase on the serosal membrane. In the present study we measured ATPase activity in the dispersed heterogeneous cell population, the MR cell, and the granular cell (Ficoll gradient centrifugation). The mitochondrial rich cell (MR) of turtle bladder is thought to be the proton secretory cell; the granular cell (G) is believed to be the sodium transporting cell. In the whole cell population Na-K ATPase activity was 1524±239 pmols/μg protein/hr, N=13; H-ATPase activity was 873±79 pmols/μg/hr, N=15; and Ca-ATPase activity was 61.3±9.8 pmols/μg/hr, N=8. As expected, H-ATPase was highest in MR cell; its activity was 7 times higher than found in G cell (2200±345 pmols/μg/hr). Na-K ATPase activity was highest in G cell (2073±204 pmols/μg/hr). This value was 3 times higher than that found in MR cell. Ca-ATPase activity was 4 fold greater in MR cells as compared to G cells (148.3±18.7 pmols/μg/hr). These results demonstrate that Ca-ATPase activity is present in turtle bladder, and furthermore, that the enzyme is preferentially located in the MR cells. These data suggest that the MR cell of turtle bladder not only modulates proton secretion, but that it also mediates calcium absorption in this membrane.

DESENSITIZATION TO PTH IN THE AGED RAT IS ASSOCIATED WITH A DECREASED NUMBER OF RECEPTORS: PARTIAL REVERSAL AFTER PARATHYROIDECTOMY (PTX). B. Sacktor, H. Hanai,* M. Goldman,* L. Cheng,* and M. Chorev.* NIA, NIH, Baltimore, MD 21224 and Merck Sharp & Dohme Res. Lab., W. Point, PA 19486

We previously reported that renal cells from aged (24 mo) vs mature (6 mo) rats exhibit decreased PTH-responsive Na-Ca exchange, PTH-induced cAMP formation and PTH-stimulated adenylylate cyclase activity and less G-protein function, as probed by cholera and pertussis toxins. The aged rat has a higher level of midmolecule iPTH. In this study we examined whether the PTH receptor in purified renal cortex basolateral membranes is altered in the aged rat. Binding to the receptor was estimated with the synthetic analog, ^{125}I -[Nle⁶,Nle¹⁰,Tyr³⁴]bPTH(1-34)amide. We found:

	6 mo		
	Control	Sham	PTX
Bmax (fmol/mg)	92.1±9.3	85.6±3.8	87.7±4.3
Kd (x10 ⁻⁹ M)	1.36±0.12	1.38±0.07	1.48±0.08
	24 mo		
	Control	Sham	PTX
Bmax (fmol/mg)	36.7±6.1	39.8±2.4	53.5±2.9*
Kd (x10 ⁻⁹ M)	1.25±0.24	1.29±0.06	1.38±0.08

*P < 0.05 compared to sham (24 mo).

- (1) The number of PTH-specific binding sites was reduced by 60% in the membranes from aged rats.
- (2) The affinity of the receptor for hormone was unchanged.
- (3) 48 hrs after PTX of the aged rat the number of PTH receptors was increased relative to the sham. These findings suggest that the age-associated blunting in the response of renal cells to PTH is due, at least in part, to the loss of PTH receptors and that this deficit can be reversed by removal of the parathyroid gland.

REGULATION OF PTH-INDEPENDENT PHOSPHATE (Pi) UPTAKE IN CULTURED RENAL CELLS BY CALCIUM (Ca). H. Sakamoto* and B. Sacktor, Lab. Biol. Chem., NIA, NIH, Baltimore, MD. 21224

Previous studies, *in vivo* and with relatively intact preparations, demonstrated that Ca, in the absence of parathyroid hormone (PTH), controlled Pi transport. The mechanism by which Ca alters this Pi uptake is unknown. In the present study, we examined the effect of altering the concentration of intracellular free Ca ($[\text{Ca}^{2+}]_i$) on the uptake of Pi in cultured opossum kidney (OK) cells which have proximal tubular-like characteristics. $[\text{Ca}^{2+}]_i$ was determined with the fluorescent indicator INDO-1/AM. Initial rate (15s) of Pi (0.1 mM) was measured in the presence of extracellular Na⁺ (150 mM). Uptake was almost completely dependent on Na⁺. In the presence of 1.0 mM extracellular Ca, $[\text{Ca}^{2+}]_i$ was 188±8 nM and Pi uptake was 131±5 pmol/mg prot./15s. Changes, both increases and decreases, in $[\text{Ca}^{2+}]_i$ were effected by different concentrations of the Ca ionophore, ionomycin, and ionomycin plus EGTA in the absence of extracellular Ca, and by varying the times of incubation with these effectors. Deviation from the basal $[\text{Ca}^{2+}]_i$ resulted in inhibition of Pi uptake, the magnitude of inhibition correlated with of the extent of change in $[\text{Ca}^{2+}]_i$. Under the same conditions, Na⁺ and D-glucose uptakes were unaffected. These finding suggest that $[\text{Ca}^{2+}]_i$ may specifically regulate Pi uptake in OK cells by a PTH-independent mechanism.

TRANSEPITHELIAL PHOSPHATE TRANSPORT BY CULTURED RENAL CELLS: INHIBITION WITH PHOSPHONOFORMIC ACID (PFA) AT THE APICAL SURFACE. Steven J. Scheinman and Susan Ford*, Dept. of Medicine, SUNY-HSC, and Dept. of Toxicology, Bristol Labs, Syracuse, NY.

To study transepithelial transport of Pi, we grew primary cultures of renal cortical tubular cells from rabbit (modified from Chung, et al., J. Cell. Biol. 95:118, 1982) on Millipore filters. When the cell layer becomes confluent it separates two compartments, one apical (A) and one basolateral (B). Layers were studied at confluence, as indicated by development of an apical-acid pH gradient; confluence was confirmed by histologic inspection of representative filters. Pi transport was studied by measuring movement of $^{32}\text{P}_i$ from one side to the other. At confluence, cell layers performed Na-dependent active transport of Pi from the A to the B side, achieving B:A ratios of 25.0 ± 4.5 (SE) for $^{32}\text{P}_i$ and .03 ± .01 for the extracellular marker (^3H -PEG). Unidirectional B to A fluxes for Pi and PEG were minimal. In contrast, these layers were highly permeable to Na.

PFA is a specific competitive inhibitor of Na-Pi cotransport in brush border membrane. When A was exposed to 5 mM PFA, % removal of Pi from A after 5 min of PFA was 7.1 ± 0.7 vs 15.7 ± 1.5 in timed controls (p<.002) and at 120 min of PFA was 30.5 ± 0.8 vs 85.5 ± 4.8 (p<.0001); % appearance of Pi at B at 10 min was 1.2 ± 0.2 (PFA) vs 5.2 ± 0.7 (p<.002) and at 120 min was 17.0 ± 1.1 (PFA) vs 67.5 ± 2.8 (p<.0001). PFA applied to the B side had no significant effects.

PFA inhibits Pi transport only when applied to the A side of a cultured cell monolayer. This preparation exhibits not only Pi uptake but also net transport of Pi, and should be valuable in studying A and B features of Pi transport.

ASSOCIATION OF UNIDIRECTIONAL CALCIUM AND MAGNESIUM FLUX WITH NaCl IN THE LOOP OF HENLE. Ihab H. Shafik,* and Gary A. Quamme. Dept. of Medicine, University of British Columbia, Health Sciences Centre Hospital, Vancouver, B.C.

Net Ca and Mg transport in the loop is thought to be principally passive and dependent on NaCl transport. Studies were designed to dissociate unidirectional Ca (Ca^{45}) and Mg (Mg^{28}) from NaCl transport. The loop of Henle was perfused at constant flow rates, 20 nl.min⁻¹, while altering either extracellular or luminal Ca or Mg concentrations. In control rats (plasma Mg 0.61±0.01; Ca 2.5±0.1 mM), Ca and Mg efflux (18.2±1.4 and 7.1±0.7 pmole.min⁻¹) was similar to net absorption (20.4±0.95 and 7.0±0.6 pmole.min⁻¹, respectively), indicating unidirectional transport of both Ca and Mg. Net and unidirectional Mg and Ca transport was highly dependent on net NaCl absorption when delivery rates were altered, 15-40 nl.min⁻¹. Elevation of either plasma Mg, (4.1±0.5 mM) or Ca (4.2±0.5 mM) decreased efflux, Mg, 3.2±0.4, and Ca, 13.2±1.6 pmole.min⁻¹, with no change in net Na transport, 1576±59 pmole.min⁻¹. In contrast, elevation of luminal Mg (3.0±0.02 mM) did not alter fractional unidirectional Mg and Ca flux. In conclusion, loop Ca and Mg transport is normally unidirectional and dependent on NaCl transport, presumably transepithelial voltage. However, elevation of extracellular Mg or Ca, but not luminal, inhibits divalent absorption with no effect on NaCl. Accordingly, Ca and Mg transport is distinctive to NaCl absorption in the loop of Henle.

FOREARM BONE MINERAL CONTENT (BMC) IN DIALYSIS PATIENTS. Charles L. Smith and Robert O. Berkseth, Hennepin County Med. Cntr., Mpls, Mn.

Non-invasive methods to evaluate renal osteodystrophy are important in the management of individuals with chronic renal failure (CRF). Single photon absorptiometry is a precise method for quantitation of forearm BMC. BMC measurements were made on the radius of the nondominant forearm at the conventional 1/3 site in 87 female and 86 male dialysis patients. No patient was receiving Vit D at the time of study. Group age ranged from 22-83 years and time on dialysis from 0 to 17 years.

BMC in women decreased with age and was lower than in men in all age groups. In men, the BMC tended to decrease with age but this was not significant even at ages over 70 years. When compared as groups to a non-renal failure, age-matched control population, dialysed males were 102% of control and females 90% of control. Accelerated loss of BMC (BMC < 80% of age-matched controls) was observed in 33 females (38%) and only 7 males (8%). No difference could be seen between diabetics and nondiabetics or between those with or without polycystic kidney disease (PCKD) or hypertension. BMC correlated negatively with time on dialysis with a 2%/year loss in both sexes.

We conclude that (1) in dialysis patients not receiving vit D, BMC decreases 2%/yr, (2) BMC is better maintained in men with CRF than in women and (3) diabetes, PCKD, and hypertension do not prevent or accelerate bone mineral loss.

EFFECT OF 1,25(OH)₂D₃ ON CYTOSOLIC CALCIUM IN DISPERSED BOVINE PARATHYROID CELLS.

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There is evidence that 1,25(OH)₂D₃ suppresses PTH synthesis and secretion. However, all the possible mechanisms have not been explored. Cytosolic calcium ([Ca]_i) is known to be an intracellular messenger in hormone action. We, therefore, examined the effect of 1,25(OH)₂D₃ on [Ca]_i levels in dispersed bovine parathyroid cells, loaded with INDO-1. At 0.5 mM extra-cellular calcium, 1,25(OH)₂D₃ exposure increased [Ca]_i in a dose-dependent manner over a 10 min period (Δ Ca: 37 + 3 nmole for ethanol control, 54 + 5 nmole for 10⁻¹⁰M, 67 + 7 nmole for 10⁻⁹M, 101 + 8 nmole for 10⁻⁸M). The increase in [Ca]_i due to 10⁻¹⁰M 1,25(OH)₂D₃ is significant from control, p < 0.01. The rise in [Ca]_i induced by 10⁻⁸M 1,25(OH)₂D₃ is nearly equivalent to that due to increasing extra-cellular calcium to 1.0 mM. 1,25(OH)₂D₃ itself had no effect on the auto-fluorescence of the cells. Neither 10⁻⁷M 25(OH)D₃ nor 10⁻⁷M 24,25(OH)₂D₃ caused a significant increase in [Ca]_i. In the absence of extra-cellular calcium, 10⁻⁸M 1,25(OH)₂D₃ had no effect on [Ca]_i. The present data demonstrate that 1,25(OH)₂D₃ rapidly increased [Ca]_i levels in parathyroid cells and this effect is specific for 1,25(OH)₂D₃. The increase in [Ca]_i is due to influx of extracellular calcium. This rapid effect of 1,25(OH)₂D₃ raises the possibility that the mechanism is independent of genome activation, perhaps attributable to a direct interaction of 1,25(OH)₂D₃ with the parathyroid cell membrane.

RED BLOOD CELL ALUMINUM (RBC-A1) AND THE DEVELOPMENT OF MICROCYTIC ANEMIA (MA) IN RATS ADMINISTERED ALUMINUM (Al). GB Sonnier*, K Abreo, ST Brown M Sella*. LSU Med. Ctr., Shreveport, LA.

Al administration has been shown to cause MA in rats (Kaiser et al., Kidney Int. 26: 269-274, 1984). To determine the relationship of RBC-A1 accumulation to the development of MA, we administered 2mg Al i.p. daily to 26 male rats (200-300 gm). Rats were sacrificed at weekly intervals; six additional rats were sacrificed for baseline data. Blood was obtained to determine RBC-A1 (μg/10⁶ cells), P-Al (μg/L), MCV (fl) RBC Count (RBC ct, 10⁶ cells) and Hct (%).

	Baseline	Week 1	Week 2	Week 3	Week 4
n	6	6	6	7	7
RBC-A1	1.8±1.3	4.0±1.1	16.3±4.9	22.2±7.7	22.5±6.9
P-Al	4.8±3.1	218±76	308±19	320±32	397±28
MCV	56.7±1.0	57.9±1.0	55.1±0.4	53.8±1.5	52.4±0.8
RBC ct	6.1±0.4	6.2±0.3	5.4±0.2	4.7±1.1	4.8±0.3
Hct	34.5±2.2	36.0±1.2	30.0±1.0	25.3±6.5	25.5±1.9

Between baseline and week 1 there was no significant change while between week 1 and week 4 there was a significant decline in MCV, RBC ct and Hct (p<.001). Between baseline and week 1 there was a significant rise in RBC-A1 (p<.01) and P-Al (p<.001). Both RBC-A1 and P-Al continued to rise between week 1 and week 4 (p<.001). There was a significant positive correlation between RBC-A1 and P-Al (r=.74, p<.001) and a significant negative correlation between RBC-A1 and MCV (r=-.77, p<.001) and between P-Al and MCV (r=-.66, p<.001).

Conclusions: (1) Elevated RBC-A1 is associated with Al induced MA in rats. (2) RBC-A1 and P-Al related in a similar manner to declining MCV. (3) Rises in RBC-A1 and P-Al preceded declining RBC indices.

CONDITIONED MEDIA FROM RAS ONCOGENE TRANSFORMED NIH3T3 CELLS INDUCES BONE RESORPTION. Vikas P. Sukhatme, Luralynn L. Wright,* and David A. Bushinsky. Department of Medicine, University of Chicago, Chicago, IL.

Tumor induced hypercalcemia is due, in part, to enhanced osteoclastic bone resorption induced by soluble factors elaborated by malignant cells. We have recently shown that RAS transformation of NIH3T3 cells results in a 50 fold induction of cathepsin L mRNA and secretion of the corresponding protein into the supernatant. Since cathepsin L is an acid proteinase we asked whether this supernatant would increase bone resorption from cultured neonatal mouse calvariae (frontal and parietal skull bones). Supernatant obtained after 72 h culture of 10⁶ NIH3T3 RAS transformed cells was subsequently used as media for a 24 h incubation of calvariae (TR); supernatant from nontransformed NIH3T3 cells was used as a control (CTL).

With TR there was net calcium efflux (JCa) from the live calvariae (JCa = 69 + 12 nmol/bone/24h, n = 23) while with CTL there was net calcium influx (JCa = -43 + 8, n = 20, p < 0.01 vs. TR). Repeat freeze-thaw cycles, to kill all bone cells, led to an influx of calcium in both groups; however, as was the case for live calvariae the difference in net flux persisted (dead TR: JCa = -120 + 11, n = 10; dead CTL: JCa = -329 ± 20, n = 8, p < 0.01 vs. dead TR).

Supernatant from RAS transformed cells enhances calcium resorption in both live and dead bone. These results suggest that cathepsin L, an acid proteinase, may act directly to enhance bone resorption.

IN VIVO EFFECT OF CHRONIC ALUMINUM (Al) ADMINISTRATION ON THE PHOSPHOLIPID CONTENT OF RENAL BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM KIDNEY CORTEX OF RATS. R.A.L. Sutton, A. Halabe, A. Elgavish, N.L.M. Wong. Dept. of Med. U.B.C., Vancouver, B.C., Canada.

Chronic Al administration has recently been reported by our laboratory to impair sodium dependent phosphate transport in brush border membrane vesicles (BBMV) isolated from kidney cortex of rats. However, the mechanism by which Al affects the Na-phosphate transport is unknown. The present experiment was done in order to clarify if chronic Al administration alters the total phospholipid content of the BBMV. Two groups of rats were studied. Group I (n=12) served as controls, receiving normal saline intraperitoneally (i.p.). Group II (n=12) were given AlCl_3 4mg/kg i.p. daily for 35 days. Plasma Al rose to 700 ± 140 ug/L in Group II as compared to 13 ± 2.5 ug/L in Group I. Phosphate excretion was significantly higher in Group II than in Group I. (Fractional excretion of phosphate was 19.1 ± 3.1 vs. $13.0 \pm 0.8\%$ respectively, $p < 0.05$). The total phospholipid content in BBMV was measured by the acid hydrolysis of the brush-border membrane as described by Elgavish et al. (J. Membr. Biol. 72:85-91, 1983). Inorganic P was determined by spectrophotometric analysis. Our results showed that the phospholipid content was similar 0.595 ± 0.034 umol P/mg prot in Group II vs. 0.636 ± 0.082 umol P/mg prot in group I ($p > 0.05$). These results indicate that chronic Al administration does not affect the total phospholipid content of the membrane at the BBMV.

1,25-DIHYDROXYVITAMIN D (1,25D) STIMULATES SODIUM DEPENDENT PHOSPHATE (Pi) UPTAKE BY BRUSH BORDER MEMBRANE VESICLES (BBMV) BY DIRECTLY AFFECTING MEMBRANE FLUIDITY AROUND Pi TRANSPORTER. M Suzuki*, Y Kawaguchi* and T Miyahara* (Intr by K Kurokawa). 2nd Dept Med, Jikei Univ Med Sch, Tokyo

Available data suggest that Pi transport of BBMV of proximal tubules is reduced in vitamin D deficiency and that 1,25D may modulate BBMV fluidity. Further, results with BBMV and liposome membrane fusion showed 1,25D stimulates Pi transport of BBMV by non-genomic liponomic mechanisms. The present study tested whether 1,25D alters membrane fluidity by its direct liponomic action and such changes are related to altered Pi transport. Thus, we examined effects of 1,25D added to BBMV suspension on Pi and glucose transport and on membrane fluidity. A majority (82%) of 1,25D added at 10^{-8} M to BBMV suspension at 0°C was incorporated to the membranes. BBMV treated with or without 1,25D were incubated for 15 min at varying temperature followed by 1 min incubation in a solution containing either ^{32}P -Pi or ^{14}C -glucose to measure Pi or glucose uptake, respectively. The 1,25 treatment stimulated Pi, but not glucose, uptake by BBMV. Arrhenius plots of the data to determine membrane fluidity showed a triphasic configuration with two transition temperatures for both Pi and glucose uptake. However, the 1,25D treatment resulted in a significant shift of the lower transition temperature for Pi uptake, but not for glucose uptake, from 15 to 13.5°C . These data indicate that 1,25D may alter membrane fluidity of BBMV through non-genomic liponomic effects and suggest that such effects are limited around the Pi transporter thus affecting Pi uptake.

NORMAL RESPONSE OF THE MINERAL HOMEOSTATIC SYSTEM TO SALMON CALCITONIN (TCT) IN HUMANS. Tamayo J.A. Walliser J.F.*, Sierra R.I.*, Peña J.C. Instituto Nacional De La Nutricion Salvador Zubiran Mexico CITY. Calcitonin is a potent inhibitor of bone resorption. At pharmacological doses it results in hypocalcemia. We studied the effect of TCT in 8 healthy ambulatory subjects, 4 females and 4 males, matched by age, \bar{x} 22.5 ± 1.3 and 27 ± 2.6 years and body surface area (BS) \bar{x} 1.42 ± 0.04 and 1.58 ± 0.04 m². The day before the study a 24 hour urine collection followed by a fasting 2 hour urine sample (Uo) and 7 ml of blood were obtained (Bo). A single i.v. bolus of TCT (1 I.U./Kg/Bw, time 0) was given, blood was obtained (7 ml) at 5, 10, 15, 30, 60, 120, 180 and 240 minutes. Urine was obtained by spontaneous voiding at 60 and 240 minutes. Calcium (Ca), Phosphate (Pi), PTH, TCT, cAMP, GLA protein and creatinine (Cr) were measured in blood, and Ca, P, cAMP, Cr in urine. $\text{TMPO} \cdot 4/\text{GFR}$, $\text{UTcAMP}/\text{mgCr}$, NcAMP (nmol/GFR) and UCa/UCr were calculated. sCa decreased in females from \bar{x} 9.1 ± 0.19 mg/dl to 8.8 ± 0.1 ($p < 0.05$) at 120 min. In males hypocalcemia occurred earlier (10-30 min), and was not significant. Pi decreased in females at 120 min from \bar{x} 4.2 ± 0.3 mg/dl to 3.4 ± 0.2 ($p < 0.025$), and at 240 min to \bar{x} 3.0 ± 0.1 ($p < 0.05$). In males Pi decreased at 180 min from \bar{x} 3.77 ± 0.4 to 3.2 ± 0.4 ($p < 0.05$). As expected there was an increment in: fractional excretion of phosphate (FEP), UTcAMP , NcAMP and a decrease in $\text{TMPO} \cdot 4/\text{GFR}$; all reflecting parathyroid gland response. Markers of bone resorption decreased. This method can be used as dynamic evaluation of patients with mineral homeostatic disorders.

CYTOSOLIC FREE Ca CONCENTRATION ($[\text{Ca}^{2+}]_i$) IN RAT PROXIMAL TUBULES: RESPONSE TO A LOW Ca DIET AND TO ACUTE CHANGES IN MEDIUM Ca. S. Tan, * P. Rodriguez, * D. Thomas, * I. Kim* and K. Lau. Michael Reese Hospital and University of Chicago, Chicago, IL.

To better understand the renal adaptation to Ca deprivation, we measured $[\text{Ca}^{2+}]_i$ in proximal tubules derived from 10 weeks old rats randomized to a normal (0.87%) or a low (0.02%) Ca diet for the preceding 14 days. After retrograde arterial perfusion with collagenase-containing Krebs-Henseleit-HCO₃ buffer, kidney cortical slices were further enzyme-treated before centrifugation to obtain proximal tubule suspension. The tubules were then loaded for 1 hour with fura 2 AM or the DMSO vehicle for autofluorescence. To avoid in-vitro Ca flooding of Ca-deprived cells, Ca was replaced by 1 mM Ba in all buffers, except as noted below. Fluorescence emitted at 505 nm was measured during dual wavelength excitation at 340 and 380 nm, to estimate $[\text{Ca}^{2+}]_i$ by the ratio method as originally described by R. Tsien. Results, tabulated below as mean \pm S.E., indicate lower plasma ionized Ca and lower $[\text{Ca}^{2+}]_i$ with diet Ca deprivation.

Diet Ca ⁺⁺	Plasma Ca ⁺⁺ (mM)	Cytosolic Free Ca Concentration (nM)				
		Medium Ca "0" mM	Acute response to 2.5 mM	3.9 mM	Δ	
Normal	1.27 ± 0.02 §	208 ± 7 §	299 ± 12 §	91 §	360 ± 13 §	151 §
Low	1.16 ± 0.02 §	179 ± 7 §	241 ± 11 §	61 §	291 ± 14 §	114 §

(§, $p < 0.05$ and vs Normal Ca diet).

Brief (40 sec) exposure to graded increases in medium Ca was buffered more avidly by tubules derived from Ca deprived rats.

We conclude (1) A low Ca diet reduces $[\text{Ca}^{2+}]_i$ in proximal tubule cells. (2) This reduction is an in vitro anamnetic expression reflecting in vivo adaptation, since it is not abolished by prolonged incubation in media with identical $[\text{Ca}^{2+}]_i$. (3) Acute Ca loading accentuates this Δ $[\text{Ca}^{2+}]_i$ suggesting increased avidity by intracellular buffers.

IN SITU MEASUREMENT OF IONIZED CA CONCENTRATION ($[Ca^{2+}]_i$) IN RAT DISTAL TUBULAR FLUID. Robert S. Vick* and Linda S. Costanzo, Medical College of Virginia, Richmond, VA.

The profile of luminal $[Ca^{2+}]_i$ along the rat distal tubule (DT) was determined and compared to the profile for total Ca ($[Ca^{2+}]_t$). In single DT segments in rats prepared for micropuncture, transepithelial potential difference and Ca^{2+} ion activity were measured simultaneously with Ling-Gerard (LG) and Ca^{2+} selective microelectrodes (Ca-ISE), respectively. The Ca^{2+} specific voltage was the difference between voltages measured with Ca-ISE and LG in a single DT segment. Ca-ISE were calibrated before and after punctures in solutions simulating early and late DT fluid. Puncture sites were determined by microdissection. $[Ca^{2+}]_i$ was 0.982 mM in earliest DT and declined rapidly along the DT to 0.036 mM, $\log [Ca^{2+}]_i = -0.018$ (% length) + 0.352, $r=0.88$. Previous data showed that $[Ca^{2+}]_t$ also fell along the DT, $\log [Ca^{2+}]_t = -0.0051$ (% length) + 0.0822. Clearly $[Ca^{2+}]_i$ fell more rapidly as a function of DT length than did $[Ca^{2+}]_t$, $p<0.001$. Comparing $[Ca^{2+}]_i$ to $[Ca^{2+}]_t$ along the DT, virtually 100% of Ca^{2+} was ionized in earliest DT, while only 10% was ionized in end DT. We conclude that: (1) Ca^{2+} reabsorption in rat DT occurs exclusively in the ionized form. (2) The sharper decline in $[Ca^{2+}]_i$ than $[Ca^{2+}]_t$ and dramatic decrease in the percentage ionized is in part caused by preferential reabsorption of ionized Ca^{2+} ; also, increased concentrations of poorly reabsorbed anions in late DT fluid, caused by water abstraction would increase Ca^{2+} complexation.

DIETARY PROTEIN RESTRICTION (PR) DECREASES RENAL REABSORPTION OF PHOSPHATE (TMPi) DURING RENAL FAILURE. S.K. Webster*, M. Einstein, and J. Borgia* Baxter Healthcare Corp., Round Lake, IL 60073

TMPI is decreased following renal failure induced by partial renal ablation. Nevertheless, phosphate retention and hyperphosphatemia may result. Since PR results in a decrease in TMPI these studies evaluated the effect of PR on TMPI in partially nephrectomized (NX) rats. One month after completion of 5/6 NX, the rats were fed 12g of low phosphate diet (less than 0.07% Pi) per day for 3 days. The protein fed group (PF) was fed diet having similar caloric content but containing no protein. The rats were then evaluated for TMPI in clearance experiments by infusing phosphate.

TMPI was significantly decreased in PR group of NX rats as compared to PF rats. Plasma Pi levels and GFR were not different between groups.

	PROTEIN FED	PROTEIN RESTRICTED
TMPI (mol/min)	4.2 ± 0.3	2.8 ± 0.4*
GFR (ml/min)	0.48 ± 0.18	0.45 ± 0.13
Plasma Pi (mM)	2.12 ± 0.21	2.32 ± 0.24

* p less than 0.05 as compared to PF

These results suggest that dietary protein restriction may alter residual nephron phosphate reabsorption after partial nephrectomy independent of plasma phosphate levels and glomerular filtration rate. Dietary protein restriction has been shown to attenuate the progression of chronic renal failure. Since dietary protein and phosphate are closely associated, it is possible that the beneficial effects of protein restriction may occur through effects on phosphate metabolism.

CHRONIC METABOLIC ACIDOSIS INDUCES CELL-MEDIATED CALCIUM EFFLUX FROM BONE IN VITRO. Luralynn L. Wright* and David A. Bushinsky. University of Chicago, Chicago, IL.

Chronic metabolic acidosis causes bone mineral dissolution. Whether the dissolution is due to alterations in physico-chemical factors alone, as in acute metabolic acidosis (AJP 248: F785, 1985), or to enhanced cell-mediated bone resorption is not clear. To study the effect of chronic metabolic acidosis on bone calcium homeostasis we cultured live (L) and dead (D), successive freeze-thaw cycles) neonatal mouse calvariae for 99 h, $pCO_2 = 40$ mmHg, in acidic (Ac, pH ≈ 7.05) or control (Ctl, pH ≈ 7.45) medium. We determined net Ca flux (JCa, nmol/bone/time interval) over 0-48, 48-96 and 96-99 h. Culture medium was replaced at 48 and 96 h with similar fresh medium.

	n	JCa 0-48h	JCa 48-96h	JCa 96-99h
L Ctl	32	-2 ± 30	-132 ± 29	-51 ± 10
L Ac	20	318 ± 62*	242 ± 121*	44 ± 10*
D Ctl	16	-97 ± 22*	-187 ± 13*	-69 ± 5*
D Ac	8	266 ± 29**	57 ± 71**	-46 ± 9*

Values mean ± SE; n, pairs of calvariae in each group; JCa, positive value indicates net Ca efflux from bone; +, $p < 0.05$ vs. L Ctl; o, $p < 0.05$ vs. L Ac; #, $p < 0.05$ vs. D Ctl.

At 96-99 h there is calcium efflux from L Ac but not from any other group. These results indicate that chronic metabolic acidosis induces cell-mediated bone resorption in vitro as measured by net calcium efflux from bone. The mechanism by which chronic metabolic acidosis stimulates bone cells, presumably osteoclasts, to resorb bone is not known.

PROSTAGLANDIN AUGMENTATION OF PTH-MEDIATED CALCIUM TRANSIENTS IN UMR-106 CELLS.

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We have previously reported that PTH causes a rise in free cytosolic calcium ($[Ca^{2+}]_i$) through both cAMP-independent (cAMP-I), and cAMP-dependent Ca^{2+} channels in the rat osteosarcoma cell line, UMR-106. Prostaglandins (PG) are autoids that may modify the actions of circulating hormones such as PTH at the local level. We studied the interaction of PG and PTH on $[Ca^{2+}]_i$ by the fluorescent Ca^{2+} dye, Fura2. PGF_2 pretreatment enhanced the effect of PTH to raise cAMP-I $[Ca^{2+}]_i$ by 2-3 fold ($ED_{50}=3.5nM$) compared to PTH alone. PGE_2 was less potent than PGF_2 in enhancing the PTH increase in cAMP-I $[Ca^{2+}]_i$. The enhancement by 10nM PGF_2 was noted over the range of PTH from 0.01 to 2.5 u/ml. Inhibition of endogenous PG production with indomethacin did not alter the rate or extent of PTH-induced rise in $[Ca^{2+}]_i$ nor the enhancement in the PTH-induced rise by exogenous PG. The enhancement was not time-dependent as simultaneous addition of PTH and PGF_2 had a similar degree of enhancement as PGF_2 pretreatment for up to 6 min. The augmented increase in $[Ca^{2+}]_i$ appeared to be mainly due to an increase in Ca^{2+} release from intracellular stores since practically the full extent of the augmented $[Ca^{2+}]_i$ rise was observed in Ca^{2+} -free medium. Conclusion: PGs increase the PTH-stimulated cAMP-I transient in $[Ca^{2+}]_i$ through an enhanced release of Ca^{2+} from intracellular stores by yet unknown mechanism(s).

ACUTE RENAL VASCULAR AND TUBULAR DAMAGE AFTER ANALGESIC DRUGS IN MAN. Mattias Aurell, Staffan Björck* and Christian T. Svalander*. University of Göteborg, Sahlgren's hospital, Dept. of Nephrol. & Pathol., Göteborg, Sweden.

Seven patients with acute renal failure after ingestion of analgesic drug combinations including paracetamol were seen. The paracetamol dose was low to moderate, but in two patients very high doses were ingested. They presented with oliguric renal failure and restitution of renal function was complete in all subjects. Five patients had no or only a minor elevation of liver transaminase levels. Two patients had severe liver damage. Renal biopsies were performed in three patients, two to five days after their maximum creatinine levels were reached. The biopsies, studied by light and electron microscopy, showed focal tubular epithelial cell necrosis, of both proximal and distal tubules. In addition, focal vascular damage was also present in all specimens, and found in various locations in the kidney. Endothelial cell necrosis was found in glomerular and peritubular capillaries and in small arterioles. In the latter vessel also focal smooth muscle cell necrosis was found. The findings may indicate that microvascular damage is an important mechanism for the acute renal failure after paracetamol and our findings support the hypothesis of a pathogenetic role of vascular injury in the origin of analgesic nephropathy.

PATHOPHYSIOLOGY OF ACUTE RENAL FAILURE AND TOXIC NEPHROPATHY

DIETARY PROTEIN RESTRICTION PRIOR TO RENAL ISCHEMIA CAN REDUCE POSTISCHEMIC UREMIC SYMPTOMS AND CELLULAR INJURY DURING ISCHEMIA. Peter M. Andrews, Department of Anatomy & Cell Biology, Georgetown University Medical Center, Washington, D.C.

Recently we have shown that maintaining rats on restricted dietary protein prior to the induction of renal ischemia will significantly improve postischemic survival regardless of the postischemic diet (*Kid.Int.*, 30:266,1986). The present investigation was undertaken to elucidate possible mechanisms which may be responsible for this protective phenomenon. Rats were pair-fed for a week on either restricted (5%) or high (60%) purified dietary protein. The diets were isocaloric and contained equal amounts of phosphorous, calcium, sodium, and mineral and vitamin supplements. Ischemia was induced by 45 minutes of renal pedicle clamping. Immediately following ischemia all rats were returned to a normal protein dietary regime (i.e., 20%). Although postischemic serum creatinine levels were similar in both groups, rats preconditioned on 5% dietary protein exhibited significantly lower BUN values ($p < 0.05$), lower serum potassium values ($p < 0.05$), less acidosis ($p < 0.05$), closer to normal serum osmolalities ($p < 0.05$), and significantly improved survival rates compared with rats preconditioned on 40% protein. An evaluation of kidney morphology immediately following renal ischemia in rats which had been maintained on a 0%, 5%, 20%, 40%, or 60% dietary protein regimes prior to the ischemic insult, revealed less necrosis of the proximal convoluted tubules, third segment proximal tubules, and medullary ascending thick segments in rats preconditioned on restricted dietary protein. These observations indicate that a decrease in renal tubule injury and a reduced postischemic uremic response result from a week of conditioning on restricted dietary protein prior to renal ischemia.

GLYCEROL-INDUCED ACUTE RENAL FAILURE (GL-RF) PROTECTS AGAINST MERCURIC CHLORIDE ($HgCl_2$) NEPHROTOXICITY: FUNCTIONAL AND PATHOLOGICAL CORRELATIONS. R. Bacenroth-Maayan,* H. Wald,* L. Schuger,* E. Rosenman,* and M.M. Popovtzer, Hadassah University Hospital, Jerusalem, Israel.

The protection conferred by GL-RF against subsequent $HgCl_2$ nephrotoxicity was examined in rats by inducing $HgCl_2$ -RF 14 days (d) after antecedent GL-RF. Serum creatinine (SCR), CR clearance (GFR), $UNaV$ and FE_{Na} were measured at 1 and 14 d after GL-RF and then 2 after $HgCl_2$ -RF. Histopathology was quantified at 14 d post-GL-RF and 2 d post- $HgCl_2$ -RF. An inverse correlation was found between the post-GL-RF and the post- $HgCl_2$ -RF GFRs ($r = -0.54$, $p < 0.05$), without correlation between $UNaV$ and FE_{Na} values. At 14 d post-GL-RF when GFR was nearly normal fraction of tubular regeneration by light microscopy was directly related with SCR at 1 d post-GL-RF ($r = 0.97$, $p < 0.01$). At 2 d post- $HgCl_2$ SCR correlated inversely with fraction of tubular regeneration ($r = -0.79$, $p < 0.05$) and directly with fraction of necrosis ($r = 0.54$). Tamm Horsfal antibody staining of cells was similar at 14 d post-GL-RF and in normal controls. We conclude that 1) there is a good correlation between function and histological quantitation in these models of RF, 2) the protection conferred by GL-RF against $HgCl_2$ -RF is directly and significantly correlated with both the severity of functional impairment and extent of tubular regeneration after the GL-RF, and 3) the protection cannot be explained by Tamm Horsfal protein depletion. These results suggest that tubular regenerative activity may play a role in renal resistance to repeated nephrotoxic insults.

EFFECT OF DIETARY PROTEIN ON MUSCLE PROTEIN SYNTHESIS (Sm) AND RENAL FUNCTION (SCr) IN 1-3/4 NEPHRECTOMIZED RATS. R. Baliga*, J. Harrah, G.T. Varghese, P.E. Ray and M.A. Holliday*. LSU Med. Ctr., New Orleans, LA and Univ of California, San Francisco, CA.

In this study we examined the effect of 14.5% and 30% dietary protein on Sm and SCr in 1-3/4 nephrectomized rats. Male Sprague Dawley rats (BW 83±6) were studied on days 5 and 10 after a second stage nephrectomy (U) or sham operation (S) (Day 0). Until day 5 all rats were fed a 17% tryptophan deficient diet to induce catabolism and enhance uremia. The U rats were paired according to their SUN before initiation of a 14.5% or 30% casein diet at 7 g/day until day 9 (anabolic phase). On day 10, maximal anabolic response defined by Sm was measured 2 hr after a standard meal (2.5 g/100 gBW) taken voluntarily after a 12 hour fast. Sm was measured by the incorporation of ³H Phenylalanine (PHE) into muscle 10 min after IV injection of PHE/³H PHE (25 uCi/100 gBW). CCr derived from 1/SCr was compared with Sm (% of average control).

There was a marked difference in Sm between the control and uremic groups P<0.005. However, the effect of the two dietary proteins on Sm in the control or uremic group was not significant. U rats fed 30% protein diet had significantly higher SCr than U rats fed 14.5% protein diet (2.05±0.5 vs 1.46±0.2), P<0.025. Regression analysis between CCr and Sm was significant, P<0.0001.

EXPERIMENTAL GENTAMICIN (G) NEPHROTOXICITY CAN BE PREVENTED BY POLYASPARTIC ACID (PAA). William M. Bennett, C.A. Wood* S.J. Kohlhepp* P.W. Kohnen* D.C. Houghton, D.N. Gilbert*, Oregon Health Sci. Univ. and Providence Med. Ctr., Portland, Oregon.

Polyamino acids such as PAA, M.W. 15,000 Daltons, inhibit G binding to renal tubular membranes while minimizing tubular necrosis in G-treated rats (Williams et al., *Journ Pharm and Exp Ther* 237:919, 1986). Since PAA is a Na salt, control for Na is vital for *in vivo* studies. Groups of male Fisher rats were given either G 20 mg/kg b.i.d., G+Na, G+PAA 380 mg/kg, PAA alone or sterile water. At 10 days BUN, creatinine clearance (CCr), urinary N-acetyl glucosaminidase (NAG), urine osmolarity (Uosm), fractional excretion of Na (FENa) and renal cortex G were obtained. Tubular necrosis was assessed by light microscopy. Results:

	BUN (mg/dl)	CCr (ml/min/100gr)	FENa (%)	NAG (U/l)	Uosm (mosm)	Renal G (µg/g)
G	39*	0.6*	0.9*	81*	788*	297*
G+Na	47*	0.5*	1.8*	151*	1258*	189*
G+PAA	18	1.4	0.6	16	1685	2866
PAA	18	1.3	0.5	19	2170	----
Control	22	1.1	0.7	19	2438	----

*p<0.01 from G+PAA, PAA and Control

There was no necrosis in G+PAA while G and G+Na had extensive damage. All PAA animals had cytoplasmic vacuoles without necrosis. G was localized within proximal tubular cells by immunoperoxidase in G+PAA despite absence of renal dysfunction.

Conclusion: PAA prevents experimental G nephrotoxicity. Protection is not dependent on Na content of PAA or exclusion of G from tubular cells.

EFFECT OF CHRONIC GENTAMICIN (G) ON GLOMERULAR HEMODYNAMICS IN 12 DAY PREGNANT (P) RATS. Christine Baylis. Univ of West Virginia, Dept. of Physiol., Morgantown, WV.

Normal pregnancy is associated with loss of vascular responsiveness to angiotensin II (ANGII) and increases in GFR. These studies were performed to assess the glomerular response of P vs virgin (V) rats to chronic G (40mg/kg/day IP for 10 days) since G-induced glomerular toxicity may be mediated by ANGII in male rats (Schor et al. *KI* 19:288,1981). Micropuncture measurements were made of single nephron (SN)GFR, glomerular plasma flow (Q_G), glomerular pressure gradient (ΔP), efferent oncotic pressure (π_e) and ultrafiltration coefficient (K_f). Data: mean ±SE. †p<0.05 G vs control (C); §p<0.05 V vs P; # minimum K_f

	SNGFR (nl/min)	Q _G (nl/min)	π _e (mm Hg)	ΔP (mm Hg)	K _f (nl/(s·mm Hg))
V+G n=8	28±2	132±14	31±2	37±1	0.048±0.006
P+G n=7	31±2	121±13	30±1	38±1	0.038±0.002
V C n=5	27±2	79±8†	37±2†	35±1	0.057±0.006#
P C n=5	33±1§	117±10§	31±2§	32±2†	0.069±0.009#†

Both V and P rats receiving G were at filtration pressure disequilibrium (ΔP>π_e) due to low values of K_f compared to C. All C rats were at filtration pressure equilibrium (ΔP=π_e) thus only minimum values of K_f could be calculated; a gestational rise occurred in both SNGFR and Q_G whereas no difference was seen between V and P rats receiving G. Surprisingly, the G induced decline in K_f was not ameliorated by pregnancy, suggesting either that the P-induced loss of vascular responsiveness to ANGII does not extend to the glomerulus or that in females, G-induced declines in K_f are not mediated by ANGII.

EARLY GLOMERULAR HEMODYNAMIC CHANGES AFTER ISCHEMIA AND REFLOW: EFFECTS OF ANTIOXIDANT THERAPY. J.E. Bird, O.W. Peterson,* and R.C. Blantz. UCSD and VAMC, La Jolla, CA.

Reduction in nephron filtration rate (SNGFR) after 1 hr ischemia and 2-3 hrs reflow in uninephrectomized rats could be the result of tubular injury and obstruction, or primary glomerular hemodynamic events. Pretreatment of ischemic (I) rats with probucol, a lipophilic antioxidant (IP), improved SNGFR after 24 hrs reflow. SNGFR, absolute proximal reabsorption (APR), and glomerular hemodynamic parameters were measured in I, IP, and control (C) rats. X±SE are given. *p<.05 vs C, † vs IP.

	SNGFR	APR	SNPF	ΔP	LpA	P _G	P _{BS}
C	44±2	11±1	136±8	36±1	.06±.006	51±1	15±1
I	21±3**	8±1*†	93±14*†	30±2*	.06±.014	55±2	25±2*
IP	36±3*	12±1	171±30	32±2*	.07±.009	54±2	22±2*

The marked decrease in SNGFR in I rats was caused by the significant decrease in the glomerular hydrostatic pressure gradient ΔP, (60%) and the decrease in single nephron plasma flow, SNPF (40%). Glomerular capillary pressure (P_G) did not increase significantly and did not contribute to the decrease in ΔP. The change in ΔP was due to an increase in Bowman's space pressure (P_{BS}). Antioxidant treatment significantly improved SNGFR, APR, and SNPF. Conclusions: 1) The major consistent factor contributing to SNGFR reduction in I rats was the decrease in ΔP, related to tubular damage and obstruction. 2) A variable reduction in SNPF also contributed to decreased SNGFR in I rats. 3) The improvement in SNGFR after antioxidant treatment was primarily due to the prevention of a drop in SNPF. Probucol therapy appears to dissociate changes in SNPF from the increased P_{BS} and tubular obstruction characteristic of I.

ROLE OF OXYGEN FREE RADICAL SPECIES (OFRS) IN *IN VITRO* MODELS OF PROXIMAL TUBULAR ISCHEMIA. S.C. Borkan* & J.H. Schwartz, B.U. Med. Center, Boston, MA

Lipid peroxidation (LP) of cell membranes by OFRS during reflow might cause proximal tubule (PT) injury following ischemia. We examined the relationship between PT function and an end product of LP, malondialdehyde (MDA), in two models utilizing a suspension of rat PT segments (PTS). Exposure of PTS to t-butyl hydroperoxide (tBHP), an oxidant, induced a dose dependent decrement in the PTS metabolic rate (QO_2); nystatin-stimulated QO_2 (NYST); and ouabain-inhibitable QO_2 (OU), while it produced a progressive increase in MDA. The relationship between increasing doses of tBHP, QO_2 and MDA as well as between MDA and QO_2 were correlated ($r > 0.86$, $n=6$). A 50% decrement in QO_2 , NYST and OU was observed with 0.75 mM tBHP ($p < 0.02$, $n=8$). MDA rose from 0.33 ± 0.03 to 0.75 ± 0.08 nmoles /mg-protein ($p < 0.01$). Dithiothreitol (1mM), an anti-oxidant, prevented the tBHP induced changes in QO_2 and MDA. Thus an exogenous oxidant causes PT injury through increased LP. In the second model, 45 min O_2 deprivation followed by 30 min of reoxygenation produced the same decrement in QO_2 , NYST, and OU as 0.75mM tBHP but there was no increment in MDA ($n=9$). Mitochondria isolated from ischemic PTS had a 50% decrement in state III respiration ($p < 0.02$, $n=4$) suggesting that mitochondrial membranes may be an OFRS target. However, ischemia and reoxygenation failed to increase mitochondrial MDA. To further evaluate the role of LP, we examined the potential protective effect of agents that reduce tissue OFRS. PTS obtained from rats ($n=6$) injected with allopurinol 18hrs before isolation and then incubated with the OFRS scavengers superoxide dismutase and catalase during ischemia and reoxygenation were not protected from functional damage. These results demonstrate that oxidant induced tubular dysfunction is associated with lipid peroxidation but that OFRS do not mediate injury in this O_2 deprivation model.

URANYL NITRATE (UN) INHIBITS CELLULAR RESPIRATION BEFORE INCREASING PLASMA MEMBRANE K^+ PERMEABILITY IN RABBIT PROXIMAL TUBULE SEGMENTS (PTS). HR Brady*, BC Kone*, RM Brenner*, and SR Gullans. Renal Div. Brigham and Women's Hosp. and Harvard Med. Sch. Boston, MA.

The site and nature of the initial cellular events in toxic nephropathy are incompletely understood. We studied the direct effects of the classic nephrotoxin UN on PTS *in vitro*. Net K^+ fluxes, measured continuously with an extracellular K^+ electrode, were used as an index of plasma membrane integrity. Oxygen consumption (QO_2), measured continuously with an oxygen electrode, was used to evaluate cellular respiration. Addition of UN to PTS produced a dose-related (K_m $5 \times 10^{-4} M$) inhibition of QO_2 , beginning at 30 seconds after drug addition, reaching 25% at 2 minutes. This inhibition was entirely ouabain-insensitive, suggesting an effect independent of Na^+ transport. UN decreased CCCP-uncoupled QO_2 by 32%, indicating mitochondrial injury. At an equivalent concentration of UN (1mM), a net K^+ efflux (66 ± 8.6 nmol/mg/min, $n=8$) did not occur until 1 minute after the changes in QO_2 . Neither effect was prevented by the concomitant addition of reduced glutathione (10mM). UN toxicity to PTS is due to inhibition of cellular respiration, followed by changes in plasma membrane K^+ permeability. These findings contrast with $HgCl_2$ injury in which enhanced K^+ permeability precedes alterations in respiration, effects prevented by glutathione. The combined measurements of K^+ transport and QO_2 provide novel insights into the cellular mechanisms of nephrotoxicity.

INCREASED PLASMA MEMBRANE K^+ PERMEABILITY PRECEDES MITOCHONDRIAL INJURY BY $HgCl_2$ IN RABBIT PROXIMAL TUBULE (PT) SEGMENTS. RM Brenner*, BC Kone*, and SR Gullans, Harvard Medical School, Boston, MA.

The initial cytotoxic effects of $HgCl_2$ in the PT have not been adequately defined. We studied the immediate effects of $HgCl_2$ on K^+ transport and respiration (QO_2) with extracellular K^+ and O_2 electrodes in PT suspensions. $HgCl_2$ inhibited basal QO_2 in a dose-dependent manner with a maximal inhibition of 80.3% ($n=5$). At $3 \times 10^{-4} M$, $HgCl_2$ decreased ouabain-sensitive QO_2 by 29%, ouabain-insensitive QO_2 by 41%, and CCCP-uncoupled QO_2 by 71% ($n=6$), indicating significant mitochondrial dysfunction. To determine whether these effects were secondary to plasma membrane injury, net K^+ fluxes were measured. $HgCl_2$ caused a dose-dependent K^+ efflux ($V_{max}=172$ nmol/mg/min, $n=6$) which was not inhibited by 5mM Ba^{++} . This efflux rate was greater than, and partially additive to, that induced by ouabain, indicating an increase in K^+ permeability. At identical $HgCl_2$ concentrations, K^+ effluxes occurred before changes in QO_2 . Furthermore the K^+ efflux was observed at a lower concentration ($K_i=70 \mu M$) than the changes in QO_2 ($K_i=160 \mu M$). Both effects were prevented by the acute addition of the sulfhydryl-reducing agents (SRA) dithiothreitol or reduced glutathione to the medium. In summary, plasma membrane K^+ permeability is more sensitive than mitochondrial QO_2 to $HgCl_2$, and external SRA prevent all cytotoxic events. Therefore, disruption of plasma membrane K^+ permeability is the initial, and potentially irreversible, site of $HgCl_2$ cytotoxicity.

ANGIOTENSIN II (AII) AUGMENTS MEDULLARY HYPOXIA AND PREDISPOSES TO ACUTE RENAL FAILURE. Mayer Brezis, Ziv Greenfeld*, Ahuva Shina*, Seymour Rosen. Dept. of Med., Hadassah Univ. Hosp., Mt. Scopus, Jerusalem, Israel & Dept. of Pathology, Beth Israel Hosp. & Harvard Med. Sch., Boston, MA.

The effects of AII upon medullary hypoxic injury and kidney function were investigated *in vitro* and *in vivo*. Synthetic AII added to perfusate ($10^{-4} M$) of isolated rat kidneys reduced perfusion flow from 48 ± 2 ml/min (+ SE) to 19 ± 1 ($p < 0.001$) without altering GFR, raising filtration fraction from 1% to 3% ($p < 0.001$). AII extended hypoxic injury to medullary thick ascending limbs (mTALs) from $66 \pm 4\%$ of tubules to 79 ± 3 ($p < 0.05$) in correlation with filtration fraction ($r=0.7$, $p < 0.001$). Addition of indomethacin ($10^{-4} M$) further extended medullary hypoxic damage to $89 \pm 2\%$ of mTALs ($p < 0.001$).

Uninephrectomized rats kept in metabolic cages were given AII by continuous infusion (0.1-0.6 μg /min) and indomethacin (10 mg/kg/day) for 24 hrs. Creatinine clearance declined from 1.3 ± 0.1 ml/min to 0.6 ± 0.06 ($p < 0.001$). Morphological examination revealed either selective necrosis of mTALs (in $12 \pm 4\%$ of tubules) or luminal collapse (in $63 \pm 8\%$). Both necrosis and collapse correlated inversely with creatinine clearance and with each other ($r=0.9$, $p < 0.001$), the latter correlation suggesting protection from hypoxic injury by cessation of solute delivery.

By increasing filtration fraction, AII decreases renal oxygen supply while maintaining oxygen consumption for solute reabsorption. AII may predispose to acute renal failure by augmenting medullary hypoxia.

QUANTITATIVE X-RAY IMAGING OF NORMAL AND GENTAMICIN INJURED PROXIMAL TUBULES. R.E. Bulger, J.D. Stockton*, B.A. Sunderland*, and A.J. Saubermann*. Microprobe Center, Univ. of Texas HSC, Houston, Texas.

New quantitative x-ray imaging methods (Gorlen et al 1984; Saubermann and Heyman, 1987) were applied to normal and gentamicin (60mg/kg for 9 days)-injured proximal tubules. Quantitative digital x-ray images (64x64 pixels) were obtained which combined a morphologically relevant image with actual elemental concentrations. Using a STEM micrograph of tissue and a computer image of P or H₂O, areas (6-210 pixels) from control (C), condensed (D), swollen (S) or necrotic (N) cells were identified for elemental analysis:

	areas	Na	P	Cl	K	H2O
C	41	37	132	44	101	72
D	81	75*	190**	86**	139**	60**
S	45	85*	110+	66*+o	100+	80*+o
N	30	100*	141	96 o	96	68 o

Sig. dif. (p<.05): from C**; DvsS**+; SvsN=o

The injured cell first appears condensed and has a significant increase (†) in Na, P, S, Cl, K which is more than expected from the decrease(+) in cell H₂O. The next step, cell swelling, demonstrates a †P, †S, †Cl, †K, and †H₂O. When compared to controls, there is an overall increase in cell Na, Cl, and water. In changing from swollen to necrotic, the cell experiences a †Cl and †H₂O. When compared to controls, however, only Na and Cl differ significantly.

EFFECT OF VERAPAMIL ON GLOMERULAR PROSTAGLANDIN PRODUCTION AND GLOMERULAR FILTRATION RATE DURING CYCLOSPORINE ADMINISTRATION. M. Bunke, L. Wilder*, Div. of Nephrology, Univ. of Louisville and Louisville VAMC, Louisville, KY.

Verapamil (V) has been reported to ameliorate the decrease in glomerular filtration rate (GFR) produced by cyclosporine (Cy). To test the hypothesis that V protects GFR by altering glomerular prostaglandin (PG) production, we administered Cy, 20 mg/kg, and V, 5 mg/kg BID (Cy+V), or Cy and saline (Cy+S) subcutaneously to pair fed Sprague Dawley rats for 7 days. Urine was collected for 24 hours on day 7 for determination of creatinine clearance (Ccr) and fractional excretion of sodium (FeNa). Kidneys were perfused free of blood and glomeruli harvested by a sequential sieving technique. PG were determined by RIA. Ccr was significantly decreased in Cy+S when compared to Cy+V (Cy+S = 0.297 ±.119 ml/min per 100 gm BW, N=13 vs Cy+V = 0.408 ±.084, N=12, p=.02). FeNa was increased in Cy+V when compared to Cy+S (Cy+V = 0.52 ±.19% vs Cy+S = 0.32 ±.14%, p=.022). The data for glomerular PG production expressed as ng per mg protein per 45 minute incubation are below (mean ± SD, N=9 each group):

	PGE ₂	6KF ₁	TxB ₂	PGF ₂
Cy+V	9.12 ±.91	1.82 ±.26	0.349 ±.047	3.85 ±.54
Cy+S	12.12 ±1.70	2.24 ±.26	0.307 ±.019	2.76 ±.34
p value	.0004	.0034	.023	.0002

We conclude: 1.) V ameliorates the decrease in GFR produced by Cy, 2.) V increases FeNa during Cy administration, 3.) V produces a decrease in glomerular PG production, 4.) V doesn't protect GFR by increasing glomerular vasodilatory PG production.

GENTAMICIN RENAL CORTICAL UPTAKE AND NEPHROTOXICITY IN THE RAT WITH A REMNANT KIDNEY. Jorge C. Busse*, Helen Alpert*, Rebecca Papendick*, Larry Spiegelman*, and Carlos Vaamonde, Dept. of Medicine, VAMC, and University of Miami, Miami, FL.

It is unclear if renal insufficiency alters the threshold for gentamicin (G) induced nephrotoxicity (G-NTX). A remnant kidney (RK) model was established (by two-stage surgical ablation) to assess renal cortical G accumulation (rG) and G-NTX in female Sprague-Dawley rats (RK, n = 18). Baseline creatinine clearance (Ccr) in RK ranged from 35 to 88% of presurgical values. All rats were fed a normal protein (24%) diet. Control (C, n = 7) rats received G (40 mg/Kg/day) sc for 9 days, whereas RK received G adjusted for Ccr.

	Baseline Ccr (ml/min)	Ccr (ml/min)	plasma G (µg/ml)	rG (µg/g)
RK	.6±.05	.3±.04†	29±5	349±26
p	<.001	<.005	NS	<.001
C	1.8±.1	.6±.1†	35±8	601±59

X±SE; †p <.001 from baseline.

After G, RK rats had less rG and a lesser decrease in Ccr than C despite similar plasma G levels. The RK group was evaluated according to the magnitude of the decrease in Ccr after surgery (> or <55% reduction). Rats with the lower GFR (n=10) had no significant G-NTX (Ccr .43±.04 to .34±.05 ml/min), whereas those with the greater baseline GFR (n=8) had significant G-NTX (Ccr .77±.04 to .34±.07; <.001) despite similar rG levels (334±22 vs 367±54 µg/g). In conclusion, rats with decreased renal mass exhibit resistance to the nephrotoxic effects of gentamicin.

EFFECTS OF EXOGENOUS ADENINE NUCLEOTIDES (EAN) ON INTRACELLULAR ATP AND ADP IN ISCHEMIC CELLS. P. Cadnapaphornchai, D. Kellner, A. Golembieski and F.D. McDonald. Department of Medicine, Wayne State U, School of Medicine, Detroit, MI.

Previous studies suggest that EAN improve kidney function and increase intracellular AN after ischemia. We studied the effects of EAN on cellular ATP and ADP levels immediately and 24 hours after the recovery of primary cell cultures of micro-dissected rabbit S3 segments from 3-6 hours of ischemia. In this study, exogenous ATP was added immediately after ischemia. Intracellular ATP and ADP were determined at 24 hours; their levels were expressed as X10 M/1000 cells. Cellular ATP levels were .77±.04, .77±.05, .86±.04 and .71±.05 (mean±SE) with the addition of 0, .05, .1 and 1mM of ATP. The ADP levels were .20±.01, .17±.02, .25±.01 and .20 ±.03 respectively. The levels of ATP for the corresponding time-control groups were 1.11±.04, .94±.08, .92±.07 and .97±.09, respectively. In a separate study, .25mM each of adenosine, AMP, ADP and ATP were added immediately after ischemia and incubated for 2 hours. Determinations of cellular ATP and ADP were done at 24 hours. The ATP levels were .83±.07, .79±.06, .77±.02 and .78±.02 after adenosine, AMP, ADP and ATP, respectively. Likewise, ADP levels did not change significantly. In the present study, % cell viability as determined by dye uptake was also not influenced by addition of EAN. Thus, in primary cell culture of S3 segments, administration of EAN does not increase intracellular ATP or ADP after ischemia.

THE PROTECTIVE EFFECT OF ATRIAL NATRIURETIC FACTOR ON CYCLOSPORIN NEPHROTOXICITY. G Capasso, C Rosati, DR Giordano, NG DeSanto. (intr. by RA DeFronzo). Chair of Pediatric Nephrology, I Facolta' di Medicina, Napoli, Italy.

Nephrotoxicity is the most common and important side effect of cyclosporin (CyA) therapy. It is characterized by a fall in Glomerular Filtration Rate (GFR) and by a decrease in Sodium and water excretion. Since Atrial Natriuretic Factor (ANF) has been shown to increase GFR and to induce a potent diuretic and natriuretic effect, the protective action of ANF on CyA induced Acute Renal Failure (ARF) was investigated. To this end 5 normal rats were studied before (period 1) and after (period 2) CyA treatment (30 µgr/Kg IV) followed by ANF administration. In period 2 both GFR (0.453 ± 0.061 vs 0.827 ± 0.055 ml/min/100 gr BW) and urinary sodium excretion (U_{NaV}) (0.201 ± 0.099 vs 0.406 ± 0.128 µEq/min/100 BW) declined significantly. This effect was coupled with a decrease in urinary flow rate (1.0 ± 0.05 vs 2.0 ± 0.07 µl/min/100 gr BW). The intravenous administration of ANF (12 µgr/Kg as a prime and then 1 µgr/Kg/min as a constant infusion) resulted in a prompt restoration of GFR (0.894 ± 0.132 vs 0.827 ± 0.055 ml/min/100gr BW) while U_{NaV} increased 20-fold and was followed by a 10-fold increase in urinary flow rate. These results show that ANF reverses experimental acute renal failure induced by cyclosporine and indicate a potential use of Atrial Natriuretic Factor to minimize cyclosporine nephrotoxicity.

ACUTE HANTAVIRUS NEPHROPATHY : A "NEW" DISEASE. J.P. Clement*, R. Verhagen*, H. Leirs*, Th. Degrez*, G. van der Groen* (intr. by George A. Porter). Univ. of Antwerp, State Univ. Center Antwerp, UCL Univ. Hosp. Woluwe, Inst. Trop. Med., Antwerp, Belgium.

Acute Hantavirus disease (HVD) is a newly recognized rodent-borne zoonose, caused by Hantavirus (HV). The clinical picture consists of thrombocytopenia and acute interstitial nephritis, giving rise to a variable degree of acute renal failure (ARF), mostly with marked proteinuria. In Europe, the clinical course is often mild and self-limiting. No cases have been reported so far from the US. A series of 7 Belgian cases is briefly described. Peak serum creat. ranged from 1.22 to 6.4 mg%, proteinuria from trace to 29 g/L. To study the prevalence of asymptomatic HVD in a standard Belgian population (mainly blood donors), a prospective case-control survey was started. The serotechnique used was an indirect immunofluorescence assay for specific IgG anti-HV antibodies. On a total of 19,890 sera screened, an overall seropositivity (titre $\geq 1/16$) of 1.35% was found, with a net preponderance for the forested regions (prevalence 2.2%). In a parallel zoological survey, a total of 3,026 wild small mammals (mainly rodents) were captured using live-trapping methods. On a total of 3,369 animal tests performed, 5.99% were positive (presence of HV-antigen in the lungs and/or seropositivity), confirming the red bank vole as the main rural host for HV.

HVD can be the cause of sporadic or clustered forms of unexplained but transient ARF which may superficially mimic leptospirosis. Only specific serology can give the clue to diagnosis.

HEPATIC GLUCOSE (G) AND AMINO ACID METABOLISM IN ACUTELY UREMIC DOGS. B Cianciaruso*, L Sacca*, V Terracciano*, F Marcuccio*, VE Andreucci*, and JD Kopple. University of Naples, Naples, Italy, Harbor-UCLA Medical Ctr and UCLA Los Angeles, CA. In acute uremia (AU) some reports indicate increased hepatic G output while others disagree, suggesting that hepatic G release is normal or low and is suppressed normally by insulin. We examined this controversy in 8 acutely anephric and 8 sham-operated dogs. Dogs had arterial, portal vein and hepatic vein catheters inserted and were studied awake during a baseline period and after infusion of insulin, 5 mU/kg/min, and G. Since plasma insulin and G are often both elevated in AU, the G was infused to maintain increased plasma G, $166 \pm SD12$ and 172 ± 8 mg/dl, in AU and sham dogs, respectively. AU dogs had a higher UNA, 3.3 ± 1.7 vs $0.5 \pm .2$ g/24h, $p < .005$. During baseline, hepatic G release was greater in shams $p < .05$. However, during insulin and G infusion, hepatic G uptake occurred in shams, whereas AU dogs had neither hepatic G uptake or release (AU vs sham $p < .05$). During G infusion, peripheral G uptake was impaired in AU vs shams $p < .001$, even though plasma insulin was greater in AU. During insulin and G infusion, there was hepatic lactate uptake in AU dogs, while hepatic lactate output occurred in shams. Fractional hepatic uptake of amino acids was greater in AU, but absolute uptake was similar in both groups. Thus, 2 factors may promote G intolerance in AU: impaired peripheral G uptake and impaired suppression of hepatic G output in response to hyperinsulinemia and hyperglycemia. This resistance to insulin and hyperglycemia may contribute to the enhanced catabolism observed in ARF.

EFFECT OF THYROID HORMONE (T4) ON RAT PROXIMAL TUBULAR LYSOSOMAL VOLUME AFTER GENTAMICIN. R. Cronin, L. Inman*, and M. Spindler*. VAMC and UTHSC Dallas, TX.

T4 decreases gentamicin nephrotoxicity, but the mechanism is unknown. Gentamicin characteristically increases the number and volume of proximal tubular lysosomes. We investigated whether T4 affected renal processing of gentamicin by studying proximal tubular lysosomal volume following 48 hours of gentamicin (30 mg/kg twice daily) in rats (GT4) that had previously received 10 days of T4 (10 µg/100 g body weight). A cortical homogenate was analyzed for the brush border enzyme Na,K-ATPase and blood and urine were analyzed for changes in renal function and renal excretion. The pair fed control group (G) received only gentamicin. At the end of the 12 day study the two groups did not differ in food intake, body weight, urine volume, U_{NaV} , or UKV. Plasma creatinine was slightly, but significantly higher in GT4 (0.46 ± 0.05 vs 0.35 ± 0.05 mg/dl, $p < .01$), but creatinine clearance was not different between the groups (0.57 ± 0.08 vs 0.73 ± 0.25 ml/min/100 g bw, NS). Na,K-ATPase in the cortical homogenate was higher in GT4 (28.2 ± 2.1 vs 22.7 ± 2.2 µM/mg protein/hr, $p < .005$). Blinded, computer-assisted electron microscopic analysis of outer cortical proximal tubules showed that lysosomes occupied a significantly smaller fraction of the cell volume in GT4 (8.1 ± 0.5 vs $11.6 \pm 1.1\%$, $p < .02$). Except for scatter foci of apical vacuolization, proximal tubular cells of both groups were otherwise normal.

The results show that chronic T4 administration alters renal tubular handling of gentamicin. Whether this effect is due to decreased cellular gentamicin uptake or enhanced gentamicin removal from the cell is unknown. The higher cortical Na,K-ATPase activity might favor the latter possibility.

GLYCINE PROTECTS PROXIMAL TUBULES FROM INJURY BY A VARIETY OF METABOLIC INHIBITORS J.A. Davis,* M. Abarzua,* T. Rajan,* and J.M. Weinberg. VA Med. Ctr. and Univ. of Mich. Ann Arbor, MI.

We have recently reported that glycine (Gly) provided as a free amino acid or derived from catabolism of glutathione (GSH) protects isolated rabbit proximal tubules from hypoxic injury (JCI, in press). To determine whether this protection is generally applicable to injury associated with inhibition of mitochondrial function and depletion of cell ATP we tested the effects of 2 mM Gly or 2 mM GSH on tubule cell injury produced by potent metabolic inhibitors under oxygenated conditions. Tubules were incubated at 37°C for 30 min. followed by exposure to inhibitor with either no further additions, Gly or GSH for 15 min. then measurement of % free LDH. *p < 0.05 or better vs. corresponding no addition group. Values are means ± SE for N=5. Untreated time controls had 12.7 ± 0.4% free LDH.

	No addition	Gly	GSH
10 μM CCCLP	38.8 ± 1.3	15.6 ± 0.7*	18.2 ± 1.5*
10 μM antimycin	41.8 ± 2.4	14.0 ± 0.3*	20.7 ± 1.6*
1 μM rotenone	28.0 ± 3.4	14.7 ± 1.0*	16.2 ± 1.2*
10 μM rotenone	40.3 ± 3.0	14.6 ± 0.5*	20.4 ± 4.1*
15 μM oligomycin	37.3 ± 3.5	17.2 ± 0.8*	16.7 ± 1.5*
2 mM cyanide	48.1 ± 3.9	16.1 ± 2.6*	23.8 ± 1.2*

Measurements of tubule respiratory rates confirmed effects of inhibitors on mitochondrial function. All inhibitors markedly reduced cell ATP levels. Protection by Gly and GSH was not accompanied by improvement of cell ATP. Thus, Gly protection is not limited to hypoxic injury. These agents will be valuable for further probing the mechanisms by which Gly protects.

RENAL FAILURE (RF) AND NEPHROGENIC DI (NDI) AS DIFFERENTIAL ISOTOPIIC EFFECTS OF LITHIUM (Li) 6 AND 7. J. Durr,* A. Mathew,* N. Miller,* and A. Alfrey. Univ. of Colo. and VAMC, Denver, Colorado.

The large difference in charge mass ratio of the two naturally occurring isotopes, Li₆ and Li₇, suggests their metabolism and toxicity might be different. To study this possibility, Li_N (natural abundance 93% Li₇ and 7% Li₆ and Li₆ (95% purity) were administered to rats with I₁₂₅ iothalamate via intraperitoneal minipumps over 8 days. At serum Li_N levels of 0.14 ± 0.01 mEq/L there was no effect on GFR (3.1 ± 0.2 ml/min) or U_{osm} (2515 ± 137 mOsm/kg). In contrast, at similar serum levels of Li₆, 0.18 ± 0.02 (ns), RF (GFR 1.76 ± 0.3 ml/min, p < .004 vs Li_N) developed, whereas U_{osm} (3135 ± 440 mOsm/kg) was not different from Li_N. With larger dosage of Li increasing serum Li_N to .52 ± 0.036 and Li₆ to .46 ± 0.02 mEq/L, Li_N animals had significantly higher urine volumes (131 ± 27 vs 55 ± 9 ml/day and lower U_{osm} (342 ± 82 vs 704 ± 101) (p < .001). Fractional excretion (FE) Li₆ and FE Li₇ were not different, and both correlated with 1/GFR (r = .90, p < .001) and rising FE_{Na} seen with RF (r = .93, p < .001).

In conclusion, tubular handling of both isotopes were similar with FE_{Li} increasing with renal failure, suggesting that the enhanced FE_{Na} in RF is due to proximal rejection. However, the nephrotoxicity of these two isotopes is dissimilar in that Li₆ has a much greater effect on renal function and less effect on concentrating ability than Li₇. These findings could have clinical relevance since comparable serum levels of Li₆ as found to be toxic in rats could be obtained in humans receiving lithium therapy.

DIETARY PROTEIN INTAKE AFFECTS THE RENAL RESPONSE TO CYCLOSPORINE IN UNINEPHRECTOMIZED RATS. Robert Feingold,* Howard Weizman,* Jonathan Winston and Robert Safirstein. Mt. Sinai School of Medicine, New York, NY.

We have previously reported that the fall in glomerular filtration rate (GFR) induced by cyclosporine (CsA) in uninephrectomized rats (UNX) occurs without a change in renal blood flow (RBF) when the animals have limited food intake. To investigate further the role of dietary factors in this model we varied the protein intake of rats prior to UNX. Male Sprague-Dawley rats were offered 30gm/day of an isocaloric high (HP:40%) and low (LP:5%) protein diet. Following ten to fourteen days of this diet, rats underwent UNX and received either CsA (30mg/kg im) or vehicle (olive oil) daily for fourteen days at which time GFR and RBF were measured. The results are: (*p < .010 CsA vs dietary control, **p < .005 vs control-HP).

	CONTROL-HP n=7	CsA-HP n=7	CONTROL-LP n=5	CsA-LP n=5
GFR (ml/min)	2.45	0.96*	1.41**	0.72*
+SEM	0.34	0.26	0.20	0.12
RBF (ml/min)	18.14	10.94*	8.03**	9.20
+SEM	5.31	1.49	2.67	0.87

In HP rats given CsA, GFR and RBF were lower than in HP controls. LP rats given CsA had a lower GFR than LP controls but there was no change in RBF. In conclusion, in the uninephrectomized rat low protein intake prevents the renal vasoconstrictive effect of CsA but does not prevent the fall in GFR.

GENTAMICIN (G) CAN PRECIPITATE HYPOPERFUSION ACUTE RENAL FAILURE (ARF). L. Gamelin*, and R.A. Zager. University of Washington, Seattle, Washington.

Septic shock is a prime indication for aminoglycoside antibiotics. Thus, the purpose of this study was to assess whether acute G therapy represents a risk factor for the development of hypoperfusion ARF. One hr of renal hypoperfusion (H) (55-60 mm Hg; renal blood flow, RBF, 1.6 ± 0.13 ml/min) was created in rats by partial aortic constriction. G, 120 mg/kg, was given s.q. at the start of the H period. Rats subjected to sham surgery/G injection or H/sham G injection served as controls. H alone was extremely well tolerated, inducing no ATP depletion, azotemia, tubular necrosis, or enzymuria (N-acetyl glucosaminidase). The only evidence that ischemic injury occurred was mild S₃ proximal tubular brush border blebbing. G alone induced no azotemia or tubular necrosis. However, H + G caused severe ARF (BUN 94 ± 16, Cr 1.74 ± 0.22 mg/dl, extensive S₃ tubular necrosis; at 24 hr). S₁ and S₂ tubular segments (the major targets of G toxicity) showed minimal damage. H resulted in an immediate, 4-fold increase in G uptake which persisted for 24 hr. However, matching this degree of uptake in control rats by increasing the G dosage or by inducing transient ureteral obstruction induced no renal damage. G did not affect RBF, MAP, or ATP during H. Conclusion: G is extremely nephrotoxic if administered under H conditions. This effect is not explained by increased drug uptake or by altered renal perfusion. Rather, it is due to G's ability to convert a sublethal ischemic S₃ tubular cell insult into a severe necrotic event.

Na-K ATPase ACTIVITY FOLLOWING RENAL ISCHEMIA.

Karen M. Gaudio, G. Thulin*, M. Kashgarian and N.J. Siegel, Depts of Peds and Path, Yale Univ Sch of Med, New Haven, CT

The role of Na-K ATPase in acute renal failure remains controversial. The functional activity of Na-K ATPase was studied by comparing O_2 consumption (O_2 , $ml/min/mg$ prot) under basal (B), nystatin stimulated (N) or ouabain inhibited (O) conditions in suspensions enriched in proximal tubules obtained from rats after either 15 (15R) or 120 (120R) min of reflow following 45 min of ischemia or non-ischemic animals (C):

	C	15R	120R
B	34±0.8	22±0.6	23±0.7
N	55±2.2	32±1.5	44±3.1
O	11±0.9	17±1.7	13±0.8

Therefore, ischemia causes a significant ($P<0.01$) reduction in a) maximal respirations (NO_2), b) reserve pump activity (difference between NO_2 and BO_2), c) functional pump activity (difference between BO_2 and OO_2) and d) maximal functional activity (difference between NO_2 and OO_2). However, when total Na-K ATPase is measured chemically, it is similar in all groups: 151.7±5 μg Pi/mg prot/10 min (C), 153.9±9.8 (15R) and 161.6±13.8 (120R).

Thus, functional Na-K ATPase activity is diminished while total Na-K ATPase activity remains unaffected by an ischemic renal insult. This would suggest that some Na-K ATPase units are present but functionally inactive immediately after ischemia.

THE ROLE OF PLATELET ACTIVATING FACTOR IN ENDOTOXEMIC ACUTE RENAL FAILURE IN THE RAT. B.Ha*, JP Tolins, G Vercellotti*, HS Jacob*, L Raij, U. of MN and VAMC, Minneapolis, MN.

In the rat, infusion of endotoxin (LPS) results in oliguric acute renal failure with decreased RBF and GFR, in the absence of changes in systemic hemodynamics. The mechanism of this specific adverse renal effect is unknown. Experimentally, intrarenal infusion of platelet activating factor (PAF) induces similar renal hemodynamic changes, also without systemic effects. In vitro, stimulated rat glomeruli have been shown to produce PAF. We, therefore, postulated that intrarenal production of PAF may mediate the adverse renal hemodynamic effects of LPS. We measured GFR and RBF (flow probe) before and after LPS (20mg/kg iv) in rats pre-treated with the PAF receptor antagonist BN 52021 (BN, 15 mg/kg iv) or vehicle (VEH). Results (±SE, $P<0.05$: † vs baseline, Δ BN vs VEH, n=6-8):

	MAP	UV	GFR	RBF
	mmHg	$\mu l/min$	ml/min	%change
BN baseline	125±5	6.2±.8	1.17±.02	----
post-LPS	126±7	3.5±.5†	0.46±.05†Δ	-39±3†Δ
VEH baseline	122±5	6.8±1.2	1.06±.06	----
post-LPS	120±6	1.5±.8†	0.15±.08†	-63±5†

Pretreatment with BN resulted in significant improvement in GFR (3 fold) and RBF (1.6 fold), as well as a tendency to less severe oliguria after LPS, when compared to VEH. Thus, pretreatment with the PAF receptor antagonist BN 52021 ameliorates LPS-induced ARF suggesting that PAF may mediate, at least in part, the specific adverse renal hemodynamic effects of LPS.

EFFECT OF IRRADIATION ON RENAL HEMODYNAMICS AFTER RELIEF OF URETERAL OBSTRUCTION: A POSSIBLE ROLE FOR INFILTRATING CELLS. K. Harris*, G. Schreiner, and S. Klahr. Renal Division, Washington University, St. Louis, MO

We have observed an influx of leukocytes, predominantly macrophages, into the cortex and medulla of the kidney following ureteral obstruction. To examine the potential contribution of these infiltrating cells on the decrease in GFR and RPF that occurs following ureteral obstruction, 14 male Sprague-Dawley rats (wt 247±4.6 g) were studied in the awake state 3 hr following unilateral release of 24 hr of bilateral ureteral obstruction (BUO). Group I (n=8) were untreated controls, Group II (n=6) received 1300 rads one day prior to obstruction. In an additional 8 rats the effect of irradiation on the leukocyte infiltrate following obstruction was studied. Irradiation increased post-obstruction GFR (1.58±0.12 vs 2.92±0.84 $ml/min/kg$ BW, $p<0.001$) and RPF (7.23±0.65 vs 12.95±0.84 $ml/min/kg$ BW, $p<0.001$) with filtration fraction remaining unchanged. Urinary excretion of thromboxane B₂ was significantly reduced in the irradiated animals (19.03±1.94 vs 32.46±4.95 pg/min , $p<0.03$). Irradiation also reduced cortical and medullary infiltration of leukocytes following BUO (27.05±3.07 vs 1.2±0.83 and 13.6±1.79 vs 0.85±0.45 $\times 10^5$ cells/gm tissue, $p<0.001$). Thus, abolition of infiltrating cells is associated with an improvement in post-obstructive renal function suggesting that infiltrating cells may modulate the changes in renal function following obstruction, possibly via the production of vasoactive substances such as thromboxane.

ATRIAL NATRIURETIC PEPTIDE (ANP) - STIMULATED PROSTACYCLIN PRODUCTION RESULTS IN INCREASED GFR IN BILATERAL (B) VS UNILATERAL (U) HYDRONEPHROSIS (UO). S.L. Himmelstein*, W.E. Yarger, and P.E. Klotman, Duke Univ. and Durham VA Medical Centers, Durham, NC

Unilateral hydronephrosis is associated with intense renal vasoconstriction even following release of the obstruction. This profound alteration in renal hemodynamics is due, in part, to increased activity of the renin-angiotensin system and altered renal eicosanoid metabolism. In recent studies, Fried et al have demonstrated an increase in ANP concentration in the blood of BUO animals, yet ANP levels in UO animals are normal. We previously reported that ANP stimulates prostacyclin (PGI₂) production by UO kidneys. Therefore, in the present study, we investigated potential differences in renal excretory function and eicosanoid production in UO vs BUO animals. Inulin clearance (C_{in}) was measured in male Sprague-Dawley rats following 24 hours of either UO or BUO. Following in vivo studies, UO and BUO kidneys were perfused in situ and renal PGI₂ and thromboxane (TX) B₂ production were measured in extracts of renal venous effluent by RIA.

	UO	BUO
C _{in} (ml/min/kg)	0.63±0.03	1.26±0.04**
PGI ₂ (pg/min)	405.5±73.6	979.6±198.3**
TXB ₂ (pg/min)	234.4±32.3	125.9±16.7*

* $p<0.025$, ** $p<0.0005$

GFR of BUO animals was significantly greater than that of UO animals. PGI₂ production was significantly increased and TXB₂ production was significantly decreased in BUO animals when compared to UO. Thus, the mechanism for improved renal excretory function by BUO animals may be related to increased production of the vasodilator PGI₂ and decreased production of the vasoconstrictor thromboxane. Higher circulating levels of ANP in BUO may account for these differences in eicosanoid metabolism.

ATTENUATION OF CYCLOSPORINE (CsA) TOXICITY IN RENAL TUBULE CULTURES BY CALCIUM RESTRICTION AND PROTEASE INHIBITION. David Hreniuk and Patricia D. Wilson. UMDNJ-RWJ Med. Sch., Dept. Physiol & Biophys., Piscataway, N.J.

A major side effect of CsA is nephrotoxicity. To determine whether renal tubule damage occurs in the absence of hemodynamic alterations, CsA (50ng to 1mg/ml) was added to cultures of human proximal convoluted (PCT), proximal straight (PST), cortical thick ascending limb (TAL) and collecting tubules (CCT). Toxicity was determined as percent lactate dehydrogenase (LDH) release and nigrosine uptake. Time dependent CsA toxicity was seen in all tubule cells at doses of 100 ng/ml and greater. In PST, 50% cell death was elicited by CsA: 100ng/ml 30hr; 1µg/ml 4hr; 10µg/ml 1hr; 50µg/ml 30min. PCT and PST were more sensitive than CCT or TAL. CsA toxicity was reduced by incubation in media lacking Ca or in full media containing the Ca channel blocker verapamil (5×10^{-7} M); or the cysteine protease inhibitor E64 (50µg/ml). Viability was:

	Control(+Ca)	0 Ca	Verapamil	E64
PST	20±5	44±9	58±11	52±15
CCT	2±1	41±11	49±13	54±9

No protection was afforded by prior depletion of cellular lysosomal enzymes by incubation in media containing NH_4Cl (10 mM, 3 days) and CsA did not induce rupture of lysosomes preloaded with the fluorescent marker Lucifer yellow CH (1mg/ml, 16hr). We conclude that CsA exerts direct toxic effects on renal tubule epithelial cells that are mediated by calcium dependent, non-lysosomal, cysteine proteases. Toxicity can be attenuated by calcium restriction and cysteine protease inhibition.

DISSOCIATION OF CELL CALCIUM (Ca^{++}) OVERLOAD FROM PROXIMAL TUBULE (PT) CELL INJURY. HD Humes, JM Messana*, DA Cieslinski*, K. Gulyas*, VAMC and Univ. of Mich., Ann Arbor, MI.

Cell Ca^{++} overload is thought to play a major role in ischemic cell injury. We have reported that incubating rabbit renal PT segments (PTS) with 2 mM ATP-Mg Cl_2 protects them against hypoxic injury, despite causing greater than 5x increases in PTS Ca^{++} content. Experiments were done to demonstrate the location of excess Ca^{++} in PTS treated with ATP. PTS incubated with ATP and 95% $\text{O}_2/5\% \text{CO}_2$ for 105 min were spun through bromododecane into sucrose (S) containing one or more of the following: CCLCP (C), EGTA (E), A23187 (A), digitonin (D), and Ruthenium Red (R). Amount of Ca^{++} released into supernatant was measured as (nmole Ca^{++} /mg protein); n=8; *, p<0.05 vs preceding value or S, respectively.

S	C	E	E+C	E+A+C	D+R	D+R+C	D+C
6.3	26.4*	27.3	32.4*	50.8*	27+	30*	34*

ATP incubation led to an increase in PTS Ca^{++} content from control of 10 to 62 nmoles/mg prot (p<0.001) without declines in PTS viability. E and D, at concentrations used, probably act to release plasma membrane bound Ca^{++} . C-releasable Ca^{++} is of mitochondrial origin. Difference between C and A releasable pools represents non-mitochondrial sequestered Ca^{++} pools. Thus, increased cell Ca^{++} seen after incubation with ATP is associated with mitochondria, endoplasmic reticulum, and probably plasma membrane. Despite significant increases in these Ca^{++} pools, tubule cell injury did not occur, thereby dissociating cell injury from both mitochondrial and non-mitochondrial Ca^{++} overload.

ROLE OF CYTOSOLIC FREE CALCIUM (Ca_f) IN RENAL TUBULE DAMAGE DURING ANOXIA. William R. Jacobs* and Lazaro J. Mandel, Dept. of Physiology, Duke Univ., Durham, NC 27710.

Numerous investigators have suggested that elevated Ca_f during oxygen deprivation contributes to subsequent renal tubule injury. While some studies have reported an increase in Ca_f during anoxia, the relationship between changes in Ca_f and cell injury is not clear. In the present study, Ca_f and cell damage were simultaneously measured in Fura 2 loaded suspensions of rabbit cortical tubules subjected to prolonged anoxia. In preliminary studies, Fura 2 loading was found to have no effect on the severity of anoxia induced damage. For measurement of Ca_f during anoxia, suspensions were incubated at 37°C in the cuvette with argon equilibrated medium and an O_2 electrode, placed just below the surface of the medium. The onset of anoxia was verified by recording zero O_2 in the medium and simultaneously confirming tubule NAD reduction. No significant change in Ca_f was observed following 2 min (110 ± 15 vs 103 ± 13 nM, n=6) 20 min (132 ± 23 vs 119 ± 24 nM, n=5) or 40 min (145 ± 10 vs 134 ± 14 nM, n=6) of anoxia. Parallel measurements of tubule damage revealed a significant increase in LDH release following 40 min of anoxia (from 2.5 ± 1.0 to $20 \pm 5\%$ of the total). To rule out the possibility that intracellular Fura 2 is Ca-insensitive during anoxia the effect of ionomycin (Iona) on Ca_f was examined. Iona increased Ca_f within 2 min from 130 ± 20 to 495 ± 60 nM, n=3, in oxygenated samples and from 120 ± 25 to 660 ± 60 nM in paired samples subjected to 20 min of anoxia. These results demonstrate that large changes in Ca_f do not precede renal tubule damage during anoxia.

DJENKOLIC ACID (DA) INDUCED ACUTE RENAL FAILURE (ARF). C. Jarusiripipat*, J.I. Shapiro, and L. Chan. Univ. Colorado Sch. Med., Denver, CO.

Djenkol-bean (DB), a food relished in Southeast Asia, can occasionally cause a syndrome of djenkolism which is characterized by loin pain, hematuria and anuria. The purpose of this study is to establish an experimental model of DB-induced ARF and to examine the effect of DA, an active ingredient of DB, on renal function. Injection of 20mg DA i.p. daily resulted in acute renal dysfunction, oliguria and formation of needle-crystals in the urine. To examine the direct effect of DA on the kidney and to dissociate direct renal toxicity from the systemic effect of djenkolism, isolated rat kidneys were used. The kidney was perfused for 90min at 37°C at 100mmHg using albumin 6.7% in Krebs-Henseleit saline supplemented with amino acids and 5mM glucose. Physiologic parameters of renal plasma flow (RPF), urine flow (V), inulin clearance (Cin), Na reabsorption and K excretion were measured. Kidneys that were perfused under normal control conditions (C1) were compared with perfusion in which DA (1µg/ml) added to the perfusion medium (D1). D1 has lower Cin when compared with C1 (240 ± 80 vs 570 ± 80 µl/min, p<.01, n=8). To demonstrate the acute toxic effect of DA, a second protocol was used in which DA (1µg/ml) was added to the perfusion medium after a control period of perfusion for 45min. Addition of DA resulted in a similar fall in Cin (319 ± 70 vs 490 ± 99 µl/min, p<.05, n=5), but without any significant changes in Na reabsorption, K excretion, RPF and V. These results indicate that DA may be the main determinant of the syndrome of djenkolism and ARF after ingestion of DB.

INCORPORATION OF AMPHOTERICIN B (AMB) INTO LIPOSOMES ALTERS AMB-INDUCED ACUTE RENAL TOXICITY IN RABBITS. *Véronique Joly*, *Janine Barge*, *Françoise Dromer*, *Patrick Yeni*, *Nathalie Seta*, *Christiane Courreau*, and *Claude Carbon*. Inserm U 12, Paris, France.

The acute nephrotoxicity consecutive to the infusion of free AMB was compared to that of liposomal AMB in rabbits. Animals were infused with saline (0.5 ml/kg/min) with inulin for 2h until equilibration before one of the following regimens was infused over 45 min: 1) AMB (10mg/kg), 2) AMB (4mg/kg), 3) AMB (10mg/kg) intercalated in liposomes (Lip-AMB), 4) liposomes alone and 5) saline. Glomerular filtration rate (GFR), fractional excretion of Na⁺ (Na FE) and K⁺ (K FE), and enzymuria (NAG) were studied before and after each treatment. AMB urinary excretion rate was measured and renal histology was performed. AMB (10 mg/kg) was immediately lethal. AMB (4mg/kg) reduced GFR and increased K FE and Na FE ($p < 0.05$), but NAG remained unchanged. Lip-AMB increased NAG ($p < 0.05$), but K FE, Na FE and GFR were unchanged. The same amounts of AMB were excreted during both regimens. Saline or liposomes alone induced no alteration of urinary parameters. Tubular necrosis was never observed. Our results show that liposomes prevent the lethal toxicity of 10 mg/kg AMB. They suggest that (i) AMB (4 mg/kg) exerts immediate nephrotoxicity through alteration of proximal tubular cell membrane and reduction of GFR, and (ii) liposomes suppress the cell membrane toxicity of AMB but could increase interactions between AMB and lysosomes leading to a different mechanism for cellular injury.

FLUORESCENCE STUDIES OF RENAL BRUSH BORDER MEMBRANES EXPOSED TO GENTAMICIN. *Barry Kirschbaum*. Medical College of Virginia, Dept. of Medicine, Richmond, Virginia.

The initial event in the development of aminoglycoside (AG) nephrotoxicity is binding of the polycationic drugs to negatively charged head groups of phospholipids on the surface of renal brush border membrane (BBM). Using the fluorescent probe, 8-anilino-1-naphthalene sulfonic acid (ANS), purified rat kidney BBM, and concentrations of gentamicin (G) at or below the levels reached in tubular fluid by customary pharmacologic doses, we have measured the change in fluorescence intensity (FI) that occurs when ANS partitions into the membrane. FI increased sharply at pH values below 6.1 which is the pH optimum for G-induced membrane aggregation and the pH routinely used during these studies. In the absence of added Ca²⁺, fluorescence of the ANS-BBM complex increased with the concentration of G. A double reciprocal plot of FI vs G gave a calculated K_d of 1.0 $\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{G}$. Membranes exposed to G, then washed to eliminate free G, still manifested increased FI with ANS even though the calculated G concentration was 0.1 $\mu\text{g}\cdot\text{ml}^{-1}$. The magnitude of FI increase seen with 1.0 and 5.0 $\mu\text{g}\cdot\text{ml}^{-1}$ levels of G was in the range observed for 1.0mM Ca²⁺. Both EGTA and EDTA exposed membranes exhibited enhanced FI with ANS. G and Ca²⁺ caused even further increases of FI of the chelator-treated membranes. Therefore, fluorescence methods utilizing ANS and other membrane probes provide a sensitive way of analyzing toxic effects of drugs and metals on renal cell membranes.

GENTAMICIN INDUCES A GENERALIZED DISRUPTION OF THE PHOSPHATIDYLINOSITOL (PI) CASCADE IN PRIMARY CULTURES OF RABBIT PROXIMAL TUBULAR CELLS (PCRPTC). *G.J. Kaloyanides*, *L. Ramsamy*, and *C. Josepovitz*. Dept. of Medicine, SUNY-Stony Brook and VAMC, Northport, NY.

We reported previously that gentamicin (G) inhibited generation of inositol triphosphate (IP₃) by PTH in PCRPTC. In the present experiment we tested the hypothesis that G inhibits the generation of IP₃ by all agonists known to activate the PI cascade in PTC. We examined the effect of G (10⁻³Mx24 hr) on the generation of IP₃ in response to PTH, angiotensin II (AII), phenylephrine (PE), bradykinin (BK) and arginine vasopressin (AVP) at 10⁻⁶M in PCRPTC preincubated with [³H]-inositol to label the PI pool.

Agonist	IP ₃ (dpmx10 ² /plate)		IP ₃ (dpmx10 ² /plate)	
	Control	G	Control	G
baseline	116±6	NS	127±6	15±1 NS
PTH	209±25**	p<.05	151±6*	25±1 p<.01
AII	228±16**	p<.01	159±10*	30±3 p<.01
PE	198±8**	p<.05	177±5*	27±1 p<.01
BK	225±10**	p<.01	156±3*	38±3 p<.01
AVP	243±18**	p<.01	158±12*	31±3 p<.01

*, **, significantly different from baseline, p<.05 and p<.01, respectively. All agonists stimulated significant increases of IP and IP₃ but not IP₂ (data not shown). G completely inhibited agonist generation of IP₃ and IP₂ and greatly reduced agonist generation of IP. The results are consistent with the hypothesis that G binds to phosphatidylinositol, 4,5 bisphosphate and prevents its hydrolysis by phospholipase C in response to agonist stimulation. Disruption of the PI cascade may contribute to the pathogenesis of aminoglycoside nephrotoxicity.

THE VULNERABILITY OF THE THIN DESCENDING LIMBS OF HENLE'S LOOP IN ISOLATED PERFUSED RAT KIDNEY.

*J. Kopolovic**, *M. Brezis*, *K. Spokes**, *P. Silva*, *F.H. Epstein*, *S. Rosen*. Depts. of Pathology and Medicine, Harvard Medical School & Beth Israel Hospital, Boston, MA, and Hebrew Univ.-Hadassah Medical School, Jerusalem, Israel.

In the isolated rat kidney (IPK) perfused without erythrocytes, the medullary thick ascending limb (mTAL) shows extensive injury. Damage to the thin descending limbs of Henle's loop (DL) has been mentioned only briefly. The DL was examined in IPK under a variety of conditions altering oxygenation and active transport in the medulla and known to affect injury to the mTAL. Necrotic DLs and mTALs were counted in an intersecting line 350-600 μ from the border of the outer stripe of the outer medulla. The short loops of DL were preserved in all experimental groups. The long loops were affected only in the upper portion (LDL) where the epithelium is more complex. In oxygenated kidneys, the necrosis involved 55% + 4.4% of the mTAL and 31% + 11.1% of the LDL. Under hypoxic conditions, the necrosis involved 84.4% + 6.0% of mTAL and 89% + 2.3% of LDL. Ouabain, furosemide and absence of filtration completely prevented the necrosis. A low protein diet, known to cause partial atrophy of these segments, reduced damage to the LDL ($p < 0.025$) and mTAL ($p < 0.001$) compared to control kidneys. The patterns of injury in LDL resembled that of the mTAL in all experiments, i.e., maneuvers that inhibit active transport limit injury and perturbations that decrease oxygen availability maximize injury.

EFFECTS OF CYCLOSPORINE A (CyA) ON CALCIUM SIGNALLING AND PROSTAGLANDIN PRODUCTION IN GLOMERULAR MESANGIAL CELLS (MC). S.G. Kremer*, B. Margolis*, H. Goldberg* and K.L. Skorecki*. Membrane Biology Group, Univ. of Toronto, CANADA.

Nephrotoxicity limits the use of CyA in organ transplantation. The acute manifestation of renal toxicity is a decrease in renal blood flow and glomerular filtration rate. We therefore investigated the effects of CyA on the cellular signalling response of vasopressin (VP) in cultured rat MC. We have previously reported (Kidney Int. 31:172, 1987) that VP causes a transient rise in cytosolic calcium in MC using the fluorescent calcium probe Indo-1. The effects of CyA were determined using adherent MC treated with CyA (10µg/ml) or CyA carrier for 2 hours. Under these conditions CyA treatment did not alter basal cytosolic calcium, nor the peak response to VP. CyA did however cause a significant prolongation of the calcium signal. As a quantitative measure of the effect, percent maximal fluorescence at 4 min. post 0.5nM VP addition was $40 \pm 4.5\%$ (n=7) in control cells vs. $66 \pm 3.5\%$ (n=6) in CyA treated cells. In addition CyA significantly inhibited both basal (control 3.6 ± 0.5 ; CyA treated, 1.9 ± 0.2 ; ng/mg protein/30min) and VP stimulated (control, 7.9 ± 0.6 ; CyA treated, 5.7 ± 0.4) prostaglandin E_2 production. We conclude that the increased renal and systemic vasoconstriction as well as the fall in glomerular filtration rate which accompanies CyA treatment, may be the result of an exaggerated rise in intracellular calcium combined with diminished production of vasorelaxant prostaglandins in response to vasoconstrictor hormones.

EPISODIC HYPERCHLOREMIA AND HYPERKALEMIA IN CYCLOSPORIN (CSA) TREATED HUMAN CARDIAC ALLOGRAFT RECIPIENTS: Mohsen Lachaal*, Susan Michalek*, Jacob Bergsland* and Basab K. Mookerjee; Dept. of Nephrology and Cardiovascular Surgery, SUNY at Buffalo, NY.

In renal allograft recipients receiving CSA, episodes of hyperchloremic renal tubular acidosis and hyperkalemia have been reported and attributed to CSA-nephrotoxicity (Bantle et al., Am.J. Med. 83:59, 1987). However, this interpretation is clouded by our previous report that allograft rejection itself may lead to similar findings (Mookerjee et al., Ann.Int.Med. 71:47, 1969). Here we report the occurrence of hyperchloremia (> 105 meq/L) and hyperkalemia (> 5.5 meq/L) in eight cardiac allograft recipients receiving CSA. All patients developed hyperchloremia (Mean 4.8 episodes per pt. ± 3 SD) and hyperkalemia (3.2 episodes per pt. ± 3 SD) which reversed spontaneously without reduction in CSA dosage. Hyperkalemia occurred only in the simultaneous presence of hyperchloremia but the opposite was not true. Serum creatinine concentration (Scr) was invariably above normal range during hyperchloremia but the latter did not predict further reduction in GFR nor indicate the need to reduce CSA dosage. Serum chloride concentrations did not correlate either with serum CSA levels (radioimmunoassay) or with Scr. Neither hyperchloremia nor hyperkalemia could be attributed to simultaneous therapy with other drugs or to other known causes. Unlike observations of Bantle, plasma renin activity tended to be elevated. These results suggest the long term CSA therapy in cardiac allograft recipients may be associated with episodes of hyperchloremia and hyperkalemia.

ALTERATIONS IN CALCIUM COMPARTMENTATION DURING ANOXIA IN PROXIMAL RENAL TUBULES. A. LeFurgey, L. J. Mandel, P. Ingram*, and P. Schreiner*. Dept. of Physiology, Duke University Medical Center, Durham, NC and Research Triangle Institute, Research Triangle Park, NC.

Many investigators have suggested that the movement and redistribution of cellular ion contents, especially calcium, play a key role in the pathophysiology of irreversible cell injury induced by oxygen deprivation. The objective of the current studies was to determine the *in situ* mitochondrial and cytoplasmic calcium content in kidney proximal tubule cells subjected to anoxia.

Quantitative electron probe x-ray microanalysis was performed on rapidly frozen, cryosectioned and freeze-dried thin sections of a suspension of rabbit proximal tubules incubated for 40' in the absence of oxygen. A heterogeneity in calcium compartmentation was observed in the anoxic tubules, with two main populations of cells discernible: 1) cells with morphological structures intact but with a decreased ratio of K:Na (~2:1) displayed an elevated cytoplasmic calcium content relative to control (11.3 ± 2.0 [n=17] vs 4.1 ± 1.4 [n=23] mmol Ca/kg dry wt), and a mitochondrial calcium content which was unchanged (3.5 ± 0.6 [n=15] vs 3.1 ± 1.1 [n=23] mmol Ca/kg dry wt); 2) cells with disrupted brush borders, swollen mitochondria and increased vacuolization exhibited a reversed K:Na ratio (0.4:1), a cytoplasmic calcium content which was not significantly altered (7.2 ± 1.7 [n=19] mmol Ca/kg dry wt), and a mitochondrial calcium content which was equal to or slightly less than control (1.0 ± 0.9 [n=20] mmol Ca/kg dry wt).

In conclusion: 1) net accumulation of calcium occurs in the cytoplasm of some of the proximal cells during anoxia; 2) no mitochondrial calcium accumulation is observed; 3) the dominant calcium buffering compartment is in the cytoplasm; and 4) calcium accumulation can be found without visible structural cellular damage.

FUROSEMIDE INHIBITS RENAL MITOCHONDRIAL OXIDATIVE METABOLISM AND Ca^{2+} UPTAKE. IMPLICATIONS FOR ITS PROTECTIVE EFFECTS IN ISCHEMIC ACUTE RENAL FAILURE. Charles D. Malis, Joseph V. Bonventre, and Horacio F. Cantiello. Mass. Gen. Hosp. and Harvard Medical School, Boston, MA.

Furosemide (F) has been found to be protective in various experimental models of ischemic renal injury. Since mitochondrial (mito) Ca^{2+} loading has been implicated in the pathophysiology of ischemic cell death, we examined whether F had any effect on mito Ca^{2+} metabolism. We found that F inhibits respiration and Ca^{2+} uptake of isolated renal mitochondria. F inhibits mito respiratory control ratio (RCR) in a bimodal fashion with K_i 's of 10^{-9} and 10^{-5} M. F also inhibited FCCP-uncoupled respiration. The inhibition of RCR and uncoupled respiration was greater with site I as compared with site II mito substrates. F directly inhibited the site I enzyme, NADH CoQ reductase. Effects of F were reversible and independent of extra mito K^+ or Cl^- concentrations. F decreased the mito transmembrane potential and inhibited Ca^{2+} uptake. Depressed mito respiration could also be observed in intact LLC-PK₁ cells exposed to 10^{-6} M F, a concentration which has little effect on the Na^+ , K^+ , Cl^- cotransport process.

In conclusion: 1) F inhibits mito respiratory function, particularly affecting site I of the electron transport chain; 2) The inhibition is reversible and independent of K^+ or Cl^- ; 3) F inhibits mito Ca^{2+} uptake possibly due to a F-induced depolarization; 4) The inhibition of mito Ca^{2+} uptake may explain the protection afforded by F in experimental models of acute renal failure.

DISSOCIATION OF LOW HEMATOCRIT FROM THE PROTECTIVE EFFECT OF SPLENECTOMY IN ISCHEMIC ACUTE RENAL FAILURE. Anil K. Mandal, Christiana E. Hall* and Jon M. Miller,* Dept. of Med. VAMC & Wright State Univ., Dayton, OH & Univ. of Oklahoma, Oklahoma City, Oklahoma.

Chronic splenectomy is protective against ischemic acute renal failure (ARF) in the dog. This study examines whether low hematocrit (HCT) mediates this functional and histopathologic preservation. Group I (n=7) was sham-splenectomized and groups II (n=5) and III (n=4) splenectomized 2 wks prior to bilateral renal artery occlusion (RAO) for 120 mins. In group III HCT was increased to 60% by infusion of packed RBCs during ischemia. Blood and urine samples were collected 1h pre(1); 1h(2); 24h(3); 48h(4); 72h(5) and 6d(6) post-RAO. HCT, serum creatinine (Scr), creatinine clearance (Ccr) and kidneys, examined by light and electron microscopy, were evaluated. T-tests determined differences between groups at each time period. HCT between I & III was not different at any time period; but group II HCT was lower than I at times 2-4(p<.05) and less than in III at all post-RAO periods(p<.05). Scr in group I was higher than group II(p<.05) at times 3&4; and higher than in group III at times 3-6(p<.05). Group III Scr was lower than group II(p<.05) at times 3,4&6. Ccr was not different between I and II; but III was higher than I at times 3&4(p<.01); and also higher than in II at time 4(p<.05). Histopathologic changes included tubular necrosis and dilatation in group I, blebs in some tubules in group II, but essentially no change in group III kidneys. The data indicate recovery from ischemic ARF is not mediated by low HCT; high HCT, in the absence of the spleen, is beneficial in RAO induced ARF.

SUBSTRATE DEPRIVATION, BUT NOT HYPOXIA, POTENTIATES CALCIUM-INDUCED MITOCHONDRIAL INJURY. JM Messana*, DA Cieslinski*, HD Humes. VA Med. Ctr. and Univ. of Mich., Ann Arbor, MI.

Mitochondrial injury and ATP depletion are critical processes in ischemic cell injury. We examined the effect of an ischemic environment on mitochondria (M) isolated from rabbit renal cortex. Ischemic conditions were simulated by exposing M to 75 nmoles $\text{CaCl}_2/\text{mg prot}$ (P) for 20 min with or without hypoxia. After 20 min, 5 mM pyruvate/malate (PM) was added and M incubated for an additional 20 min under oxygenated conditions. State 3 and 4 respiration and acceptor control ratio (ACR) were measured in presence of PM and ADP and expressed as natoms O/mg P/min. Uncoupled respiration was measured after addition of 2,4-DNP (DNP). n=4; * p<.05 vs O_2 control (C) + p<.05 vs N_2 C. Data reported as means.

Condition	ACR	State 3	State 4	DNP
O_2 Control	5.8	116	17	108
$\text{O}_2/\text{Ca}^{++}$	2.9*	84*	29*	68*
N_2 Control	5.4*	115	22*	114
$\text{N}_2/\text{Ca}^{++}$	2.6**	78**	30**	66**

To assess the role of substrate deprivation, M were incubated for 20 min with or without PM followed by addition of PM to all samples. Respiration measured as described above. n=3; * p<.05 vs O_2/S ; + p<.05 vs O_2/Ca .

Condition	ACR	State 3	State 4	DNP
O_2 /substrate	5.5	95	17	90
$\text{O}_2/\text{Ca}^{++}$	1.2*	43*	38	40*
$\text{O}_2/\text{Ca}^{++}/\text{substrate}$	3.8+	77+	21	72**
$\text{O}_2/\text{no substrate}$	4.5+	104+	23	99+

Thus, small amounts of calcium injure mitochondria and this M injury is markedly potentiated by substrate depletion but not by hypoxia.

THE EFFECT OF L-THYROXINE (T4) ON RENAL FUNCTION AND MEDULLARY THICK ASCENDING LIMB (TAL) MORPHOLOGY IN THE ISOLATED KIDNEY. S. Mills,* J. Shapiro, P. Shanley, G. Johnson,* and L. Chan. Univ. Colorado Sch. Med., Denver, CO.

In virtually all previous studies, physiologic or pharmacologic maneuvers that decreased TAL transport were noted to decrease morphologic evidence of injury to that segment in the isolated perfused kidney. As T4 is known to increase energy turnover in most tissues yet has been shown experimentally to protect against acute renal failure (ARF) in both ischemic and toxic models, we decided to examine its effects on renal function and TAL morphology in the isolated kidney. Rats were treated with T4 [1 $\mu\text{g/g b.w.}$ given i.p.] (E, n=7) or saline (C, n=7) for 3 days. Following this, the animals right kidney was perfused for 1 hr at 37°C at 100 mmHg perfusion pressure using albumin 6.7% in Krebs Henseleit saline supplemented with 5 mM glucose. Physiologic parameters of renal plasma flow (RPF), inulin clearance (Cin), net Na reabsorption (T_{Na}) and O_2 consumption (QO_2) were measured. Following perfusion, kidneys were studied histologically grading percentage TAL segments showing fragmentation (F) and nuclear pyknosis (P). No differences in RPF, Cin or T_{Na} were noted between E and C. E has higher QO_2 (2.4±0.2 vs 1.9±0.3 $\mu\text{mole/min/g}$, p<.05) but lower F (3.5±2.5 vs 20.4±8.1%, p<.05) and P (10.3±3.6 vs 17.6±3.1, p<.05). These data which show morphologic evidence of protection despite increased energy turnover may help explain the mechanism by which T4 ameliorates experimental ARF.

PROPERTIES OF PHOSPHOLIPASE C (PL-C) ALONG THE PROXIMAL TUBULE AND ITS INHIBITION BY GENTAMICIN (G). T. Moriyama*, H. Nakahama*, S. Shin*, A. Wada*, Y. Fujiwara*, Y. Fukuhara*, Y. Orita*, and T. Kamada*. Osaka Univ. Med. Sch., Osaka, Japan (intr. by J. S. Handler).

Phospholipidosis in renal membranes is a consequence of G nephrotoxicity. Therefore, we tested for G inhibition of membrane-bound PL-C. We prepared outer cortical (OC) and outer medullary (OM) brush border membranes (BBM) from rabbit kidney, and estimated the effects of G on PL-C activity in the two preparations. PL-C activity was assayed as ^3H -inositol 1-monophosphate (IP_1), hydrolyzed from phosphatidyl- ^3H -inositol in the presence of 3 mM Ca^{2+} , 3 mM deoxycholate and appropriate constituents. IP_1 was determined by an anion-exchange HPLC system developed in our laboratory (Biochem. Biophys. Res. Commun. 142; 70, 1987). In both preparations, G inhibited PL-C activity in a concentration-dependent manner. As shown in the table, 3 mM G decreased PL-C activity to less than 30% of control values.

	$^3\text{H-IP}_1(\text{dpm})/5 \text{ min}/10\mu\text{g BBM protein (Mean}\pm\text{S.D.)}$	
	OC BBM	OM BBM
Control	17517± 72	23251±350
3 mM G	4924±120	6927±452

We suggest that the inhibition of BBM PL-C by G leads to the increased phosphoinositides in renal membranes and contributes to G nephrotoxicity.

FUROSEMIDE ENHANCES THE GENTAMICIN INHIBITION OF Na⁺-DEPENDENT D-GLUCOSE TRANSPORT IN RABBIT RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV). H.Nakahama*, T.Moriyama*, Y.Fukuhara*, Y.Orita* and T.Kamada*. Dept. of Med., Osaka Univ. Med. Sch., Osaka, Japan. (intr. by J.S.Handler).

Furosemide (F), a potent loop diuretic, is known to potentiate gentamicin (G) nephrotoxicity

In our previous study, we demonstrated that G inhibits Na⁺-dependent D-glucose transport in BBMV. The outer cortical (OC) transport site is more vulnerable to G toxicity than the outer medullary (OM) transport site (Biochim. Biophys. Acta, 858:163, 1986). In this study, we investigated the effects of G and F in vivo on Na⁺-dependent D-glucose transport in BBMV. Rabbits received either 1 ml normal saline (group-C), G (20 mg/kg) alone (group-G), F (10mg/kg) alone (group-F) or combination of G and F (group-GF) intravenously.

	OC		OM	
	K _m (mM)	V _{max} (nM/min/mg prot)	K _m	V _{max}
C	3.5±0.3	97.5±25.3	0.80±0.16	38.1±7.4
G	6.3±0.2*	135.2±32.5	0.84±0.25	42.7±17.6
GF	6.3±0.4*	155.3±40.0	1.21±0.14*	47.6±12.2
F	4.1±0.4	119.5±29.8	0.71±0.17	30.8±6.8

*: p<0.05 as compared with C

While in group-G, only the OC transport site was impaired, in group-GF both OC and OM transport sites were impaired. F may potentiate G toxicity by enhancing G accumulation (74.3±12.4 µg/g wet tissue, G alone, vs 112.7±26.9, G+F, in OC, 82.4±18.3, G alone, vs 153.4±37.3, G+F, in OM).

EFFECT OF HUMAN ATRIAL NATRIURETIC FACTOR (ANF) ON POST-ISCHEMIC ACUTE FAILURE IN CONSCIOUS DOGS. H.-H. Neumayer, U.Seher-Thos, M.Blossei, K.Wagner. Dept. of Nephrology, Klinikum Steglitz, Free University of Berlin, FRG.

Human atrial natriuretic factor (ANF) ameliorates norepinephrin-induced acute renal failure (ARF) in rats. (Nephron 44:240-244, 1986). We therefore studied the effect of ANF (a-hANaP) on the course of postischemic ARF in chronically instrumented conscious dogs, subjected to 120 min of ischemia. In contrast to control group A (n=7, infusion of 0.9% saline), the investigational group B (n=7) received an intraaortic bolus injection of 100µg a-hANaP immediately after ischemia, and an additional infusion of 0.3 µg/min/kg over a period of 4 hours. At day 2 and 3 after ischemia only the bolus injection was repeated. Results are given as mean±SEM, *p<0.05, ***p<0.001 vs controls.

Group	before ischemia	after ischemia			
		day 1		day 3	
		A	B	A	B
RBF(ml/min)	158±19	163±27 / 208±20	127±22 / 148±11		
GFR(ml/min)	44±3	18±4 / 33±2 ***	20±4 / 34±2*		
P _{creat} (µmol/l)	80±5	204±37 / 124±14	173±34 / 103±7		
V _u (ml/min)	0.8±0.06	0.5±0.1 / 0.8±0.06	0.6±0.1 / 0.7±0.06		

Our data clearly demonstrate that bolus application in combination with a short-term infusion of a-hANaP does ameliorate postischemic ARF. This beneficial effect on renal function might be in part due to an improvement of renal perfusion by counterbalancing renal vasoconstrictors. But an enhancement of glomerular capillary pressure and an increase in the filtration coefficient (K_f) must also be considered as preferential action of ANF.

EFFECT OF GLUTATHIONE (G) DEPLETION ON HYPOXIA (H)-INDUCED INJURY TO RABBIT RENAL PROXIMAL TUBULE SEGMENTS (PTS). VD Nguyen*, JM Messana*, DA Cieslinski*, HD Humes. VA Med. Ctr. and Univ. of Mich., Ann Arbor, MI.

Hypoxic cell injury is associated with increased lipid peroxidation (LP) and depletion of cellular glutathione levels. We have previously reported that maintenance of PTS G levels with exogenous G supplementation protects against H-induced injury to PTS. To further investigate the role of G and LP in H-induced cell injury, suspensions of PTS were incubated with 1.5 mM diethyl malate (DEM) and 100 µM BCNU for 15 min, then subjected to 30 min H (95% N₂/5% CO₂) followed by 30 min of reoxygenation (95% O₂/5% CO₂) and measured for tubule total G (nmol/mg prot (P)), LP (nmol MDA/mg P), K⁺ (nmol/mg P), and CCCLP-uncoupled respiration (R) (natoms O/mg P/min). Data are presented as means; * denotes p<0.05 compared to control (C), + p<0.05 compared to H. n=4-6.

Conditions	G	LP	K	R
C	6.9	1.4	320	222
H	4.9*	1.9	233*	147*
H+DEM	1.4*+	1.9	216*	140*
H+BCNU	2.0*+	1.9	200*	130*

DEM and BCNU led to significant declines in total G levels. G depletion with these agents, however, did not potentiate the degree of cell injury produced by H, thereby suggesting that endogenous free radical generation during reoxygenation is not a critical factor in H-induced cell injury. The protection afforded by G supplementation and non-potentialiation of G depletion may, however, be explained by differential effects on the mitochondrial G pool.

PROTECTIVE EFFECT OF DEFEROXAMINE IN GLYCEROL-INDUCED ACUTE RENAL FAILURE IN THE RAT. Mark S. Paller, University of Minnesota, Minneapolis, Minnesota.

Glycerol injection produces myoglobinuria and hemoglobinuria and consequent acute renal failure. We tested the possibility that iron released from hemoglobin and myoglobin might mediate this renal injury by promoting formation of the free radical hydroxyl radical (OH·). Male Sprague-Dawley rats (220-290 g) were deprived of water for 15 hr and given an intramuscular injection of 50% glycerol (5 ml/kg). To chelate any released iron, deferoxamine (DFO) was given as a constant infusion for 7 hr (25 mg/kg x 1 hr, 12 mg/kg x 3 hr, 6 mg/kg x 3 hr). Control animals received an equivalent volume of 5% dextrose. Single kidney GFR (inulin clearance) was then determined. In control animals GFR fell to 133 ± 34 ul/min (n=5). DFO-treated animals had a 3-fold greater GFR: 374 ± 56 ul/min (n=5; p<0.01). Free radical-mediated lipid peroxidation was estimated by measuring renal malondialdehyde (MDA) content 4 hr after glycerol injection. Kidneys from animals not given DFO had a significant increase in MDA content from baseline: from 0.336 ± 0.058 to 0.842 ± 0.120 nmol/mg prot (p<0.05). In DFO-treated rats glycerol caused less lipid peroxidation (0.562 ± 0.137 nmol/mg prot; NS vs baseline or control). To further examine the role of iron in heme pigment nephrotoxicity, hemoglobin or methemoglobin was infused in separate animals and caused a decrease in GFR (from 1160 ± 120 to 842 ± 54 and 893 ± 93 ul/min, respectively). DFO prevented this hemoglobin-induced fall in GFR. We conclude that following glycerol injection iron is released from hemoglobin and myoglobin and promotes free radical formation and lipid peroxidation. Deferoxamine prevents renal injury in this form of acute renal failure by binding free iron and rendering it "non-toxic".

NEPHRON MODEL OF NEPHROTOXIC ACUTE RENAL FAILURE: EFFECT OF LOOP DIURETICS AND GLOMERULAR CAPILLARY HYDROSTATIC PRESSURE (P_G) RESPONSE. O.W. Peterson* and R.C. Blantz. UCSD and VAMC, La Jolla, CA.

We recently demonstrated that nephron filtration rate (SNGFR) in late proximal (P) and distal tubules (D) decreases within 5 min after 0.5 ng of uranyl nitrate (UN) is microperfused into the early proximal tubule of the same nephron (ICN, 1987). The effects of continuous addition of saline (SC), $2 \times 10^{-5} M$ (FL), $2 \times 10^{-4} M$ furosemide (FH), and $2 \times 10^{-5} M$ Bumetanide (B) to the early P of the same nephron on this UN protocol were examined. Controls (C) utilized saline perfusion and no UN. V_D = distal tubular flow rate. Data are expressed as pre-UN values and change (Δ) after UN- $t_p < 0.05$ paired from pre-UN (nl/min).

	SNGFR-P	Δ	SNGFR-D	Δ	V_D	Δ
C	42 \pm 2	+3 \pm 2	35 \pm 2	-1 \pm 3	13 \pm 1	-1 \pm 2
UN-SC	38 \pm 1	-9 \pm 2 ⁺	31 \pm 2	-5 \pm 2 ⁺	8 \pm 1	-2 \pm 1
UN-FL	34 \pm 2	-6 \pm 2 ⁺	30 \pm 3	-7 \pm 4 ⁺	12 \pm 1	-4 \pm 1
UN-FH	34 \pm 2	-0.3 \pm 2	34 \pm 3	-2 \pm 3	17 \pm 2	-1 \pm 2
UN-B	37 \pm 2	+2 \pm 2	33 \pm 2	-2 \pm 2	15 \pm 1	-1 \pm 2

Studies demonstrated no tubular backleak of inulin after UN such that SNGFR represents filtration. P_G was also measured directly and continuously before (51 \pm 3 mmHg) during (53 \pm 2), immediately (49 \pm 2) and 5 min after UN perfusion (53 \pm 3 mmHg) and did not change. Conclusions: 1) The reduction in SNGFR after UN microperfusion to early P tubules is a functional response to tubular damage. 2) This functional reduction in the absence of glomerular exposure to UN occurs without a change in P_G , similar to the systemic effects of UN. 3) Tubuloglomerular feedback activation logically causes SNGFR changes after UN since both FH and B prevent this response.

CULTURE OF VIABLE CELLS FROM URINE OF PATIENTS WITH "ACUTE TUBULAR NECROSIS" (ATN). L.C. Racusen, B. Fivush, K. Solez. Johns Hopkins Univ. School of Medicine, Baltimore, Maryland.

In "acute tubular necrosis" in man, renal biopsy usually reveals gaps along the tubular basement membrane where tubular cells have been lost and cell regeneration without overt focal necrosis (Medicine 58:362, 1979). Intact cells may be seen in the tubular lumina and in urine, and EM of urine sediment reveals intact ultrastructure in many voided calls (Kidney Int. 28:58, 1985). We have studied urine from patients with ATN in native or transplanted kidneys to further assess characteristics of voided tubular cells. In native kidney ATN, all patients voided viable tubular cells, viabilities ranging from 5-80%, as assessed by trypan blue exclusion. In patients with transplant ATN, 88% voided viable tubular cells, up to 1×10^5 cells/ml urine. Viabilities ranged from 8-100%. Some patients voided viable tubular cells for several weeks. Stains for brush border enzymes (alkaline phosphatase, gamma-glutamyl transpeptidase) confirmed proximal origin and helped differentiate tubular cells and leukocytes. Cells were seeded for culture at 1×10^5 - 1×10^6 cells/ml and grown in hormone-supplemented Hams F12:DMEM with tapered amounts of fetal calf serum (10-5-2.5%). Epithelial cell monolayers were grown from 33% of patients with native kidney ATN and 30% with transplant ATN. These results suggest that many tubular cells in "acute tubular necrosis" detach from the tubular basement membrane while still viable (and culturable). It may be possible to develop new strategies to prevent this detachment phenomenon, thus preventing or ameliorating clinical ATN.

NaCl BUT NOT MANNITOL REVERSES RENAL FAILURE INDUCED BY Na DEPLETION (LNa) AND CAPTOPRIL (CEI) IN THE RAT. M. Rathaus, A. Pomerantz, E. Podjarny and J. Bernheim. Meir Hospital, Kfar-Saba and Tel-Aviv University, Israel. (Introduced by M. Chabellan).

We have previously described that LNa-CEI induces renal failure in the rat, which is reversible with NaCl replacement. To evaluate the role of volume expansion in this reversal, clearances of inulin (CIN) and PAH (CPAH), ml/min/100 g bw, were measured in control, LNa and LNa-CEI rats on either a hydroperic (NaCl, 1.3% bw/h) or a volume expansion (NaCl, 3% - bw/h) protocol.

In hydroperic control rats (n=5), CIN was $.93 \pm .08$, CPAH $1.93 \pm .13$ and mean arterial pressure (MAP) 109 ± 7 mmHg. In 5 LNa rats, either hydroperic or volume expanded, CIN, CPAH and MAP values were similar to control rats. In 5 hydroperic LNa-CEI rats, CIN was reduced ($.53 \pm .10$, $p < .01$ vs control), but CPAH was normal ($1.56 \pm .31$). In 9 LNa-CEI rats, volume expansion with NaCl induced an increase above control (CIN, $1.56 \pm .19$, $p < .02$ and CPAH, $3.55 \pm .46$, $p < .02$). In 5 other LNa-CEI rats given isotonic mannitol 2.5% of bw/h, CIN was $.61 \pm .11$, ($p < .02$ vs control and not different from hydroperic LNa-CEI), and CPAH was $1.52 \pm .60$, MAP was 94 ± 13 mmHg, similar to control and higher than both LNa-CEI groups (70 ± 4 and 80 ± 6 mmHg respectively, $p < .05$).

The findings suggest that NaCl depletion may play an important role, independent of volume and systemic hypotension, in the glomerular hemodynamic changes characteristic of this form of renal failure.

POLYETHYLENE GLYCOL EFFECTS ON HYPOXIC INJURY IN THE ISOLATED PERFUSED RAT KIDNEY. S. Rosen, J. Kopolovic*, M. Brezis, K. Spokes*, P. Silva, F.H. Epstein. Depts. of Pathology and Medicine, Harvard Medical School and Beth Israel Hospital, Boston, MA, and Hebrew Univ.-Hadassah Medical School, Jerusalem, Israel.

Polyethylene glycol (PEG) protects against O_2 deprivation after clamping of the renal artery or norepinephrine infusion and in hypoxic primary cell culture. Isolated perfused kidneys under hypoxic conditions develop morphological alterations in all segments of the proximal tubule and medullary thick ascending limb (mTAL). In an attempt to ameliorate the effect of hypoxia, rat kidneys were perfused for 90 min. with regularly oxygenated (95% O_2 + 5% CO_2) or hypoxic perfusate (95% N_2 + 5% CO_2) supplemented with 8-12% PEG (MW ~ 8000). In oxygenated and hypoxic kidneys PEG produced changes in S1-S2 segments consisting of reduction of cell thickness and organelle compaction with internalization of brush border (BB) into the tubulo-vesicular system. In the S3 segment, the cellular volume loss was more limited; the BB was transformed to membranous whorls and the cytoplasm contained large, irregular, clear zones. Mitochondrial swelling was pronounced in the hypoxic proximal tubules. PEG quantitatively increased and emphasized the damage in the mTAL. Inclusion of 10^{-2} M ouabain preserved the mTAL from hypoxic injury and PEG had no effect on this undamaged epithelium. Thus, PEG affects renal tubules on the basis of their known water permeability and does not protect against but rather worsens hypoxic injury in the mTAL.

HgCl₂ and K₂Cr₂O₇ INDUCED REGIOSELECTIVE ACUTE TUBULAR NECROSIS IN RENAL CORTICAL SLICES: LOCALIZATION AND TRANSPORT STUDIES. Charles E. Ruegg*, A. Jay Gandolfi*, and Klaus Brendel*. Univ. of Arizona, Dept. of Pharmacology and Toxicology, Tucson, Arizona. Intr. by Lazaro J. Mandel.

To investigate the mechanisms underlying selective necrosis to the pars recta (PR) or pars convoluta (PC) following *in vitro* exposure of slices to HgCl₂ or K₂Cr₂O₇, respectively, localization and transport studies were performed. Following *in vitro* exposure, electron probe x-ray analysis or silver amplification techniques demonstrated that K₂Cr₂O₇ and HgCl₂ accumulate selectively within the PC or PR respectively. The transport of PAH, TEA, phosphate, sulfate, glutathione, and cysteine were examined as potential mechanisms for selective accumulation of either metal. Both metals reduced the steady state accumulation of PAH and TEA but HgCl₂ and K₂Cr₂O₇ do not seem to utilize these carriers to enter the slice as the uptake rate of PAH and TEA was unaltered by increasing metal concentrations. K₂Cr₂O₇ (1 μ M - 1 mM) caused a dose dependent reduction in the uptake rate of sulfate into cortical slices, while phosphate uptake was mostly unaffected. Although HgCl₂ has a high affinity for sulfhydryl groups, its uptake into slices was not enhanced by complexing HgCl₂ with glutathione or cysteine. These *in vitro* results demonstrate that K₂Cr₂O₇ and HgCl₂ accumulate within different regions of the proximal tubule and that innate cellular functions localized to the susceptible region may be responsible for this specific distribution independent of *in vivo* mechanisms.

EXPRESSION OF GENES ENCODING GROWTH FACTORS IN CISPLATIN INDUCED NEPHROTOXICITY. Robert Safirstein, Arthur Zelent, and Peter Price, Mt. Sinai School of Medicine, NY, NY.

Increased renal DNA ³H-thymidine incorporation and regeneration of renal cells occurs 4 days after cisplatin (CP) administration. To determine whether increased renal ³H-thymidine incorporation after CP is preceded by changes in growth factors expression, we evaluated the relative abundance of proto-oncogene and epidermal growth factor (EGF) transcripts in renal cortical tissue from Sprague Dawley rats 12, 24, 36, 48, and 72 after CP, 5 mg/kg, or 0.9% NaCl (control) injection. After intravascular perfusion of the kidneys, poly A⁺mRNAs were isolated by guanidinium isothiocyanate extraction and oligo-dT chromatography. Northern blot hybridization revealed increased levels of c-fos and TGFbeta transcripts beginning 24 hours after CP and continuing through 72 hours. By contrast, a striking decrease in abundance of EGF mRNA was observed at 12 hours, and extended through 72 hours. No change in the levels of other proto-oncogene or beta actin gene transcripts was noted.

The data show specific renal proto-oncogene regulation during CP-induced nephrotoxicity. The early and opposite change in EGF mRNA abundance as compared to changes in c-fos and TGFbeta mRNA, suggests a regulatory role for the EGF gene in the kidney.

LOW MOLECULAR WEIGHT PROTEIN (LMWP) TOXICITY IN THE PROXIMAL TUBULE OF THE RAT. PW Sanders, GA Herrera*, and JH Galla. Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, AL.

We have previously shown that a human kappa Bence Jones protein (BJP) can produce proximal tubule (PT) cell toxicity after 20 min of perfusion. To examine the potential toxicity of LMWPs, we perfused individual nephrons of Sprague-Dawley rats *in vivo* with each of 3 human BJPs - 2 kappa (k), 1 lambda (l) myoglobin (MYG) or beta-lactoglobulin (BLG). Either artificial tubule fluid (ATF) or ATF containing 5 g/dl protein was perfused for 20 min.

ABSORPTIONS

GROUP	pI	n	VOLUME nl/min/ mm	CHLORIDE pEq/min/ mm	GLUCOSE pmol/min/ mm
ATF1		13	2.30±0.27	198±23	44±4
BJP1	7.7	16	1.37±0.11 ^a	138±16 ^a	32±3 ^a
ATF2		10	2.00±0.23	179±20	39±3
BJP2(k)	7.1	11	1.76±0.22	195±37	37±3
ATF3		16	1.40±0.16	145±16	42±3
BJP3(l)	5.7-6.1	15	1.62±0.32	175±24	38±4
ATF4		11	1.98±0.18	164±19	37±3
BLG	5.1	13	1.29±0.14 ^a	112±16 ^a	28±2 ^a
ATF5		9	1.48±0.29	120±20	36±5
MYG	7.3	8	1.41±0.21	164±49	44±3

^ap<0.05 compared to ATF values; results are mean ±SE.

PTs with functional defects showed vacuolation, enlarged and bizarre endolysosomes, disruption of the brush border, and cellular debris in lumen.

The data suggest that certain LMWPs produce acute functional and structural damage to rat PTs. Toxicity is apparently unrelated to isoelectric point (pI).

EFFECT OF AN ALTERED GLUTATHIONE CONTENT ON THE FUNCTION AND MORPHOLOGY OF POSTISCHEMIC KIDNEY. Russell Scaduto, Jr.*, Louis Martin*, Steve Slusser*, and Vincent Gattone III, Departments of Surgery and Anatomy, Hershey Medical Center, Hershey, PA.

The glutathione (GSH) reductase system serves to protect cells against damage caused by oxidative stress. Since ischemia and blood reflow causes an increase in tissue free radical generation and lipid peroxidation, we examined the role of GSH in renal ischemia. After 35 min of renal artery occlusion (RAO), the content of renal GSH decreased to 40% of control.

To assess if this decrease affected functional recovery, rats were pretreated with buthionine sulfoximine or with GSH-monoethylester to either deplete or elevate renal GSH, respectively. Controls and treated rats were subjected to right nephrectomy and 35 min left RAO and 90 min reflow. Ischemia and reflow caused a decrease in ATP and total adenine nucleotide content to 65 and 67% of control. Isolated cortex mitochondria displayed a decreased respiratory control to 61% of control values. These changes were not affected by prior alteration of renal GSH content. However, GFR was more markedly decreased in rats with an elevated GSH compared to control. Morphologic analysis indicated enhanced cast formation. Depletion of GSH had no effect.

The data suggest that the ischemic decrease in renal GSH does not affect functional recovery of the kidney and that elevation of renal GSH enhances ischemic injury.

ATRIAL NATRIURETIC PEPTIDE PROTECTS AGAINST GENTAMICIN INDUCED ACUTE RENAL FAILURE IN THE RAT. K. Schafferhans, E. Heidbreder, R. Schmatz, A. Heidland (intr. by Robert W. Schrier)

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The aim of our study was to investigate the protective effect of α -atrial natriuretic peptide (ANP), in comparison with Verapamil (V) on the gentamicin (G) induced acute renal failure (ARF). Studies were performed on female Sprague Dawley rats. After surgical procedure and an equilibration period of 1 hour, V or ANP was infused i.v. (both at 10 μ g/kgBW/h over a 60 min. period). Controls received isotonic saline (NaCl) only. For induction of GARF, G was administered as an i.v. bolus injection at 15mg/kgBW and kidney function was investigated over a 90 min. period. Inulin clearance, diuresis, electrolyte excretion and blood pressure was measured. In controls, G administration induced an early nonoliguric ARF (GFR, 0.50 \pm 0.05 ml/min). In those rats, pretreated with V, G application led to a less pronounced decrease of GFR, significantly different from controls. The most beneficial effect was obtained from the ANP group. G had no effect on GFR. Moreover, GFR remained markedly elevated (GFR, 2.63 \pm 0.37 ml/min), significantly different from the V group. V and ANP are capable of increasing renal blood flow, this is especially true for ANP in states of renal vasoconstriction. In a recent study it was demonstrated, that ANP prevents the ischemic ARF. Moreover, ANP has been shown to decrease renin, intracellular calcium and dilates mesangial and glomerular epithelial cells and hence increases ultrafiltration coefficient. These findings may contribute to the powerful effect of ANP in GARF.

HISTOPATHOLOGY OF HUMAN ISCHEMIC ACUTE RENAL FAILURE (ARF). P.F. Shanley, Dept. Path., U. Colo. Health Sci. Ctr., Denver, CO

The histopathology of human ischemic ARF is notable for the absence of the extensive tubular necrosis universally seen in animal models of renal ischemia. It has been suggested that the pathophysiology of the human syndrome might, therefore, be distinct from that of animal models. Against this is the present case: a 21 year old man with a variant of Wiskott-Aldrich syndrome who died in shock 16 hours after an in-hospital episode of massive gastrointestinal hemorrhage. Following the bleeding there was hemodynamic instability, plasma creatinine rose from 0.9 to 1.7 mg/dl and anuria developed. At autopsy the kidney showed extensive coagulative necrosis confined largely to the lower portions of medullary rays and the immediate subcortex corresponding therefore to near total loss of pars recta of the proximal tubule. There was focal necrosis in proximal convoluted tubules, numerous pigmented casts in the medullary collecting ducts, and no significant damage in the thick ascending limb of Henle. This case represents a rare opportunity to observe human renal histology acutely after an ischemic insult. The striking similarity of the extent and distribution of tubular necrosis in this case and that of ischemia-reflow in the rat, supports the idea that study of the rat model has relevance for at least some clinical situations. Since most studies of the renal pathology of human ischemic ARF have been made days or even weeks after an insult it is possible that extensive necrosis does occur in anuric ARF but is not reported because of the timing of observations.

THE LONG-TERM COURSE OF CYCLOSPORINE-ASSOCIATED CHRONIC NEPHROPATHY. Richard Sibley, Lynne Newton* and Bryan D. Myers. Stanford Univ., Depts. of Medicine and Pathology, Stanford, California.

We evaluated a chronic renal injury in 37 cardiac transplant recipients treated for 12-24 months with cyclosporine (CsA). Twenty-four cardiac transplant recipients treated with azathioprine for 24 months served as controls. Despite equivalent cardiac performance, GFR in those treated with CsA was depressed, 47 \pm 3 vs 94 \pm 4 ml/min/1.73m² (p<0.001). CsA therapy was associated also with 2-fold elevation of renal vascular resistance (RVR), proteinuria, and arterial hypertension. Histopathological changes included an obliterative arteriopathy with deposition of proteinaceous material in necrotic arteriolar walls, and associated tubulo-interstitial damage. A minority of glomeruli exhibited either ischemic collapse or sclerosis. Area perimeter analysis revealed significant expansion of the mesangium and enlargement of the remaining glomeruli. Longitudinal examination of 15 subjects over a 48 month period during which CsA was reduced (6.8 to 3.1 mg/Kg/day) or withdrawn (N=4) revealed persistent hypofiltration, increasing RVR (p<0.05) and increasing urinary excretion of albumin (162 \pm 70 to 546 \pm 300, p<0.01) and IgG (6 \pm 2 to 53 \pm 43, p<0.05). Further histopathological deterioration was observed when renal tissue was sampled a second time in 6 patients, and two members of the experimental group developed end-stage renal disease. We conclude that continuous CsA therapy for more than 12 months causes a chronic injury to renal microvessels that is rarely reversible and potentially progressive.

ALTERATIONS OF GLOMERULAR DEVELOPMENT INDUCED BY GENTAMICIN (G). H Smaoui, JP Mallié*, M Cheignon, J Schaefferbeke, Laboratoire de Biologie Cellulaire, Université Paris and *Laboratoire de Néphrologie, Université Nancy FRANCE. (Introduced by W.M. Bennett)

Aminoglycosides lead to a well-known nephrotoxicity. Recently, Mallié et al. (Ped Pharmacol 5:229, 1986) described morphologic evidence of nephrotoxicity in the deep cortex of rat neonates born to females given G during renal organogenesis and nephron differentiation. In this study, female Wistar rat received G-sulfate (75 mg/kg/day) i.p. on days 7-11 and 14-18 of gestation. Pregnant controls received i.p. saline. In neonates prenatally exposed to G, electron microscopy showed alterations in glomerular development whatever the stage of differentiation even in the earliest stages. These changes consisted of increased thickness of glomerular basement membrane material lined by anionic sites (disclosed by polyethyleneimine) and defective permselectivity to proteins, revealed by passage of anionic ferritin into the urinary space. In addition, decreased endocytosis was observed in proximal tubules. After delivery, mothers exhibited increased permeability of the GBM to high molecular weight proteins without any obvious alterations of glomerular structure or anionic-site distribution. In both neonates and mothers, many inclusions were observed, within lysosomes as previously described.

We conclude that G can lead to abnormal maturation involving both glomeruli and tubules starting from the earliest stages of nephron development.

EFFECT OF NIFEDIPINE, PROPRANOLOL AND OTHER ANTI-HYPERTENSIVE DRUGS ON RENAL FUNCTION OF TRANSPLANT RECIPIENTS TREATED WITH CYCLOSPORINE (CsA). K. Solez, L.C. Racusen, W.K. Vaughn*, Johns Hopkins Hosp.(JHH), Baltimore, MD, S.E. Organ Procurement Foundation (SEOPF), Richmond, VA.

Antihypertensive drugs such as clonidine (Cl) lessen the severity of ischemic and toxic acute renal failure in animal models and may lessen CsA-induced impairment of function in native kidneys. The 6 JHH renal transplants treated with Cl and CSA from time of transplant have had more rapid and better function than other JHH CsA pts. To determine whether Cl has a significant effect on function of CsA-treated transplants, we investigated the SEOPF data base. The 500 Cl-CsA SEOPF pts. did not differ from the remaining 3,711 SEOPF CsA pts. in initial peak serum creatinine (Cr), rate of decline of Cr, discharge Cr, or number of rejection episodes. Many transplant recipients require antihypertensive meds initially. A similar investigation of the effect of other such meds on function of the CsA-treated transplants was therefore undertaken. Captopril and prazosin had no significant effects. Nifedipine resulted in fewer initial rejection episodes (.92±.05 vs. 1.15±.02 for other meds), whereas metoprolol resulted in a higher serum Cr at discharge (3.48±.20). Nifedipine was the only agent with a significant effect on 1-yr graft survival (82.6% vs 72.6%). Propranolol resulted in a higher maximum serum Cr (11.2±0.2 vs 10.6±0.1) and an increase in number of rejection episodes (1.19±.03 vs 1.08±.02). These data suggest that the calcium channel blockers have a beneficial effect on function and outcome of CsA-treated renal allografts while beta blockers have an adverse effect.

INHIBITION OF ADENOSINE DEAMINASE ENHANCES RENAL RECOVERY FROM ISCHEMIA. Michael E. Stromski*, A. van Waarde*, G. Thulin*, K. Gaudio, R. Shulman* and N. Siegel, Depts of Peds and Mol Bioph and Bioch, Yale Univ Sch of Med, New Haven, CT.

The postischemic infusion of exogenous adenosine nucleotides accelerates the restoration of renal ATP and enhances the recovery of renal function. To determine if endogenous nucleotides would have a similar effect, rats were injected with 2'-deoxycoformycin (DCF, 1 mg/kg). In the kidney, this produced an 80% inhibition of adenosine deaminase (ADA) and resulted in a 20% increase in the residual nucleotide pool and a 2.5 fold increase in adenosine (inosine and hypoxanthine were undetected) after 45 min of ischemia.

As determined by ³¹P-NMR *in vivo*, animals given DCF, as compared to untreated rats, had a) greater initial recovery of ATP (63±2% of control vs 53±2, P<0.01), b) an accelerated rate of ATP resynthesis (0.18±0.03% control/min vs 0.10±0.03, P<0.05) and c) a higher renal ATP level after 2 hrs of reflow (84±5% of control vs 62±3, P<0.01). This augmentation of cellular metabolism was associated with an enhanced inulin clearance 24 hrs after ischemia (330±15 μ l/min/100gm BW vs 215±10, P<0.05).

Inhibition of ATP degradation during ischemia will ameliorate the metabolic and function consequences of the renal insult in a manner similar to that seen with infusion of exogenous nucleotides.

REESTABLISHMENT OF EPITHELIAL POLARITY FOLLOWING ISCHEMIA: A REQUIREMENT FOR NORMAL CELL FUNCTION. D.M. Spiegel* and B.A. Molitoris, VAMC Denver, CO.

Ischemic injury results in proximal tubular dysfunction (PTD) and loss of surface membrane (SM) polarity. Since vectorial Na⁺ transport requires SM NaK-ATPase polarity, we set out to determine if correction of PTD following ischemia is dependent upon reestablishment of SM polarity. ARF was induced using a bilateral 50 min pedicle clamp. Cellular morphology normalized by day 3. SCr and FE_{Na} (Table, Mean ± 1 S.D.) were maximal on day 1, remained elevated on day 3 and returned toward baseline by day 10. Fractional Li clearances (58 ± 12 vs 29 ± 14; p < .05) suggested the elevated FE_{Na} resulted from PTD. Reduced Na⁺ reabsorption correlated with the abnormal localization of NaK-ATPase to the BBM.

	Creat (mg/dl)	FE _{Na} %	NaK-ATPase Enrichment	SPH/PC Ratio
Cont	0.4 ± .1	0.4 ± .1	1.6 ± .6	1.8 ± .4
Day 1	3.0 ± .7	3.0 ± .7	3.0 ± 1.6	1.1 ± .1
Day 3	1.2 ± .4	0.8 ± .6	3.2 ± 1.4	1.2 ± .3
Day 10	0.6 ± .1	0.5 ± .1	1.6 ± 0.4	1.4 ± .1

Tubular reabsorption of glucose (TR_{glu}), which is modulated by the sphingomyelin/phosphatidylcholine (SPH/PC) ratio (JCI 9/87) decreased on post-ischemic day 1 (95 ± 2.6 vs 99.7 ± .3; p < .01) remained abnormal on day 3 and returned to baseline on day 8. Glucose transport in BBM decreased on post-ischemic day 3, (107 ± 23 vs 459 ± 102; p < .01) confirming a BBM defect. SM lipid polarity (reduced SPH/PC) was abnormal on days 1 and 3, and correlated with the defect in TR_{glu}. In summary recovery of PT glucose and Na⁺ reabsorption correlated with the reestablishment of SM polarity and not cellular morphology.

2-BROMOETHYLAMINE HYDROBOMIDE (BEA) DOES NOT INDUCE RENAL PAPILLARY NECROSIS (RPN) IN BRATTLEBORO RATS RECEIVING ADH. Susumu Takahashi* and Tetsuo Shimamura, Dept. of Path. UMDNJ- Robert Wood Johnson Med. Sch., Piscataway, NJ.

A single i.v. administration of BEA (250mg/kg) induced RPN in rats (Fisher, Holtzman, & Sprague-Dawley strains). Toxic papillary hyperconcentration is a prevailing view of the pathogenetic mechanism. We examined the effects of BEA in Brattleboro rats (N:12) manifesting congenital diabetes insipidus. Rats were injected daily with either 0.1 U Pitressin tannate in oil or with oil. On day 3, all rats were injected BEA (i.v., 50mg/100gm). Metabolic balance studies were carried out and papillae examined on day 8. Statistically significant differences were observed in urine flow rate, U_{osm}, and water intake between 2 groups, but neither group developed a classic RPN. In separate experiments, rats were injected either with BEA or saline. Examination of urinary excretion of bromine revealed significant amounts of it in experimental rats but not in controls indicating that BEA or its metabolites reached the target organ. Renal tissue distribution of BEA is currently under examination. The data strongly indicate a strain difference in susceptibility to BEA. We speculate that drug resistance genes to BEA are operative in Brattleboro rats.

INHIBITION OF HYPOTONIC CELL VOLUME REGULATION BY CYCLOSPORINE. D.A. Terreros, E.R. Ashwood.* Veterans Administration and University of Utah Medical Center, Salt Lake City, Utah.

We reported that cyclosporine (Cya) inhibits the ability of proximal tubule cells to osmoregulate in hypotonic solutions (Kind Int 31:376,87). Colombani first demonstrated that Cya is an inhibitor of calmodulin (Science 228:337,84). We observed that other inhibitors of calmodulin, i.e. trifluoroperazine, also inhibit cell volume regulation (CVR). We think, thereby, that the effects of Cya are probably related to alterations in calmodulin mediated osmotic unloading (Fed Proc 46:348,87). It has been suggested, however, that this effect may also be due to Cya induced reduction of water permeability. To study that possibility, proximal renal tubules from Carassius auratus were incubated with or without 5 uMol Cya for 3 minutes in isotonic fish Ringer's solution (290 mOsm) and then exposed to fish Ringer's made hypotonic by decreasing NaCl (110 mOsm). Experiments were videotaped and external diameters were measured at 10 seconds intervals. Using least mean squares curve fitting, we found that the simplest equation that describes hypotonic CVR is $\Delta D = A(1 - e^{-Jt}) - B(1 - e^{-kt})$; where D is diameter (um) and t is time (min). Means and standard errors are as follows:

	N	A	J	B	k
Control	15	24.4(2.9)	1.60(.11)	22.2(2.8)	.57(.06)
5 uMol	11	18.1(2.5)	2.18(.25)*	12.9(2.0)*	.42(.10)

*p<.05, unpaired t-test

These results indicate that Cya associated inhibition of CVR is not due to decreased water permeability but to an inhibition of the cellular ability to unload osmotic particles.

FUNCTIONAL EFFECTS OF CHRONIC CYCLOSPORINE ON GLOMERULAR HEMODYNAMICS. S. Thomson,* B.J. Tucker,* and R.C. Blantz., Dept. of Medicine, UCSD and SDVAMC, La Jolla, CA.

We evaluated the effects of chronic cyclosporine (CsA) administration on the determinants of nephron filtration rate (SNGFR) using micro-puncture (mp) techniques in Munich-Wistar rats. Animals received CsA (30 mg/kg SQ) in olive oil daily for 8 days prior to mp. Controls (PFC) were pair-fed. We studied 13 euvolemic (EUV) and 12 plasma volume expanded (PVE) rats. SNGFR, glomerular hydrostatic pressure gradient (ΔP), nephron plasma flow (SNPF), plasma oncotic pressure (π_a), and ultrafiltration coefficient (LpA) were quantitated. Results are: (mean \pm SEM, *p<.05 vs. EUV/PFC, †p<.05 vs. EUV/CsA).

	SNGFR nl/min	ΔP mmHg	π_a	SNPF nl/min	LpA nl/sec/mmHg
EUV/PFC	32 \pm 2	37 \pm 2	20 \pm 1	121 \pm 5	.06 \pm .01
EUV/CsA	23 \pm 2*	25 \pm 1*	16 \pm 1*	100 \pm 7*	.11 \pm .02*
PVE/CsA	40 \pm 1†	34 \pm 1†	19 \pm 1	142 \pm 8†	.09 \pm .02

The role of renal nerves in accounting for the above was explored in 13 rats subjected to ipsi-(DNX) or contralateral-(INX) renal denervation prior to 8 days of CsA and mp. Results follow: (\S =p<.05 compared to INX/CsA).

	SNGFR	ΔP	π_a	SNPF	LpA
INX/CsA	23 \pm 2	27 \pm 1	15 \pm 1	85 \pm 5	.06 \pm .01
DNX/CsA	32 \pm 2 \S	32 \pm 1 \S	16 \pm 1	106 \pm 7 \S	.07 \pm .01

Conclusions: As opposed to acute infusion of CsA which lowers SNGFR by lowering LpA and SNPF (KI 32:19-25, 1987), the functional decrease in SNGFR associated with chronic CsA administration results primarily from a fall in ΔP and less importantly, SNPF. DNX or PVE restores effective filtration pressure and SNGFR.

MITOCHONDRIAL TOXICITY (MITO TOX) OF CEPHALOSPORIN ANTIBIOTICS (C's) - AN INHIBITION OF SUBSTRATE UPTAKE. Bruce M. Tune, Richard K. Sibley, and Chieh-Yin Hsu.* Dept of Pediatrics, Stanford University, Stanford, CA.

The C's produce renal cortical mito respiratory tox after in vitro (vt) or in vivo (vv) exposure. Vt tox is immediate, nonselective among toxic (Tx) and nonTx C's, and reversed by substrate excess. Vv tox is delayed, specific to the nephroTx C's, and irreversible. Both exposures affect respiration (resp) with succinate (S) more than glutamate/malate (G/M) as substrates. Because G and M access the intramito enzyme chain proximal to S, this pattern suggests decreased mito substrate uptake. It has therefore been proposed that all C's fit the carriers for mito substrate entry, but in the intact kidney this causes limited or transient inhibition of resp with nonTx C's; in vivo tox, which is seen after isolation and washing of mito exposed in situ, develops with the more sequestered and reactive C's, that acylate these transporters. To test this model, the net uptake of isotopic S and ADP by rabbit renal cortical mito was measured by the method of sieve filtration after vv and vt exposure to cephaloglycin (Cgl, Tx) and cephalixin (Cix, nonTx). Cgl vv (300 mg/kg 1 hr before sacrifice) and vt (1000 μ g/ml) decreased State 3 mito resp with S by 44% and 59%, respectively (P < .005 ea). In nonmetabolizing mito (5 μ g/ml antimycin A) these same vv and vt Cgl exposures reduced the net uptake of S (ambient conc 1.2×10^{-6} M) from $10.31 \pm SE 1.89$ to 3.25 ± 0.40 and $3.27 \pm 0.40 \times 10^{-6}$ M in mito H₂O, respectively (n = 10-14, P < .005 ea), but had little or no effect on ADP uptake. Cix inhibited resp and S uptake only with vt exposure. S washout (slope of log conc vs t (min)) from Cgl-Tx mito (-0.074 ± 0.012 (10)) was not different from control (-0.075 ± 0.017 (10)) (t_{1/2} = 4 min ea), ruling out increased efflux as a cause of decreased uptake. Freeze-thaw fragmentation, which both uncouples resp and allows direct substrate access to the intramito enzymes, increased resp in Cgl-Tx (by $40 \pm 11\%$ (10), P < .005) but not normal ($0 \pm 5\%$ (10)) mito, an effect not seen with uncoupling with CCCP. Conclusion: the resp tox of C's can be explained by a primary reduction of the mito substrate transport, consistent with the proposed acylation of substrate carriers.

POTENTIAL ROLE OF HYDROXYL RADICAL IN GLYCEROL-INDUCED ACUTE RENAL FAILURE. Patrick D. Walker, Sudhir V. Shah. Depts. of Pathology and Medicine, Tulane Medical School, New Orleans, Louisiana.

The role of reactive oxygen metabolites, in particular hydroxyl radical, has been previously demonstrated in several models of tissue injury including ischemic and gentamicin-induced acute renal failure. The aim of the present study was to examine the role of hydroxyl radical in glycerol-induced acute renal failure. To induce acute renal failure, rats were dehydrated for 24 hr and then injected with 8 ml/kg 50% glycerol i.m.. The hydroxyl radical scavenger dimethylthiourea (DMTU) was administered 500 mg/kg i.p. just before the glycerol injection and 125 mg/kg i.p. 8 hrs later. Because the generation of hydroxyl radical in biological systems requires the presence of a trace metal such as iron, we also examined the effect of the iron-chelator, desferoxamine (DFO, 30 mg i.v. just before the glycerol injection and 30 mg/day delivered by Alzet pump) on glycerol-induced renal failure. The mean \pm SEM of BUN and creatinine measured 24 hours after the glycerol injection were as follows:

	Cont	Glycerol	+DMTU	+DFO
BUN	20 \pm 1	136 \pm 16	48 \pm 11*	22 \pm 3*
Creat	0.5 \pm 0.1	3.5 \pm 0.5	1.1 \pm 0.3*	0.6 \pm 0.1*
(n)	(7)	(8)	(8)	(8)

*p<.0001 compared to glycerol alone group.

In contrast to the marked protective effect of DMTU, urea (which is not an effective scavenger of hydroxyl radical) failed to protect against acute renal failure. In a separate experiment, a second hydroxyl radical scavenger, sodium benzoate, was also protective. The beneficial effects of two hydroxyl radical scavengers as well as an iron chelator strongly implicate a role for hydroxyl radical in glycerol-induced acute renal failure.

ATRIAL NATRIURETIC FACTOR PREVENTS ACUTE RENAL DYSFUNCTION INDUCED BY HYPOTENSIVE HEMORRHAGE IN THE DOG. DJ Yasmineh,* JA Schirger,* BS Edwards*, TR Schwab, DM Heublein*, and JC Burnett Jr., Mayo Medical School, Rochester MN.

This study was designed to evaluate the effect of atrial natriuretic factor (ANF) on renal hemodynamic function in a clinically relevant model of acute renal dysfunction (ARD). ARD was produced by hypotensive hemorrhage (HH, arterial pressure reduced to 35-40 mmHg) in anesthetized dogs pretreated with indomethacin (10 mg/kg, iv). Renal function was measured prior to HH. Blood was then re-transfused (RT) and renal function measured immediately post-RT (Imm-RT) and 2 hours post-RT. In the ANF group, ANF was administered (0.3 µg/kg/min, iv) during RT and continued for the duration of the experiment. Dogs were anuric during the period of HH.

†p<.05 compared to baseline.

	Baseline	Imm-RT	2 hr-RT
GFR, ml/min	27±3	27±5	14±4†
RVR, RU	0.70±.07	0.70±.06	1.22±.25†
Group II (HH+ANF, n=7)			
GFR, ml/min	29±4	33±4	33±4
RVR, RU	0.78±.09	0.63±.09	0.67±.07

Following HH, glomerular filtration rate (GFR) progressively declines and renal vascular resistance (RVR) increases. In contrast, ANF prevents these alterations in renal hemodynamic function. We conclude that atrial natriuretic factor importantly serves to preserve glomerular filtration rate and prevents the marked renal vasoconstriction in this clinically relevant model of acute renal dysfunction.

VASA RECTA BLOOD FLOW IN ACUTE RENAL FAILURE IN THE RAT Yoram Yagil*, Masaaki Miyamoto* and Rex L. Jamison, Stanford Univ., Stanford, CA and Univ. of Rochester, Rochester, NY.

It has been proposed that alterations in intrarenal blood flow play a role in the pathogenesis of hemodynamically mediated acute renal failure. To assess the effect of renal ischemia on the circulation of the inner medulla, capillary diameter (Diam, µ) and blood flow (Q_{VR}, nl/min) in descending and ascending vasa recta were studied by fluorescent videomicroscopy in the uninephrectomized rat after renal artery clamp lasting 45 min. Animals were studied 90 min. (Group I, n=7) and 24 hrs. (Group II, n=7) after end of ischemia (EXP). Control rats (CON) were studied 90 min. (Group I, n=5) and 24 hrs. (Group II, n=5) after uninephrectomy alone. Results (means):

Group	Asc. Vasa Recta		Desc. Vasa Recta	
	Diam	Q _{VR}	Diam	Q _{VR}
CON I	14.5	5.4	13.4	6.9
EXP I	16.2	9.6*	14.6	18.9*
CON II	16.1	5.6	14.9	9.5
EXP II	18.2	12.3*	17.0	23.0*

*p<0.05 when compared to CON

In coronal slices, congestion of the outer medulla was observed in the EXP but not CON kidneys. These results indicate that blood flow in vasa recta in the inner medulla, which originates from the efferent arterioles of juxtamedullary nephrons and crosses the outer medulla, is markedly enhanced after renal ischemia in the uninephrectomized rat.

COURSE AND PATHOGENESIS OF POST-ISCHEMIC ACUTE RENAL FAILURE IN THE RAT. Yoram Yagil,* Bryan D. Myers, and Rex L. Jamison, Stanford University Medical Center, Div. of Nephrology, Stanford, CA.

The renal artery was clamped for 45 min in the chronically cannulated uninephrectomized rat to reproduce the syndrome following renal ischemia in humans and to determine transtubular backleak using the fractional dextran clearance technique. Twenty-four hours after ischemia, creatinine and inulin clearances were reduced by 90%; fractional excretion (FE) of Na⁺ and H₂O were increased markedly. Over the next 5 days, creatinine clearance rose in a ramp-like pattern to normal values; FENa⁺ and FEH₂O declined reciprocally. Fractional clearances of dextran (radii 20-44 Å) were not different after 24 or 48 hrs of renal failure from control. To test for backleak, ³H-methoxy-inulin and ¹⁴C-dextran were microinjected directly into the proximal nephron in anesthetized rats. Their recovery in the final urine was markedly lower 24 and 48 hrs after renal ischemia than in controls; in a few tubules of ischemic animals, recovery was normal. In cross-transfusion experiments with normal rats, the recovery of radioactivity in recipient urine demonstrated backleak of inulin and dextran microinjected into ischemic kidneys. We conclude that in this model of renal ischemia in the conscious rat (1) the course is analogous to self-limited acute renal failure in man, (2) the fractional dextran clearance technique is not sensitive enough to detect the amount of backleak encountered, and (3) a minority of intact and patent nephrons contribute significantly to the formation of the final urine.

REACTIVE OXYGEN SPECIES (ROS) MEDIATE THE REDUCTION IN ULTRAFILTRATION COEFFICIENT (K_f) AND INCREASE IN EFFERENT ARTERIOLAR RESISTANCE INDUCED BY ACTIVATED POLYMORPHONUCLEAR LEUKOCYTES (PMN). T. Yoshioka, K.F. Badr & I. Ichikawa, Vanderbilt Univ., Nashville, TN.

Depletion of PMN in rats with nephrotoxic serum nephritis was demonstrated previously to totally abolish the profound constrictive responses of the glomerular microcirculation, i.e., increased afferent (RA) and efferent (RE) arteriolar resistances, and decreased K_f. A specific peptidyl leukotriene receptor antagonist was recently shown by us to be capable of substantially, but only partially, normalizing K_f without effect on RA and RE. We therefore examined the *in vivo* effects of ROS, another biologically highly active substance known to be released from activated PMN, on the glomerular microcirculation.

To induce local activation of PMN, phorbol myristate acetate (PMA, 25 µg) was infused unilaterally into the renal artery in 11 Munich-Wistar rats. Micropuncture study was performed 12 hours later.

	AP	SNGFR	QA	PGC	RA	RE	K _f
	mmHg	nl/min	mmHg	mmHg	min-mmHg/nl	nl/min-mmHg	nl/min-mmHg
PMA	115	29*	122*	59*	0.29	0.23*	1.6*
PMA + CAT	108	41‡	163‡	48‡	0.20	0.11‡	4.2‡
Sham	111	40	151	45	0.25	0.11	4.1

[* and ‡ denote p < 0.05 vs. Sham and PMA, respectively]

Successful achievement of local PMN activation was evidenced by glomerular PMN accumulation and a reduction in GFR, which were seen only in the PMA-infused but not in the contralateral kidney. PMA infusion led to a fall in SNGFR and an elevation in PGC in association with a decrease in K_f and an increase in RE. Pretreatment with catalase (CAT), a scavenger of hydrogen peroxide, ameliorated these changes. In a separate group of 7 rats depleted of PMN by nitrogen mustard, PMA administration did not cause any significant changes in glomerular microcirculatory parameters.

These results indicate: 1) ROS, released from PMN, evoke a profound constrictive response in the glomerular microcirculation. 2) The effects of ROS are localized primarily on the efferent arteriole and the glomerular capillary.

EVIDENCE AGAINST REACTIVE OXYGEN SPECIES (ROS) AS MEDIATORS OF ISCHEMIC ACUTE RENAL FAILURE (ARF). R.A. Zager, Univ. of Washington, Seattle, WA.

There has been intense interest in the role of ROS as mediators of ischemic ARF. This is based on observations using the renal artery occlusion (RAO) model of ARF that renal malondialdehyde (MDA) concentrations, an index of lipid peroxidation, rise in the post ischemic period, and that antioxidants confer protection. Clinical ischemic ARF is due to hypoperfusion (H), not blood flow interruption. Therefore, we assessed whether ROS mediate H-induced renal injury, using the criteria established for the RAO model. H-ARF was induced in rats by suprarenal partial aortic ligation, lowering renal perfusion pressure to 20 mm Hg for 45 min. Renal MDA concentrations were measured 15 min after ligation release. Renal function (BUN, creatinine (Cr)) and morphology were assessed 24 hr later in control rats and in rats pretreated with antioxidants (allopurinol, superoxide dismutase, dimethylthiourea, reduced glutathione; in doses previously suggested to lessen RAO-ARF). H caused no rise in renal MDA ($p=0.54$). Control ARF rats developed severe azotemia (BUN 119 ± 6 ; Cr 3.3 ± 0.37) (mg/dl) and widespread tubular necrosis. Antioxidants, either alone or in combination, did not lessen the ischemic damage (range: BUN 124-141; Cr 2.9-3.7; comparable histology). In additional experiments, an IV infusion of reduced glutathione which doubled renal glutathione concentrations did not protect against 40 min of RAO (BUN 125 ± 6 ; Cr 3.9 ± 0.2 ; controls 123 ± 6 ; 3.7 ± 0.3). These findings challenge the notion that ROS are critical mediators of experimental ischemic ARF, whether induced by H or total blood flow interruption.

URANYL FLUORIDE-INDUCED NEPHROTOXICITY FOLLOWING A REDUCTION OF RENAL MASS. Rudolfs K. Zalups*, Robert M. Gelein*, Paul E. Morrow* and Gary L. Diamond* (Intr. by James Strand) University of Rochester Medical Center, Depts. of Pharmacology and Biophysics, Rochester, New York 14642.

It has recently been demonstrated that certain types of toxic nephropathy are more severe in animals with reduced renal mass than in animals with two normal kidneys. The objective of the present study was to determine whether or not the nephropathy induced by the uranium containing compound uranyl fluoride (UO_2F_2) is enhanced in rats with reduced renal mass. Uninephrectomized (NPX) and sham-operated (SO) rats were given single intravenous injections of UO_2F_2 at doses delivering 100 or 250 μ g U/kg sixteen days after surgery. By the fifth day after the administration of either dose of UO_2F_2 , the urinary excretion of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) albumin and glucose had increased significantly in both the NPX and SO rats. During the fifth day the excretion of these compounds (except for albumin) was greater in the SO rats than in the NPX rats. Moreover, creatinine clearance was lower and the fractional excretion of glucose was higher in the SO rats than in the NPX rats. Furthermore, the level of histologically demonstrable damage to the pars recta of proximal tubules in the inner cortex and outer medulla was slightly greater in the SO rats than in the NPX rats. Therefore, the findings of this study indicate that the nephropathy in rats induced by UO_2F_2 is not enhanced after unilateral nephrectomy. If anything, unilateral nephrectomy may provide some protection against the nephrotoxic effects of UO_2F_2 .

PATHOPHYSIOLOGY OF CHRONIC RENAL DISEASE

SHORT-TERM EFFECTS OF SUBTOTAL NEPHRECTOMY (Nx) ON GLOMERULAR PROCOLLAGEN mRNA EXPRESSION.

SG Adler, JR Lombet,* PS Anderson,* AH Cohen, RJ Glasscock. Harbor-UCLA Med Center, Torrance, CA. Sclerosis after Nx is partly characterized by increased mesangial matrix (MM). Type IV collagen is a major constituent of MM. We measured glomerular mRNA procollagen $\alpha 1$ (IV) levels in normal and Nx rats and assessed the effects of short-term enalapril administration. 31 male Sprague-Dawley rats underwent 1-2/3 Nx or sham Nx (SNx, n=7). After 3 weeks, Nx rats were matched by BP and serum creatinine (Scr) (T=0) and assigned to receive no therapy (Nx, n=14) or enalapril (NxE, n=10). Rats were pair-fed and sacrificed after 10 days (T=10). At T=0, Scr and BP were higher in the Nx rats than in SNx ($p<0.05$). Urinary protein did not differ. At T=10, Scr was unchanged in all groups. BP was unchanged in SNx and Nx but decreased in NxE from 184 ± 21 to 142 ± 14 mm Hg ($p<0.001$). Proteinuria was unchanged in SNx and NxE but increased in Nx from 5.2 ± 2.3 to 32.9 ± 59.8 mg/24^h ($p<0.05$). Glomerular mRNA for procollagen $\alpha 1$ (IV) did not differ between the Nx groups, Units/ μ gRNA, (91.2 ± 30.3 Nx; 75.4 ± 37.2 NxE; $p>0.05$), but were higher than in SNx (34.0 ± 19.0 , $p<0.05$). In conclusion: 1) Glomerular mRNA for procollagen $\alpha 1$ (IV) increases after subtotal Nx in rats. 2) Under these experimental conditions, after short term enalapril administration, there is modulation of proteinuria but little modulation of glomerular mRNA levels for procollagen $\alpha 1$ (IV). 3) The differential acute response to enalapril on proteinuria and mRNA levels suggests that the underlying pathophysiology for proteinuria and sclerosis may differ at least in part.

ACUTE INFUSION OF CALCIUM CHANNEL BLOCKERS (CCB) REDUCES GLOMERULAR CAPILLARY PRESSURE (\bar{P}_{GC}) IN RATS WITH REDUCED RENAL MASS. S. Anderson, L.E. Clarey,* S.L. Riley,* and J.L. Troy*. Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Successful antihypertensive therapy does not always reduce \bar{P}_{GC} or glomerular injury in rats with renal ablation. To evaluate the glomerular hemodynamic consequences of blood pressure reduction with CCB, we measured mean arterial pressure (AP) and single nephron glomerular filtration rate (SNGFR) before, and glomerular hemodynamics after, intravenous infusion of diltiazem (DILT) (50 μ g/kg/min), verapamil (VER) (15 μ g/kg/min), or saline vehicle (SAL) in male Munich-Wistar rats 4 wks after 5/6 nephrectomy. Results:

Group (n)	\bar{AP} mmHg	SNGFR ----nl/min----	Q_A	\bar{P}_{GC} mmHg
SAL (5)				
Pre	140 \pm 8	91 \pm 7		
Post	139 \pm 10	92 \pm 11	373 \pm 67	69 \pm 3
DILT (4)				
Pre	153 \pm 10	87 \pm 7		
Post	101 \pm 1*	58 \pm 5*†	210 \pm 35+	49 \pm 2+
VER (5)				
Pre	142 \pm 6	96 \pm 12		
Post	103 \pm 3*†	66 \pm 2*†	195 \pm 30+	52 \pm 2+

(Mean \pm SE; * $p<0.05$ vs Pre, † $p<0.05$ vs SAL). Normalization of \bar{AP} with both DILT and VER normalized \bar{P}_{GC} , with reduction of SNGFR and plasma flow rate (Q_A) toward but not to normal levels. Whether these acute effects of CCB will be sustained with chronic therapy, and will protect against injury, await further study.

"HYPERFILTRATION INJURY" DOES NOT OCCUR IN A NORMOTENSIVE RAT REMNANT KIDNEY (RK) MODEL. A. Bidani, K. Mitchell, M. M. Schwartz, L. G. Navar, E. J. Lewis. Rush Medical College, Chicago, IL. and Univ. of Alabama, Birmingham, AL.

The progressive glomerular injury seen by 8 wks in the RK model has been attributed to adaptive hyperfiltration. The contribution of systemic hypertension (HTN) to progressive nephron loss was examined in the RK model (>80% ablation) in normotensive male Wistar-Kyoto (WKY) rats fed a standard (25%) protein diet (body wt. 311 ± 13 g, mean \pm SEM). After 14-16 wks, GFR and RPF were measured and the kidneys processed for morphology. The 10 normotensive rats showed no evidence of progressive morphologic injury to the glomeruli. Only 2/12 rats developed HTN (systolic BP >150 mmHg). These 2 rats with HTN showed segmental and global necrosis and/or sclerosis in 6-10% of the glomeruli. SCr was significantly increased by 4 wks (pre-ablation, 0.5 ± 0.03 vs 1.0 ± 0.05) and stayed unchanged through the course in the 10 normotensive rats (0.98 ± 0.05 at 14-16 wks), but increased to 2.0 and 2.7 mg/dl in the 2 rats with HTN. Protein excretion (mg/24h) increased in all rats (pre-ablation 8.0 ± 0.9 to 33.5 ± 4.2 at 14-16 wks). Micropuncture studies in 5 additional WKY remnant kidneys at 6 wks after ablation demonstrated the SNGFR to be markedly elevated (72.8 ± 7.6 nl/min) but without increased glomerular capillary pressures (PSF 36.4 ± 1.5 mmHg). These data show that hyperfiltration in these remnant kidneys does not result in morphologic injury and therefore may not be maladaptive in the absence of systemic HTN. Proteinuria in this model occurs in the absence of morphologic injury and may be the consequence of altered hemodynamics.

RENAL HANDLING OF METHYLGUANIDINE IN ACUTE AND CHRONIC RENAL FAILURE. D.P. Brooks*, G.R. Rhodes*, P. Woodward*, V.K. Boppana*, H.E. Griffin*, F.M. Mallon*, and L.B. Kinter, Smith Kline & French Labs., Dept. of Pharmacology, Swedeland, PA, USA.

Methylguanidine (M) is a suspected uremic toxin that accumulates in renal failure. We measured M in the plasma of dogs with ischemic-induced renal failure (ARF) and in the plasma and urine of dogs with spontaneous renal insufficiency (CRF), using HPLC with post-column fluorescence detection following solid-phase extraction. ARF was induced in 4 uninephrectomized mongrel dogs by constricting the remaining renal artery for 90 min under isoflurane anesthesia. 24 hr following constriction, the plasma creatinine concentration (P_{Cr}) had increased from 1.28 ± 0.03 to 3.43 ± 0.56 mg/dl, and blood urea nitrogen (BUN) from 24 ± 3 to 81 ± 15 mg/dl. P_M increased from 12 ± 3 to 63 ± 23 ng/ml. All 3 parameters returned towards control values over the next 14 days. Three dogs with CRF were placed in metabolism cages and 24 hr clearance (C) measurements made (see table):

	DOG 1	DOG 2	DOG 3
P_{Cr} (mg/dl)	3.8	3.8	1.2
BUN (mg/dl)	65	69	20
P_M (ng/ml)	198	178	11
C_{Cr} (ml/min)	2.6	4.6	9.4
C_M (ml/min)	8.7	14	46

The data indicate that in ARF, P_M increases rapidly and follows a similar pattern as P_{Cr} . In CRF, P_M increases by a greater % than either P_{Cr} or BUN. M is apparently secreted by the dog kidney, and this appears to be impaired in renal failure.

CATION TRANSPORT IN RAT THYMOCYTES: Na/H EXCHANGE IN CHRONIC RENAL FAILURE (CRF). Canessa ML*, Druml W*, Kelly RA*, Mitch WE. Dept. Medicine, Harvard Med. Sch., Boston, MA and Emory Sch. of Med., Atlanta, GA.

Chronic metabolic acidosis (as in CRF) increases Na-H exchange in renal tubule cells but the impact of acidosis and CRF on Na-H exchange in non-secretory cells is unknown. We studied cation flux and Na-H exchange in thymocytes of 8 pairs of CRF and pair-fed, sham-operated (SO) rats. After 6 weeks, CRF rats (SUN, 104 ± 4 vs 26 ± 2 mg/dl, $\bar{x} \pm SE$) were acidemic (pH: 7.25 ± 0.02 CRF vs 7.37 ± 0.02 , SO; HCO_3^- : 16.6 ± 0.03 , CRF vs 23.5 ± 1.4 , mM, SO; $p < 0.01$). Ouabain-sensitive ^{86}Rb influx was slightly lower in CRF (9.2 ± 1.2 , SO vs 8.2 ± 1.1 , CRF mmol/L cells/hr; $p < 0.01$) but Na/K/Cl cotransport and passive permeability were unaltered. However, amiloride-sensitive cell swelling in the presence of Na propionate was 50% greater in CRF thymocytes ($p < 0.05$). When intracellular pH was varied with nigericin, amiloride-sensitive ^{22}Na uptake was higher in CRF at pH 6.7 and 7.0 ($p < 0.05$), though maximal ^{22}Na uptake at pH 6.0 was only slightly higher (13.4 ± 0.5 , SO vs 14.8 ± 2.4 , CRF umol/L cells/min; $p < 0.10$). Thus, CRF impairs the sodium pump and enhances Na-H exchange in rat thymocytes.

DETERMINATION OF FREE RADICALS IN THE TISSUES IN EXPERIMENTAL PYELONEPHRITIS. Vitaly G. Maidannik, Vera D. Chebotareva, Alexander P. Pecheny. A.A. Bogomoletz Med. Inst., Kiev, USSR.

It was shown recently that the free radicals (FR) may be involved into the tissues damage in the inflammatory diseases. But it is known a little about the FR level in kidneys, liver and other tissues in animals with experimental pyelonephritis (PN). The mice CBA with 16-18g weight were used. The experimental PN was induced by the direct injection of Staph. aureus into the surgically exposed right kidney under the hexenalum anaesthesia. The determination of FR electronic level in the tissues was fulfilled with the paramagnetic resonance method. The results show that the development of experimental PN is accompanied with the significant increase of FR with $g=2,005$ and half-width of signal 25G in liver. On the 7th day of disease the FR content in liver increase on 300% in comparison with control group. Insignificant increase of FR level on 25-30% was observed in the tissue of undisturbed kidney. In the other organs (heart, spleen and other) the FR contents decrease significantly. The most decrease of FR level in these organs was registered on the 7th day of disease. These findings show that the FR plays a highly important role in pathogenesis of PN.

EFFECT OF GALACTOSE ON RENAL HEMODYNAMICS.

María Coco*, Norman Bank. Montefiore Hospital, Dept. of Medicine, Bronx, NY.

Glomerular filtration rate and renal plasma flow can be elevated in early insulin dependent diabetes. In order to determine whether these hemodynamic abnormalities may be related to increased polyol pathway metabolism (PPM) via aldose reductase, we studied non-diabetic rats fed a high galactose diet since galactose also is metabolized by this pathway. Normal SD rats were fed a 50% by weight galactose (G) diet. The total protein content was half that given to control animals fed a regular diet. A third group of rats received the G diet with the addition of sorbinil (S), an aldose reductase inhibitor (ARI), at 20 mg/kg/d. After 10-14 days, inulin and PAH clearances were obtained, as well as serum glucose and osmolality. The results are as follows:

DIET	C _{IN}	C _{PAH}	GLUCOSE	Osm
	ml/min/kg		mg%	mOsm
G	14.8±2*	41.6±5*	61±6*	295±3
G+S	7.2±1.7	22.3±2	116±11	291±6
control	6.4±1.2	22.5±2	96±6	292±2

*p<.05 vs. control diet

Sorbinil added to control diet did not affect normal hemodynamics. These observations indicate that galactose feeding results in hyperfiltration and increased plasma flow, in the absence of diabetes. Sorbinil, an ARI, prevents these hemodynamic changes. We conclude that increased PPM can cause marked hemodynamic changes in the kidneys of normal rats. This pathway may be responsible for the hyperfiltration seen in diabetes mellitus.

ROLE OF MYO-INOSITOL IN RENAL Na-K-ATPase CHANGES PRODUCED BY DIABETES. R.A. Cohen,* L.C. MacGregor,* K.C. Spokes,* P. Silva and F.H. Epstein. Department of Medicine, Harvard Medical School and Beth Israel Hospital, Boston, MA

The activity of Na-K-ATPase in renal medulla is increased by experimental diabetes mellitus. Because the medulla is rich in inositol, and abnormal inositol metabolism has been implicated in early neural complications of diabetes, we studied the effect of myo-inositol supplementation on Na-K-ATPase in medullary homogenates of rats with streptozotocin diabetes. Inositol (650 mg/kg) was given by gavage daily for 2 weeks after induction of diabetes. Medullary Na-K-ATPase (umol/mg prot/hr) was increased by 50% in diabetic rats (n=9) vs. control (n=6) (24.5±0.8 vs. 16.1±0.5) while cortical activity was unchanged. The increase was completely prevented by inositol (17.7±0.8; n=7), though hyperglycemia persisted. Similar results were seen when diabetic rats were treated with Sorbinil, an aldose-reductase inhibitor. Inositol content of outer medulla was 5x that of cortex but was unaltered by the diabetic state. Inositol and sorbinil did not reduce Na-K-ATPase activity of non-diabetic control rats, nor did they prevent the rise in medullary Na-K-ATPase in compensatory hypertrophy following nephrectomy. The effect of inositol supplements on Na-K-ATPase appeared independent of GFR, since the increase in GFR measured in diabetic rats (0.63±0.05 ml/min/100 g vs 0.42±0.004 in controls) was unaffected by inositol. The results indicate that disturbed inositol metabolism may play a role in the renal changes produced by diabetes, possibly through an effect on the peripheral or central nervous system.

ERYTHROPOIESIS AND ERYTHROPOIETIN BLOOD LEVELS (Ep) IN RATS WITH RENAL FAILURE (RF) AND INDUCED ANEMIA. N.E.I.R. Contreras,* A. Sanchez,* J. Cajias,* P. Durrego,* J. Chacón,* E. Bellorin-Font, V. Paz-Martínez and J.R. Weisinger. Centro Nacional de Diálisis y Trasplante and Renal Division, Hospital Universitario. Caracas, Venezuela.

The anemia of renal insufficiency has been related to impaired Ep secretion and/or decreased bone marrow response. In the present study, acute anemia was produced in rats, after 10 days of surgically induced RF (60-70% reduction of GFR) or in sham operated controls (C). Rats were bled by heart puncture (1.6 ml/100 g BW) and volume replaced with 0.9% saline. Blood hematocrit (Htc), % reticulocytes (Ret) and Ep (J. Caro, Cardeza Foundation, Philadelphia, PA.), determined before, 2, 5, and 10 days after bleeding were:

DAYS	0	2	5	10
Htc C	38±0.5	31.3±0.3	35.2±0.5	38.7±0.6
Htc RF	37±0.8	27.4±0.6	32.1±1.0	38.1±0.7
	n.s.	p<0.01	p<0.02	n.s.
Ret C	2.6±0.2	6.2±0.5	13.5±0.3	8.3±0.4
Ret RF	1.8±0.3	3.9±0.8	9.0±0.6	5.7±0.6
	n.s.	p<0.05	p<0.01	p<0.02
Ep C	16.5±0.8	46.7±4.5	42.3±3.8	25.3±2.5
Ep RF	23.2±1.2	53.0±8.3	37.6±8.9	30.4±2.6
	p<0.01	n.s.	n.s.	n.s.

Daily treatment with arachidonate (2 ml/Kg BW) starting the day of the bleeding, did not improve the erythropoietic response. In summary, after acute bleeding, rats with RF, developed a larger degree of anemia and a slower recovery compared with C animals, indicating an impaired erythropoietic response, in spite of adequate Ep levels. These results suggest the occurrence of resistance to Ep in RF rats.

CHANGES IN SODIUM TRANSPORT IN UREMIC RED BLOOD CELLS WITHOUT DISRUPTION IN INTRACELLULAR SODIUM HOMEOSTASIS. D.B. Corry, M.L. Tuck* and D.B.N. Lee. Olive View and Sepulveda VA Med. Ctrs. and UCLA Sch. of Med., Los Angeles, California.

We examined unidirectional cationic fluxes in RBC from 8 dialysis patients (D) and 8 normal subjects (N). Na-K pump (P)-mediated fluxes were measured as ouabain-sensitive Na²² efflux (Ex) and rubidium⁸⁶ (Rb) influx (Ix). Na,K cotransport (CoT)-fluxes were measured as bumetanide-sensitive Na Ex and Na Ix. Na fluxes, resistant to both ouabain and bumetanide, were termed "residual" Ex and Ix. RBC Na ([Na]_i), in mmole/L RBC, were not different between D and N both before (7.7±0.8 vs 8.4±0.4) and after (13.9±0.6 vs 14.0±0.7) nystatin-induced Na loading. Fluxes in mmole/L RBC/hr were:

FLUXES	D	N
P Na Ex	4.10±0.40	4.30±0.40
P Rb Ix	1.80±0.40	1.60±0.10
CoT Na Ex	0.16±0.04	0.40±0.08*
CoT Na Ix	0.54±0.14	0.49±0.12
Residual Na Ex	0.67±0.08	0.82±0.15
Residual Na Ix	4.10±0.38	1.85±0.23*

* D vs N, P<0.01.

Thus, in uremic RBC: 1. [Na]_i was normal both in fresh and under Na-loading states. 2. P function was indistinguishable from N. 3. CoT Na Ex was inhibited. 4. Non-P, non-CoT, residual Na Ix was increased. The changes in fluxes favoring Na accumulation in uremic RBC may represent adaptative responses to loss of other, unidentified mode(s) of Na entry. This counter-vailing adaptation could explain the observed normal [Na]_i in uremic RBC.

URINARY (U) UREA NITROGEN (UN), CALCIUM (CA) AND PHOSPHATE (P) IN MALES AND FEMALES. B. Cristal*, J. Rudoy*, R. Kohan*, S. Shasha*, N. Griner*, U. Makov*, J. Ben Ari* (Intr. by J. Levy) Dept. of Nephrology, Biochemistry Lab., Carmel Medical Center, Nahariya Government Hospital, and Statistics Dept., Haifa University.

Castration may prolong life of male rats with chronic renal failure (Levy, ASN 1986). Protein (Pr) and P intake may affect renal function. The purpose of this study was to evaluate UN, Ca, and P excretion as indicators of Pr, Ca and P intake. We studied 125 normal males and 127 females with normal renal function. 24 hrs excretion of U UN, U Ca and U P were measured and expressed in mmol. per Kg. body weight (KgBW). Statistical analysis used t test.

Results:

	Male	Female	p values
UUN/KgBW	0.02735	0.02499	0.026
UP/KgBW	3.7876	3.5002	0.033
UCa*UP/KgBW	197.8689	132.7034	0.0001

The results indicates higher amount of U UN/kgBW, U P/KgBW and U Ca*UP/KgBW in males, this may result from higher intake due to appetite stimulation effect of androgens. We suggest that the observed difference between human males and females may help to understand also the effect of castration in renal failure in rats.

UREMIA CHANGES CATION TRANSPORT IN RAT MYOCARDIUM. Druml W,* Kelly RA*, O'Hara DS*, Mitch WE, Dept. Med. Harvard Med. School, Boston, MA. and Emory Sch. Med., Atlanta, GA.

As abnormal cation transport occurs in several tissues of uremic rats, we investigated transport in myocardium because it might affect the genesis of uremic cardiomyopathy. Rats with acute (ARF, n=8) or chronic (CRF, n=7) renal failure were compared to pair-fed, sham-operated (SO) rats. Only CRF rats had hypertension (HBP) and left ventricular hypertrophy (LVH) (+33%, p<0.01). Intracellular sodium, and ouabain-sensitive, furosemide-sensitive and insulin-stimulated ^{86}Rb influx were all normal in myocardial slices from ARF and CRF rats. [^3H] ouabain binding was 45% lower in CRF (58.1±8.2 vs 81.6±15.3 pmol/g, \bar{X} ±SE, p<0.05), and was negatively correlated with LVH (r=-0.82; p<0.01). Na pump number was normal in ARF. High and low affinity ouabain binding sites (K_A of 8.0×10^6 and 2.9×10^6 L/M respectively) were present; decreased binding in CRF could be attributed to loss of low affinity sites. Thus, CRF reduces myocardial Na pumps but cation flux and intracellular sodium are normal indicating increased Na pump turnover. This response was most closely related to LVH and hence, HBP.

CONVERTING ENZYME INHIBITION (CEI) LESSENS TUBULOINTERSTITIAL INJURY (TII) IN PUROMYCIN AMINONUCLEOSIDE (PA) NEPHROSIS. J.R. Diamond, S. Anderson. Brigham & Women's Hosp.; Department of Pathology, Harvard Medical School, Boston, MA

Central venous injection of PA produces late tubular as well as glomerular injury. Since CEI lessens glomerular injury in this model (ISN, 494, 1987), we investigated whether late TII may also benefit from this therapy. Male Munich-Wistar rats received a single jugular venous injection of PA, 50 mg/kg or saline (S). One nephrotic group (PA) served as controls, while another (PA/E) received the CEI enalapril (50 mg/L drinking water). PA rats developed striking interstitial fibrosis, tubular casts and dilatation, and a mononuclear cell infiltrate after 70 wks. In contrast, despite comparable initial proteinuria (UprotV) and early tubulointerstitial abnormalities, the late development (70 wks) of interstitial fibrosis (p<0.01) and tubular casts and dilatation (p<0.001) were much less severe by quantitative scoring in PA/E rats. (Mean ±SE, *p < .05 vs S, †p < .05 vs PA):

70 Weeks	S(n=12)	PA(n=8)	PA/E(n=8)
UprotV (mg/d)	16±4	52±9*	10±4†
LMW UprotV (mg/d)	8±2	21±10	6±1

Late UprotV was higher in PA rats, whereas low molecular weight (LMW) UprotV, an index of tubular function, was only numerically higher in PA rats, perhaps because of the focal nature of the TII. Thus, CEI effectively lessens tubular as well as glomerular injury in this model. Moreover, severity of early proteinuria and morphologic injury does not necessarily predict late glomerular or tubulointerstitial injury.

ATRIAL NATRIURETIC PEPTIDE (ANP) AND ADAPTATION OF SODIUM URINARY EXCRETION IN PATIENTS WITH CHRONIC RENAL FAILURE. Jean-Claude Dussault, Stanislas Czekalski, Catherine Michel, Françoise Mignon, and Raymond Ardailou. (Intr. by K.F. Badr). INSERM U.64, Hôpital Tenon, Paris, France.

In order to examine the potential role of ANP in modulating the increased Na excretion per nephron in chronic renal failure (CRF), we studied 5 healthy subjects with normal renal function and 10 patients with moderate or severe CRF prior to, during and after administration of an i.v. Na load. All subjects had been for 5 days before the study on a controlled Na diet (120 mmol/day). Under basal conditions, plasma ANP and fractional Na excretion (FENa) were significantly correlated (p<0.01) and increased in proportion to the reduction of GFR. Acute Na loading resulted in plasma ANP increase in the 3 groups and in FENa increase in the patients with CRF. A highly significant correlation was found between both parameters in the latter 2 groups (p<0.001). In a further study we measured FENa and plasma ANP in 4 patients with severe CRF on the last day of two successive 6 days period during which they ingested diets containing 100 and 20 mmol Na/day respectively. There was a parallel decrease from 5.9 ± 1.6 to 2.2 ± 0.4 % for FENa and from 234 ± 111 to 76 ± 27 pg/ml (erect position) or 412 ± 189 to 121 ± 42 pg/ml (supine position) for plasma ANP. Both parameters were significantly correlated (p<0.001). Plasma aldosterone and renin activity increased during the Na-depleted period but were not correlated with FENa. These results suggest an important role for plasma ANP in promoting adaptation of Na excretion in patients with CRF both chronically and following an acute Na load.

NIFEDIPINE PREVENTS GLOMERULAR INJURY WITHOUT REDUCING GLOMERULAR PRESSURE (P_{GC}) IN RATS WITH DESOXYCORTICOSTERONE-SALT (DOC-SALT) HYPERTENSION. L. D. Dworkin, J. Benstein,* H. D. Feiner, M. Parker.* New York Univ. Med. Ctr., N.Y., N.Y.

Increasing calcium intake dramatically worsens glomerular injury in DOC-SALT rats. In this model, kidney damage is associated with glomerular hypertension, yet calcium feeding causes only a modest, further elevation in P_{GC} . To further clarify the roles of calcium and intrarenal hypertension in the genesis of hypertensive nephrosclerosis, we compared uninephrectomized (UNX) Munich-Wistar rats given DOC, saline for drinking and fed chow containing the calcium channel blocker, nifedipine (NIF, n=6) with DOC-SALT rats fed standard chow (CON, n=6). Eight weeks after UNX, awake systolic blood pressure (SBP) was elevated in CON (193 ± 5 mmHg, Mean \pm SE). CON rats also had proteinuria (77 ± 12 mg/24h) and morphologic evidence of glomerular injury. In contrast, SBP was normal (122 ± 7), proteinuria reduced (37 ± 8) and glomerular sclerosis completely prevented in NIF rats. Micropuncture revealed:

GROUP	N	AP ---mmHg---	P_{GC} ---	SNGFR nl/min	R_{A5} dyne's \cdot cm 5 $\times 10^{10}$
CON	6	136 ± 3	59 ± 1	70 ± 5	1.2 ± 0.1
NIF	6	104 ± 2 *	62 ± 2	62 ± 5	0.8 ± 0.1 *

Mean \pm S.E.; * $P < 0.02$ vs CON

Compared to values in intact animals, P_{GC} is elevated in DOC-SALT rats. Despite normalization of mean arterial pressure (AP), glomerular hypertension persists in rats given nifedipine due to a reduction in afferent resistance (R_A). Therefore, nifedipine prevents glomerular injury by a mechanism not dependent on reduction in P_{GC} . Taken together with the observation that calcium feeding promotes injury, these findings suggest that entry of calcium into one or more cell types is required for progressive hypertensive glomerular injury to develop in DOC-SALT rats.

DEEP NEPHRONS ARE MORE VULNERABLE TO RENAL MASS ABLATION. E. Fernandez-Repollet, E. Tapia* and M. Martínez-Maldonado. VA Center and Depts. Pharmacol. & Med., UPR School Med. San Juan, PR.

Reduction of renal mass in the rat results in progressive glomerular injury. To determine the vulnerability of deep nephrons to renal mass ablation we developed a model of unilateral cortical necrosis (CN) by thermal injury in the rat. Wistar-Furth rats (75+8 g) were divided as follows: Sham (n=8); 2/3 renal infarction (n=8); CN (n=8); 2 weeks later the right kidney was removed (NPX) in all rats. Clearance studies were performed in awake rats 14 weeks after NPX. The results are Mean \pm SEM; * $p < 0.05$ CN or 2/3+NPX vs Sham. + $p < 0.05$ CN vs 2/3+NPX.

	GFR ml/min	RPF ml/min	RVR dynes	Purea mg%	Uprot mg/ml
S	2.2 ± 0.1	7.3 ± 0.4	8 ± 1	30 ± 1	14 ± 1
2/3	1.4 ± 0.1 *	4.7 ± 0.4 *	16 ± 2 *	45 ± 7 *	67 ± 8 *
CN	0.9 ± 0.1 *+	3.7 ± 0.2 *+	25 ± 2 *+	60 ± 19 *+	102 ± 6 *+

Mean arterial pressure was not different between CN (178+7) and 2/3 NPX (168+7) and was higher than that of sham (140+6) rats. Glomerular counts were not different between CN and 2/3+NPX and were approximately half the Sham values. Calculated SNGFR was not different in any of the groups. By contrast, calculated prostaglandin excretion/nephron was lower in CN and 2/3+NPX than in Sham. In conclusion, the study suggests that deep nephrons are more susceptible to damage after renal mass ablation in the absence of superficial nephrons than in models where remnant kidney have both superficial and deep nephrons. This appears to result from glomerular hypertension more than from hyperperfusion.

GROWTH IN THE EXPERIMENTAL ANIMAL WITH CHRONIC RENAL FAILURE (CRF): USE OF ENALAPRIL (E).

AL Friedman, and Rita Pityer*, Department of Pediatrics, University of Wisconsin, Madison, Wisconsin.

Growth failure is a major complication of CRF in the growing animal. Preservation of renal function has been accomplished by dietary protein restriction and by the use of angiotensin converting enzyme inhibitors such as E. We undertook to study the effect of E on growth and renal function in animals with CRF. 3 wk old rats were divided into 2 groups following 3/4 nephrectomy to create CRF. 1 group, animals were fed a 22% protein diet and received E (20 mg/L) in the drinking water. The 2nd group was fed the same diet but no E in the water (Reg). Wt (g), Lt (cm), CrCl (ml/min/100g BW, Urinary Protein (mg/24 hrs) were followed for 20 wks. Starting with 15 animals in each group, 11 CRF rats on E survived to 20 wks whereas only 7 of the reg diet animals survived to 20 wks. At 20 wks of age other data included:

	CrCl	U Prot mg/24 hrs.	Wt (g)	Lt (cm)
E	$.14 \pm .08$	22.7 ± 7.3	210	21.5*
reg	$.16 \pm .04$	67.9 ± 9.5 *	227*	23.5*

* $p < .05$

Although E led to improved survival and lower protein excretion it also resulted in poorer growth. The exact mechanism for E induced growth retardation is unknown. This observation puts in question the use of angiotensin converting enzyme inhibitor as a strategy for preserving renal function and improving growth in the young animal.

GLOMERULAR HYPERTROPHY AND EPITHELIAL CELL INJURY ARE DETERMINANTS OF PROGRESSIVE GLOMERULOSCLEROSIS IN THE RAT. J.U. Fries*, D. Sandstrom*, T.W. Meyer, and H.G. Rennke. Brigham and Women's Hospital, Dept. of Pathology, Boston, MA, and Stanford Univ. and VA Med. Ctr. Palo Alto, CA.

Recent studies have implicated an elevation of the glomerular capillary pressure (Pgc) in the pathogenesis of glomerulosclerosis. The modulating role played in this process by structural determinants, such as epithelial cell damage and glomerular size, was explored in MW rats given either the epithelial cell toxin Adriamycin (Ad) or saline, followed 4 weeks later by 4/5 nephrectomy (Nx) to induce glomerular hypertrophy, or sham operation. Mean values (\pm SEM) for protein excretion (UprotV, mg/day), mean arterial pressure (AP, mmHg), glomerular volume (Vglom $\times 10^{-6}$, μm^3), and frequency of segmental sclerosis (%SS) were measured 7 weeks after Nx. Pgc was measured 3 weeks after Nx in separate groups of animals.

	Ad/Nx	Ad/sham	saline/Nx	saline/sham
AP	140 ± 9	129 ± 2	159 ± 8	126 ± 3
UprotV	156 ± 14	70 ± 13	48 ± 7	10 ± 2
%SS	14.2 ± 5.8	0.7 ± 0.2	5.1 ± 1.3	0.2 ± 0.1
Vglom	2.8 ± 0.16	1.4 ± 0.05	2.5 ± 0.15	1.3 ± 0.04
Pgc	63 ± 3	60 ± 1	68 ± 2	53 ± 1

2-way ANOVA indicates a significant effect of Nx but not Ad on Vglom and AP, additive effects and interaction of Ad and Nx on UprotV and %SS. We conclude that epithelial cell injury exacerbates hemodynamically-mediated glomerulosclerosis. High Pgc is most damaging to hypertrophied glomeruli, where an increased capillary diameter is expected to result in greater tension transmitted to the peripheral capillary wall (Laplace's law).

CREATININE (CR) DEFICIT AND TOXIN PRODUCTION RESULTING FROM BOWEL BACTERIAL CREATININASE ACTIVITY (CRase) IN CRF. G. Gabuzda*, K. Superdock*, R. Kolecki*, S. Dunn*, and M. Simenhoff. Dept. of Med., Jefferson Medical College, Phila., PA.

This study extends observations on CR degradation in feces to define effects of various types of microorganisms, influence of previous antibiotic administration, and inhibition of CRase activity on creatinine deficit and toxin production in CRF. Forty studies in 34 patients were performed (13♂ 21♀; ages 16-68). Six healthy subjects acted as controls (30 studies). Methylamines were analyzed by gas chromatography and CRase activity was measured in stool as % CR consumption/5 hours (% CR/5) (of added CR incubated in an anaerobic (AN) chamber). Results ± SEM (p value):

CR < 6 mg/dl	CR > 6 mg/dl	Control
27.2±13.9	64.3±6.6 (< 0.001)	12.5±2.2
+ Fecal Antibiot.	12.9±2.4 (< 0.002)	6.8±2.8

When oral antibiotics were taken within 3 months of test, % CR/5 was reduced to 14.7±3.8% (p<0.001). Some aerobic % CR/5 was shown (37.4±9.0%) with p<0.05 when compared with AN conditions. Normal stool incubated with CR for 12-24 hours could be induced to CRase activity (12.8±3.6 to 77.2±11%, p<0.001). Five patients with demonstrated small bowel bacterial overgrowth by intubation also showed significant methylamine production (310±63ug/dl) and fecal CR degradation (69.0±30%). We conclude that CR activity is inhibited in the bowel by oral and/or fecal antibiotics suggesting a bacterial cause and that normal stool can be induced to produce CRase. These findings account for the CR deficit (and large extra-renal CR clearance) and potential toxin production from CR degradation in CRF.

URINARY ABNORMALITIES IN MICE WITH SURGICALLY-INDUCED CHRONIC RENAL FAILURE. Raymonde Gagnon. Montreal General Hospital, Montreal, Canada. (intr. by P. Somerville).

We have previously demonstrated that stable and severe renal failure can be achieved in the mouse by surgical diathermy of the surface of one kidney combined with contralateral nephrectomy. We studied urinary (U) parameters in normal (N) 13 week female C57BL/6J inbred mice as well as in mice with surgically-induced renal failure (RF) of 6 weeks' duration and in sham-operated (S) controls (diathermy only). Results (mean±SD) in groups of more than 12 mice were:

	Normal	Sham surgery	Renal failure
BUN ^a	26±5	25±4	119±22
Body weight ^b	22±2	22±3	20±3
Hemoglobin ^c	13±1	13±1	8±2
U volume ^d	1.5±0.8	2.2±0.8	3.0±1.3
U osmolarity ^e	418±164	308±87	339±107
U protein ^f	0.1±0.1	0.1±0.1	0.5±0.4

a:mg/dl; b:g; c:g/dl; d:ml/24 hr; e:mOsm/kg H₂O; f:mg/24 hr.

Growth retardation and anemia directly paralleled the azotemia. RF mice developed significant proteinuria. U osmolarity was reduced significantly in both S and RF mice, suggesting that diathermy is associated with loss of U concentrating ability.

ANEMIA LESSENS SYSTEMIC/GLOMERULAR HYPERTENSION AND PROTEINURIA IN RENAL ABLATION. DL Garcia*, S Anderson, KJ Sandquist*, HG Renke, and BM Brenner. Brigham & Women's Hosp., Boston MA.

Glomerular hemodynamic factors play a key role in progressive renal disease, and their modification affects the pace of progression. One such potential modifier in clinical renal disease is progressive anemia. Two groups of Munich-Wistar rats (n=5 each) received isocaloric diets differing only in iron content: group C received standard chow (35 mg iron/kg chow), and group A received the same chow but with only 6 mg iron/kg. 5/6 nephrectomy (NX) was performed after 7 wks on diets when hematocrits (Hct) averaged 53±1% and 31±1%, respectively (p<.05). Micro-puncture studies were performed 4 weeks later: (Mean±SEM; *p<0.05 vs C).

	Hct %	AP ---mmHg	P _{GC} ---mmHg	SNGFR ---nl/min	Q _A ---ml/min	R _A ×10 ¹⁰ dyn·s·cm ⁻⁵	R _E ×10 ¹⁰ dyn·s·cm ⁻⁵
C	42±1	147±8	61±1	80±6	257±16	1.6±.2	1.0±0.7
A	25±1*	124±2*	47±1*	88±4	358±12*	1.3±.1	0.6±0.7*

NX further lowered Hct in both groups, and in group A the elevated mean arterial pressure (AP) did not occur. Anemia led to further elevation of glomerular plasma flow rate (Q_A) without affecting the single nephron glomerular filtration rate (SNGFR). A proportionately greater reduction in efferent (R_E) than afferent (R_A) arteriolar resistance, a presumed viscoelastic effect, prevented the rise in mean glomerular hydraulic pressure (P_{GC}) in Group A. Anemia also limited proteinuria in group A (19±7 mg/d) compared to group C (60±16 mg/d) at 6 wks post-ablation (p<.05). Thus, anemia may serve to lessen risks of systemic/glomerular hypertension and progressive injury after loss of renal mass.

AZOTEMIA AND NEPHROPATHOLOGY CORRELATE IN THE EXPERIMENTAL CYSTIC NEPHROPATHY OF ENDOTOXIN-INJECTED CONVENTIONAL RATS. K.D. Gardner, A.P. Evan, and W.P. Reed*. Depts. of Medicine and Anatomy, Univ. of New Mexico, Albuquerque, New Mexico, and Indiana University, Indianapolis, Indiana.

Endotoxin (LPS) induces the appearance of interstitial leukocyte infiltrates and the development of cysts in kidneys of germfree rats fed nordihydroguaiaretic acid (NDGA) (Kidney Int., 1987). To establish the relationship of these events to each other and to azotemia in conventional rats, we graded renal pathology on a 0-4+ scale and related the results to blood urea nitrogen concentrations (BUNs) in 18 180 gm male Sprague-Dawley rats fed 2% NDGA for 37 days and injected i.p. with 40-160 nanogm E. coli O113 endotoxin q 2 days over the final 8 days. Significant correlations (r ≤ 0.56; p ≤ 0.01) were recorded between BUNs and final body weights (negative) and between BUNs and 9 of 15 selected parameters of renal pathology. Interstitial infiltrates and fibrosis, tubular dilation (cyst formation), and micropolyp formation were significant predictors of BUN; vascular and glomerular changes were not. Normal vessels in 14/18 (78%) kidneys excluded LPS-mediated endothelial damage as a contributor to azotemia. Neither BUNs nor nephropathology correlated significantly with LPS dosage. The statistical relationships demonstrated here are predicted by the hypothesis that cytokines and toxic oxygen radicals, released locally from LPS-stimulated inflammatory cells, are linked to cyst development, micropolyp formation, and azotemia in this model of experimental renal cystic disease.

DECREASED ACTIVITY OF INTRALYSOSOMAL PROTEOLYTIC ENZYMES AS POSSIBLY RELEVANT FACTOR FOR TUBULAR HYPERTROPHY IN THE EARLY DIABETIC KIDNEY. B.Geissinger*, C.J.Olbricht, K.M.Koch*. Med. School Hannover, Federal Republic of Germany

In renal hypertrophy of early diabetes mellitus (DM) the kidney protein mass is increased. This may be due to increased protein synthesis or decreased protein breakdown. The lysosomal proteases cathepsin B and L are major enzymes involved in intracellular protein breakdown. Hence, to evaluate the second hypothesis we measured the activity of Cathepsin B and L in microdissected glomeruli and in the single segments S1, S2, and S3 of proximal tubule by ultramicro-assay. Three groups of age matched female rats were studied: 1. Sham injected controls (C). 2. Ten days after induction of DM by i.v. streptozotocin, 60mg/kg BW (DM). 3. DM-rats treated with insulin (INS). Enzyme activities are given as pmol/min incubation/mm tubule length or /glomerulus. Values are mean±SD.

Group	n	Kidney Weight,mg	Cathepsin B+L			
			Glom.	S1	S2	S3
C	8	798±90	3±2	16±5	16±4	6±2
DM	8	985±30*	2±1	10±4*	10±4*	4±2*
INS	10	847±66§	3±2	14±4§	18±5§	5±2

* = p < 0.05, C vs DM. § = p < 0.05, DM vs INS
The increase of kidney weight in diabetic rats was prevented by insulin. Cathepsin B+L activity was decreased in S1, S2, and S3 of diabetic rats. In S1 and S2, the decrease of enzyme activity was prevented by insulin. Cathepsin activity in glomeruli remained unchanged. Hence, reduced intracellular proteolytic activity may partially explain tubular hypertrophy but not glomerular hypertrophy in early diabetes mellitus.

ADAPTIVE RESPONSES TO LOW-PROTEIN (LP) DIETS: WHAT IS THE RISK IN CHRONIC RENAL FAILURE (CRF)? Goodship, THJ*, Young VR*, Steinman TI and WE Mitch. M.I.T. and Harvard Med. Sch., Boston, MA and Emory Med. Sch., Atlanta, GA.

LP diets could cause malnutrition in CRF if patients could not activate normal responses including suppression of amino acid oxidation (AAox) and protein breakdown during feeding. To assess adaptation in CRF, we measured the kinetics of constantly infused L[15N, 1-¹³C] leucine and nitrogen balance (BN) in 6 CRF (mean Scr 5.6 mg/dl) and 4 normal subjects (C) during 1 week of 1.0 (HP) vs 0.6 (LP) g protein/kg/d diets. Leucine turnover was analyzed after an overnight fast and with feeding. During HP, feeding stimulated AAox but LP suppressed this response 26% in C vs 33% in CRF (P=NS). Feeding inhibited protein breakdown equally in C and CRF; protein synthesis (PS) was unaffected by protein intake or CRF. HP was better for BN and for the difference between PS and protein breakdown (CRF: 1.8±0.7 vs -0.2±0.7 gN/day, LP; p<0.01 and C: 1.9±0.3, HP vs 0.6±0.5 gN/d, LP; p<0.05) Thus, adaptive responses to LP seem unimpaired by moderate CRF since leucine flux, the AAox response to feeding and BN were the same as in C. With an adequate diet but no added catabolic stimulus, the risk of malnutrition is not increased by CRF.

EFFECT OF DIETARY PROTEIN AND UNINEPHRECTOMY (Unx) ON URINARY EPIDERMAL GROWTH FACTOR EXCRETION (uEGF). A Gung*, KF Badr, DN Orth* and RC Harris*. Vanderbilt University, Nashville, TN.

Immunocytochemical staining for EGF and in-situ hybridization of mRNA for preproEGF have localized EGF to TALH and early DCT. Since protein feeding is specifically associated with hypertrophy of TALH (Bouby, Trinh, & Bankir. KI:31:430, 1987), we examined the effect of varying protein intake on uEGF and compared it to that seen following Unx.

Urine was collected from adult male rats fed a standard (24%) protein diet and uEGF measured by radioimmunoassay. 24h after Unx, uEGF fell from 1605 to 910 pg/min, with no increase over the subsequent 5 days. Thus, uEGF/kidney was not increased: 1332±346 (control; n=5) vs. 821±108 (Unx; n=5). Micropuncture revealed that, 4-6 days post-Unx, SNGFR increased from 27.3±4.8 (n=4) to 40.2±4.2 (n=5) (p<0.05). In additional studies, uEGF levels were obtained in control (2K) or Unx rats after 6-8 days of 6, 24 or 40% protein diets.

Dietary protein	uEGF (pg/min/kidney)	
	2K	Unx
6%	414±150 (n=4)*	954±244 (n=5)
24%	803±68 (n=28)	623±54 (n=3)
40%	698±105 (n=5)	770±203 (n=4)

Thus: 1. In rats with two kidneys, an increase in protein intake from 6 to 24% is associated with doubling of uEGF/kidney, an effect abolished by prior Unx. 2. In normal rats on 24% protein diet, Unx is not associated with increased uEGF/kidney despite an increase in SNGFR. These results show that increased dietary protein, a stimulus of TALH hypertrophy, is also associated with increased uEGF, suggesting a possible causal relationship. This association is lost following reduction of renal mass. * : p<0.025 vs 2K(24%)

CHOLESTYRAMINE RESIN (CR) LOWERS ACUTE AND RECURRENT PROTEINURIA IN CHRONIC PUROMYCIN AMINONUCLEOSIDE (PA) NEPHROSIS. N.A. Hanchak*, M.J. Karnovsky, J.R. Diamond, Dept. of Pathology, Harvard Medical School, Boston, MA

Dietary cholesterol supplementation exacerbates progressive PA glomerulopathy (KI, 31:383A) presumably by aggravating the hypercholesterolemia of nephrosis. To further assess the putative aggravating role of cholesterol, we studied the effect of a serum cholesterol-lowering agent, CR, in the chronic PA nephrotic model (Am J Pathol, 122:481).

Chronic PA nephrosis was induced in male SD rats by a single jugular venous injection of PA (5 mg/100 g BW). Immediately following PA injection, one group received a standard rodent diet supplemented with 5% CR, while another received the standard diet supplemented with 5% cellulose (C). Proteinuria (UprotV), fasting total cholesterol (FTC), fasting triglycerides (FTG), and serum creatinine (Scr) were measured (mean ± SE; †p<0.05; *p<0.01; **p<0.005 v PA/C):

	UprotV	Scr	FTC	FTG
	mg/d	mg/dL	mg/dL	mg/dL
1 Week				
PA/CR(n=10)	38±10†	.4±.03†	114±17*	163±23**
PA/C(n=9)	69±7	.6±.04	186±17	395±63
15 Weeks				
PA/CR(n=10)	4±1†	.6±.03	60±3	57±5†
PA/C(n=10)	9±1	.5±.03	59±3	74±6

This data suggests that reduction in FTC and/or FTG by CR may be important in the amelioration of acute nephrosis. Also, CR blunts the recurrent UprotV at 15 wks. The importance of this late protection and its relation to serum lipids awaits investigation at later intervals.

STEROIDS (S) INDUCED PROGRESSIVE RENAL DAMAGE (PRD) IN RATS WITH 5/6 RENAL MASS ABLATION. Bertha Herrero*, Guillermo Feria*, Felipe Zárate*, Edgar-do Reyes*, Juan A. Tamayo and José Carlos Peña. Instituto Nac. de Nutrición Salvador Zubirán, Deptos de Nefrología y Patología, México, D.F., México.

S, Methyl-prednisolone, 1 mgr/day, was given intramuscularly to male Wistar rats, 250 g body weight (BW) with 5/6 kidney mass reduction 4 weeks after surgery. Animals were divided in 4 study groups: G-A) Normal protein diet (NPD) (23%) 9 rats BW 282±9; G-B) NPD + S 10 rats, BW 234±7; G-C) low protein diet (LPD) (8%) 9 rats, BW 261±15; G-D) LPD diet + S 9 rats, BW 254±11 B Urea (BU), creatinine, Na, K and phosphate (P), were measured in blood, drawn from tail and in 24 h urine collected every 2 for 24 weeks. At 24 weeks surviving animals were sacrificed and kidneys perfused with gluteraldehyde and phosphate buffer, for histopathological studies.

Results are summarized as mean ±se; P<0.05 is significant*.

Groups	Death rate	BW g	Urea mg/dl
A 4w	1/9 11 %	325±13	103±38
20w	3/9 33 %	454±18	96± 6
B 4w	2/10 20 %	234±10	122± 7
20w	9/10 90 %	254±	271
C 4w	3/9 33 %	260±18	30± 5
20w	5/9 44 %	256±16	39± 5
D 4w	1/9 11 %	211± 9	38± 5
20w	3/9 33 %	171±11	21± 3

S increased kidney damage and death rate in G-B; BW was 50% less in G-B G-A; Urea was different (*) from G-A,C,D from week 2 to week 8. The S induced PRD in rats with renal ablation and NPD. LPD protect against the S action. Use of S in renal disease may be hazardous.

THE ABILITY OF DIETARY PROTEIN RESTRICTION OR ENALAPRIL TO REDUCE ALBUMINURIA IN NEPHROTIC RATS IS ADDITIVE AND INCREASES WITH LENGTH OF TREATMENT. F.N.Hutchison, V. Martin *, H.Jones Jr.*, and G.A.Kaysen, Martinez V.A.M.C., University of California at Davis, Department of Medicine, Martinez, CA.

Both enalapril (EN) and dietary protein restriction (LPD) cause a decrease in urinary albumin excretion (UalbV mg/day) in rats with passive Heymann nephritis (HN). An additive effect of EN with LPD was not detected during the short periods previously studied. To determine what interactive effect EN therapy and LPD might have on albumin homeostasis, 4 groups of rats (N = 45) with HN were fed either 8.5% (LP) or 40% protein (HP). On day 14 after injection with anti serum, half of each dietary group had EN (40 mg/kg/d) added to their drinking water (HPE and LPE). Serum albumin concentration (Salb mg/ml), UalbV, plasma and total albumin mass (PAM and TAM, mg/100g bw respectively), were measured using ¹²⁵I albumin during days 17 to 21 or 25 to 28. (later time tabulated below, * P<.05 vs LP)

N	Salb	UalbV	PAM	TAM
HP 6	13.9±3*	515±63*	87±10*	196±25
HPE 5	31.6±1*	66±38	172±9*	296±18*
LP 5	24.0±2	57±11	116±12	195±15
LPE 5	33.2±1*	20±6*	158±3*	300±18*

Neither UalbV nor Salb changed in HP with time. UalbV was significantly less (P<.001), and Salb and PAM significantly greater (P<.05 for each) in LP and in HPE, than in HP at each time. UalbV decreased (P<.01) and Salb increased (P<.001) with time in LP, LPE and HPE. The combination of LP and EN resulted in the lowest UalbV and highest Salb and TAM.

GLOMERULAR HYPERFILTRATION, HYPERPERFUSION OR HYPERTENSION DOES NOT MEDIATE THE HYPERTROPHY OF GLOMERULI WHICH PREDISPOSES TO SCLEROSIS.

L.Ichikawa, Y. Yoshida* & A. Fogo*. Dpts. of Pediatrics & Pathology Vanderbilt University School of Medicine, Nashville, Tennessee.

Unilateral ureteral diversion into the peritoneal cavity (UD) is known to induce glomerular hyperfiltration in the contralateral kidney, but unlike after unilateral nephrectomy (NPX), without renal hypertrophy. We examined the long term effect of UD vs NPX on the function and structure of the glomeruli. Prior to study, 2/3 of renal mass was removed from the left kidney in all three groups of Munich-Wistar rats (n=32 rats). UD or NPX was performed on the right kidney in the first and second groups of rats, respectively, while the right kidney was left untouched in the third group of rats to serve as controls (CONT). At 2 wks, UD and NPX rats had similarly elevated BUN levels (42 and 49 mg/dl). Also, the left kidneys of UD and NPX rats had marked and comparable degrees of glomerular hypertension, hyperperfusion and hyperfiltration, when compared to CONT, i.e., glomerular capillary pressure averaging 71 mmHg and 73 vs 52, glomerular plasma flow rate 197 nl/min and 193 vs 175, SNGFR 63 nl/min and 61 vs 47. Whereas the maximum glomerular corpuscular planar area (GA) of UD rats assessed by serial section histological analysis remained at the level equal to that of CONT rats, averaging 7.8 μm² vs 7.4, GA was markedly increased in NPX (10.4 μm²). Similar degrees of glomerular hypertension, hyperperfusion and hyperfiltration were also demonstrated at 4 wks in the left kidneys of UD and NPX rats. Whereas average glomerular sclerosis index (GSI: 0-4 scale) assessed at 4 weeks was 0.14 in UD and 0.01 in CONT, significantly and substantially higher GSI (0.42) was noted in NPX rats. Moreover, among the glomeruli of NPX rats a highly significant positive correlation (p<0.025) was present between GA and mild GSI (GSI<1.5) determined by serial section histological analysis for the same glomerulus. Thus, while glomerular hypertrophy appears to be an important step preceding the subsequent glomerular sclerosis, glomerular hyperfunction plays no role for the induction of this hypertrophy. The results also indicate that physical removal of nephrons, and not the loss of their excretory function alone, is required to trigger the accelerated processes of glomerular hypertrophy and sclerosis.

THE COMBINED EFFECTS OF REDUCED NEPHRON MASS AND DIET-INDUCED HYPERCHOLESTEROLEMIA ON GLOMERULAR INJURY. B.L. Kasiske, M. O'Donnell, and W.F. Keane. Henn. Co. Med. Ctr., U. of MN, Mpls, MN.

Both hyperlipidemia and compensatory changes that accompany a reduction in functioning nephrons, e.g., hyperfiltration, influence the progression of focal glomerulosclerosis (FGS). The present study was designed to assess the independent and/or synergistic effects of reduced renal mass and increased serum cholesterol (C) on FGS using 2-way analysis of variance (ANOVA). Ten week old male rats fed a 4% C diet underwent unilateral nephrectomy (UNX) (C-1K, n=18) or sham surgery (C-2K, n=10). Rats fed standard (S) diet also underwent UNX (S-1K, n=8) or sham surgery (S-2K, n=8). Blood pressure (BP), C (mg/dl), and urine albumin excretion (UalbV, mg/24h) were measured throughout the study. Results 15 weeks after surgery (mean ± SD, p values for 2-way ANOVA):

	Serum C	KW(1)	UalbV	FGS(%)
C-1K	407±274	2.6±0.6	100±105	7.8±10.0
C-2K	256±93	1.7±0.2	59±28	3.5±4.6
S-1K	73±15	2.4±0.4	15±7	1.0±1.9
S-2K	66±10	1.6±0.3	5±8	0.8±1.6
p(C vs S)	0.00	0.16	0.00	0.03
p(1K vs 2K)	0.11	0.00	0.21	0.22

BP was not affected by UNX or high C. Diet-induced hypercholesterolemia markedly accelerated FGS in both 1K and 2K rats. At 15 weeks, UNX had little impact on FGS in rats fed S diet. More importantly, UNX failed to cause a significant worsening of C-induced FGS (1K vs 2K). Thus, with this degree of nephron reduction, hyperlipidemia was the more important determinant of the degree of glomerular injury.

ALBUMIN SYNTHESIS IS RESTRICTED BY A LOW PROTEIN DIET IN NEPHROTIC RATS. G.A.Kaysen, V.Martin*, H.Jones Jr. *, F.N.Hutchison, Martinez V.A.M.C., University of California at Davis, Department of Medicine, Martinez, CA.

Urinary albumin excretion (UalbV, mg/100g/h) and the rate of albumin synthesis (AlbSyn, mg/100g/h) increase, and serum albumin concentration (Salb, mg/ml) decreases when dietary protein intake is changed from 8.5% (LP) to 40% (HP) in rats with passive Heymann Nephritis (HN). To determine if HP would increase AlbSyn independent of its effect on UalbV, enalapril (40mg/kg/d) (E) was administered to 22 HN rats eating 40% (HPE) or 8.5% (LPE) protein. Plasma albumin mass (PAM, mg/100g), total albumin mass (TAM, mg/100g), and AlbSyn were measured using ¹²⁵I albumin. Similar measurements were made on 23 HN rats not receiving E (LPNE and HPNE). AlbSyn was 15.5 ± 1.3 in all HP and increased with decreasing Salb (AlbSyn = 22 - 0.38 x Salb, r = .5253, P<0.01). AlbSyn was 8.4 ± .5 in all LP and did not increase at reduced Salb. HPE and LPNE had similar UalbV, 1.62 ± .07 and 1.45 ± .31 respectively, yet Salb, PAM and TAM were greater in HPE (31.6 ± .91 vs 24 ± 2.3, P<0.02, 172 ± 9 vs 116 ± 12, P<0.01, 296 ± 18 vs 195 ± 15, P<0.005 respectively) AlbSyn also tended to be greater in HPE than in LPNE (7.5 ± .4 vs 6.8 ± 1) despite a lower Salb in LPNE. HPNE had the greatest UalbV (12 ± 1.4) and the lowest Salb (13.9 ± 2.7), although AlbSyn was greatest (20 ± .7). The paradoxical decrease in Salb in HN rats fed HP is due to the increase in UalbV, not an inability of HP to stimulate AlbSyn. Although LP reduces UalbV, it also prevents maximization of albumin pools at any given UalbV in HN rats.

TWO-KIDNEY, ONE-CLIP (2K1C) HYPERTENSION ALTERS GLOMERULAR (GLOM) PROSTAGLANDIN (PG) RESPONSE TO DIABETES. R.T. Kopecky, D. Patchin*, E.T. Schroeder. SUNY Health Science Ctr., Syracuse, NY.

The pathophysiologic significance of enhanced glom PG production in diabetes is uncertain. We studied in vitro glom PG production in rats with combined 2K1C hypertension and streptozotocin-induced diabetes (HD) since in this model unclipped kidneys (UnCK) have increased and clipped kidneys (ClK) decreased susceptibility to diabetic glom injury. Age-matched normal (N), diabetic (D) and 2K1C (H) rats served as controls. Systolic BP in HD and H (171 ± 3 vs 165 ± 3 mmHg) and glucose in HD and D (542 ± 18 vs 503 ± 15 mg%) were comparable. Isolated glom PG production expressed as ng/mg protein/30min, ± SE, n=10-14. D produced more PGE₂ (4.0 ± .4 vs 2.4 ± .2, p<.005) and 6-Keto PGF_{1α} (1.9 ± .3 vs 1.2 ± .1, p<.05) than N. UnCK of HD produced more PGE₂ (4.2 ± .5) than ClK (2.5 ± .3, p<.05) and were similar to D, while UnCK and ClK of H produced the same amount of PGE₂ (2.5 ± .3 vs 2.4 ± .3, NS) and were not different from N. Similar results were obtained for 6-Keto PGF_{1α}. Addition of 3μM arachidonate significantly increased PGE₂ in H-UnCK, H-ClK and HD-ClK, but not in HD-UnCK, indicating maximum basal substrate utilization in HD-UnCK. Euglycemic insulin treatment reduced PGE₂ (2.2 ± .2) and 6-Keto PGF_{1α} (.9 ± .2) production of D (n=7) to normal, eliminated difference in PG production between UnCK and ClK of HD (n=4) and prevented diabetes associated renal hypertrophy. Enhanced glomerular PG production is not an invariable metabolic consequence of diabetes. A correlation between vasodilator PG production and susceptibility to diabetic glom injury is suggested.

EFFECT OF UREMIC PLASMA ON RBC INSULIN RECEPTOR. Hi Bahl Lee, Seung Ho Baick*, Myung Hi Yoo* and Seung Duk Hwang*. Dept. of Intern. Med., Soon Chun Hyang Univ. Hosp. Seoul, Korea

We have previously demonstrated that RBC ¹²⁵I-insulin binding and high affinity receptor concentration were both decreased in uremic patients and that these abnormalities were not corrected by longterm dialysis (ASN 1986, P231 A). In order to further investigate the role of uremic plasma in the RBC insulin receptor defect in uremic patients, we measured RBC insulin receptor before and after 4hours of hemodialysis (HD) and also after incubating normal RBC in autologous and uremic plasma and uremic RBC in autologous and normal plasma.

RBC ¹²⁵I-insulin binding was significantly lower in uremia than in normal control (8.60±2.67 vs 10.19±2.54%, P<0.05). High affinity receptor concentration was also lower in uremia than in normal control (0.47±0.07 vs 0.71±0.08ng/ml, P<0.05). After 4hours of HD RBC ¹²⁵I-insulin binding did not change (8.60±2.67 vs 8.31±2.10%, P>0.05). After incubation for 18hrs at 40°C in autologous plasma, normal or uremic RBC ¹²⁵I-insulin binding did not change significantly. Normal RBC insulin binding after incubation in uremic plasma was not different from that after incubation in autologous plasma (autologous 10.39±4.57 vs uremic 10.04±0.30%, P>0.05). No difference was observed in uremic RBC insulin binding between values after incubation in normal plasma and in autologous plasma (autologous 9.20±3.97 vs normal 9.45±2.91%, P>0.05).

These results suggest that mechanisms other than uremic serum factor(s) may be responsible for the RBC insulin receptor defect in uremia.

SEX VULNERABILITY IN THE SUBTOTAL NEPHRECTOMY (Nx) MODEL OF GLOMERULOSCLEROSIS. JR Lombet*, SG Adler, PS Anderson*, CC Nast, D Olsen*, RJ Glasscock. Harbor-UCLA Medical Center, Torrance CA and Jefferson Medical College, Philadelphia, Pennsylvania.

Most studies of Nx induced glomerulosclerosis in rats were performed in males. This study shows relative resistance of females to the development of glomerulosclerosis after Nx. 14 male rats underwent 1-2/3 Nx (n=6, NxM) or sham Nx (n=8, SxM). 15 females underwent 1-2/3 Nx (n=7, NxF) or sham Nx (n=8, SxNF). Rats were pair-fed for 6 weeks. BP, body weight (BW), serum and urine creatinine and urine protein were measured 1, 3 and 5 weeks after surgery. At sacrifice (week 6), RNA was extracted from sieved glomeruli and mRNA levels for procollagen α1 (IV) measured. Renal biopsies for light and immunofluorescence microscopies were obtained and scored blindly. In the Nx groups, BP, serum creatinine, and creatinine clearance/100 gm BW were equal at 1, 3 and 5 weeks (p>.05). At 5 weeks: BP, mmHg (188±20, NxM; 188±28, NxF); serum creatinine, mg/dl (0.8±0.1, NxM; 1.1±0.3, NxF); creatinine clearance/100 gm BW (0.3±0.12, NxM; 0.3±0.12, NxF, p>.05). Proteinuria was greater in NxM than NxF at 3 weeks (29±21 vs 6±3 mg/24 hrs, p<.05), and 5 weeks (79±55 vs 30±35, p<.05). Proteinuria in both control groups was <8±4. Procollagen α1 (IV) mRNA levels were higher in NxM than all other groups (p<.05). mRNA levels in NxF, SxNF & SxNM did not differ significantly, (p>.05).

mRNA (U/ug RNA)	NxM	SxNM	NxF	SF
	71±18	40±18	42±24	44±17

18-20% of glomeruli exhibited light microscopic changes in both Nx groups. The NxF group had 1/3 the mesangial expansion and 1/10 the glomerulosclerosis exhibited in the NxM group, (p<.05). In conclusion, although systemic hypertension occurs equally in NxM & NxF rats, proteinuria, mesangial expansion, glomerulosclerosis, and procollagen α1 (IV) mRNA levels indicate that males are more vulnerable to glomerular damage after Nx than females.

CHLORIDE CONDUCTANCE DEFECT IN HUMAN UREMIC RED CELL GHOSTS IS CORRECTED BY DIALYSIS. R.D. London, M.S. Lipkowitz, and R.G. Abramson. Mt. Sinai School of Medicine, N.Y., N.Y.

Electroneutral (non-conductive) and conductive components of RBC ghost chloride permeability were evaluated in 3 groups of nontransfused adults: uremic patients pre-dialysis (n=12), stable asymptomatic CAPD patients (n=8), and normals (n=15). The kinetics of the electroneutral Cl/HCO₃ antiporter were measured with an acridine orange fluorescent assay: uremia did not affect the Km for internal Cl, external Cl or external HCO₃. The conductive component of Cl permeability was determined by measuring Cl conductance relative to that of K (GCl/GK) with the PD sensitive fluorescent probe DiS-C₂-(5). In contrast to their unaltered electroneutral Cl permeability, uremic ghosts demonstrated a significantly higher GCl/GK than normals (0.55±0.03 vs 0.35±0.02; p < 0.001). In well dialyzed patients, however, GCl/GK (0.35±0.04) was indistinguishable from that in normals.

These studies demonstrate a selective, reversible increase of the conductive component of chloride permeability in RBC membranes of uremic patients. Insofar as uremia may affect Cl conductance in other membranes, then an increased GCl/GK would tend to depolarize those cells in which Cl activity is at or above electrochemical equilibrium. Reversal of the increased GCl/GK with dialysis would repolarize the cells. Such phenomena may explain the prolonged nerve conduction and depolarization of muscle cells in uremic patients which may be reversed by dialysis. It is suggested that a generalized increase in cell membrane Cl conductance in uremic patients may also significantly affect the magnitude of all electrogenic transport processes in intestinal and residual renal epithelial cells.

REVERSAL OF PROTEINURIA AND EARLY SCLEROSIS IN CHRONIC PUROMYCIN AMINONUCLEOSIDE NEPHROPATHY (CPAN) BY LOW PROTEIN DIET. G.N. Marinides, G.C. Groggel, A.H. Cohen, W.A. Border. Univ. of Utah Med. Center, Salt Lake City, UT, Harbor-UCLA Med. Center, Torrance, CA.

We studied effects of dietary protein on the course of CPAN by inducing the disease through 7 SQ injections of puromycin aminonucleoside 20 mg/kg over 10 weeks in male Sprague-Dawley rats divided into a 22% protein diet group (Gr I, n=24) and a 6% protein group (Gr II, n=24). Group III (n=12) rats served as age-matched controls. Both diets had the same phosphorus content and were isocaloric. Animals from each group were sacrificed at 12, 18, and 24 wks.

Proteinuria was significantly less in Gr II vs Gr I at all times, and this difference became striking after 12 weeks (289±180 vs 135±63 mg/day at 12 wks, p<0.05, 261±134 vs 20±14 at 18 wks, p<0.05, 174±89 vs 27±22 at 24 wks, p<0.05). Serum creatinines were higher in Gr I vs Gr II but this difference was significant only at 18 wks (1.1±0.3 vs 0.8±0.1, p<0.05). Early lesions of focal segmental glomerular sclerosis/hyalinosis (FSH) were equally present in Gr I and II by 12 wks (16% vs 15% vs 1.5% in controls) but by 18 wks a reversal in FSH was seen in Gr II (3% vs 14% in normal protein diet group). Immunofluorescence for rat albumin, fibrin, C3, IgG and IgM was not different between the two groups although there was a trend for more deposits in Gr I.

In summary, low protein diet for 24 wks initially ameliorated proteinuria and seemed with time to reverse both proteinuria and early FSH lesions in CPAN.

REDUCED ALBUMIN SYNTHESIS WITH DIETARY PROTEIN RESTRICTION IN NEPHROSIS IS INDEPENDENT OF CHANGES IN URINARY ALBUMIN EXCRETION. V. Martin*, B. Don*, F.N. Hutchison, M. Schambelan, G.A. Kaysen, Martinez VA and U.C. Davis, San Francisco General Hospital and U.C.S.F., Martinez and San Francisco, CA.

We previously reported that both albumin synthesis rate (ASR g/1.73m²/d) and urinary albumin excretion (UalbV g/1.73m²/d) decreased in nephrotic patients during dietary protein restriction (DPR). To determine whether decreased ASR resulted from decreased UalbV or directly from DPR, we analyzed measurements of ASR, serum albumin concentration (Salb g/dl) and plasma albumin mass (PAM g/1.73m²) in 12 patients ingesting high (1.6 g/kg) and low (0.8 g/kg) protein diets. The decrease in UalbV during DPR was significant in 6 [responders], was accompanied by a decrease in ASR, and an increase in Salb and PAM. No significant change occurred in UalbV in the other 6 [non-responders] but ASR still decreased by a similar amount.

	RESPONDERS	NON-RESPONDERS
Δ UalbV	- 3.7 ± 1.1*	- 0.01 ± 0.3
Δ ASR	- 5.4 ± 1.7*	- 3.9 ± 1.1*
Δ Salb	+ 0.2 ± 0.07*	+ 0.1 ± 0.1
Δ PAM	+ 7.2 ± 2.2*	- 0.6 ± 5.0

*difference from control of P<.025

Therefore a component of the decrease in ASR that accompanies DPR in nephrotic patients is independent of decreased UalbV. Although neither Salb nor PAM were reduced, even in non-responders, the decrease in ASR may reflect insufficient amino acid supply to assure maximal ASR.

LONG-TERM CLINICAL EFFECTS OF ENALAPRIL (E) ON PROGRESSION OF CHRONIC RENAL FAILURE (CRF). G. Maschio, L. Oldrizzi*, C. Rugiu*. Div. Nephrology. Verona, Italy

We prospectively followed for 22 months (m) two groups of patients (p) with CRF. Group 1 had 14 p with serum creatinine (SCr) 1.5-2.6 mg/dl (mean 1.9). Group 2 had 17 p with SCr 2.8-6.0 mg/dl (mean 4.4). The two groups were matched for age, sex and underlying renal disease. All p were hypertensive and were treated in the first 12 m (period A) with propranolol, clonidine or prazosin, with apparent good control of blood pressure (BP). In the following 10 m (period B) all p received E (10 to 20 mg/day), again with good control of BP. During both periods the dietary intakes of calories, protein and phosphate remained stable (35 Kcal/kg, 0.6 g/kg, and 600 mg/day, respectively).

In group 1 the monthly increase in SCr did not change (0.034 mg/dl in period A vs 0.042 mg/dl in period B), whereas in group 2 it rose significantly from 0.107 to 0.246 mg/dl (p < 0.001). A direct relationship was observed between SCr before E and the monthly increase after E (r: 0.65; p < 0.01).

Conclusions: the degree of functional deterioration is critical in modulating the effect of E. At SCr levels exceeding 2.6 mg/dl the renal hemodynamic changes induced by inhibition of converting enzyme may be harmful to residual renal function.

RENAL AND SYSTEMIC RESPONSES TO ANGIOTENSIN II (AII) IN ACUTE AND CHRONIC UNTREATED STREPTOZOTOCIN-INDUCED DIABETES MELLITUS (DM): ROLE OF AII RECEPTORS. Peter Mento*, Barry Wilkes and Sabrina Silverman*. Div of Nephrol. and Hypertension, North Shore Univ. Hosp. and Cornell Univ. Medical College, Manhasset, NY.

Studies were performed to measure renal and systemic responses to AII in untreated DM rats. Each rat was studied twice: with and without AII infusion (12.5ng/kg/min). In the absence of AII infusion GFR was increased at 1 wk (control, 0.63 ± 0.03 vs 1 wk DM, 0.94 ± 0.09 ml/min/100gBW, $p < .01$) but returned to normal by 2 mo (0.77 ± 0.09 ml/min/100gBW). These changes paralleled changes in glomerular AII receptors which were reduced by 26.5% at 1 wk but returned to normal by 2 mo. If the AII receptor deficit contributed to hyperfiltration, increasing plasma AII would lead to increased receptor occupancy and reversal of hyperfiltration. During AII infusion in 1 wk DM rats GFR was restored to control levels (0.70 ± 0.07 ml/min/100gBW). In contrast to the normalization of GFR in chronic DM, systemic responses to AII diminished with time. AII caused a 24 ± 5 mm Hg rise in MAP in control rats, a 28 ± 5 mm Hg rise in 1 wk DM rats, but only a 9 ± 3 mm Hg rise in 2 mo DM rats ($p < .05$ vs control or 1 wk DM). We conclude that (1) hyperfiltration is limited to early DM, (2) the time course of increased GFR parallels the changes in glomerular AII receptors and (3) AII infusion reverses hyperfiltration in 1 wk DM rats possibly by increasing the number of occupied receptors. (4) The acquired resistance to the pressor actions of AII in chronic DM are likely to reflect either altered vascular reactivity or cardiac function.

THE EFFECTS OF DIETARY PROTEIN ON DNA AND PROTEIN SYNTHESIS AFTER SHAM(S), UNILATERAL (UNI) AND 5/6 NEPHRECTOMY (R). CA Miskell* and DP Simpson, University of Wisconsin, Madison, WI

To study the effects of dietary protein intake and the amount of tissue removed on cellular hypertrophy and hyperplasia in compensatory renal growth (CRG), we measured renal protein (P), DNA content and ^3H -thymidine ($^3\text{H-T}$) uptake by DNA in rats fed 8,20 or 35% protein diets for 4 or 8 weeks after (S), (UNI) or (R) operations. Animals were infused with ^{14}C -inulin, PAH and $^3\text{H-T}$. Nephron number (N) was determined from glomerular counts.

	GFR/N	$\mu\text{gP/N}$	$\mu\text{gDNA/N}$	$^3\text{H-T}/\mu\text{gDNA}$
4 wks n = 8				
8% S	59 ± 4	7 ± 1	$.10 \pm .00$	$1.1 \pm .4$
R	83 ± 11^d	22 ± 3^d	$.29 \pm .03^d$	$4.2 \pm .7^c$
35% S	81 ± 6	9 ± 1	$.11 \pm .01$	$.9 \pm .3$
R	151 ± 27^b	28 ± 5^c	$.27 \pm .04^c$	4.3 ± 1.5^b
8 wks n = 8				
8% S	58 ± 8	7 ± 0	$.11 \pm .19$	$1.0 \pm .2$
R	97 ± 7^d	21 ± 2^d	$.26 \pm .03^d$	$2.5 \pm .5^b$
35% S	120 ± 14	10 ± 1	$.13 \pm .01$	$.8 \pm .1$
R	113 ± 13^a	29 ± 4^d	$.35 \pm .04^d$	$1.6 \pm .3^b$

a = NS, b = $p < .05$, c = $p < .01$, d = $p < .001$, c/w S
Results in UNI and 20% protein intake were intermediate to those in table. These results indicate 1) hyperfiltration and CRG are related to the amount of tissue ablated, 2) high dietary protein increases GFR/N and hypertrophy but not hyperplasia, 3) persistent hyperplasia contributes significantly to CRG in R at both 4 and 8 weeks, 4) the degree of hyperplasia in R kidneys does not correlate with increase in GFR/N stimulated by high protein.

DIETARY PROTEIN RESTRICTION REDUCES GLOMERULAR VOLUME AND PROTEINURIA IN RATS WITH ESTABLISHED ADRIAMYCIN NEPHROSIS. PL Miller*, HG Rennke, and TW Meyer. Stanford University, Stanford, CA and Harvard University, Boston, MA.

Protein restriction reduces proteinuria in patients with the nephrotic syndrome. We studied the effect of low protein intake in Munich Wistar rats with adriamycin (A) nephrosis. Rats with similar albumin excretion (UalbV) and serum albumin (Salb) 5 weeks after A (3 mg/kg) were maintained for 10 days on 25% casein (C, n=8) or 8% casein (LP, n=7) after which micropuncture and morphologic studies were performed. LP reduced UalbV without reducing Salb. (Results: mean \pm 1SE, \dagger , $p < .05$ LP vs C)

	Baseline		10 days	
	UalbV mg/d	Salb g/dl	UalbV mg/d	Salb g/dl
C	838 ± 51	$1.06 \pm .11$	923 ± 63	$0.94 \pm .14$
LP	908 ± 52	$1.00 \pm .13$	$597 \pm 21^\dagger$	$1.10 \pm .08$

Reduction of albuminuria by LP feeding was associated with significant reduction of kidney weight (kw) and glomerular volume (gv) while arterial pressure (MAP) remained unchanged and single nephron GFR (SNGFR) and transcapillary hydraulic pressure (ΔP) were only slightly reduced.

	kw g	gv $10^3 \mu^3$	SNGFR nl/m	MAP mm Hg	ΔP mm Hg
C	$1.8 \pm .1$	$0.94 \pm .01$	36 ± 2	118 ± 2	46 ± 1
LP	$1.3 \pm .1^\dagger$	$0.81 \pm .01^\dagger$	32 ± 2	118 ± 3	43 ± 1

Since reduction of ΔP with a converting enzyme inhibitor does not reduce UalbV in adriamycin nephrosis (KI 31:393A), we conclude that a structural change associated with reduction of glomerular volume is essential to reduction of proteinuria with low protein intake in this model.

SORBINIL CORRECTION OF DIABETIC HYPERFILTRATION UNASSOCIATED WITH CHANGES IN A II RECEPTORS. Patricia Mower*, Barry Wilkes, Norman Bank, Montefiore Med. Ctr. Dept. of Med. Bronx, NY and North Shore Hosp., Manhasset, NY.

It has been postulated that the decrease in Angiotensin II receptors (AIIr) in the glomeruli of diabetic (DM) rats plays a role in the hyperfiltration of early DM. Aldose reductase inhibition corrects the GFR in DM rats. In order to determine whether this is due to an increase in the number of AIIr we studied the effects of Sorbinil 20mg/day (S), an aldose reductase inhibitor, on the AIIr density in DM rats. The Sorbinil was fed for 7-14 days. Four groups of rats were studied--streptozotocin diabetics, DM+S, normals (N), and N+S. Inulin and PAH clearances were measured and AIIr, renin and aldol levels were done.

Group	C_{In} ml/min/kg	C_{PAH}	AIIr/glomerulus $\times 10^6$
DM	$9.7 \pm .8$	33.3 ± 4	37.3 ± 4
DM+S	$5.9 \pm .6^*$	$27.7 \pm 2^{**}$	$43.5 \pm 5^{**}$
N	$6.6 \pm .3$	18.3 ± 1	81.8 ± 11
N+S	5.8 ± 1	17.9 ± 2	74.4 ± 10

* $p < .05$ DM vs DM+S ** $p < .05$ DM+S vs N

C_{In} and C_{PAH} were elevated in DM rats. Sorbinil caused a fall in C_{In} to normal. AIIr were decreased in DM glomeruli, and were not increased by Sorbinil. There was no difference in the renin or aldosterone levels. We conclude that the correction of hyperfiltration of early DM by S cannot be attributed to any change in the number of AIIr in the glomeruli of those kidneys.

MECHANISM OF ANTIPROTEINURIC EFFECT OF INDOMETHACIN IN NEPHROTIC HUMANS. Bryan D. Myers, Virginia Black,* and Helen Golbetz.* Stanford Univ., Div. of Nephrology, Stanford, California.

The mechanisms by which indomethacin lowers proteinuria were studied in 20 patients with the nephrotic syndrome. We performed differential macromolecule clearances and electrofocused urine and plasma prior to and after 3 days of therapy (150 mg/24 hr). Urinary excretion rate of albumin and IgG decreased by 53 and 59%, respectively ($p < 0.01$). Whereas cationic subclasses (pI 7.5 and 8.8) accounted for 76% of total urinary IgG initially, anionic subclasses (pI 4.8 and 5.8) predominated (55%) after treatment. Indomethacin also elevated the fractional clearance of dextrans of radius 28-44 Å, while depressing those of dextrans of radius 50-60 Å. A heteroporous model which depicts the major portion of the glomerular capillary wall as an isoporous membrane (pore radius = 56 Å) and the minor portion as a nondiscriminatory shunt, revealed the former to be unchanged and the latter to be less prominent following indomethacin. A lower fraction of total filtrate volume permeating the shunt (0.0068 ± 0.0007 to 0.0056 ± 0.0007 $p < 0.01$), together with a concomitant lowering of overall glomerular filtration rate (GFR) by 24%, reduced the absolute rate of flux of macromolecule-rich fluid through the shunt pathway from 0.40 to 0.25 ml/min per 1.73m² ($p < 0.01$). We conclude that indomethacin lowered the filtered protein load by restoring barrier size-selectivity while reducing the GFR. Enhanced reabsorption of cationic and perhaps other proteins at the less saturating load may have contributed further to the antiproteinuric effect of indomethacin.

INFLUENCE OF FLUID INTAKE ON PROGRESSION OF CHRONIC RENAL FAILURE IN 5/6 NEPHRECTOMIZED RATS.

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Even with declining urine concentrating ability in CRF, a non negligible amount of solute-free water is reabsorbed to raise urine osmolality (Uosm) above that of plasma. To test the influence of this process on the progression of chronic renal failure (CRF), water intake (WI) was altered in male Sprague Dawley rats with 5/6 nephrectomy (NX). Eighteen rats were paired by BW and creatininemia (Pcreat) one week after NX. All rats had water ad lib and ate a 23% casein diet (16g/day). "High WI rats" (HWI) (1 of each pair) received the food in an agar gel (4 ml water + 60 mg agar per g food). "Normal WI rats" (NWI) received the dry food + 60 mg agar/g. Three NWI rats died of CRF on wks 6-7 after NX. Reducing free water reabsorption (T_{H2O}) 2.5 fold, by increasing WI, lowered proteinuria (Prot) and blood pressure (SBP) with no change in Pcreat, over the whole study period (10 wks). Results at 5 wks after NX were:

(means ± SEM, * = $p < 0.05$ or less by paired test)

	Uflow ml/d	Uosm mosm/kg	T _{H2O} ml/d	Prot mg/d	Pcreat μmol/d	SBP mm Hg
NWI	17.0	946	31.4	23.1	93	167
n=9	±1.7	122	3.8	6.2	10	10
HWI	41.7*	390*	12.4*	8.6*	91	142*
n=9	±1.3	9	1.2	1.8	5	8

Hypertrophy was lower in the remnant kidney of HWI (1.62 ± 0.08 g) than in that of NWI (2.04 ± 0.14 , $p < 0.02$) (at 10 weeks post NX, after perfusion fixation). Evaluation of glomerulosclerosis is in progress. These results suggest that operation of urine concentrating mechanism (and/or ADH) may participate in loss of glomerular permselectivity and in hypertrophy of remaining nephrons in early CRF.

EFFECT OF DIETARY PROTEIN RESTRICTION ON OXYGEN CONSUMPTION (QO₂) AND OXIDANT STRESS IN THE REMNANT NEPHRON. K.A. Nath, A. J. Croatt, and T.H. Hostetter. University of Minnesota, Minneapolis, MN.

The loss of renal mass evokes increased QO₂ in surviving nephrons. Such augmented QO₂, by imposing oxidant stress may be injurious in remnant nephrons (Xth ICN, 510, 1987). Since protein restriction reduces renal damage in the remnant kidney model we examined the effect of protein restriction on renal QO₂ and cortical content of malondialdehyde (MDA), an index of oxidant-induced lipid peroxidation, in 1 2/3 subtotaly nephrectomized rats (SNX) maintained on isocaloric diets of 30% or 6% protein. Remnant kidney QO₂ was determined under euvoletic conditions 3 wks after renal ablation. Results: means ± SE. * $p < 0.05$. N, nephron number by colloidal carbon counting.

	GFR ml/min	QO ₂ μmoles/min	N	QO ₂ /N pmoles/min	QO ₂ /TNa ⁺ nmoles/μEq
30% P	1.00	7.67	8800	859	57
n=6	±0.14	±1.67	±1400	±83	±13
6% P	0.45*	3.71*	8450	490*	59
n=6	±0.05	±0.52	±1150	±85	±7

Thus, the lower protein diet was associated with reduced whole kidney and average nephron QO₂. In separate groups of SNX rats maintained on 6% P or 30% P for 6-9 wks, 6% P decreased cortical hypertrophy and total cortical content of MDA (17.3 ± 1.9 vs 11.4 ± 1.3 nmoles*); MDA/N was thus decreased with 6% P. Urinary excretion of MDA also declined with 6% P (21.5 ± 1.5 vs 12.9 ± 1.7 nmol/24 h*). We suggest that the oxidant stress imposed on surviving nephrons is decreased with protein restriction and this effect may contribute to the beneficial effects of reduced protein intake.

INDOMETHACIN IMPROVES GLOMERULAR PERMSELECTIVITY IN NEPHROTOXIC SERUM NEPHRITIS (NSN) BY DECREASING UTILIZATION OF THE SHUNT PATHWAY.

J. Neugarten, A. Kozin*, P. Alfino*. VAMC, NY, NY and Montefiore Med. Center, Bronx, NY.

Dextran sieving studies were performed before and after intravenous administration of indomethacin (INDO) in 9 NSN rats with heavy proteinuria (184 ± 27 mg/24 h) and 8 normal control rats (CON):

	MAP mm Hg	GFR ml/min	U _v /GFR mg/ml GFR
NSN	135±6	2.58±.50	.059±.033
NSN-INDO	136±5	1.39±.27	.031±.013
NS	NS	<0.001	<0.05
CON	121±3	2.84±.73	.011±.002
CON-INDO	122±3	2.75±.76	.017±.006
NS	NS	NS	NS

The fractional clearances of neutral dextrans (θ_{ND}) with molecular radii (MR) >40 Å were elevated above control values in NSN ($p < 0.05$). After indomethacin, θ_{ND} in NSN declined toward control values and was elevated only for MR > 54 Å. In control rats θ_{ND} was not affected by INDO. Micropuncture studies were performed before and after INDO:

	MAP mm Hg	SNGFR nl/min	Q _A nl/min	P _{GC} mm Hg
NSN	145±2	30±3	102±8	50±2
NSN-INDO	147±3	15±1	62±4	48±2

The fraction of filtrate permeating the shunt pathway in NSN declined from .86% to .45% after INDO. Pore radius was 50 Å before and 49 Å after INDO. These data suggest that INDO reduces proteinuria and improves impaired glomerular size-selectivity in NSN by reducing Q_A and decreasing utilization of the shunt pathway.

VITAMIN C SUPPLEMENTATION AGGRAVATES OXALATE DEPOSITIONS IN 5/6 NEPHRECTOMISED RATS. Keiji Ono*, Hiroko Ono*, and Kazuhiko Kikawa*. (Intr. by Dr. F. Marumo). Ono Geka Clinic and Fukuoka Tokushukai Hospital, Fukuoka and Kasuga, Japan

Our recent study suggested that hyperoxalemia in regular hemodialysis patients is aggravated by routine vitamin C supplementation (Clin. Nephrol. 26:239,1986). This study was undertaken to confirm this findings in experimental animals. Fifty 5/6 nephrectomised rats, 200 gm, were divided into two groups: 30 rats were allowed to freely drink water containing 8mg/ml of vitamin C (oxalate precursor) and remaining 20 rats were given tap water without vitamin C. All animals received standard diet with 26% protein. Serum creatinine, Hct, & body weight were measured monthly for 12 months. Rats were killed at appropriate intervals. Plasma vitamin C & oxalate levels were analysed. Kidneys were examined histologically in all rats. Creatinine increased and Hct decreased gradually, however, there was no difference between two groups. Plasma vitamin C, oxalate and urinary oxalate levels were higher in vitamin C treated groups than non-treated rats. Histological study revealed the glomerular and interstitial fibrosis and round cell infiltration, as well as the tubular cyst formation. Oxalate deposits in renal tubules were found only in vitamin C treated rats with advanced renal failure and non-treated rats with equally advanced renal impairment showed no oxalate deposits. Our results confirm the previous clinical findings that vitamin C supplementation aggravates the secondary oxalosis in chronic renal failure. Therefore, vitamin C supplementation should not be given to hemodialysis patients.

ADENOSINE NUCLEOTIDES IN EXERCISED AZOTEMIC RABBITS. A.E.Parrish, C. Tomaselli, M.Zikria, and R.A.Kenney. George Washington Medical Center, Depts of Medicine & Physiology, Washington, DC.

Previous studies have suggested abnormalities in uremic muscle function. This study was undertaken to determine if adenine nucleotide metabolism was affected.

Rabbits were made azotemic by subtotal nephrectomy. The soleus muscle of operated and sham operated animals were exercised by electrical stimulation until the amplitude of contraction was reduced to near zero. The muscle was frozen in situ, lyophilized and analysed for ATP, ADP, AMP, IMP by enzymatic methods and HPLC.

ATP and ADP levels were not significantly different before exercise. With exercise both nucleotides decreased with no significant difference between the post exercise values. AMP and IMP levels did not change with exercise in experimental animals but decreased significantly in control animals. It is proposed that the failure of removal of these two compounds in the azotemic animals resulted in a decreased cell "charge" and could impair muscle function.

DEMONSTRATION THAT GLOMERULAR HYPERTENSION IN REMNANT HYPERFILTERING NEPHRONS IS ANGIOTENSIN II DEPENDENT. Juan C. Pelayo, Albert G. Quan*, Robin G. Coombs*, and Paul F. Shanley. Univ. of Colo., Sch. of Med. Dept. of Peds. & Path., Denver, CO.

Chronic angiotensin converting enzyme inhibition with enalapril in rats with reduced renal mass normalizes glomerular hypertension, the glomerular ultrafiltration coefficient (LpA) and lessens glomerular injury (JCI 76,1985). Critical to determining a biological role for angiotensin II (AII) in mediating these alterations would be documentation of normalization by acute AII inhibition of the glomerular hypertension and the diminished LpA associated with nephron loss. Thus, we studied glomerular dynamics before and during the acute infusion of enalapril (1 mg·kg⁻¹·h⁻¹, i.v.), in pair-fed Munich-Wistar rats subjected to either 3/4 (n=8) or sham nephrectomy (n=8), 2 weeks after ablation. AII inhibition, in control rats, had no effect on mean arterial pressure (MAP), glomerular dynamics, and urinary protein excretion (Uprot). In contrast, in hyperfiltering remnant nephrons acute AII inhibition normalized both glomerular hypertension (64.1±1.4 to 54.9±1.6 mmHg, p<0.0001), the result of efferent arteriole dilation (p<0.05), and the decreased LpA (0.039±0.005 to 0.054±0.007 ml/(s·mmHg), p<0.005); these responses were associated with constancy in MAP, single nephron GFR and plasma flow. Of note, this maneuver also attenuated the augmented Uprot by 35% (p<0.01). Segmental glomerular sclerosis was present in <2% of remnant nephrons. Thus, endogenous AII activity is responsible for the glomerular hypertension and diminished LpA observed in the early phase of hyperfiltration of remnant nephrons.

EFFECT OF CAPTOPRIL (CEI) AND DILTIAZEM (CCB) ON RENAL FUNCTION AND GLOMERULAR PROSTANOIDS (P) IN ADRIAMYCIN (A) NEPHROPATHY. E.Podjarny, M.Rathaus, A.Pomerantz and J.Bernheim. Meir Hosp. Kfar-Saba and Tel-Aviv Univ. Israel. (Intr. by M.Chambelan).

Increased glomerular P synthesis may be related to changes in creatinine clearance (CCL) and proteinuria (UP) in chronic renal disease. After subtotal nephrectomy, the use of CEI or CCB protects renal function. We compared their effect on the course of A nephropathy and P synthesis. Rats were divided into 4 groups: control (C, n=10), A (2 mg/kg/IV, d -21 and 0, n=14), A+CCB (30 mg/kg/d, n=13) and A+CEI (10 mg/kg/d, n=13). At week 2, no change was noted in CCL, UP and P synthesis. At 7 weeks, a marked increase in UP appeared in A and A+CCB, and less marked (p<.001) in A+CEI. Systolic blood pressure (SBP) increased only in A. At week 21, SBP (mmHg), CCL (ml/min/100 BW), UP (mg/d), glomerular P (6-keto-PGF_{1α}, PGE₂, ng/mg pr/30 min) and glomerular ⁴⁵Ca incorporation (cpm 10⁻³/mg pr) were:

Group	C	A	A+CCB	A+CEI
SBP	109 ± 4	131 ± 5*	107 ± 3	108 ± 2
CCL	.84 ± .09	.43 ± .01*	.50 ± .04*	.76 ± .1 [^]
UP	13 ± 4	616 ± 90*	596 ± 52*	149 ± 42 [^]
6-keto	.88 ± .2	2.1 ± .2*	2.2 ± .2*	1.8 ± .2*
PGE ₂	2 ± .2	3.8 ± 1.9	4.4 ± .7*	3.9 ± .4*
TXB ₂	.7 ± .08	1.8 ± .6	.7 ± .06	.85 ± .1
⁴⁵ Ca	22 ± 2	42 ± 5*	13 ± 3*	27 ± 2

* = p<0.01 vs C; [^] = p<0.01 vs A.

A nephropathy is characterized by an increase in SBP, glomerular 6-keto PGF_{1α} synthesis and ⁴⁵Ca incorporation. The beneficial effect of captopril on CCL and UP appears to be unrelated either to the decrease of SBP or changes in P synthesis and Ca incorporation.

ENHANCEMENT OF Na,K-ATPase ACTIVITY BY ANTIDIGOXIN ANTIBODY. A.L. Quintarilla, S.K. Mujais and K.M. Johnson,* Section of Nephrology, VA Lakeside Medical Center and Northwestern University Medical School, Chicago, IL.

The observation that Na,K-ATPase (the Na-pump) is decreased in several models of volume-expanded hypertension has led to the theory that a ouabain-like factor (OLF) is released by volume expansion. We tested further this hypothesis by examining the effect of a specific antidigoxin antibody on Na,K-ATPase activity in red cells and kidney cortex in the rat. Normal and uremic rats (uremia induced by 7/8 nephrectomy) were treated with an antidigoxin antibody raised in sheep (Digibind®), 15 mg/kg injected I.V. over 10 minutes. Na,K-ATPase, expressed as inorganic phosphate (Pi) released per µg of membrane protein per hour, was found significantly higher in red cells and kidney cortex in the antibody-treated animals. Means and SEM were as follows:

	Erythrocyte (nmoles/ng/hr)		Kidney Cortex (µmoles/ng/hr)
	Normal	Uremic	Normal
Controls	332±9	456±64	20.7±2.3
Antibody	431±40	615±50	54 ±11.3
P	<0.02	<0.05	<0.01

The higher Na,K-ATPase in antibody-treated animals suggests that the antibody binds an OLF, inhibitory of the Na-pump, which probably circulates in both normal and uremic animals but is higher in the uremic state. This observation is also consistent with the presence of a digoxin-like material, causing the inhibition of the Na,K-pump reported in several volume-expanded states.

HYPERLIPIDEMIA AND RENAL INJURY. STUDIES IN ATHEROSCLEROSIS PRONE WATANABE RABBITS WITH HEREDITARY HYPERLIPIDEMIA. L. Raij, JP Tolins and T. Luscher, Veterans Administration Medical Center and University of Minnesota, Minneapolis, Minnesota and Department of Medicine and Research, University Hospital, Basel, Switzerland.

It has recently been suggested that plasma lipids may be important risk factors in the development of focal glomerulosclerosis. Hence, we hypothesized that the Watanabe heritable hyperlipidemic rabbit (WHHL) which develops pronounced hyperlipidemia on a normal diet would be ideal to study the relationship between blood lipids and glomerular injury. In 300 day old WHHL and age matched New Zealand white (NZW) rabbits we determined awake intra-arterial MABP (mmHg) and measured serum creatinine (Cr, mg/dl), triglycerides (Trg, mg/dl), cholesterol (Chol, mg/dl), urine protein, mg/dl/Cr mg/dl (U. Pro/Cr) and renal histology. Mean±SD (n=7-10)

	MABP	Cr	Chol	Trg	U Pro/Cr
WHHL	100±5	1.0±0.4	512±131	203±115	8±1
NZW	90±8	1.2±0.9	39±19	80±44	7±0.7
P	NS	NS	<0.01	<0.05	NS

Neither WHHL nor NZW rabbits had focal glomerulosclerosis and/or mesangial expansion although intrarenal vessels appeared thicker in WHHL. These studies suggest that in normotensive rabbits with normal renal mass severe hyperlipidemia does not lead to glomerular injury.

ACUTE ANGIOTENSIN II (AII) BLOCKADE REDUCES PROTEINURIA IN RATS ON A HIGH BUT NOT A LOW PROTEIN DIET. Mark E. Rosenberg* and Thomas H. Hostetter. University of Minnesota, Minneapolis, MN.

Both chronic dietary protein restriction and converting enzyme inhibition can favourably influence the course of chronic renal disease. To determine whether the higher plasma renin activity previously observed with increased dietary protein influences glomerular permselectivity, we examined the effect of acute AII blockade in subtotally nephrectomized (1 2/3 Nx) male Sprague-Dawley rats. Two weeks following 1 2/3 Nx the rats were stratified according to their serum creatinines to either a 30% or a 6% protein diet. After two weeks clearance studies were performed. Responses to an intra-renal infusion of the AII antagonist Sar¹ Gly⁸-AII (10 ng/kg/min) were examined. Results (mean ± SEM; †P<0.05 compared to base line; ⊖-Fractional clearance x10⁻⁴):

	MAP (mmHg)		⊖ Albumin		⊖ IgG	
	Baseline	Sar-Gly	Baseline	Sar-Gly	Baseline	Sar-Gly
30% Protein (n=9)	139 ±8	135 ±9	40.5 ±9.1	30.6† ±8.0	9.6 ±1.5	8.0† ±1.1

6% Protein (n=7)

	Baseline	Sar-Gly	Baseline	Sar-Gly	Baseline	Sar-Gly
MAP (mmHg)	147	139	24.4	21.9	8.9	8.1
⊖ Albumin	±9	±7	±4.9	±4.6	±1.3	±1.2

GFR did not change with Sar-Gly infusion. Thus, AII blockade improved glomerular permselectivity only in the rats on the 30% diet as evidenced by the decreased fractional clearance of albumin and IgG. In a third period (not shown) the simultaneous infusion of exogenous AII did not change GFR, MAP, or proteinuria, confirming blockade of AII action. The improvement of glomerular permselectivity after AII blockade on the 30% diet implicates AII as an injurious component of the higher protein diet. Hence, the stimulation by dietary protein of the renin-angiotensin system may contribute to the deleterious effects of dietary protein on chronic renal injury.

BODY MASS INDEX (BMI) URINARY UREA NITROGEN (UUN) AND PHOSPHATE (P) EXCRETION. Jacob Rudoy*, B. Cristal*, S. Shasha*, R. Kohan*, N. Griner*, U. Makov*, J. Ben-Ari* (Intr. by J. Levy), Dept. of Nephrology, Biochemistry Lab., Carmel Medical Center, Nahariya Government Hospital, and Statistics Dept., Haifa University.

Patients with low BMI reach end stage renal failure ten years earlier than those with high BMI (EDTA Report, 1983). Protein (Pr) and P affect negatively renal function. We studied the correlation between BMI and UUN, Calcium (Ca) and P excretion in 252 normal renal function volunteers (G1), 42 renal failure patients on a free diet (G2) and 41 renal failure patients on a low Pr low P diet (G3). For each of them, blood samples and 24 hours urinary (U) values for Ca, P, UN, and Pr were determined. Results were expressed per kg. body weight (KgBW). Correlation tests (Ct) and Multiple linear regression (LR) were used.

In G1 Ct between BMI and UUN/KgBW = -0.12861 (p=0.0475), BMI and UP/KgBW = -0.20798 (p=0.0012). LR of BMI and UP/KgBW = -0.3987 (p=0.0003). BMI and Tubular Reabsorption of Phosphate (TRP) = 0.000011 (p=0.0001). In G2, LR between BMI and UP/KgBW = -1252 (p=0.0086), BMI and TRP = 0.000027 (p=0.0082). In G3, LR between BMI and TRP = 0.00016 (p=0.0488).

If we accept excretion of UUN as a marker for Pr intake, higher Pr/KgBW and higher P/KgBW in low BMI patients can be considered as a renal risk factor and may explain the EDTA results.

GLOMERULAR PROCOAGULANT (GPCA) AND FIBRINOLYTIC (GFA) ACTIVITIES ON THE RAT REMNANT KIDNEY. P. Ruedin, B. Mougnot, E. Rondeau, JD. Sraer, A. Kanfer. Hôpital Tenon, Paris, France. (Intr. by D. Schlondorff).

The glomerular hemostatic system was investigated in rats with reduction of renal mass (RRM). Sprague-Dawley rats were killed at day 12 or at day 30 after 3/4 RRM: mean serum creatinine was 118 $\mu\text{mol/l}$ at day 12, 112 $\mu\text{mol/l}$ at day 30 after RRM, vs 43 $\mu\text{mol/l}$ in sham-operated controls. the glomerular hypertrophy (GH) was less pronounced at day 12 than at day 30 post RRM. Glomerular fibrin deposits were never detected at day 12 but always found at day 30 by immunofluorescence. For each experimental period glomeruli from 2 to 3 remnant kidneys were isolated; tissue factor-like GPCA was assessed by the ability of disrupted glomerular suspensions to reduce clotting time of normal plasma (units of tissue factor/mg protein), and GFA by the release of 125 I fibrin from coated tubes (μg fibrin lysed/mg protein).

	GPCA	GFA
Day 12 RRM (6)	125 \pm 19	8.02 \pm 1.53
Controls (6)	224 \pm 48	11.26 \pm 2.21
Day 30 RRM (6)	107 \pm 22*	8.95 \pm 1.14
Controls (6)	209 \pm 18	9.03 \pm 1.06

Conclusion 1) Despite a progressive GH between day 12 and day 30 post RRM the renal function did not improve, suggesting a pathogenic role for the fibrin deposits which appeared during this period.

2) Since GFA was not modified and GPCA progressively decreased after RRM, a role for an extraglomerular PCA or an inappropriate GFA is questionable.

* $p < .01$

A FISH OIL DIET (FOD) SUPPRESSES GLOMERULAR PGE2 AND ENHANCES THE RENAL CONSTRICTOR RESPONSE TO ANGIOTENSIN II (AII) IN RENOPRIVAL NEPHROPATHY. L. Schar Schmidt, N. Gibbons*, H. Aynedjian*, and N. Bank, Albert Einstein Coll. of Med. and Montefiore Hosp. and Med. Ctr., Bronx, N.Y.

We previously found that a FOD accelerates renal disease induced by 1 1/3 nephrectomy (NX) in rats despite reducing serum lipids and glomerular TXB2 (K.I., in press). To better understand the mechanism by which this occurs we performed micropuncture studies early in the course of the disease. Rats were 5 weeks post 1 1/3NX and had eaten either a beef tallow diet (BTD, n=8) or FOD (n=9) for only 3 weeks (20% w/w), compared to 3 months in our earlier study. FOD had already reduced serum lipids, glomerular synthesis (ng/mg prot/10 min) of PGE2 (BTD 4.3 \pm 4 vs FOD 1.8 \pm 4) and TXB2 (BTD 1.6 \pm 3 vs FOD .7 \pm 1) ($p < 0.02$), but there were no differences in histology, BP, basal RPF, GFR or glomerular capillary pressures (Pgc) between BTD and FOD rats. Intrarenal AII infusion induced similar declines in GFR, but RPF decreased significantly only in FOD rats. AII-induced increments in Pgc of FOD rats were twice that of BTD rats ($p < 0.02$).

	Basal		AII	
	BTD	FOD	BTD	FOD
RPF (ml/min)	20 \pm 3	15 \pm 2	18 \pm 3	11 \pm 1*
GFR (/kg)	3.7 \pm 0.4	3.6 \pm 0.3	2.9 \pm 0.2*	2.7 \pm 0.2*
Pgc (mmHg)	41 \pm 1	39 \pm 1	46 \pm 1*	51 \pm 2*

* $p < 0.01$ AII vs Basal on same diet.

These results suggest that PGE2 is necessary to maintain renal hemodynamics in 1 1/3NX rats stressed by AII. The decrease in glomerular PGE2 caused by FOD causes an exaggerated constrictor response to AII and may contribute to the accelerated glomerulosclerosis in 1 1/3NX rats fed FOD.

ACUTE BLOCKADE OF ANGIOTENSIN II (AII) ACTIVITY DOES NOT REPRODUCE THE EFFECT OF CONVERTING ENZYME INHIBITION IN RATS WITH REDUCED RENAL MASS. JW Scholey* and TW Meyer. Stanford University and VA Medical Center, Palo Alto, California.

Chronic converting enzyme inhibitor (CEI) therapy lowers glomerular capillary pressure (Pgc) in rats with reduced renal mass. We sought to relate control of remnant glomerular capillary hypertension by CEI to control of systemic blood pressure and to blockade of intrarenal AII activity.

Munich Wistar rats underwent micropuncture study 4-6 weeks after 5/6 nephrectomy. A first group (CON) received no drug, a second group (CEI) received the CEI RS10085 (400 mg/l in the drinking water) for 18 hours prior to study, and a third group (SAR ILEU) received the angiotensin II antagonist, Sar¹Ileu⁸AII, 1000 ng/kg/min IV during micropuncture. Drug effects were verified by measuring the pressor response (ΔAP) to 200 ng angiotensin I. Results: (mean \pm 1SE, * $p < .05$ vs CON)

	AP	Pgc	GFR	SNGFR	Qa	ΔAP
	mm Hg	ml/min	nl/min	mm Hg	mm Hg	mm Hg
CON (n=14)	144	64	.81	102	375	44
CEI (n=7)	6	2	.05	4	24	7
SAR ILEU (n=8)	133	56*	.84	96	396	10*
	5	2	.04	9	44	3
	137	63	.92	95	327	5*
	5	2	.09	6	21	2

Short term CEI reduced remnant Pgc without reducing arterial pressure (AP) or single nephron plasma flow (Qa). GFR and single nephron GFR (SNGFR) remained stable despite reduction of Pgc. The effect of CEI on remnant glomerular hemodynamic function could not be reproduced by acute blockade of AII activity with Sar¹Ileu⁸AII.

CHOLESTYRAMINE NORMALIZES LDL CATABOLISM IN UREMIC GUINEA PIGS. R. Jean Shapiro, Dept. of Medicine, University of British Columbia, Health Sciences Centre Hospital, Vancouver, B.C.

Chronic renal failure results in hyperlipidemia in humans and in a 1 2/3 nephrectomy model of uremia in the guinea pig. We have previously shown that plasma clearance of low density lipoprotein (LDL) was reduced in uremic guinea pigs compared to control animals (Kidney Int. 27:250, 1986). To determine if this was due to a decrease in LDL receptor-mediated catabolism, fractional catabolic rates (FCR) of radioiodinated native guinea pig LDL and methylated guinea pig LDL (a tracer of receptor-independent degradation) were measured simultaneously in 7 control (C) and 9 uremic (UR) recipient guinea pigs. The FCR of native LDL was lower in UR compared to C animals (1.60 \pm 0.24 vs. 1.95 \pm 0.21 pools/day, $\bar{X} \pm \text{S.D.}$; $p < 0.01$). There was no significant difference in the FCR of methylated LDL between UR and C animals (0.73 \pm 0.13 vs. 0.65 \pm 0.19 pools/day). Clearance by the LDL receptor-dependent pathway (calculated as the difference between the FCR of native LDL and methylated LDL) was lower in UR guinea pigs (0.87 \pm 0.33 vs. 1.30 \pm 0.34 pools/day; $p < 0.03$). Treatment of UR animals with cholestyramine-supplemented chow (2% W/W) increased the FCR of LDL by the receptor-mediated pathway from 0.86 \pm 0.18 to 1.79 \pm 0.05 pools/day ($p < 0.01$). These results suggest that hypercholesterolemia in uremia results in part from impaired LDL clearance by receptor-dependent mechanisms, and that treatment with cholestyramine restores this toward normal.

DIETARY INTAKE PATTERN, HIGH PROTEIN DIET, AND GLOMERULOSCLEROSIS

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Influence of dietary intake pattern on accelerated loss of renal function remains unknown. This is to examine effects of dietary intake pattern on the course of an experimentally-induced chronic renal disease. Rats with 1&3/4 nephrectomy were fed either *ad libitum* 40 % protein diet everyday or 2 out of every 3 days. On the 13th week, clearances, serum creatinine, blood pressure, Uprotein, BUN, and body and kidney weights were determined. Statistically significant difference was observed in Inulin and creatinine clearances, and serum creatinine levels. No statistically significant difference was observed in the average body weights of two groups. Values of the other parameters indicated a trend towards enhanced retention of renal function in the scheduled diet group. The data indicate that dietary intake pattern influences the progression of chronic renal disease. Further studies are needed to determine whether the dietary intake pattern is responsible for amelioration of progressive renal disease in this experiment, not the total dietary amount consumed.

EFFECTS OF FISH OIL (FO) ON GFR, GLOMERULAR PROSTAGLANDIN (PG) PRODUCTION, AND SERUM LIPIDS IN RATS WITH DIABETES MELLITUS (DM). A. Sinha*, L. Scharschmidt, R. Neuwirth, H. Holthofer* and D. Schlondorff, Dept. of Med., Albert Einstein Coll. Med., Bronx, N.Y.

The mechanisms responsible for hyperfiltration in D.M. and for the diabetic nephropathy have not been fully elucidated. Enhanced PGE2 production has been invoked in the former and thromboxane and hyperlipidemia in the latter. Fish oil (F.O.) enriched diets can favorably alter eicosanoid synthesis and serum lipid profiles. Groups of 5-8 rats with streptozotocin DM were maintained on low insulin and then pair-fed with isocaloric diets enriched with either F.O. (20% w/w) or beef tallow (BT; 20% w.w.). GFR was determined at onset of diet and after 20 wks by [¹⁴C] inulin clearance using implanted osmotic mini-pumps each time.

Onset: DM/BT | DM/FO At 20wks: DM/BT | DM/FO
Blood Sugar: 426±28 | 432±44 378±68 | 376±56
GFR/ml/min: 2.6±0.2 | 2.5±0.1 2.6±0.3 | 2.8±0.2
Significant hyperfiltration (Normal Control GFR: 1.5±0.2ml/min) was maintained on both diets for 20 wks, in spite of a significant (P<0.02) and greater than 50% decrease in all PGs (PGE2, TXB2, PGF2a, 6 keto PGF1a) produced by glomeruli isolated from DM/FO as compared to DM/BT or Controls. FO diet completely corrected the hypertriglyceridemia from 1162±325 mg% in DM/TG to 150±42 mg% in DM/FO. Conclusion: Hyperfiltration persists in DM/FO rats in spite of 50% decrease in PG indicating independence from full PG synthesis. FO diet did however prevent the hypertriglyceridemia of DM, which may be of long-term benefit.

MESANGIAL IMMUNOGLOBULIN (mes Ig) DEPOSITION AND ALCOHOL ABUSE (AA): A CLOSE RELATIONSHIP *SM Smith, WE Hoy. Univ. of New Mexico and Lovelace Medical Foundation, Albuquerque, New Mexico.

Mesangiopathic glomerulonephritis with Ig deposition has been described in subjects with alcoholic cirrhosis in other countries, but there are no US series. Relationships between less severe evidence of AA and renal changes are not generally recognized.

AA is documented in half the sudden deaths in New Mexico. We studied 60 autopsies from sudden deaths to probe relationships of AA and renal changes. 43 subjects were American Indians and 17 were Caucasian. 45 subjects had measurable blood alcohol, (35 to 409 mg/dl). AA was confirmed in subjects with cirrhosis (5), alcoholic hepatitis or fibrosis (20), or linear IgA in hepatic sinusoids (9). AA was suspected in subjects with fatty liver (21), or normal liver or nonspecific hepatic change (9), if accompanied by alcohol levels ≥ 100 mg/dl or a clinical history. We cannot exclude AA in those without these findings, however.

42 of 49 subjects with confirmed or suspected AA had renal mes Ig deposition (86%), vs 6 of 11 subjects without evidence of AA (55%) (p=0.022). Both ethnic groups showed this association. IgM was present alone in 4 cases, IgA alone in 3, and IgA and IgM together in 41. Intensity ranged from 1+ to 3+. Ig positive kidneys showed small mes deposits on EM and 4 had mes proliferation by light microscopy.

This confirms findings from other countries. It also demonstrates a much broader relationship between AA and mes Ig deposition than previously reported, involving AA subjects with much milder hepatic change, and even some with normal livers. This observation has broad implications. It might contribute to population differences in the prevalence of mes GN. The phenomenon deserves further exploration.

ABNORMAL NOREPINEPHRINE (NE) METABOLISM IN BRAIN SYNAPTOSOMES (SY) OF RATS WITH CHRONIC RENAL FAILURE (CRF). ROLE OF PARATHYROID HORMONE (PTH). M. Smogorzewski*, Vito M. Campese, and Shaul G. Massry, Div. Nephrol., Univ. of So Calif, Los Angeles, CA.

Abnormalities of central nervous system (CNS) exist in CRF and are related to excess PTH which causes a rise in brain Ca. The latter may affect neurotransmitters metabolism in brain SY. We measured NE content, uptake and release in brain SY of CRF rats and studied whether excess PTH affects these parameters. SY from CRF rats compared to SY of control rats have higher (p<0.01) calcium content (11.4±0.9 vs 7.8±0.4 μ mole/mg protein and lower NE content (11±0.6 vs 14±0.5 pmole/mg protein), NE uptake (1.6±0.15 vs 3.1±0.2 pmole/mg protein) and Na-K ATPase activity (6±0.9 vs 11.4±0.8 pmoleP/mg protein). Parathyroidectomy in CRF rats kept normocalcemic, reversed these abnormalities. Administration of 1-84 PTH for 3 weeks to normal rats produced abnormalities in brain SY similar to those seen in CRF rats. The data show that abnormalities in brain SY function occur in CRF and are due to the excess PTH. The latter may mediate its effects by enhanced entry of calcium into the SY and by inhibition of their Na-K ATPase activity. These actions of PTH on neurotransmitter metabolism of brain SY may contribute to the CNS dysfunction in CRF.

RENAL RESPONSE TO ACUTE PROTEIN MEAL: DISSOCIATION OF UREA AND ELECTROLYTE EXCRETION INDEPENDENT OF PROSTAGLANDINS. CP Swainson, RJ Walker, Royal Infirmary, Edinburgh, United Kingdom

Acute protein meals induce significant increases in GFR and RBF in normal humans, probably mediated by intrarenal prostaglandins (PG). 9 subjects with normal renal function were studied twice after a 24 h food fast. GFR and RBF clearances were collected before, 2 h and 4 h after a meal containing 1.5 g/Kg body weight of protein and 1488 Kcal, on a control (C) day, and after indomethacin (I) 50 mg before and during experiment. Baseline GFR was significantly reduced after I (92 vs 109 ml/min) and at 2 h (104 vs 134 ml/min) and 4 h (103 vs 114 ml/min) after the test meal. There was no increase in RBF after I at 2 h (1031 vs 1024 ml/min) and renal resistance did not change. Baseline MAP was elevated after I (89 vs 85 mmHg). Urea excretion paralleled changes in GFR. CH₂O was lower after I at baseline but no different from C after the test meal. NA and K excretion did not parallel the changes in GFR, were maximal at 4 h and were not affected by I. Plasma NE and PRA and urinary PGE after a meal were suppressed by I. These results suggest that renal haemodynamic and hormonal responses to acute protein meal are mediated by PGE. Glomerular balance is preserved and the mechanisms controlling sodium excretion after meals are independent of those that control GFR.

CALORIE RESTRICTION (CR) RETARDS THE PROGRESSION OF CHRONIC RENAL FAILURE (CRF) IN RATS. D. Tapp, W. Wortham*, J. Addison, J. Barnes, and M. Venkatchalam. Brooke Army Med. Ctr. and Univ. Texas Health Science Center, San Antonio, Texas.

To evaluate the effect of CR on CRF, rats subjected to 5/6 nephrectomy were assigned to one of the following diets: Control (CONT) - ad lib consumption of a 21% protein diet; CR - 40% less calories (via reduced sucrose, dextrin and corn oil) and sodium than CONT; Sodium Restriction (SR) - 40% less sodium than CONT; Protein Restriction (PR) - 40% less protein than CONT; Calorie/Protein Restriction (CPR) - 40% less calories, protein and sodium than CONT.

Proteinuria, inulin clearance and renal histology were evaluated 20 weeks post-ablation. GS is % of glomeruli with sclerosis. TI is the number of points falling on the interstitium divided by the sum of points falling on the interstitium plus points on tubular epithelial cells (by morphometric analysis). A score of 25 represents minimal tubulo-interstitial disease, but a score of 45 is the equivalent of far advanced tubular atrophy and interstitial fibrosis.

	C inulin (ml/min/100 g)	Proteinuria (mg/24 h)	GS (%)	TI (%)
CONT (n=8)	0.05±0.008	86±10	72±8	48±3
CR (n=9)	0.19±0.039*	41±8*	27±7*	20±3*
SR (n=7)	0.08±0.02	78±10	55±6	42±4
PR (n=7)	0.08±0.014	63±12	55±10	44±7
CPR (n=11)	0.16±0.035	31±6*	27±9*	25±5*

*P < .05 vs. CONT. (mean±SE)

In conclusion, we have found that CR, irrespective of protein intake, protects renal function and structure in the renal ablation model of CRF in rats. The mechanism of this effect is unknown.

DISPARATE EFFECTS OF CONVERTING ENZYME INHIBITION (CEI) AND DIETARY PROTEIN RESTRICTION ON GLOMERULAR INJURY. INFLUENCE OF GENETICALLY DETERMINED RENAL HEMODYNAMIC RESPONSES. JP Toling, K Coffee*, L Raij, U. of MN and VAMC, Minneapolis, MN.

In both patients and experimental animals, low protein diet and CEI have each been found to prevent or delay progression of chronic renal failure. Experimentally, in SHR and Munich-Wistar rats both treatments reduce glomerular (glom) hypertension; protein restriction predominantly induces pre-glom vasoconstriction while CEI predominantly decreases efferent resistances. We hypothesized that protein restriction may not be as effective as CEI in preventing glom injury in hypertensive DAHL-S rats (DS), in whom pre-glom vasoconstriction is defective. DS with established post-salt hypertension (190±8 mmHg) underwent 1/5/6 nephrectomy and were randomized into 3 treatment groups matched for serum creatinine (CONT: 24% protein diet; CEI: 24% protein diet and Captopril+HCTZ in drinking water; LoPro: 6% protein diet). Rats underwent functional and histologic studies after 2 wks. Results (mean±SE, n=8, P<.05: †CONT vs CEI, * CONT vs LoPro, Ω CEI vs LoPro):

Group	MAP (mmHg)	UproV (mg/d)	CIN (ml/min)	CPAH (ml/min)	FF	Glom Injury (score)
CONT	208† ±7	240†* ±37	0.31†* ±0.04	1.20† ±0.16	0.28* ±0.03	184†* ±15
CEI	117Ω ±7	57 ±11	0.65 ±0.09	4.04Ω ±1.04	0.21Ω ±0.04	35Ω ±6
LoPro	200 ±7	59 ±7	0.54 ±0.06	1.24 ±0.14	0.45 ±0.04	96 ±11

CEI treated DS had significantly less glom injury than both LoPro and CONT. CEI and LoPro had equivalent reduction in proteinuria and preservation of GFR but compared to LoPro, CEI was associated with low renal vascular resistance and filtration fraction (FF). Thus, in a strain of hypertensive rats lacking effective pre-glom vasoconstriction, protein restriction afforded only partial protection from glom injury after renal ablation, while CEI exerted a markedly beneficial effect. Clinically, CEI but not protein restriction may offer universal protection from progressive glom injury in patients with chronic renal failure independent of genetic factors which determine glom hemodynamic responses.

ALTERATION OF MDCK CYST ENLARGEMENT IN HYDRATED COLLAGEN GEL BY INHIBITORS OF SOLUTE TRANSPORT. M.E. Uchic*, V.S. Donoso, J. Kornhaus*, E.J. Cragoe* and J.J. Grantham. Univ. of Kansas Med. Ctr., Kansas City, KS and Merck, Sharp & Dohme, West Point, PA

We previously reported that in an in vitro model of cystic disease, amiloride, and amiloride analogs which alter Na, Ca, and H transport, slowed the rate that MDCK cysts enlarged when grown in hydrated collagen gels. We recently evaluated, in relation to amiloride, the effects of compounds that alter additional transport processes. Ouabain and vanadate (inhibit Na, K, ATPase), ethacrynic acid and bumetanide (inhibit Na, K, 2Cl co-transport) and a non-diuretic ethacrynic acid derivative L-645, 695 (inhibits Cl/NaCO₃ antiport) were added to medium bathing hydrated collagen gels where MDCK cells grew in cyst configuration. Cyst growth rate was determined from the diameter of individual cysts. Low concentrations of drugs stimulated and higher concentrations inhibited cyst enlargement.

COMPOUND	IC ₅₀ (uM)	STIM INDEX	STIM CONC (uM)
Amiloride	800	1.0	10-250
Ouabain	0.03	0.6	.0001-.01
NaVO ₃	34	1.1	.10-1.0
Bumetanide	50	0.3	.1
Ethacrynic Acid	23	1.1	.001-1.0
L-645, 695	9	0.4	.01-1.0

Biphasic effects suggest that parallel absorptive and secretory processes may influence the absolute rate of cyst growth. We conclude that drugs which potentially inhibit cyst growth without promoting cyst enlargement may be useful in the management of polycystic kidney disease.

ROLE OF EPIDERMAL GROWTH FACTOR (EGF) IN COMPENSATORY RENAL HYPERTROPHY IN MICE. S. Uchida*, O. Tsutsumi*, M.K. Hise* and T. Oka* (intr. by M. Burg). NIDDK and NHLBI, Bethesda, MD., Univ. Texas Med. Sch., Houston, TX.

A number of "renotropic factors" have been proposed to cause compensatory renal hypertrophy. We examined the possible role of EGF as such a factor by using the combination of sialoadenectomy (Sx), which decreased plasma EGF in mice, and EGF replacement. Two weeks after Sx mice were subjected to unilateral nephrectomy (Nx). Eight days after Nx the following indicators of renal hypertrophy were measured:

Treatment	Kidney wt.(mg)	Protein (mg/kid.)	Tubule diameter, μ m	
			Proximal	Distal
Control	134	12.9	35.0	31.5
Nx	163*	15.8*	38.8*	34.9*
Sx	128	12.4	34.2	32.8
Sx+Nx	130	12.7	34.3	31.5
Sx+Nx+EGF	145*	14.8*	39.0*	35.8*

* $p < 0.05$ vs. control (sham-operated), $n = 8-12$.

Plasma EGF in Nx mice was essentially the same as in control, while it was undetectable in Sx and Sx+Nx mice. Urinary excretion of EGF per kidney was virtually constant under all conditions. In Sx+Nx mice, plasma creatinine and BUN were 15% and 38% greater respectively than in Nx alone. After EGF replacement in Sx+Nx mice these values were the same as in Nx mice.

CONCLUSION: EGF deficient mice failed to undergo compensatory renal hypertrophy, resulting in renal insufficiency, whereas EGF replacement alleviated these abnormalities. Circulating EGF may have an important role in compensatory renal hypertrophy.

BICARBONATE TRANSPORT IN COLLECTING TUBULES (CT) OF REMNANT KIDNEYS. V.M. Vehaskari, K. Hering-Smith*, and L.L. Hamm. Depts. of Peds. and Med., Washington Univ. Med. School, St. Louis, MO

Previous studies of ours have demonstrated a surprising lack of functional adaptation in HCO_3^- transport in cortical CT from remnant kidneys (RK) compared to normal kidneys (NK): no differences in unidirectional HCO_3^- secretion in HCO_3^- -loaded animals or in unidirectional HCO_3^- reabsorption in acid-loaded animals. The present studies were performed to further explore whether the capacity for HCO_3^- transport in the CT of RK had undergone functional changes. Cortical or outer medullary CT (CCT and MCT respectively) from either NK or RK (three weeks after 3/4 to 7/8 reduction in renal mass) were studied using *in vitro* microperfusion. Addition of 10^{-4} M cAMP to CCT from RK did not increase HCO_3^- secretion (-8.3 vs -9.3 $\text{pmol}/\text{mm}^2\cdot\text{min}$, $n=6$, NS); in contrast, in CCT from NK, cAMP increased HCO_3^- secretion from -12.3 to -16.7 $\text{pmol}/\text{mm}^2\cdot\text{min}$ ($n=6$, $p < 0.05$). However, transepithelial voltage did respond to cAMP in CCT from both RK (mean -54.4 to -41.3 mV with cAMP) and NK (-20.3 to -3.4 mV). HCO_3^- reabsorption was measured in MCT from NK, RK, and RK from acid-loaded animals; no differences were found (mean fluxes of 9.8, 10.1, and 9.7 $\text{pmol}/\text{mm}^2\cdot\text{min}$ respectively). In conclusion, no adaptive increase in HCO_3^- secretion or HCO_3^- reabsorption can be demonstrated in CT from RK. Also, there may be a defect in the HCO_3^- secretory response to cAMP in CCT from RK.

EFFECT OF UREMIA ON Na,K-ATPase IN THE RAT RED CELL. M. I. Weffer*, S. K. Mujais, K. M. Johnson and A. P. Quintanilla. VA Lakeside Med. Center and Northwestern Univ. Med. School, Chicago, IL.

Na,K-ATPase is found decreased in a variety of tissues in uremic animals. There is controversy, however, regarding ATPase activity in the red cell (RBC) of the uremic rat. The issue is important because studies of the Na,K-pump are often conducted in RBC, under the assumption that changes in the RBC reflect similar changes in other tissues. To clarify this issue, uremia was induced in Sprague-Dawley rats by a 2-stage, 7/8 nephrectomy. Matched controls underwent a sham operation. The rats were subsequently pair-fed to prevent significant differences in body weight.

The animals were studied after 1, 2, 3, 4 and 6 weeks of uremia. Mean SUN in controls and uremic animals was 17 and 138 mg/dl respectively, and did not vary significantly between 1 and 5 weeks. Each week a group of matched pairs (uremic-control) were studied. After light pentothal anesthesia, the animals were exsanguinated, the red cells separated, and Na,K-ATPase measured in RBC membranes of control and uremic animals. The results (in nmoles/mg/hr) were:

	1 week	2 weeks	3 weeks	4 weeks	6 weeks
Control	276	274	378	290	338
Uremia	392	504	436	501	829
P =	0.0973	0.0019	0.1471	0.0029	0.0064

In contrast to other tissues, Na,K-ATPase was significantly higher in the RBC of uremic rats and the difference increased at 6 weeks. We conclude that the activity of Na,K-ATPase is increased in the uremic RBC and that studies of the Na-pump in the RBC can not be assumed to reflect the pump activity in other tissues.

METABOLIC FACTORS IN THE DEVELOPMENT AND REVERSAL OF HYPERFILTRATION IN DIABETIC (DM) RATS. Barry Wilkes and Sabrina Silverman*. Div. of Nephrology/Hypertension, North Shore Univ. Hospital and Cornell Univ. Medical College, Manhasset, New York.

The effects of duration of DM, plasma glucose concentration, hypoinsulinemia and intracellular myoinositol (MYO) depletion were studied in separate groups of SD rats with streptozotocin DM. In DM rats not receiving insulin (glucose, 425 ± 30 mg/dl), GFR was elevated at 1 wk (control, 0.63 ± 0.03 vs 1 wk, 0.94 ± 0.09 ml/min/100g BW, $p < .01$), but with longer duration of DM (≥ 2 mos) GFR returned to normal (2mo DM, 0.77 ± 0.09 ml/min/100gBW). The effects of glucose infusions were studied in 1 wk DM rats given insulin to normalize blood glucose. In euglycemic DM rats, GFR was restored to control levels (0.54 ± 0.05 ml/min/100gBW). Elevations in blood sugar to 467 ± 48 mg/dl did not increase GFR suggesting that insulin depletion rather than hyperglycemia was the important factor for hyperfiltration. Since aldose reductase inhibition reverses hyperfiltration, intracellular sorbitol accumulation and MYO depletion, additional DM rats were given MYO (670 mg/kg) daily and studied at 1 wk. MYO administration did not reverse the hyperfiltration (GFR: 0.91 ± 0.05 mg/dl; $p < .01$ vs control). We conclude that (1) insulin depletion rather than hyperglycemia is more directly related to hyperfiltration, (2) hyperfiltration is limited to a brief period following the induction of diabetes and (3) the reversal of hyperfiltration by aldose reductase inhibitors is independent of MYO depletion.

THE EFFECT OF VITAMIN B6 DEFICIENCY ON IMMUNE FUNCTION IN CHRONICALLY AZOTEMIC AND SHAM RATS. M. Wolfson, R. Jones*, VAMC and OHSU, Portland, OR.

Vitamin B6 deficiency (B6-) occurs in chronically uremic patients who do not receive B6 supplements. B6- and chronic uremia are associated with decreased immune responsiveness, but the etiology of these findings is unknown. We examined the T-lymphocyte subset composition of splenocytes from B6- or replete (B6+) and 5/6 nephrectomized chronically azotemic (AZ) or sham operated (S) rats. AZ or S was performed as previously described (J. Nutr. 116: 1865-72, 1986). Rats were then paired for 6 weeks to receive a diet devoid of vitamin B6 or a B6+ diet which contained 22mg/kg pyridoxine HCl. AZ rats had decreased average of the urea and creatinine clearances, an estimate of GFR, as compared to S rats (0.29 ± 0.12 SD cc/min/100g vs 0.54 ± 0.10 , $p < 0.01$). B6- rats had higher EGOT indices than B6+ rats (2.9 ± 0.7 vs 1.5 ± 0.04 , $p < 0.01$), indicating B6 deficiency. Significant differences in monoclonal antibody staining of splenic mononuclear cells were observed between B6+ and B6- rats in the presence or absence of AZ. W13/13 (total T), W3/25 (T helper), and Ox-8 (non-T helper) monoclonal antibody staining was lower in B6- than in B6+ rats (19.7 ± 9.5 vs 37.0 ± 8.6 , 13.2 ± 6.6 vs 26.9 ± 8.1 , 6.8 ± 3.2 vs 19.3 ± 6.3 , respectively, $p < 0.01-0.03$). These data suggest that B6 deficiency influences the composition of the splenic lymphocyte population in the presence or absence of AZ. Nutritional factors may be important in the function of the immune system in chronic uremia through an influence on the presence of functional lymphocyte subpopulations in the spleen.

RENAL METABOLISM

HIGH SALT DIET REDUCES $\alpha 1$ - AND $\alpha 2$ -ADRENERGIC STIMULATION OF DIACYLGLYCEROL (DAG) PRODUCTION IN RAT RENAL PROXIMAL TUBULES. Andrew D. Baines, and Patrick Ho*. Dept. of Clin. Biochemistry, Univ. of Toronto, Toronto, Ontario, Canada.

We have previously shown that phorbol esters mimic the vasoconstrictor and proximal-antinatriuretic actions of α -adrenergic agonists in isolated perfused rat kidneys (Fed. Proc. 46:1283). Phorbol esters may act like endogenously produced DAG to stimulate protein kinase C; therefore, we have measured the effect of α -adrenergic agonists on DAG production by rat proximal tubules. Proximal tubules were separated by centrifugation through Percoll and incubated at 37°C in oxygenated glucose-alanine-salt solution with norepinephrine (NE), cirazoline (C) or guanabenz (Q). DAG was measured with DAG kinase. 1 μ M NE increased DAG content of proximal tubules; at 0.5 min. by 18±3%, at 1 min. by 15±5%, at 5 min. by 21±6% (SEM). Both $\alpha 1$ - and $\alpha 2$ -antagonists (0.1 μ M prazosin and 0.1 μ M yohimbine) inhibited the 13±3% increase produced by 0.1 μ M NE. Both $\alpha 1$ - (0.1 μ M C and 1 μ M C) and $\alpha 2$ -agonists (1 μ M C) increased DAG production by +21±10, +34±10, and +28±8 respectively. A high salt diet increases $\alpha 2/\alpha 1$ adrenoceptor ratio; therefore, rats were given 1% saline to drink for 5-8 days; unstimulated DAG content increased from 5.6 ± 0.3 to 7.1 ± 0.2 nmol/100 nmol phospholipid and the adrenergic responses were obliterated; 1 μ M C, -2±1%; 0.1-1 μ M C, -6±2 and +4±2. These results indicate that $\alpha 1$ - and $\alpha 2$ -adrenergic stimulation rapidly increases DAG content of rat proximal tubules and that a high salt diet inhibits this response while raising the basal DAG content.

INTERACTIONS IN THE RENAL REABSORPTION (R) AND UTILIZATION (Q) OF MONOCARBOXYLATES IN VIVO. M. Barac-Nieto. A. Einstein Coll. Med. Depts Pediatrics, Physiology & Biophysics. Bronx N.Y.

Experiments in renal brush border membranes indicate that monocarboxylates share a Na⁺ dependent transporter. We now report on the interactions between hydroxybutyrate (HB) isomers (L+ and D-) and L+lactate in the rat kidney in vivo. The L+HB isomer decreased the apparent Tm for D-HB from 1.7 to 0.7 μ mol/g min ($p < 0.01$) without altering D-HB Q or R through non-saturable pathways. L+lactate (12 mM), inhibited R of D-HB and of acetoacetate (AA) (from 98 to 80%, $P < 0.01$ and from 99 to 95%, $p = 0.1$ respectively), without altering D-HB Q. The effect of L+ lactate infusion on D-HB R was independent of the associated alkalosis since similar degree of alkalosis ($pH_a = 7.6$) induced by NaHCO₃ failed to alter D-HB R. This pH independence indicates little contribution of non ionic diffusion to the renal R of D-HB.

At 1 mM blood L+lactate, renal Q of D-HB (0.8 mM), AA (0.2 mM) and lactate can account for 100% of the renal QO₂ (7 ± 0.4 μ mol/g.min), if these three monocarboxylates are completely oxidized. At 12 mM lactate, renal Q of lactate and ketoacids must also occur through synthetic pathways, since not enough oxygen is consumed by the kidney to effect complete oxidation of these metabolites in the amounts used by the kidney.

ALTERED PROXIMAL TUBULE (PT) GLUCOSE (G) METABOLISM IN X-LINKED HYPOPHOSPHATEMIA (HYP/Y). A.W. Capparelli*, O.D. Jo* and N. Yanagawa. Nephrology Div., Sepulveda VAMC, UCLA Sch. of Med., Los Angeles, CA

Altered PT brush border membrane (BBM) phosphate (Pi) transport in Hyp/Y has been well studied. Less is known on PT cellular metabolism in Hyp/Y. Since we have previously shown that pentose cycle (PC) in PT may interact with BBM Pi transport, PT G metabolism in HYP/Y was examined. PT were purified with Percoll gradient from Hyp/Y and normal littermate +/Y mice, and PC quantitated by method described (J. Biol. Chem. 238:517, 1963). While total G utilization was similar in both groups, the amount of G metabolized through PC was markedly lower in Hyp/Y (3.48 ± 0.69 vs. 12.0 ± 2.6 nmol/mg/hr, $n=5$, $p < 0.01$). Addition of phenazine methosulfate (0.1 mM) stimulated PC in both groups. In contrast, the rate of G production from α -ketoglutarate (3 mM) was significantly higher in Hyp/Y (25.4 ± 2.6 vs. 11.4 ± 1.8 nmol/mg/hr, $n=4$, $p < 0.01$). G production rate from other substrates (glutamin, malate, fructose) was also higher in Hyp/Y. PTH (1 uU/ml) and Ca (3 mM) stimulated G production in both groups. Furthermore, the difference in PC and G production rates was found to persist in primary cultured Hyp/Y and +/Y PT cells. These results thus suggest the presence of a genetically determined intrinsic abnormality in Hyp/Y PT G metabolism.

PARATHYROID HORMONE STIMULATES AMMONIAGENESIS IN CANINE RENAL PROXIMAL TUBULAR SEGMENTS.

M.C. Chobanian,* and M.R. Hammerman. Univ. of Wisconsin Sch. of Med., Dept. of Peds., Madison, WI and Washington Univ. Sch. of Med., Dept. of Internal Med., St. Louis, MO.

Parathyroid hormone (PTH) is known to regulate metabolic and transport processes in proximal tubule. Gluconeogenesis is stimulated by PTH at this site. Gluconeogenesis in proximal tubule is linked to ammoniagenesis in that L-glutamine, the major ammoniagenic precursor, is a gluconeogenic substrate. To determine whether PTH affects ammoniagenesis in proximal tubule, we measured ammonia productions in suspensions of canine renal proximal tubular segments incubated with 10 mM L-glutamine \pm PTH. Productions of ammonia were linear functions of time for 120 min and averaged 131 ± 24 and 197 ± 40 $\mu\text{mol}/\text{gram protein}/120$ min in the absence and presence of 10^{-7} M PTH respectively ($p < 0.02$). Half-maximal stimulation of ammonia production occurred between 10^{-8} and 10^{-9} M PTH. Incubation of segments with 10^{-7} M PTH markedly stimulated cAMP production. Incubation of basolateral membranes isolated from segments with PTH activated adenylate cyclase. Half-maximal activation occurred between 10^{-8} and 10^{-9} M PTH, corresponding to half-maximal stimulation of ammonia production in segments. Ammonia production was significantly enhanced by incubation of segments with the cAMP analog, 8BrcAMP, (10^{-4} M) as well as by PTH. We conclude that PTH stimulates ammonia production in canine renal proximal tubular segments. This effect appears to be mediated, at least in part, through cAMP. Such stimulation could reflect an action of PTH on the proximal tubule to enhance ammoniagenesis in vivo.

ADENOSINE TRIPHOSPHATE (ATP) STIMULATES THYMIDINE (T) INCORPORATION BUT DOES NOT PROMOTE CELL GROWTH IN PRIMARY CULTURES OF RABBIT PROXIMAL TUBULE (PT) CELLS. DA Cieslinski*, JM Messana*, HD Humes, VAMC & Univ. of Mich., Ann Arbor, MI.

It has been shown that ATP is an acute mitogen in an established kidney epithelial cell line. To assess whether ATP is also an acute and chronic mitogen in PT cells, we investigated the effects of ATP on $^3\text{H-T}$ incorporation in confluent, quiescent primary cultures of PT cells and on the growth rate of subconfluent PT cell cultures grown on plastic. Acute addition of 2 and 0.2 mM ATP increased T incorporation in PT cells by 179 and 146%, respectively, over control levels. However, the effect of additions of ATP on days 3, 5, 6, 7, and 9 on PT cell growth demonstrated ($n=3-4$):

ATP (mM)	Day	Cell Counts ($\times 10^6$)			
		5	7	8	9
Control	.257	.366	.446	.624	.769
0.002	.226	.393	.631	.822	.898
0.2	.184	.230	.273	.251	.243
2.0	.145	.059	.031	.021	.021

Thus, 0.002 mM ATP mildly stimulated PT cell growth but 0.2 and 2.0 mM ATP suppressed growth. Importantly, 0.2 mM ATP caused a 7-fold increase in cells released into the culture media compared to controls. These cells, however, excluded Trypan blue. In addition, LDH release into the media after ATP addition was unchanged from control plates. Thus, low dose ATP mildly stimulates PT cell growth but at high doses acutely stimulates T incorporation probably due to detachment of cells from confluent plates. This high dose ATP effect results in a suppression of long term growth of PT cells in culture on plastic.

EFFECTS OF PROTEIN INTAKE ON GLOMERULAR NUCLEOTIDE METABOLISM. P. Cortes, F. Dumler and N.W. Levin, Henry Ford Hospital, Detroit, Michigan.

Optimal protein glycosylation of glomerular basement membrane components is probably necessary for maximum rates of extracellular deposition. Protein glycosylation requires uridine diphosphosugars (UDP-S) which are the product of the *de novo* and salvage pathways for pyrimidine nucleotide synthesis, with orotate (O) and uridine (U) being the respective metabolic intermediates. To investigate if exogenous O or U are effective precursors of glomerular UDP-S, isolated rat glomeruli were incubated with 1 or 10 μM labelled O or U, and UDP-S measured by chromatography. Incorporation rates were expressed as pmole/min/mg DNA and results as mean \pm SD. At 1 μM concentrations, O and U incorporation into UDP-S were 1.6 ± 0.1 and 4.3 ± 0.8 , respectively. Increased substrate concentrations were associated with a 4.8-fold and a 1.7-fold greater incorporation rate for O and U, respectively (O: 7.7 ± 0.5 and U: 7.3 ± 1.2). To determine if the availability of exogenous O or U for glomerular metabolism could be altered by dietary protein intake, plasma O and U levels were measured after 4 days of feeding diets containing 5 or 60% protein. Plasma O and U were significantly greater ($p < .001$) in animals on a high protein diet (O: 95 ± 10 vs 54 ± 9 nM; U: 3.1 ± 0.6 vs 1.7 ± 0.2 μM). It is concluded that high protein diets will enhance glomerular UDP-S synthesis by increasing plasma concentrations of O and U. This may facilitate maximum rates of protein glycosylation and basement membrane synthesis.

LOSS AND RECOVERY OF PARA-AMINOHIPPURIC ACID (PAH) IN GLYCOSURIC URINE OF DIABETIC PATIENTS.

N Dalton, M J Wiseman, C Turner and G C Viberti. Introduced by F Pugliese. Dept. of Paediatrics and Unit for Metabolic Medicine, UMDS, Guy's Campus, LONDON, UK.

Loss of PAH in glycosuric urine of hyperglycaemic subjects has long been recognised and is generally ascribed to competition between tubular secretion of PAH and reabsorption of glucose. We collected urine samples during PAH clearance studies in 6 hyperglycaemic and glycosuric insulin-dependent diabetics. Urine aliquots were stored at -20° for between 8 and 26 weeks with and without the addition of 6 $\mu\text{l}/\text{ml}$ of 4 M NaOH. PAH concentrations were lower in the untreated compared with alkalinised urine (13.0 ± 3.3 vs 90.6 ± 7.0 mg/dl; $p < 0.001$). Acid hydrolysis of untreated urine restored PAH concentrations to levels similar to those of alkalinised urine (92.5 ± 7.1 mg/dl). Addition of NaOH to untreated urine after storage did not lead to recovery of PAH. HPLC analysis in untreated urine revealed the presence of a PAH-glucose conjugate which was absent in urine collected in NaOH and abolished by acid hydrolysis. When PAH and glucose in known amounts were added in vitro to urine stored at -20° , measurable PAH concentration declined by 40% by week 8 and 70% by week 26, with formation of PAH-glucose conjugate. This process was prevented by addition of NaOH to the urine and reverted by acid hydrolysis. Loss of PAH in glycosuric urine is due to a post-renal reaction between PAH and glucose during storage. This reaction can be prevented by alkalinisation and reversed by acid hydrolysis.

INTERACTION OF GLUTAMINE (Gln) AND GLUTATHIONE (GSH) IN NONACIDOTIC (NA) AND ACIDOTIC (A) RAT RENAL PROXIMAL TUBULES. P. Dass*, S. Hardman*, R. Budden* and I. Kurtz. Div. of Nephrology, UCLA School of Medicine, Los Angeles, CA.

The role of γ -Glutamyltransferase (γ -GT) in renal ammonia production from Gln in acidosis remains controversial, prompting the current investigation. GSH has been previously demonstrated to stimulate Gln utilization in NA however its effect in A is unknown. We studied the interaction of GSH and Gln in rat proximal tubule suspensions incubated in KRB media with 2mM substrates, either Gln, GSH, or Gln+GSH in A and NA. Rates of Gln utilization and ammonia production were measured and are expressed as nmoles/min/mg protein (Mean \pm SEM, n=5-6)

	Gln Utilization		NH ₄ ⁺ Production	
	NA	A	NA	A
Gln	7.6 \pm 0.5	13.0 \pm 0.4	10.0 \pm 0.6	17.7 \pm 0.7
GSH	- - - - -	- - - - -	5.9 \pm 0.5	7.5 \pm 0.5
Gln +GSH	11.0 \pm 0.9 ^b	17.3 \pm 0.5 ^b	10.4 \pm 0.4 ^a	23.6 \pm 0.6 ^b

a, NS vs. Gln; b, P<0.005 vs. Gln. Furthermore, γ -glutamylglutamine increased 400% in the presence of GSH +Gln as compared to Gln alone.

In conclusion: 1) Glutathione increases glutamine utilization in both nonacidosis and acidosis. 2) Glutathione stimulates ammoniogenesis from glutamine in acidosis but not in nonacidosis.

CONTRASTING METABOLIC RESPONSES OF RENAL PROXIMAL TUBULES (RPT) TO HYPOXIA AND ANOXIA. Kathleen G. Dickman* and Lazaro J. Mandel. Duke Univ. Med. Ctr., Dept. of Physiol., Durham, NC.

Complex alterations in renal metabolism and transport function are elicited in response to oxygen deprivation. Using a suspension of purified rabbit RPT, we have characterized the metabolic and functional properties of RPT maintained under hypoxic (1% O₂), anoxic (0% O₂), and normoxic (air) conditions for 45 min, followed by reoxygenation in air (REOX) for 45 min. The following parameters were examined and the data summarized below: ATP and K⁺ contents (nmol/mg pro), lactate (LAC) production (nmol/min-mg), oxygen consumption (QO₂, nmol/min-mg), LDH release (%).

	LDH	ATP	K ⁺	QO ₂	LAC
0% O ₂	12 \pm 3	2 \pm 0.4	48 \pm 5	ND	1.9 \pm 0.6
1% O ₂	3 \pm 1	3 \pm 0.2	110 \pm 11	ND	10.4 \pm 1.9
AIR	3 \pm 1	12 \pm 1.0	292 \pm 42	ND	1.3 \pm 1.1
0% REOX	18 \pm 5	5 \pm 0.8	139 \pm 41	19 \pm 1.2	ND
1% REOX	5 \pm 1	8 \pm 0.5	333 \pm 29	33 \pm 0.3	ND
AIR	4 \pm 1	13 \pm 0.4	282 \pm 36	38 \pm 1.3	ND

Examination of responses to longer exposure periods revealed that the above parameters were maintained in hypoxic RPT for at least 90 min, while anoxic RPT exhibited further deterioration as indicated by increased LDH release (28 \pm 7) and a further drop in ATP content (0.9 \pm 0.4). These results indicate that 1) prolonged anoxia, but not hypoxia, compromises RPT cellular integrity as judged by LDH release; 2) ATP and K⁺ contents are better preserved during hypoxia; 3) hypoxia, but not anoxia, elicits LAC production, suggesting a protective role for glycolytic ATP provision; 4) recoverability from hypoxia is greater than that from anoxia.

BASOLATERAL GLUCOSE UPTAKE AND OXIDATION ARE REQUIRED FOR ARGININE VASOPRESSIN (AVP)-STIMULATED HYDRAULIC CONDUCTIVITY (Lp) IN THE RAT CORTICAL COLLECTING TUBULE (CCT). M.A. Dillingham. V.A. Med. Ctr., Denver, Colorado.

The metabolic requirements necessary to support AVP-stimulated hydroosmosis in the mammalian CCT are unknown. The present studies were therefore undertaken to evaluate the role of basolateral glucose uptake in supporting AVP-stimulated Lp. All studies were performed in rat CCT perfused at 37°C in vitro. Removal of basolateral glucose decreases significantly both AVP (50 μ U/ml) (348 \pm 52 to 114 \pm 27 cm/atm \cdot s \times 10⁻⁷, p<.02) and ClPheScAMP (10⁻⁴M) (320 \pm 33 to 207 \pm 42 cm/atm \cdot s \times 10⁻⁷, p<.05) stimulated Lp. To determine the cellular metabolic pathways that utilize glucose to support AVP- and ClPheScAMP-stimulated Lp, the effect of inhibitors of oxidative phosphorylation (rotenone 10⁻⁸M) and glycolysis (iodoacetate 10⁻⁶M) on AVP- and ClPheScAMP-stimulated Lp were examined. Both rotenone and iodoacetate significantly (p<.05) inhibit AVP- and ClPheScAMP-stimulated Lp in rat CCT. Rotenone at 10⁻⁸M also decreased rat CCT ATP concentration by 54 \pm 6%. These studies demonstrate that basolateral glucose uptake with its subsequent oxidation to ATP is required to maintain AVP-stimulated Lp in the mammalian CCT by acting at least in part, at post-cyclic AMP steps.

DIFFERENCES IN PYRIMIDINE NUCLEOTIDE METABOLISM BETWEEN RAT GLOMERULAR EPITHELIAL AND MESANGIAL CELLS IN CULTURE. F. Dumler and P. Cortes. Henry Ford Hospital, Detroit, Michigan.

Pyrimidine nucleotides (PN) are necessary coenzymes for the synthesis of glomerular basement membrane and mesangial matrix. We have studied the cellular pools of PN rat glomerular epithelial (E) and mesangial (M) in tissue culture. Cells were incubated in RPMI 1640 media supplemented with 20% fetal calf sera and studied at confluency during a 4 hour incubation period using 3H-uracil as precursor. The following PN were analyzed by liquid chromatography: UTP, UDP-N-Ac-Glucosamine (UDPAG), UDP Glucose (UDPG), UDP Glucuronic Acid (UDPGA), and RNA. Results are expressed per mg DNA as mean \pm SD:

	nmole				ug
	UTP	UDPAG	UDPG	UDPGA	RNA
E (n=10)	92 \pm 64	29 \pm 14	24 \pm 11	7 \pm 3	200 \pm 31
M (n=7)	255 \pm 15	131 \pm 13	87 \pm 17	43 \pm 10	238 \pm 52

Synthesis rates measured as pmol of substrate incorporated into PN per mg DNA were:

	UTP	UDPAG	UDPG	UDPGA	RNA
E	197 \pm 40	66 \pm 55	66 \pm 17	26 \pm 5	192 \pm 30
M	299 \pm 41	171 \pm 37	203 \pm 43	48 \pm 12	359 \pm 43

M cells showed greater PN pools (P<.001) and precursor incorporation rates (P<.001) than E cells. This difference in synthetic activity by M may underline a greater capacity for protein glycosylation and deposition of glomerular basement membrane and mesangial matrix.

CONTRIBUTION OF INCREASED UREA APPEARANCE TO DEHYDRATION-INDUCED AZOTEMIA. D.E. Kamm, S. Chin, and B.L. Kuchmy. Roch. Gen. Hosp., U Roch Sch. of Med., Rochester, NY.

Azotemia is commonly seen in dehydrated patients and is usually attributed to a reduced urea clearance (C_{urea}). To examine the contribution of urea appearance to dehydration-induced azotemia, we examined urea metabolism in 7 dehydrated (D) and 7 hydrated (H) pair-fed Brattelboro rats during 1 control day followed by 3 experimental days. D animals were tube-fed 30 ml/day of a 170 mM NaCl + KCl solution containing 10 G of ground rat chow. H animals received the same diet with sufficient NaCl and KCl to maintain similar Na and K balances in both groups. When compared with H, 3 days of free water deprivation in D, resulted in greater weight loss (Δ weight $-31 \pm 2.1^*$ vs -16 ± 3.6 G/3 days), an elevated plasma [Na] ($161 \pm 2.5^*$ vs 152 ± 1 mEq/L) and plasma [urea] ($56 \pm 4.9^*$ vs 28 ± 1.1 mg/dl), increased Δ urea excretion (exper days 2 and 3 - control day) ($67 \pm 16^{**}$ vs -7.6 ± 24 mgN/day) and a lower C_{urea} on exper day 3 ($0.37 \pm 0.03^*$ vs 0.61 ± 0.05 ml/min/100 gbw).

It is concluded that in dehydrated rats the azotemia is secondary to both decreased C_{urea} and increased urea appearance (increased plasma and urinary urea). The increase in urea appearance may be related to decreased ECF volume which is known to enhance urea appearance (Kid Int 32: 47, 1987). These studies also suggest that the increased urea appearance during ECF volume depletion is not mediated by enhanced ADH secretion.

Different from H * $p < 0.01$ ** $p < 0.05$

FREE CYTOSOLIC Ca^{2+} IN RENAL PROXIMAL TUBULES UNDERGOING KIDNEY GROWTH. J.Llibre* and DC. Batlle. Northw. Univ. and Lake Side VA, Chicago, Illinois.

An increase in cytosolic Ca^{2+} (iCa $^{2+}$) has been suggested to mediate growth of cells in culture and has also been found to antedate the activation of Na $^+$ /H $^+$ exchange seen immediately after exposure to growth factors. This study was designed to investigate whether iCa $^{2+}$ is affected by normal somatic growth and accelerated compensatory kidney growth following uninephrectomy (Ux). Free iCa $^{2+}$ was measured in renal proximal tubules prepared by standard density gradient centrifugation procedures which were incubated with Fura-2AM for 30 min. at room temperature. Fura-2 fluorescence was monitored at excitation wavelengths of 340 and 380nm. Kidney growth was estimated from the increase in kidney wt. In young Sprague-Dawley rats of 5wks of age (Group II, n=10), body and kidney wt, increased markedly as compared to rats of 4wks of age (Group I, n=8). This increase in somatic growth was associated with a significant increase in iCa $^{2+}$. (Table)

Group	Body wt.	Kidney wt.	iCa $^{2+}$
I	96 \pm 3	0.53 \pm 0.02	119 \pm 7
II	143 \pm 6*	0.91 \pm 0.03*	167 \pm 19*
III	140 \pm 3*	1.18 \pm 0.05*	123 \pm 27

* denotes $p < 0.01$ as compared to Group I
In Ux rats (Group III, n=5) kidney wt increased further but iCa $^{2+}$ did not. Since compensatory kidney growth following Ux is due mainly to cell hypertrophy our data suggest that an increase in iCa $^{2+}$ is not a feature of this form of kidney growth.

AUGMENTATION OF PROTEOLYSIS BY THYROID HORMONE IN PATIENTS WITH CHRONIC RENAL FAILURE (CRF). VS Lim, E Tsalikian and MJ Flanagan, Depts. Med. and Ped., Univ. of Iowa College of Med., Iowa City, IA.

To examine if thyroid hormone adversely affect protein metabolism in CRF patients. Leucine flux (F) was measured during primed-constant infusion of 2H_3 leucine and ^{15}N leucine (representing leucine carbon and nitrogen F, respectively) in the basal state (B) and after 7 days of treatment with triiodothyronine (T_3), 0.8 ug/Kg/day, in 7 normal (C) and 11 CRF (dialysis) patients. Studies were performed 14 hrs post absorption and identical constant diet was taken during both periods. Serum T_3 (ng/dl) rose from 130 \pm 7 to 229 \pm 19 in C and from 105 \pm 9 to 197 \pm 9 in CRF.

Results, means \pm SEM, are as follows:

	2H_3 leucine		^{15}N leucine	
	MPE %	Flux umol/Kg/min	MPE %	Flux umol/Kg/min
CB	2.32 \pm 0.08	1.22 \pm 0.05	4.40 \pm 0.29	2.10 \pm 0.15
CT3	2.06 \pm 0.08	1.40 \pm 0.05#	3.91 \pm 0.26	2.48 \pm 0.14
RB	2.14 \pm 0.10@	1.40 \pm 0.09@	4.48 \pm 0.24@	2.54 \pm 0.23@
RT3	1.76 \pm 0.09*	1.72 \pm 0.09*	3.46 \pm 0.21	3.44 \pm 0.22*

MPE=Isotopic moles percent enrichment in plasma. #, @ and * represent $p < 0.05$ by t test comparing CB/CT3, RB/RT3 and CT3/RT3, respectively. Leucine, being an essential amino acid, derives only from tissue breakdown during fasting. Increased leucine F in these studies indicates proteolysis. In B state leucine F was not different between the 2 groups of subjects suggesting that CRF patients were not more catabolic. T_3 treatment increased leucine F in both C and CRF subjects; in the latter, the proteolytic effect was augmented.

GAMMA GLUTAMYLTRANSFERASE, γ -GT, CONTRIBUTION TO AMMONIAGENESIS IN MAN. Ann Lockett, Proveen Dass and T.C. Welbourne. Dept. of Physiol. & Biophys., LSUMC, Shreveport, LA.

The contribution of γ -GT to luminal ammoniogenesis was assessed during hippurate modulation by administering an oral benzoate load. Studies were performed on 6 healthy individuals in a post absorptive state. After voiding, 9:00 AM, the subjects were given 0.5L H_2O followed by urine collections over 2 consecutive hours; benzoate, 100mg/Kg, was then given dissolved in 0.5L H_2O and urine collections obtained over 3 consecutive hours. Urinary hippurate concentration rose from the control value of 2.6 ± 0.3 mM to 34.6 ± 0.5 , 30.4 ± 5.6 and 29.7 ± 8.0 mM at the end of 1, 2 and 3 hours. The corresponding values for urinary NH_4^+ excretion, expressed as umol/mg creatinine were, control = 22.9 ± 6.4 and 40.9 ± 9.3 , 56.5 ± 16.6 and 48.8 ± 11.8 after 1, 2 and 3 hours respectively, $p < 0.05$; urinary pH and flow were not significantly different. Because glutamine likely enters the lumen via the paracellular pathway, one expects urinary glutamine concentrations to fall; they did: control 417 ± 138 nmol/mg creatinine vs 144 ± 52 nmol/mg creatinine. On the other hand, luminal glutamine hydrolysis obligates the formation of glutamate. Excretion of glutamate rose, but to only the borderline of significance, $p < .10$, indicating a large luminal reabsorption. The ratio of excreted gln to glu, as an index of luminal γ -GT activity fell from 2.85 ± 0.4 to 0.61 ± 0.22 . γ -GT appears to support renal function consequential ammoniogenesis.

EFFECTS OF OSMOLALITY ON CARBACHOL-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN RENAL MEDULLA. Lal C. Garg, Elzbieta Kapturczak*, and Shari McArdle*. Univ. of Florida Coll. of Med., Gainesville, FL.

Recently, we reported that carbachol (a cholinergic agonist) produces a concentration-dependent increase in phosphoinositide (PI) hydrolysis in the rabbit renal medulla and the effect was blocked by atropine, a cholinergic antagonist (Fed. Proc. 45:427, 1986). Because cholinergic agents increase renal blood flow and decrease the osmolality of the renal medulla, we determined the effect of osmolality (increased with a 50:50 mixture of urea and NaCl) on carbachol-stimulated PI hydrolysis in the inner medullary slices of the rabbit kidney. PI hydrolysis was measured by: a) incorporation of ^3H -inositol into PI for 1 hr at 37°C; b) release of ^3H -inositol phosphates (IPs) in the presence and absence of 1 mM carbachol for 1 hr at 37°C in the presence of 10 mM LiCl; c) separation of ^3H -IP from ^3H -PI by treatment with chloroform-methanol and Dowex columns; and d) determining the radioactivity of ^3H -IPs (aqueous layer) and ^3H -PI (organic layer). The release of ^3H -IPs in each buffer is expressed as % of total ^3H -inositol incorporated (into PI) in the same buffer. The osmolalities of buffers I, II, III and IV were 300, 600, 900 and 1200 mOsm/kg H_2O .

	I	II	III	IV
Control	0.1 ± 1.9	2.1 ± 0.4	0.2 ± 0.4	1.7 ± 2.6
Carbachol	25.1 ± 1.1*	16.2 ± 4.1*	8.9 ± 1.7*	2.2 ± 1.5**

Values are mean ± S.E.M. of 3-5 animals.

* P < 0.05 vs control in the same buffer.

** P < 0.05 vs carbachol in buffers I, II and III.

There was an inverse linear relationship between the osmolality of the incubation media and carbachol-stimulated release of IPs in the inner medullary slices. Our results suggest that vasodilator action of carbachol may increase its action on PI hydrolysis by decreasing the osmolality of the renal medulla.

α -ADRENERGIC AGONIST STIMULATION OF OXYGEN CONSUMPTION (QO_2) IN PROXIMAL AND DISTAL NEPHRONS. FA Gesek*, DW Wolff*, JW Strandhoy. Wake Forest University Medical Center, Winston-Salem, NC.

α_1 and α_2 agonists produced a rapid (<60 sec) non-linear increase in QO_2 of pooled cortical or separated proximal tubules. This rapid phase was then followed by a linear steady state (SS) QO_2 at a steeper slope than control. Increased SS but minimal rapid phase was observed in distal tubules. We tested the hypothesis that the transient, rapid phase was due to drug transport and metabolism and that the new SS phase was receptor related. Both α_1 (phenylephrine, cirazoline, norepinephrine) and α_2 agonists (guanabenz (GBZ), UK 14,304) produced dose-related increases in SS QO_2 in proximal and distal tubules. The SS but not the rapid, transient phase was blocked by ouabain. Acetate and tartrate mimicked the transient but not SS increase in QO_2 of GBZ acetate and Epi bitartrate. Active (-), but not inactive (+), Epi bitartrate increased SS QO_2 whereas both increased the rapid phase. GBZ and UK (1 μM) both increased distal QO_2 by 30% but only UK substantially increased proximal QO_2 . These data suggest that the rapid phase of QO_2 is likely due to drug transport and metabolism. The SS stimulation of QO_2 is receptor related and can be blocked by ouabain. Differences between agonists for QO_2 stimulation reflect both α -receptor affinities and differences in rates of drug transport and metabolism. Measurements of pharmacologic effects of α -adrenoceptor agonists in proximal tubules must consider the drug as an organic substrate as well as a receptor stimulator.

BENZOATE (B) STIMULATES RENAL GLUTAMINE (Gln) AMMONIAGENESIS VIA γ -GLUTAMYLTRANSFERASE (γ -GT). S. Hardman*, P. Dass* and I. Kurtz. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

Hyperammonemia is a characteristic feature of children born with a congenital urea cycle enzyme deficiency. B has been used clinically to stimulate hepatic channeling of glycine N_2 to Hip. Recent studies have also demonstrated that B increases the renal synthesis of Hip, which in turn stimulates Gln. ammoniagenesis via γ -GT. The present study was designed to determine the effect of B on renal

γ -GT glutaminase activity and urinary ammonia excretion. The effect of B (4mM) on Gln (2mM) metabolism was studied in rat renal proximal tubule suspensions and fasted rats. AT-125 was used to inhibit γ -GT. Rates are expressed as nmol/mg protein/30 min. (Mean ± SEM, n=4).

	utilization	production	
	glutamine	glutamate	ammonia
Gln	312.6±6.9	130.2±3.5	389.8±8.2
Gln+B	383.0±5.0 ^b	158.0±2.5 ^b	517.0±10.0 ^b
Gln+B +AT-125	184.0±9.1 ^{bc}	44.0±0.8 ^{bc}	270.3±14.3 ^{bc}

a, NS vs. Gln; b, p < 0.05 vs. Gln; c, p < 0.05 vs. Gln+B.

In separate whole animal experiments, B (1mmole/kg) stimulated urinary ammonia excretion by 35%. In conclusion: benzoate has a dual effect, it not only removes glycine N_2 by synthesis of hepatic and renal Hip, but it also stimulates renal Gln ammoniagenesis via γ -GT.

POTENT-INTRINSIC GROWTH PROMOTING FACTORS IN MOUSE KIDNEY FOLLOWING NEPHRON LOSS. M.K. Hise and K.V. Chacko*. Univ. of Maryland Hosp., Dept. of Medicine, Baltimore, MD.

Studies of some cells transformed by viruses and certain tumors indicate that they secrete autocrine or paracrine growth promoting factors. In order to examine the possibility that the kidney, following nephron loss, has similar capacities, adult male mice underwent sham (SNx) or unilateral (UNx) nephrectomy. Cytosol, 100,000g supernatant, was prepared from whole kidney, cortex, or liver 24 hrs following surgery. Newborn male mice received subcutaneous injections of cytosol, 1.1 mg, daily for up to 10 days. After 7 days, the wet weight of SNx injected mice averaged 4.442±.051g while that of UNx injected mice averaged 4.930±.073g (n=7, p<.001). Whole animal protein at 7 days averaged 441.8±2.7 mg in SNx injected mice and 550.3±8.6 mg in UNx injected mice (n=8, p<.001). Both SNx and UNx injected mice were larger than liver cytosol or saline injected mice. Similar data were observed at 2, 5, and 10 days following cytosol injection. The growth promoting activity appeared rapidly and was present 4 hrs following nephrectomy; it persisted for at least 48 hrs. Cortical cytosol also possessed significant growth promoting activity. Dialysis of the cytosol in high salt was used to identify the mol. weight of the stimulating activity. SDS-PAGE chromatography was performed to examine the presence of new proteins.

The studies demonstrate that following nephron loss, the mammalian kidney possesses potent growth promoting activity. It is hypothesized that acting locally, these factors serve as a positive feedback mechanism regulating renal cell mass.

REGULATION OF CALCIUM/PHOSPHOLIPID DEPENDENT PROTEIN KINASE C (PKC) DURING DIABETIC RENAL GROWTH. P.S. Mehta* and M.K. Hise. Univ. of Maryland Hosp., Dept. of Medicine, Baltimore, MD.

Studies of animals and humans indicate that proceeding the characteristic histologic lesions of diabetic nephropathy, significant renal growth occurs. PKC plays an important role in cell growth. The purpose of these studies were to examine the activity of PKC during diabetic renal growth and during rat kidney development. Adult male rats were made diabetic by the IV injection of streptozotocin, 35 mg/kg. Four weeks following the induction of diabetes, whole kidney proteins averaged 215.7±6.8 mg in diabetic animals and 166.1±4.8 mg in control animals (n=5, p<.001). Cytosolic PKC activity in control animals averaged 46.0±4.2 pmol mg⁻¹ min⁻¹ and 57.8±2.3 pmol mg⁻¹ min⁻¹ in diabetic animals. Basolateral membrane PKC activity also did not differ between the two groups. Brush border membrane PKC activity averaged 83.8±4.6 pmol mg⁻¹ min⁻¹ in control animals and 107.3±5.5 pmol mg⁻¹ min⁻¹ in diabetic animals (n=5, p<.02). Cytosolic PKC activity in rats 14 and 21 days of age was high when compared to animals 60 days of age. On the other hand, brush border membrane PKC activity was low on a day 14 when compared to activity on day 21 and day 60.

The data indicate that the brush border membrane has a selective increment in PKC activity in diabetic rats. Furthermore, during the period in the developing rat that electrolyte transport processes are maturing, there is an increment in brush border membrane PKC activity. In both models, the change in PKC activity may have important consequences for luminal membrane transport functions.

¹⁵N GLUTAMINE METABOLISM IN EXPERIMENTAL FANCONI SYNDROME Itzhak Nissim and Stanton Segal. Univ. of Penna. Sch. of Med., Dept. of Peds., Philadelphia, PA

Our aim was to evaluate the metabolism of ¹⁵N labelled glutamine by rat renal tubules of either maleic acid (M) or succinylacetone (SA) induced animal model of the human Fanconi syndrome. To this end, either M or SA was given intravenously in a dose of 2mM/kg and 4mM/kg respectively, two hours before killing the rats. Tubular incubations were conducted in Krebs buffer (pH 7.4) containing either 1mM [2-¹⁵N]glutamine or [5-¹⁵N]glutamine. In a separate series of experiments, tubules obtained from untreated rats were incubated as above in the presence of either 5mM SA or M. Measurements in freeze-clamped kidney show that both M and SA significantly reduced ATP level and ATP/ADP ratio. Similarly, both agents reduced ATP/ADP ratio and glucose formation following 60 min incubation of renal tubules obtained from untreated rats. Presence of M but not SA in the incubation system caused considerable decrease in lactate/pyruvate ratio and increased total NH₃ formation from glutamine. ¹⁵N analysis demonstrates that both M and SA significantly decreased ¹⁵NH₃ enrichment from [5-¹⁵N]glutamine. However, ¹⁵NH₃ from [2-¹⁵N]glutamine was 5-fold higher with M and 2-fold lower with SA, compared with control tubules. In addition both M and SA significantly inhibit formation of ¹⁵N labelled amino acids and 6-amino group of purine nucleotides (PNC) from [2-¹⁵N]glutamine.

The observations indicate that (a) both M and SA inhibit PNC activity (b), M stimulates NH₃ production, mainly via glutamate dehydrogenase activity (GLDH) and (c), SA inhibits both GLDH and phosphate-dependent and/or independent glutaminase activity and increases NH₃ formation from non-glutamine sources. The effect of M and SA on renal glutamine metabolism is presumably due to a depletion of energetic state and/or to a shift in mitochondrial redox state.

ACTIVITIES OF THE ENZYMES INVOLVED IN THE PURINE NUCLEOTIDE CYCLE IN THE RAT KIDNEY CORTEX AND MEDULLA. Tadeusz Pawelczyk* and Stefan Angielski*. Chair and Dept. of Clin. Biochemistry, Medical School, Gdansk, Poland. (intr. by J.I. Kreisberg)

The activities of adenylosuccinate synthetase (AMPS synthetase), adenylosuccinate lyase (AMPS lyase) and AMP deaminase were measured in cytosolic fractions from rat kidney cortex and medulla prepared as Bogusky *et al.* (J. Clin. Invest. 58, 326, 1976). The activities of AMPS synthetase, AMPS lyase and AMP deaminase were 4.1-, 22.7- and 1.7- fold higher in medulla than in cortex, respectively. The maximal activity of AMPS synthetase in medulla was 1.24 nmol x min⁻¹ x mg⁻¹ and S_{0.5} for IMP was 43 μM. IMP at concentrations higher than 150 μM inhibited this enzyme. The activity of AMPS synthetase in cortex was only 0.32 nmol x min⁻¹ x mg⁻¹ in the presence of 150 μM IMP. The activity (V_{max}) of AMPS lyase was 1.1 and 25 nmol x min⁻¹ x mg⁻¹ and K_m for AMPS was 4.8 and 3.1 μM in cortex and medulla respectively. AMPS at concentrations higher than 10 μM inhibited AMPS lyase from kidney medulla; AMPS (100 μM) inhibited AMPS lyase by 60%. AMPS lyase from cortex was not inhibited by AMPS. The activities of AMP deaminase were 17 and 29 nmol x min⁻¹ x mg⁻¹ in kidney cortex and medulla, respectively. These results suggest that the purine nucleotide cycle operates primarily in the medulla of rat kidney.

MECHANISM FOR REDUCED PHOSPHOLIPID ARACHIDONIC ACID (AA) IN RENAL CORTEX OF DIABETIC RATS. L. Ramsammy*, C. Josepovitz* and G.J. Kaloyanides. Dept. of Medicine, SUNY-Stony Brook and VAMC, Northport, NY.

AA is reduced in virtually all tissues including renal cortex of diabetic (D) man and rats. The mechanism is not known but decreased synthesis and/or increased consumption can be postulated. AA (20:4) is synthesized from linoleic acid (18:2) as follows:
18:2 → Δ⁶-desaturase → 18:3 → elongase → 20:3
20:3 → Δ⁵-desaturase → 20:4. AA and its precursors can be utilized for eicosanoid production, for generation of fuel (β-oxidation), or converted to malondialdehyde (MDA) during free radical induced lipid peroxidation. To identify the mechanism(s) by which AA is reduced in the renal cortex, we measured the desaturases and elongase activities, lipid peroxidation and β-oxidation in the renal cortex of control (C) and D rats.

	Δ ⁵ -desat	Δ ⁶ -desat	elong.	MDA	20:4
	nmol/mg prot				pmol CO ₂ /mg
C	.72±.09	.78±.08	.81±.11	.77±.02	.23±.01
DM	.24±.04	.15±.01	1.16±.15	.63±.02	.21±.01
P	<.001	<.001	NS	<.001	NS

We conclude that decreased synthesis secondary to decreased Δ⁵ and Δ⁶ desaturase activities contributes to depression of AA in renal cortex of D rats. No evidence was found for increased consumption due to β-oxidation or lipid peroxidation.

VANADATE STIMULATES H-ATPase ACTIVITY IN RAT ASCENDING LIMB. S. Sabatini and N.A. Kurtzman, Texas Tech Univ. Health Sciences Center, Lubbock, TX.

Vanadate has been used in many cellular systems to elucidate mechanisms of enzyme action. Vanadate inhibits Na-K ATPase activity in many tissues. In isolated collecting tubule it inhibits sodium transport and vasopressin-stimulated water flux, the latter presumably distal to cyclic AMP formation. In turtle bladder vanadate inhibits both acidification and H-ATPase activity. Vanadate also stimulates a variety of enzymes including phosphodiesterase and 2,3-diphosphoglycerate phosphatase. We studied the effect of vanadate at 3 concentrations (0.01, 0.1, 1 mM) on H-ATPase activity in microdissected segments of rat nephron. In proximal convoluted tubule and in cortical, medullary, and papillary collecting ducts vanadate had no effect on H-ATPase activity. In medullary thick ascending limb, however, vanadate (1mM) significantly stimulated H-ATPase activity (241±14 vs 531±74 pmols/mm/hr, N=14, P<0.01). Our results demonstrate that vanadate selectively stimulates H-ATPase activity in rat nephron. These data suggest that the thick ascending limb enzyme is different from other segments. Taken together with our previous study, showing different H-ATPase enzyme kinetics for cortical collecting tubule and medullary collecting tubule, we postulate that the renal H-ATPase is a heterogeneous enzyme. It also is distinctly different from H-ATPase in other nonmammalian proton secretory epithelia which are vanadate inhibitable.

RELATIONSHIP BETWEEN INTRACELLULAR pH AND NH₃ METABOLISM IN LLC-PK₁ CELLS. A. Sahai, E. Laughrey, R.L. Tannen. University of Michigan, Ann Arbor, Michigan.

We have recently reported that 3 hrs incubation of subconfluent rocked LLC-PK₁ cultures in a media of low pH results in parallel increases in NH₃ and alanine production and a decrease in the intracellular levels of alpha KG and glutamate. To determine the initiating signals and the metabolic alterations responsible for altered NH₃ production, studies were performed in both still and rocked LLC-PK₁ cells. In contrast to rocked cultures NH₃ production, glutamine consumption and the intracellular levels of alpha KG and glutamate were unaltered by a low media pH in still cultures. Alanine production, however, was increased from 164 ± 9.0 nmol/mg/hr to 233 ± 15.5 (pH 7.6 vs. 7.0) in still cultures compared with 270 ± 10 to 470 ± 21 in rocked cultures. When intracellular pH was determined using (¹⁴C) DMO the response to alterations in media pH (7.0, 7.4 and 7.6) was identical in both rocked (6.95 ± 0.03, 7.42 ± 0.05 and 7.50 ± 0.06, respectively) and still (7.01 ± 0.05, 7.43 ± 0.03 and 7.47 ± 0.11, respectively) cultures. These data indicate that some alteration in the metabolic pathway or in the intramitochondrial response to a low cytosolic pH accounts for the absence of increased NH₃ production in still cultures. The data also suggest that activation of alpha KG dehydrogenase is an integral step in the acute pH stimulation of ammoniogenesis.

GLUTAMATE/H⁺ COTRANSPORT IN SUBMITOCHONDRIAL PARTICLES. S. Sastrasinh and M. Sastrasinh. VA Medical Ctr., East Orange, NJ and UMDNJ-New Jersey Medical School, Newark, NJ.

The existence of a bidirectional glutamate/H⁺ carrier was reported in both kidney and liver mitochondria. Although increases in medium pH reduce glutamate efflux from the mitochondria of both organs, neither the effect of medium pH on glutamate uptake nor the effect of matrix pH on glutamate efflux was seen in kidney mitochondria. To investigate this issue further we studied the uptake of 0.5 mM glutamate in submitochondrial particles from rat kidney. Glutamate uptake was stimulated by inwardly directed H⁺ gradient: pH_{in}/pH_{out}

pH _{in} /pH _{out}	Glutamate Uptake (pmol/mg)				
	30 sec	1 min	5 min	15min	30min
7.5/7.5	97±8	131±4	225±5	276±7	300±7
7.5/6.5	163±14	228±10	344±18	391±12	397±12
P	<0.01	<0.01	<0.01	<0.001	<0.01

Glutamate efflux was also stimulated by H⁺ gradient (pH_{in}/pH_{out} 6.5/7.5 vs 6.5/6.5; glutamate efflux 207±2 vs 141±13 pmol/mg/5 sec; P<0.05, n=3). The stimulatory effect of H⁺ gradient was maximal at 0.5 pH unit. Higher H⁺ gradient did not have any additional effect on glutamate transport.

pH _{in} /pH _{out}	Glutamate Uptake (pmol/mg/sec)			
	7.5/7.5	7.5/7.0	7.5/6.5	7.0/6.0
	23±3	59±3	49±2	48±1

We conclude that H⁺ gradient stimulates glutamate transport across renal mitochondrial membrane in both directions. The limited response of the carrier activity to pH changes may explain the findings in intact kidney mitochondria. Submitochondrial particles are useful in studying metabolite transport across mitochondrial membrane.

MEASUREMENT OF INTRACELLULAR pH IN SUSPENSIONS OF RENAL CORTICAL TUBULES FROM POTASSIUM DEPLETED RATS. Anton C. Schoolwerth, Medical College of Virginia, Richmond, VA.

To evaluate the possible contribution of intracellular acidosis as a stimulus for augmented renal ammonia and glucose production in potassium depletion (KD), experiments were performed with cortical tubules from rats fed control (C) or KD diets. Serum potassium levels were significantly less than C following two weeks of KD (2.4±0.4 vs 4.7±0.6mM, mean ±SD, KD vs C, p<.001). Muscle K⁺ content was also significantly lower in KD (285±36 vs 368±31 mmol/kg, p<.001). Ammonia and glucose production from 1mM glutamine were two-fold greater in tubules from KD rats compared to C (both p<.001). Intracellular pH (pH_i) was estimated at three different medium pH values by the fluorescence of 2',7'-bis(carboxyethyl)5,6 carboxyfluorescein (BCECF). pH_i at medium pH 7.0, 7.4, and 7.7 in C was 6.96, 7.23, and 7.45, respectively, no different from values of 6.99, 7.31 and 7.49 in KD. In parallel incubations, mitochondrial pH (pH_m) was estimated utilizing labeled DMO. pH_m was approximately 0.5-0.6 pH units higher than pH_i at each medium pH value and no different in KD vs C. These experiments demonstrate that increased ammonia and glucose production of tubules from KD rats occurs in the absence of demonstrable changes in pH_i, pH_m, or pH gradients across cell and mitochondrial membranes. The results suggest that some stimulus other than intracellular acidosis maintains a high steady-state rate of renal ammoniogenesis and gluconeogenesis in potassium depletion.

GLUCONEOGENIC METABOLISM IN CULTURED PROXIMAL TUBULAR CELLS. K. Suresh*, M.J. Tang*, A. Sahai*, R.L. Tannen. University of Michigan, Ann Arbor, Michigan

We have reported that primary culture of rabbit proximal tubules exhibit heightened glycolytic metabolism during the proliferative phase as reflected by increased glucose consumption and lactate production along with enhanced activity of glycolytic enzymes. Unexpectedly the activity of the gluconeogenic enzyme PEPCK appears to increase in parallel with pyruvate kinase enzyme activity. Furthermore, LLC-PK₁ cells which also are highly glycolytic have been reported to exhibit high activity of PEPCK along with undetectable amounts of fructose 1,6 bisphosphatase (FBPase). To further examine this dichotomy, we analyzed PEPCK radioisotopically and also performed FBPase assays in the primary cultures and LLC-PK₁ cells. Earlier measurements of PEPCK had been performed spectrophotometrically, a methodology that can yield aberrantly high values when pyruvate kinase activity is high in the tissue. Fresh rabbit proximal tubules exhibited high PEPCK activity (30.03±2.97 nmol/min/mg). However, the enzyme activity was significantly reduced to 18.23±3.70 by day 6 in culture and further decreased to 14.71±2.8 by day 9. FBPase activities were 14.11±3.95 nmol/min/mg in fresh tubules and 4.96±1.20 and 4.10±2.13 by day 6 and 9, respectively. Both PEPCK and FBPase were undetectable in LLC-PK₁ cells. Thus, there is coordinate regulation of glycolytic and gluconeogenic enzymes in primary cultures as well as in established cell lines, with glycolytic metabolism predominating. The factors that account for the conversion to a glycolytic profile remain to be determined.

GLYCOLYTIC METABOLISM IN CULTURED RABBIT PROXIMAL TUBULES. M.J. Tang*, R.L. Tannen. University of Michigan, Ann Arbor, MI.

Primary cultures of rabbit proximal tubules grown in hormonally defined, serum free media revert to glycolytic metabolism during the proliferative phase (day 1-6) reflected by increased glucose consumption, lactate production, and activity of pyruvate kinase (PK) and lactate dehydrogenase (LDH). This behavior is not secondary to hypoxia and occurs in cells with elevated rather than suppressed ATP levels. The present study was designed to extend these observations to the confluent stage. Daily glucose uptake increased significantly from day 4 to day 6 and remained unchanged thereafter. Lactate production, however, was increased further from day 6 (4.23±0.09 μ mol/mg/day) to day 9 (6.47±0.21). PK activity 31.5±8.8 mU/mg in fresh tubules increased to 159±38 by day 6 and rose further to 363±44 on day 9. LDH activity was 5 fold higher than fresh tubules on day 6 and continued to rise by day 9 (day 6 = 372±68 mU/mg and day 9 = 521±12). To determine whether hypoxia is responsible for enhanced glycolysis cultures were grown on a rocker platform to provide adequate O₂. Rocked cultures exhibited metabolic and enzymatic profile similar to standard cultures during growth phase (day 1 - day 6). In contrast to standard still cultures lactate formation and PK activity was significantly decreased by rocked cultures during the confluent phase (day 7 - day 9). Day 9 values for lactate formation was 5.12±0.26 and PK activity 170±30. Thus during the differentiated phase inadequate O₂ appears to partially account for enhanced glycolysis.

METABOLISM OF LACTATE BY THICK ASCENDING LIMBS (TAL) OF THE DOG KIDNEY IN VITRO: RELATION WITH TRANSPORT. A. Tejedor, J. Noël, P. Vinay, Y. Boulanger, A. Gougoux. Departments of Medicine and Physiology, Université de Montréal, Montréal, Canada.

Dog TAL in suspension respire rapidly in vitro (337 μ mol O₂.g⁻¹.hr⁻¹), and respond to nystatin (0.5 mM) (877 μ mol O₂) in a ouabain-dependent fashion, and to the uncoupler CCCP (0.5 mM) (1313 μ mol O₂). More than 90% of the Na,K-ATPase activity in situ is related to Na transport through furosemide-(F)-sensitive luminal transporters. The addition of substrates increases the respiration (lactate(L), 21%; glutamine(Gln), 11%; glucose(G), 4% in a saturable (above 2 mM) fashion. More than 70% of this stimulation is ouabain-sensitive (ouabain 1 mM) with L, but only 15% with Gln and 0% with glucose. Thus L specifically stimulates the Na entry and transport by the Na,K-ATPase in TAL. F, but not acetazolamide nor amiloride, inhibits this stimulation (70%). Thus the lactate-driven Na entry occurs with Cl and K through F-sensitive structures. If F or SITS is applied before L, or if the TAL are incubated in a Cl-free medium, no stimulation of respiration is observed. Thus L entry in TAL appears to be coupled with the transepithelial flux of Cl. Furthermore, L uptake occurred against a concentration gradient. F and SITS also specifically impairs the metabolism of L (as compared with G). Our results suggest that a large fraction of basolateral L entry in TAL is coupled to the Cl efflux by a SITS-sensitive anionic exchanger. This mechanism insures a stoichiometry between the transepithelial flux of NaCl and substrates availability to support the energetic needs to TAL cells.

ACCELERATION OF ALANINE FLUX BY THYROID HORMONE IN CHRONIC RENAL FAILURE PATIENTS (CRF). E Tsalkian, VS Lim and MJ Flanigan, Depts. Med. & Ped., Univ. of Iowa College of Med., Iowa City, IA.

To assess if thyroid hormone affects protein metabolism in CRF patients, alanine flux was measured during primed-constant infusion of ²H₃ alanine in the basal state (B) and after 7 days of treatment with triiodothyronine (T₃), 0.8 ug/Kg/ day, in 7 normal (C) and 11 CRF (dialysis) subjects. Studies were performed 14 hrs post absorption and identical constant diet was taken during both periods. Serum T₃ increased and TSH decreased in both C and CRF. Alanine flux, mean± SEM, are as follows:

MPE (%)	Flux	Prot. Deg	Syn	
	(umol/Kg/min)			
CB	2.45±0.31	6.26±0.67¶	1.35±0.05	4.91±0.66¶
CT3	2.23±0.18	6.57±0.53	1.54±0.05#	5.03±0.50
RB	1.74±0.19	9.50±0.71@	1.54±0.10@	7.95±1.00@
RT3	1.20±0.19*	16.68±1.54*	1.89±0.09*	14.79±1.51*

MPE=isotopic moles percent enrichment in plasma. Prot. Deg=alanine flux derived from protein degradation and Syn=alanine flux derived from de novo synthesis. ¶, #, @ and * represent p<0.05 by t test comparing respectively, CB/RB, CB/CT3, RB/RT3 and CT3/RT3. Plasma alanine (uM), determined from the peak area ratio of ¹³C¹⁵N²H₄ and natural alanine were 284±32 and 391±26, respectively, in C and CRF (p<0.01).

Alanine flux was increased in CRF patients in the B state. T₃ treatment further enhanced alanine flux in CRF but not in C. The increased alanine flux was derived mostly from de novo synthesis. Increased alanine flux and elevated plasma alanine are consistent with enhanced gluconeogenesis.

INSULIN INHIBITS ACCELERATED PROTEOLYSIS IN SERUM-DEPRIVED CULTURED KIDNEY CELLS.

T. Tsao*, G.E. Mortimore*, E. Cragoe* and R. Rabkin. Dept. Medicine Stanford Univ. & VAMC Palo Alto, Ca, Dept. Physiol. Penn State Univ., Hershey and Merck Sharp Dome Res. Lab West Point, Penn.

Intrinsic cell proteins are key cellular constituents. Cell protein content is influenced by a balance between rates of synthesis and degradation. However little is known about the regulation of cell protein degradation in the kidney in general and in tubular epithelium in particular. As insulin is a potent physiologic regulator of proteolysis in other tissues, we set out to determine whether it influences proteolysis in cultured kidney epithelial cells. Monolayers of the proximal-like opossum kidney cell line were grown in culture medium containing 10% serum and amino acids. Cell proteins were labelled with ^{14}C -valine for 20 hours prior to study. Proteolysis, determined from the release of acid soluble radioactivity, averaged $1.3 \pm 0.4\%/hr$. After removing serum, proteolysis increased by 29% ($p < .05$). Addition of physiologic levels of insulin (10^{-9}M) to serum deprived cells partially inhibited the accelerated proteolysis by 11% ($p < 0.02$). Complete inhibition was achieved with supraphysiologic insulin concentrations (10^{-6}M). When amiloride (0.1mM) was added inhibition was still evident. Accordingly it appears that insulin mediated inhibition is not due to changes in intracellular pH, that might follow insulin stimulated NA^+/H^+ exchange.

We conclude from this study that insulin modulates cellular protein breakdown in kidney epithelial cells.

REGULATION OF HEXOSE MONOPHOSPHATE SHUNT (HMPS) ACTIVITY IN PRIMATE RENAL TUBULE CELLS, AND ITS RESPONSE TO OXIDANT STRESS. **JV Vadgama***, **JH Kluwata***, **WD Davidson**, Harbor-UCLA Medical Center, Torrance, CA.

We have demonstrated the presence of HMPS in cultured renal tubular cells (BSC), derived from the African Green Monkey. The primary objective of our study was to elucidate the importance of the shunt in protecting the renal cells from oxidant stress. Oxygen free radicals (OFR) have been implicated in the pathogenesis of many pathologic conditions including acute tubular necrosis. Inactivation of OFR involves glutathione reductase (GSH-R) which requires NADPH as a cofactor. Since the thiol redox state of the cell is important, we studied the HMPS as the source of NADPH for GSH-R Activity. HMPS activity was measured as $^{14}\text{CO}_2$ production from glucose 1- ^{14}C metabolism, in the presence and absence of the oxidant, t-butyl hydroperoxide (TBH). Our results show a dose dependent stimulation of glucose-1- ^{14}C metabolism by TBH. HMPS activity was dependent on the rate of cell growth. Post confluent cells showed increased HMPS activity. HMPS activity was regulated by (a) amino acid availability and (b) serum in the growth media. The basal activity declined significantly on removal of amino acids. However, when the cells were exposed to TBH, the shunt was stimulated by 15 fold during amino acid starvation. The presence of serum during amino acid starvation stimulated the TBH induced HMPS activity to only 3 - 5 fold. These observations show that growth conditions play an important role in regulating the HMPS activity, and in turn, its response to oxidant stress.

ISCHEMIA/REFLOW-INDUCED CHANGES IN CORTICAL & MEDULLARY ADENINE NUCLEOTIDES: EFFECT OF ALLOPURINOL

B.K. Urbaitis, **C.H. McRoy*** & **D. Leeper***. Depts of Physiology (Dental School) & Medicine (Nephrology) Medical School. Univ. of MD, Balto, MD.

Experiments were done to determine the effect of allopurinol (A) on changes in ATP (T), ADP (D), AMP (M) & Pi concentration in renal cortex and red medulla that occur as a result of 60 min of ischemia alone (I) or ischemia followed by 15 min of reflow (IR).

The femoral vein was cannulated for the delivery of saline or (A). The left renal artery was exposed via a midline incision and occluded for 60 min (I) or following this time, blood was allowed to flow for 15 min (IR). In (A) experiments 50 mg/kg (A) was administered 15 min prior to occlusion. At the end of each experiment kidneys were quick frozen and analyzed for nucleotides by enzymatic fluorometry. Right kidneys were used as controls. Data ($\mu\text{m/g.d.w.}$) obtained from 12 experiments are:

	CORTEX				MEDULLA							
	CONTROL	A	IR	C	CONTROL	A	IR	C				
T	9.0	1.1	4.3	9.2	1.3	4.0	8.6	1.3	4.9	9.3	1.1	4.5
D	2.3	1.0	1.3	1.7	0.5	0.9	2.6	1.0	1.4	2.3	0.8	1.0
M	0.6	1.4	0.7	0.7	1.2	0.4	0.5	1.1	0.3	0.4	1.5	0.3
Pi	22	55	27	18	54	30	26	39	29	25	47	25

Ischemia led to a drastic decrease in ATP concentration which was partly restored by 15 min of reflow. Pi increased with ischemia and returned to control levels at 15 min of reflow. (A) data are identical to control. In conclusion: (A), at this dose does not moderate ischemia-induced changes in adenine nucleotide or Pi concentration in cortex or red medulla.

INHIBITION OF 5'-NUCLEOTIDASE ENHANCES POSTISCHEMIC RESTORATION OF RENAL ATP. **Arez van Waarde***, **M. Stromski***, **G. Thulin***, **K. Gaudio**, **R. Shulman*** and **N. Siegel**, Depts of Peds and Mol Bioph and Bioch, Yale Univ Sch Med, New Haven, CT

As determined by ^{31}P -NMR *in vivo*, postischemic recovery of renal ATP is a biphasic process. A rapid initial recovery upon reperfusion is followed by a slow return toward preischemic levels. The magnitude of the initial recovery is strongly correlated with the residual nucleotide pool at the end of ischemia. Therefore, we attempted to enhance the postischemic recovery of renal ATP by inhibition of nucleotide catabolism during ischemia.

Rats were injected with β -methylene adenosine diphosphate (AMPCP, 4 mg/kg BW, i.m.), which led to a consistent 70% reduction in renal 5'-nucleotidase activity. Analysis of HClO_4 -extracts of kidneys from AMPCP treated rats by ^{1}H -NMR showed a 45% increase in the residual nucleotide pool ($3.24 \pm 0.33 \mu\text{mol/g}$ vs 1.53 ± 0.17 in control rats, $P < 0.01$). *In vivo* ^{31}P -NMR of AMPCP-treated rats had a) higher initial recovery of ATP following 45 min of ischemia ($69.6 \pm 3.7\%$ of the preischemic level vs. $53.0 \pm 2.7\%$ in untreated rats, $P < 0.01$), b) accelerated rate of ATP resynthesis ($0.21 \pm 0.05\%$ of control-min vs. 0.10 ± 0.03 , $P < 0.05$) and c) significantly higher ATP level after 2 hrs of reflow ($93.9 \pm 6.3\%$ of control values vs $61.4 \pm 3.0\%$, $P < 0.005$). Thus, inhibition of 5'-nucleotidase enhances postischemic restoration of renal ATP.

IMAGE-LOCALIZED ^{31}P NUCLEAR MAGNETIC RESONANCE SPECTRA OF THE ORTHOTOPIC AND TRANSPLANTED HUMAN KIDNEY. M. W. Weiner & G. B. Matson*. Magnetic Resonance Unit, Depts. Med. & Radiol., VA Medical Center & Univ. Ca., San Francisco, CA.

The goal of these experiments was to obtain localized ^{31}P NMR spectra of human kidneys, guided by MRI images. Experiments were performed in a MRI/MRS whole body system at 1.5 T using a surface coil placed over the region of the kidney. Normal volunteers and patients with well functioning kidney transplants were placed into the magnet and an MRI was obtained to precisely define the renal location. The ISIS technique was used to obtain ^{31}P NMR spectra from a region defined from the MRI image. Spectra were obtained in 30 min showing peaks for ATP, Pi, phosphomonoesters, and phosphodiesteres. A peak for phosphocreatine was either very small or absent, indicating the absence of muscle contamination. Spectra from orthotopic and transplanted kidneys were similar. Examination of an irreversibly obstructed kidney showed no ATP. The results show that NMR spectroscopy, combined with MRI, provides reliable measurements of renal metabolites in human subjects. This approach will be used to investigate the metabolic disturbances associated with renal failure, acid-base disorders, and transplant rejection.

PARAMINOHIPPURATE STIMULATION OF GAMMA GLUTAMYLTRANSFERASE AMMONIAGENESIS. T. C. Welbourne, Dept. of Physiol. & Biophys., LSU-MC. Shreveport, LA.

The role of γ -GT in renal ammoniagenesis was studied in post absorptive male Sprague Dawley rats weighing 350-400g. Glutamine extraction and total NH_4^+ production was determined across the left kidney with cannulas placed in the left renal vein and ureter. Renal plasma flow was determined using tracer levels of ^3H -PAH; blood flows were derived from the hematocrit factor. Total NH_4^+ production was 1001 ± 197 in control period and rose to 1591 ± 230 nmol/min, $p < 0.025$, with the infusion of cold PAH, 0.4 $\mu\text{mol}/\text{min}/100\text{g}$; arterial and urinary PAH concentrations were 0.21 ± 0.08 and 73.7 ± 15.0 mM respectively. Increased ammoniagenesis was attributable to a rise in luminal NH_4^+ release, 278 ± 48 to 694 ± 164 nmol/min, $p < 0.025$, supported by increased gln extraction, 279 ± 94 to 876 ± 188 nmol/min. The rise in gln extraction was associated with a large drop in urinary gln and rise in the blood to urine gln concentration gradient from 16 ± 94 to 381 ± 41 nmol/ml; urinary glutamate excretion rose and the ratio of excreted gln to glu fell from 2.4 ± 0.8 to 0.8 ± 0.4 . The results are consistent with PAH stimulation of luminal γ -GT's hydrolysis of gln and enhanced paracellular gln flux down a steeper diffusion gradient. To confirm this supposition, γ -GT was eliminated by administration of acivicin 30 mg/Kg. After inhibition of γ -GT, PAH failed to increase the blood to urine gln diffusion gradient, ammoniagenesis or gln extraction.

RENAL PHYSIOLOGY— ACID BASE

GLUCOCORTICOID PARTIALLY REGULATES Na/H ANTI-PORTER OF RABBIT RENAL CORTICAL BRUSH BORDER MEMBRANE VESICLES (BBMV) IN METABOLIC ACIDOSIS. T. Akiba, S. Sasaki, K. Tomita, Y. Iino, and N. Yoshiyama. Tokyo Med. & Dental Univ. Tokyo, Japan.

Adaptive increase of V_{max} of Na/H antiporter of BBMV in metabolic acidosis is mediated by glucocorticoid in rat (Kinsella et. al. 1984). Our experiments tried to study the role of glucocorticoid on adaptation of Na/H antiporter of rabbit BBMV to metabolic acidosis. Each five female NZW rabbits were treated with ammonium chloride for 2 days (ACID), with 2mg dexamethasone two times for three days (DEX), or both (ACID+DEX). Five rabbits were adrenalectomized and maintained with 0.9% saline for 7 days (ADX). BBMV were prepared by Mg precipitation methods. Na/H antiporter were assayed by acridine orange method.

Plasma $[\text{HCO}_3^-]$ (mM)	V_{max} (AFU/sec/mg protein)
CONTROL	18.8(1.1)* 21.2(1.8)*
ACID	9.7(1.1) 37.2(6.2)*
DEX	19.8(0.8)* 21.9(1.0)*
DEX+ACID	4.0(1.7) 27.5(1.7)*
ADX	20.2(3.5) 15.3(1.8)

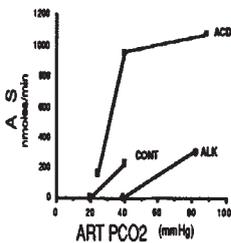
MEAN(S.E.M.) * $p < 0.05$ compared to control. Adx suppressed V_{max} but large dose of Dex did not increase V_{max} compared to control. V_{max} of DEX+ACID was increased compared to DEX ($p < 0.05$). K_{ms} for Na did not change significantly. These data indicate that adaptation of Na/H antiporter to metabolic acidosis in rabbit does not require an increase in glucocorticoid

ELECTROPHYSIOLOGICAL IDENTIFICATION OF CELLS IN MONOLAYERS OF RABBIT CORTICAL COLLECTING TUBULE (CCM). Elsa Bello-Reuss. University of Texas Medical Branch, Dept. of Med., Galveston, Texas.

Double barrel microelectrodes were used to determine apical cell membrane voltage (V_a) and intracellular pH (pH_i) in CCM grown in the absence of aldosterone. Some of the cells bind fluorescein-labeled peanut lectin. Electron microscopy showed two different cells resembling intercalated and principal cells. Culture purity was 96%. Electrophysiologically 3 cell types were identified. In cell 1, V_a was -64 ± 4 mV. The apparent ratio of membrane resistances ("a") was 0.54 ± 0.01 , and pH_i 7.20 ± 0.03 . Exposure to 50mM K^+ on the apical side depolarized V_a by 30 ± 1 mV. V_a and pH_i did not change when Cl^- was replaced by cyclamate. In cell 2, V_a was -39 ± 2 mV, "a" was 8 or more, apical increase of $[\text{K}^+]$ did not change V_a . Apical Cl^- removal hyperpolarized V_a by 6 mV and increased pH_i from 7.08 ± 0.02 to 7.30 ± 0.05 . Cell 3 was infrequently found (3 out of 41 cells). V_a was -49 ± 4 mV, pH_i was 7.12 ± 0.02 . Apical high K^+ depolarized V_a by 26 ± 3 mV and Cl^- removal depolarized V_a by 27 ± 5 mV while pH_i increased slightly to 7.17 ± 0.01 . In conclusion: 1. Three different cell types can be identified electrophysiologically in CCM. 2. Cell 1 has the electrical characteristics of principal cells while cell 2 resembles the intercalated cells. Its response to Cl^- removal suggests the existence of apical $\text{HCO}_3^-/\text{Cl}^-$ exchanger. 3. Cell 3 appears to have both K^+ and Cl^- conductive pathways at the apical membrane. The identity of cell 3 is uncertain.

ACIDIFICATION ADAPTATION ALONG THE INNER MEDULLARY COLLECTING DUCT(IMCD). H.H. Bengel, E.R. McNamara*, J.H. Schwartz, and E.A. Alexander. Renal Sect., Boston City Hosp. Boston Univ School of Med, Boston, MA.

Chronic acid feeding (ACD) stimulates and chronic alkali feeding (ALK) suppresses acid secretion (AS) along the IMCD. Our purpose was to determine whether these stimuli produce adaptation. Would IMCD acidification differ quantitatively when a comparable acute stimulus or suppression was applied to ACD or ALK rats? To answer this we produced alterations in systemic PCO₂, which we have previously shown stimulate (↑PCO₂) or suppress (↓PCO₂) IMCD acidification. Utilizing microcatheterization, IMCD pH and PCO₂ were measured directly with electrodes. Fluid was obtained for measurement of HCO₃⁻, titratable acid and NH₄⁺ and net AS was calculated as the difference between paired proximal and tip samples. These data were then compared with data previously obtained in control, ACD and ALK rats with varying systemic PCO₂s.



We interpret the results (Fig) to indicate that alterations in systemic PCO₂ produce physiologically appropriate responses in IMCD acidification. Dietary acid/alkali manipulations alter the intrinsic capacity of the IMCD so that with a comparable stimulus

(Δ arterial PCO₂) IMCD AS is greater in ACD rats than in ALK rats. We conclude that the IMCD demonstrates acidification adaptation.

H⁺ TRANSPORT IN CULTURED INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. Jac P. Brion*, John H. Schwartz, Herbert M. Lachman*, Ed A. Alexander, and George J. Schwartz. Dept. of Peds. & Med., Albert Einstein Coll. of Med., Bronx, N.Y., and Dept. of Med., Boston Univ. School of Med., Boston, MA.

The papillary portion of the IMCD contains a morphologically uniform cell type analogous to the principal cell, yet can secrete H⁺ (J_{H+}) against steep pH gradients. To determine if a specific sub-group is specialized for J_{H+}, cultured IMCD cells derived from the rat papilla were assayed for peanut lectin (PNA) binding, cell pH (pH_i), and carbonic anhydrase (CA). Fluorescent PNA stained 48±4% of cells (PNA+). When loaded with BCECF-AM, PNA+ cells accumulated 225±51% (n = 7) more BCECF (450 excitation) than PNA- cells, and this intensity was reduced by pre-incubation in acetazolamide (CA involved in hydrolysis). Northern blots of IMCD total RNA showed a specific mRNA band for CA II (cDNA probe provided by Dr. P. Curtis). Also pH_i of PNA+ cells (5-10 cells per monolayer) was (7.25±0.07, n=7) more alkaline than PNA- cells (6.56±0.06, n=7). Subcultures were enriched (>90%) with PNA+ cells using gel-immobilized PNA. Enriched monolayers had a greater pH_i (ΔpH_i = 0.3±0.1, n = 11) than controls and a 3-fold greater CA activity. More than 85% of enriched monolayers were capable of NEM sensitive, Na⁺ independent J_{H+} after an acid load (33% for controls). Thus, IMCD cultures contain a subpopulation of PNA+ cells that is more alkaline, enriched with CA and may express specific proteins required for H⁺ secretion.

PURIFICATION OF A PROTEIN FROM RAT RENAL BRUSH-BORDER MEMBRANES INVOLVED IN Na⁺/H⁺ EXCHANGE.

G. Burckhardt*, J. Sablotni*, and T. Friedrich* (intr. by P.S. Aronson). Max-Planck-Institute for Biophysics, Frankfurt/Main, Fed. Rep. Germany

In previous photoaffinity and affinity labeling studies with an amiloride analog and with dicyclohexylcarbodiimide (DCCD) we have identified a MW 65,000 protein which is most likely involved in rat renal Na⁺/H⁺ exchange (J. Membrane Biol. 94, 253-266, 1986). Additional studies reveal that this protein has an isoelectric point of ~6.2 and is glycosylated: treatment with endoglycosidase F decreases the MW to 52,000. We then attempted to purify the MW 65,000 protein using two strategies. First, we electroeluted the amiloride-protectable, [¹⁴C]DCCD-labeled MW 65,000 band from preparative SDS gels. As this band contains eight polypeptides as judged from two-dimensional gels we used reversed phase HPLC for further purification. At 41% acetonitrile a single fraction is recovered from HPLC containing amiloride-protectable [¹⁴C]DCCD-labeled polypeptides. Second, we constructed an affinity column with amiloride as a ligand. Solubilized rat renal brush-border proteins were added to the column and the specifically bound proteins were eluted with an excess of 5-N(ethylisopropyl)amiloride. A protein with MW 65,000 and IP of ~6.2 represents the major contents of this eluate. Reversed phase HPLC of affinity purified proteins yields a major protein peak at 41% acetonitrile containing the MW 65,000 polypeptide. We conclude that a combination of HPLC with either preparative gel electrophoresis or affinity chromatography is well suited to purify the protein involved in rat renal Na⁺/H⁺ exchange.

POWERFUL, DIRECT RECEPTOR-MEDIATED CONTROL OF EARLY PROXIMAL ACIDIFICATION BY ANGIOTENSIN II (A_{II}). M.G. Cogan & F.-Y. Liu. Dept. Med. & CVRI, UCSF, CA.

We recently reported that A_{II} more markedly stimulated bicarbonate absorption in the early (S₁) compared to late (S₂) proximal convoluted tubule (PCT). In the intestine, A_{II} stimulates sodium transport indirectly, by potentiating sympathetic nerve activity, but the mechanism in PCT is unknown. In vivo microperfusion (30 nl/min) was performed in Munich-Wistar rats before and after A_{II} (20 ng/kg/min i.v.). Compared to the innervated state, renal denervation obliterated small changes in chloride transport but did not prevent the A_{II}-stimulation of bicarbonate absorption in early (ΔJ_{HCO₃} 169±25 vs. 190±10 peq/mm·min) and late PCT (27±2 vs. 60±6 peq/mm·min). The greater, non-neural stimulation in early PCT suggested a high A_{II} receptor density on S₁ cells. Maximal specific [¹²⁵I]-A_{II} binding was, in fact, 10-fold higher in early compared to late microdissected PCT (4000 vs. 300-500 amol/cm) with similar K_D (5-6nM). Basolateral rather than luminal receptors had greater functional significance since peritubular A_{II} stimulated early PCT bicarbonate reabsorption two-fold more than either 10⁻¹² or 10⁻¹¹ M luminal A_{II} (94±6 and 53±5 peq/mm·min). The Na/H antiporter was involved in the A_{II}-induced transport stimulation because luminal 4mM amiloride decreased ΔJ_{HCO₃} by 86%. Kinetically, A_{II} caused a change in substrate affinity but not in antiporter V_{max} since A_{II} had no effect on the maximal acidification rate (548±9 to 557±18 peq/mm·min).

In conclusion, receptors in high density on S₁ cells directly mediate the potent stimulation of acidification by A_{II} *in vivo* and cause a change in the apparent substrate affinity, but not number, of Na/H antiporters.

BINDING OF 5-(N-METHYL-N-ISOBUTYL)AMILORIDE (MIA) TO THE Na^+/H^+ EXCHANGER AT A HIGH AFFINITY, INTERNAL SITE. G.V. Desir*, E.J. Cragoe, Jr.*, and P.S. Aronson. Yale Univ., New Haven, CT, and Merck Sharp & Dohme Research Laboratories, West Point, PA

The binding of [^3H]MIA, an inhibitor ($\text{IC}_{50} = 4 \mu\text{M}$) of the Na^+/H^+ exchanger present in rabbit renal brush border membrane vesicles (BBMV), was studied using a gel filtration assay. Scatchard analysis of equilibrium (2 hr) MIA binding indicated the presence of a single site: $K_d = 9.5 \pm 1.0 \text{ nM}$ and $B_{\text{max}} = 1.5 \pm 0.2 \text{ pmol/mg protein}$.

The following suggested that this high affinity site represented [^3H]MIA binding to the Na^+/H^+ exchanger. The rank order for inhibition of specific [^3H]MIA binding was MIA > amiloride > benzamil, the same as for inhibition of Na^+/H^+ exchange. In contrast, benzamil is known to be the most potent inhibitor of the epithelial Na^+ channel. Specific [^3H]MIA binding was progressively inhibited by increasing [H^+] in the range pH 7.5-6.0. Lastly, high affinity [^3H]MIA binding was not detected in sonicated asolectin liposomes or in rabbit basolateral membrane vesicles, which lack the amiloride-sensitive Na^+/H^+ exchanger.

High affinity [^3H]MIA binding occurred subsequent to its uptake into the BBMV via the organic cation transporter. Thus, 40 mM tetramethylammonium and 50 μM tetraphenylammonium, which inhibit organic cation transport but not Na^+/H^+ exchange, decreased the initial rate (1 min) of [^3H]MIA uptake by 90% and 75%, respectively, without affecting equilibrium (2 hr) binding.

The high affinity internal binding site for MIA was distinct from the external amiloride inhibitory site, as preloading BBMV with submicromolar [MIA] did not inhibit Na^+/H^+ exchange. In addition, high affinity MIA binding was not inhibited by 40 mM Na^+ or Li^+ , which are known to compete with amiloride at the external inhibitory site.

These data suggest that the Na^+/H^+ exchanger has a high affinity, internal site for binding of amiloride analogues.

TWO INDEPENDENT, NEUROHORMONALLY-INDUCED REACTION CASCADES FOR UP-REGULATION OF ALKALI SECRETION INTO THE URINE. J. H. Durham, E. Schneider, C. Matons, W.A. Brodsky. Mt. Sinai Med. Ctr. Depts of Physiology & Biophysics and of Medicine, New York, New York 10029.

Isolated urinary bladders from alkalotic turtles possess a discrete mechanism for the primary active and electrogenic secretion of alkali, as determined from a positive short-circuiting current (I_{sc}) or from an equivalent rate of luminal pH statting. When such bladders are incubated in Cl^- -free, Na^- -free, ($\text{HCO}_3^- + \text{CO}_2$)-containing Ringer media, the initially maximal rate of alkali secretion falls to near-zero in 1-2 hrs, but can be restored to near-maximal levels by: (i) the paracrine hormone, vasoactive intestinal peptide (VIP) or by cAMP, providing that a phosphodiesterase (PDE) inhibitor (IBMX) is present; and by (ii) the cholinergic neurotransmitter, carbachol, or by quasi second messengers of the phosphoinositide: protein kinase C (PKC) cascade [e.g., 1-oleoyl-2-acetyl-glycerol (OAG); A-23187 (the Ca^{2+} ionophore) or phorbol myristate acetate (PMA)], each of which is fully effective in the absence of IBMX. Recently, it has been shown that mucosally added diphenylamine carboxylate (DPC), at micromolar levels, nearly nullifies the carbachol supported alkali secretion, but has little or no effect on the (cAMP + IBMX)-supported alkali secretion. Evidently DPC does not interact directly with the alkali pump, but instead inhibits the PKC-mediated activation of the alkali pump, or blocks the activation of PKC.

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Na^+ -INDEPENDENT Cl^- -BASE EXCHANGE REGULATES INTRACELLULAR pH (pH_i) IN RAT GLOMERULAR MESANGIAL CELLS. M. Ferdows*, K. Golchini*, S.G. Adler, and I. Kurtz. Divisions of Nephrology, UCLA School of Medicine and Harbor-UCLA Medical Center, Los Angeles, CA.

The present study was designed to investigate Cl^- -dependent pH_i regulatory process(es) in cultured mesangial cells. Steady state pH_i measured with BCECF in the presence of 25mM HCO_3^- , pH 7.4, was 7.08 ± 0.03 (n=13). Removal of external Cl^- resulted in an acute increase in pH_i of 0.31 ± 0.03 pH units (n=9), $p < 0.001$. The increase in pH_i induced by Cl^- removal was inhibited 50% by 0.5mM DIDS. Na^+ removal and 1 mM amiloride did not alter the increase in pH_i induced by Cl^- removal. Steady state pH_i in cells bathed in 25 mM HCO_3^- , pH 7.4 in the absence of external Cl^- was significantly increased to 7.39 ± 0.02 , (n=7), $p < 0.001$. When the cells were acutely alkalinized by removal of CO_2 , pH_i increased rapidly and recovered at a rate of 0.15 ± 0.03 pH/min (n=7). In Cl^- free media, the pH_i recovery rate was significantly decreased to 0.01 ± 0.008 pH/min (n=5), $p < 0.01$. Na^+ removal and 1mM amiloride did not alter the pH_i recovery rate. In conclusion: Glomerular mesangial cells possess a Na^+ -independent Cl^- -Base exchanger. The exchanger plays an important role in regulating intracellular pH under steady state conditions and following acute alkaline loading.

IMPROVED CARBONIC ANHYDRASE STAINING REVEALS A REACTIVE SUBLUMINAL CELL AND UNIQUE DISTRIBUTION OF ENZYME ACTIVITY IN TURTLE BLADDER MITOCHONDRIAL-RICH CELLS. C. Fritsche, J.G. Kleinman, J.L.W. Bain*, R.R. Heinen* and D.A. Riley*. VA Medical Center and Medical College of Wisconsin, Depts. of Med. and Anat. and Cell Biol., Milwaukee, WI.

Carbonic anhydrase (CA) plays a significant role in acid-base transport in turtle bladder mitochondrial-rich (MR) cells. To determine the ultrastructural distribution of CA, 3 bladders from March-April turtles were processed cytochemically using the improved method of Riley et al (J. Histochem. Cytochem. 30:1276, 1982). Reaction specificity was verified with 0.01mM acetazolamide inhibition. CA positive cells comprised 12.7% of total mucosal epithelial cells and included microplicated (MP), microvillated (MV) and subluminal (SL) cells. MP cells comprised 40% of CA positive cells and displayed at least 2 distinct staining patterns: 1) Reaction product filled the luminal third of some cells including the terminal web and microplicae. These cells were characterized by very few vesicles in the terminal and subterminal web regions, a morphologic feature of ongoing H^+ secretion. 2) Reaction product was uniformly distributed throughout other cells, except for the terminal web and microplicae. These cells had a large population of subterminal web vesicles, a morphologic feature of diminished H^+ secretion. MV cells, comprising 10% of CA reactive cells, were stained throughout, with more intense staining in the apical region. Fifty percent of CA positive cells were found in the basal cell layer and termed SL cells. SL cells were uniformly stained, mitochondrial-rich and contacted the basal lamina rather than the lumen.

In conclusion: 1) CA subcellular distribution is correlated with another morphologic indicator of MP cell H^+ transport. 2) Turtle bladder contains newly described subluminal CA positive cells. They may be precursors of functioning MP and/or MV cells.

CHLORIDE-DEPLETION METABOLIC ALKALOSIS (CDMA) INDUCES ECF VOLUME (ECFV) CONTRACTION VIA INTERNAL FLUID SHIFTS IN NEPHRECTOMIZED DOGS. S. Garella, TE Northrup*, and JJ Cohen. Michael Reese Hosp., U of Chicago, Chicago, IL.

The role of ECFV in the maintenance phase of CDMA remains uncertain. Many studies of the issue have been flawed because the models of CDMA employed perturb ECFV during the generation phase and/or depend solely on external balance techniques to quantify changes in ECFV. Adding even further uncertainty is a possibility not previously examined, namely, that CDMA could induce an internal shift of Na and H₂O out of the ECFV just as HCl-acidosis induces a shift into ECFV.

To evaluate this possibility, CDMA was induced euvolemically in nephrectomized dogs using a previously described hemofiltration technique that rigorously avoids changes in external Na and H₂O balance during the generation phase (KI 29:364a, 1986). ECFV was measured in CDMA and control (C) dogs as the volume of ³H-mannitol distribution immediately before and 3 hours after hemofiltration. CDMA animals experienced the following changes ($p < 0.05$) with respect to C animals: plasma [HCO₃]⁻ rose by 15.9 mEq/L; [Cl⁻] fell by 16.8 mEq/L; and ³H-mannitol volume of distribution fell by 360 ml/20 kg body weight. As further evidence that ECFV fell in CDMA dogs, Hct increased from 45.3 to 48.2% and calculated Cl-space fell by 240 ml/20 kg body weight.

These data indicate that CDMA causes an internal shift of fluid out of the ECF. The resulting ECFV contraction, not detectable by external balance measurements, appears to be an inherent feature of CDMA and may be an important factor in maintaining CDMA in the intact animal.

ACTIVE AMMONIUM ION ABSORPTION BY THICK ASCENDING LIMB. J.L. Garvin*, M.B. Burg, M.A. Knepper, NIH, NHLBI, Bethesda, MD.

Thick ascending limbs absorb ammonium ions. We investigated whether NH₄⁺ absorption by isolated, perfused rabbit medullary thick ascending limbs (mTALs) occurs by active transport or voltage-driven passive transport. In mTALs perfused at rapid flow rates (>10 nl/min-mm) with identical bath and perfusate solutions (5 mM NH₄Cl; 130 mM NaCl), NH₄⁺ was absorbed at 11.0±2.5 pmol/min-mm and the transepithelial voltage (V_t) was +4.8±0.9 mV. When the bath NaCl concentration was reduced to 50 mM (creating a lumen negative dilution potential), NH₄⁺ was absorbed at 4.9±2.5 pmol/min-mm although V_t fell to -2.9±1.0 mV. Thus, NH₄⁺ was absorbed against an electrochemical gradient. With the identical perfusate and bath solutions, furosemide (0.1 mM in the perfusate) decreased NH₄⁺ absorption to 2.8±1.2 pmol/min-mm ($p < 0.05$ vs. zero) and decreased V_t to -0.3±0.1 mV. The residual NH₄⁺ flux was completely eliminated by addition of 0.1 mM ouabain to the bath. When NaCl dilution potentials were imposed, with furosemide present in the lumen, a linear voltage-NH₄⁺ flux relationship was obtained. The NH₄⁺ permeability estimated from the slope of this line (13×10^{-5} cm/s) was the same as was estimated from independent experiments using NH₄⁺ gradients to drive passive NH₄⁺ flux. CONCLUSIONS: 1) Medullary TALs actively absorb NH₄⁺. 2) The active transport is mediated in part by NH₄⁺ substitution for K on the apical Na-K-Cl cotransporter, and in part via another transcellular pathway not yet identified. 3) The NH₄⁺ permeability of the TAL is high, permitting a substantial voltage-driven passive NH₄⁺ flux which augments the active flux.

INHIBITION OF BICARBONATE ABSORPTION BY PEPTIDE HORMONES IN RAT MEDULLARY THICK ASCENDING LIMB (MAL). David W. Good. Univ. of Texas Medical Branch, Galveston, Texas.

Peptide hormones play an important role in regulating ion transport in the thick ascending limb but their effects on acid-base transport in this segment have not been evaluated directly. To examine effects of different hormones on bicarbonate and ammonia transport in the MAL, tubules from rats were perfused *in vitro* with 25 mM HCO₃⁻ in bath and perfusate. Arginine vasopressin (AVP, 3×10^{-10} M in the bath) reduced HCO₃⁻ absorption by 50% (7.8 to 3.7 pmol/min/mm) and increased transepithelial voltage (V_{TE}) by 2 mV. In MAL perfused with 10^{-4} M furosemide to inhibit NaCl absorption, AVP caused a similar reduction in HCO₃⁻ absorption (12 to 8 pmol/min/mm). With furosemide, V_{TE} was near zero and was not affected by AVP. Glucagon (Glu, 2×10^{-9} M in the bath) reduced HCO₃⁻ absorption from 11.7 to 7.6 pmol/min/mm. Parathyroid hormone (PTH, 2×10^{-9} M in the bath) reduced HCO₃⁻ absorption from 12.5 to 10 pmol/min/mm. In the absence of added hormone, 8 bromoadenosine 3',5'-cyclic monophosphate (8 Br cAMP, 10^{-3} M in the bath) reduced HCO₃⁻ absorption from 13.1 to 8.2 pmol/min/mm. All of the above effects were reversible and statistically significant. Glu, PTH and 8 Br cAMP did not significantly affect V_{TE}. In contrast to HCO₃⁻, total ammonia absorption by MAL perfused and bathed with 4 mM NH₄Cl was not affected by AVP. Conclusions: 1) HCO₃⁻ transport in the MAL is hormonally regulated; 2) AVP, Glu and PTH directly inhibit HCO₃⁻ absorption; 3) the inhibition of HCO₃⁻ absorption is independent of effects on net NaCl absorption; 4) the inhibition of HCO₃⁻ absorption is probably mediated by increased levels of intracellular cyclic-AMP.

INTRACELLULAR NA⁺ BINDING TO TRANSPORT AND REGULATORY SITES OF NA⁺/H⁺ EXCHANGER IN UMR-106 CELLS. Jacob Green*, Dean T. Yamaguchi, Charles R. Kleeman and Shmuel Muallem*. Lab of Membrane Biology, Division of Nephrology, Cedars-Sinai Medical Center, Los Angeles, CA.

Intracellular (IC) Na⁺ (Na_i⁺) dependency of Na⁺/H⁺ exchanger was studied in UMR-106 cells (rat osteosarcoma cell line). Exchanger activity was determined by monitoring amiloride-sensitive changes in IC pH (pH_i), using the pH sensitive dye, BCECF. Na_i⁺ and pH_i were set by suspending the cells in solutions containing variable concentrations of Na⁺, K⁺, nigericin and monensin. Under conditions of pH_i=pH_o=7.1, when Na⁺-loaded cells were diluted into Na⁺-free medium, a sigmoidal Na_i⁺ dependency of Na_i⁺/H⁺ exchange (reverse), was demonstrated. The calculated Hill coefficient (HC) was about 2. H⁺ dependency of Na_i⁺/H⁺ exchange was saturable with a HC of about 1. The reverse Na⁺/H⁺ exchange is electroneutral with Na⁺:H⁺ stoichiometry of 1. Thus, Na_i⁺ activated the exchanger. The relationship between Na_i⁺ and pH_i during Na_o⁺/H_i⁺ exchange was examined at pH_i=6.3 and 7.1. Inhibition of forward exchange by Na_i⁺ was pH_i dependent, and was due to simple competition between at H_i⁺ and Na_i⁺ for binding to the transport site. In parallel with the inhibition of Na_o⁺/H_i⁺ exchange, Na_i⁺ stimulated Na_o⁺/Na_i⁺ exchange. Na_i⁺ dependency of the two activities was similar. Conclusions: 1) Na⁺ can bind to both the transport and the regulatory IC sites of the exchanger; 2) The competition between Na_i⁺ and H_i⁺ for binding to the transport site appears to govern exchanger activity under physiological conditions.

PROSTAGLANDIN (PG)-AMMONIAGENESIS INTERACTIONS IN ACUTE RESPIRATORY ALKALOSIS (ARA). M. Heifets, S.Kapoor* and R.G. Narins. Temple Univ. Health Sci Ctr., Philadelphia, PA.

PG synthesis inhibition increases total renal ammoniogenesis (TRA). Inhibition of TRA by ARA may be mediated by PG. Accordingly, TRA was measured under control conditions and during ARA in Sprague-Dawley rats receiving either meclofenamate (M) or vehicle (C). Following basal measurement of C_{in} , C_{PAH} , TRA, urinary PGE_2 excretion, ARA was induced by reduction of F_iCO_2 to decrease pCO_2 from 40 to 20mmHg. TRA was calculated as the sum of urinary ammonia (NH_3) excretion and the product of C_{PAH} and the renal arteriovenous difference of NH_3 . TRA is expressed as nmole/min per ml of GFR. Data are expressed as means \pm SEM.

	ARTERIAL pH		URINARY pH		TRA	
	base-line	ARA	base-line	ARA	base-line	ARA
C	7.382	7.592*	6.35	6.99*§	386.7§	228.7*§
(n=10)	+0.03	+0.017	+0.09	+0.09	+63.1	+42.12
M	7.385	7.595*	6.43	6.66	723.12	689.72
(n=10)	+0.02	+0.02	+0.09	+0.12	+108.2	+178.95

*significantly higher than baseline;
§different from M group ($p < 0.05$)

PG excretion in Group C was not changed by ARA and was undetectable in Group M. We conclude that ARA inhibits basal but not stimulated TRA. PG inhibition maximizes TRA in ARA but not in acute metabolic alkalosis (JCI 74:992, 1984). ARA may inhibit TRA by sensitizing the kidney to basal PG synthesis. Removal of PG would then allow for maximal TRA. PG's therefore may act as mediators of TRA suppression in ARA.

ROLE OF ALDOSTERONE (A) ON RENAL ACIDIFICATION DURING CHRONIC ACID LOADING: PRIMACY OF THE Na-INDEPENDENT EFFECT. M Hizon* and DC Battle, Northw. Univ. and Lakeside VA, Chicago, IL.

In vitro studies have shown that A stimulates Na-dependent and Na-independent RA in the collecting tubule. The regulatory effect of A on KA in vivo, however, has been suggested to be solely mediated by stimulation of the Na-dependent component. We approached this issue by examining RA in response to NH_4Cl loading in rats placed on a low Na diet and given amiloride to completely inhibit Na-dependent RA (Group I). The administration of NH_4Cl for 3 days to Group I resulted in an increase in acid excretion which was not different from that seen in controls (Group II) on a normal Na diet (0.19 ± 0.03 vs 0.23 ± 0.02 uEq/min.). The increase in RA in Group I took place in association with a 10-fold increase in plasma A (PA). To determine whether this rise in PA had mediated the increase in acid excretion seen despite blockade of Na-dependent RA, studies were performed in adrenalectomized (ADX) rats (Groups III and IV) treated as Group I but given fixed A replacement to achieve the levels seen in Groups I and II, respectively. (Table) * $p < 0.01$

	PA (ng/dl)	Blood pH	Net acid (uEq/min)
G III	717 ± 159	7.24 ± 0.03	0.21 ± 0.03
G IV	$47 \pm 4.9^*$	$7.18 \pm 0.01^*$	$0.10 \pm 0.02^*$

The blood pH and acid excretion of rats with normal PA (Group IV) were lower than those seen in similarly PA treated ADX rats (Group III) whose PA levels were elevated. Thus, despite blockade of Na-dependent RA in the cortical collecting tubule the level of PA determined the rate of acid excretion thereby demonstrating the primacy of the Na-independent effect of A on RA in vivo.

CHRONIC NEUTRAL PHOSPHATE INFUSION INDUCES SUSTAINED RENAL METABOLIC ALKALOSIS. P. Houillier*, A. Prigent*, P. Borensztein*, M. Bichara*, and M. Paillard. Lab. de Physiologie Rénale, Hôpital L. Mourier, 92 Colombes, France.

The urinary phosphate concentration is a potent acute stimulus to the proton secretion in collecting ducts (A.J.P. 251:F802-F809, 1986). To determine whether renal metabolic alkalosis occurs during chronic phosphate administration, 5 adrenalectomized rats supplemented with physiological doses of aldosterone and dexamethasone through chronic indwelling catheters and fed a fixed amount of standard rat chow were studied during three consecutive 7-days periods: 1) control (C); 2) neutral phosphate infusion (NPI), 3.8 mmol/day as Na and K salts that only doubled normal phosphorus intake, with phosphate replacing chloride; and 3) recovery (R). During the three periods, administered amounts of Na and K were constant. Results (means \pm SEM) are shown in the table.

	C	NPI	R
Plasma $[HCO_3^-]$, mM	29.5 ± 0.2	$40.5 \pm 2.3^*$	26.7 ± 0.7
Arterial pH	7.46 ± 0.01	$7.57 \pm 0.01^*$	7.46 ± 0.01
Urinary pH	7.23 ± 0.01	$7.00 \pm 0.01^*$	7.34 ± 0.01
$\Delta N.A.E.$, mmol/day	0	$+0.28 \pm 0.04^*$	-0.39 ± 0.03

($\Delta N.A.E.$, difference in net acid excretion between experimental and control periods. * $p < 0.001$, compared with control or recovery periods).

The glomerular filtration rate (GFR) was constant during the three periods at 0.90 ± 0.02 , 0.89 ± 0.01 , and 0.84 ± 0.01 ml/min/g kidney wt, respectively. In two of these rats studied during a fourth period, neutral sodium phosphate infusion, while chloride administration did not change, also resulted in metabolic alkalosis of renal origin despite extracellular fluid volume expansion and increase in GFR to 1.14 ± 0.02 ml/min/g kidney wt. The same sequence of events during the 4 periods was observed in 2 intact animals.

In conclusion, this study shows for the first time that chronic neutral phosphate administration induces renal metabolic alkalosis independent of changes in Na, K, and Cl supplies and in adrenal hormones.

EVIDENCE FOR A CRITICAL LYSINE GROUP AT THE AMILORIDE BINDING SITE ON THE RENAL Na/H ANTI-PORTER. Z.-Q. Huang* and D.G. Warnock. Depts. of Medicine, Hua Shan Hosp., Shanghai Med. Univ., Shanghai, PRC and VA Med. Ctr., UCSF, San Francisco, CA.

External buffer chloride interferes with the effect of amiloride on the Na/H antiporter in brush border vesicles (KI 31:443a, 1987). Amiloride inhibition and the interference by chloride were more marked when the external buffer pH was 8.5, suggesting that a site with an alkaline pK interacts with chloride and amiloride. Group specific reagents reacted at pH 9.2 with brush border vesicles showed that this site was more likely to be a lysine group than a guanidinium: phenyl isothiocyanate (PITC) at 0.2 μ moles/mg protein irreversibly inactivated Na/H exchange by 40% while phenylglyoxal had little effect. Amiloride protected against inactivation by PITC in a concentration dependent manner, with complete protection observed at equimolar concentrations. Similar antiporter inactivation was obtained with Fluorescein isothiocyanate (FITC) at 4 nmoles/mg protein; amiloride again protected against inactivation. Pretreatment of brush border vesicles with 100-fold excess PITC reduced FITC labelling by 80%

Conclusions: 1). Chloride blunts the effect of amiloride at a positively charged site on the Na/H antiporter. 2). Group specific reagents suggest that lysine is critical for antiporter activity. 3). Sequential treatment with PITC and FITC, using amiloride protection may be useful for labelling the antiporter.

FACTORS THAT DETERMINE PHCO_3 IN K-DEPLETION ALKALOSIS IN THE RAT. Allen M. Kaufman, and Thomas Kahn. Bronx VA Med. Ctr. and Mt. Sinai School of Medicine, New York, New York.

Selective dietary K depletion (K-DEPL) results in metabolic alkalosis with quantitatively normal net acid excretion (NAE). This suggests that K-DEPL increases the set point at which the kidney maintains PHCO_3 . To test this, the response of K-DEPL alkalotic rats to a chronic base or acid load was evaluated. The response to a reduction of PHCO_3 with acetazolamide was also assessed.

Rats were made K-DEPL and alkalotic with a K-deficient diet for 4 weeks. Eight rats then had NaHCO_3 , 4000ueq/d, added to the diet for 7d. Urine HCO_3 increased and NH_4 decreased so that PHCO_3 increased minimally from 32.8 to 35.8meq/l. In 5 rats NH_4Cl , 2000ueq/d, was added for 7d. Urine NH_4 increased comparably to normals so that PHCO_3 decreased minimally from 34.7 to 33.2meq/l.

Data shown is mean daily values from 10 K-DEPL alkalotic rats before, during 2d of acetazolamide, 2.5mg IP/d, and for 2d after. Blood was obtained at the end of each period.

	pH	HCO_3	TA	NH_4	NAE	PHCO_3
Baseline	7.00	283	139	1504	1360	31.7
Acetazolamide	7.26	1504	80	1787	363	27.7
Post-Acetazol	6.80	366	288	2403	2330	36.5

Acetazolamide produced a HCO_3 diuresis and a fall in PHCO_3 . However, PHCO_3 remained elevated (normal=21.6meq/l). Post-acetazolamide, despite high PHCO_3 , NH_4 and NAE increased markedly and PHCO_3 rose sharply. Thus when PHCO_3 is lowered the K-DEPL rat will increase NAE to restore the original PHCO_3 . Taken together these studies suggest that in K-DEPL the kidney will alter acid excretion to defend an increased set-point of PHCO_3 .

ROLE OF CHLORIDE DEPLETION IN POTASSIUM CHLORIDE DEPLETION ALKALOSIS IN THE RAT. Allen M. Kaufman, and Thomas Kahn, Bronx VA Med. Ctr. and Mt. Sinai School of Medicine, New York, New York.

In K+Cl depleted rats with metabolic alkalosis acute NaCl administration markedly decreases PHCO_3 suggesting that Cl depletion plays a major role in the alkalosis. The present studies were performed to further evaluate the extent to which the metabolic alkalosis produced by K+Cl depletion in the rat relates to the availability of dietary Cl.

I. Rats were deprived of either K+Cl intake or of K alone by replacing KCl with NaCl. PHCO_3 increased similarly and progressively over 35 days (21.9 to 37.6 meq/l) and net acid excretion was similar in both groups. Thus Cl depletion did not enhance the alkalosis of K depletion.

II. Selective Cl depletion, produced by replacing dietary KCl with K_2SO_4 , did not increase PHCO_3 .

III. When Cl as NaCl was added to the diet of K+Cl depleted rats PHCO_3 fell from 28.5 to 23.7 meq/l in 1 day. By day 7 and 14 PHCO_3 rose to 29.7 and 31.9 meq/l, averaging only 2.3 meq/l less than rats not receiving Cl. Thus the chronic provision of Cl to K+Cl depleted rats results in only a mild amelioration of alkalosis.

IV. In rats with K+Cl depletion K depletion was gradually corrected with dietary K_2SO_4 or KCl. In both groups PHCO_3 fell progressively and similarly to normal levels and urine composition in terms of pH, NH_4 and TA also became normal. Thus the correction of PHCO_3 and urine composition did not require Cl administration.

In summary these studies suggest that concomitant Cl depletion has only a minor effect on the acid-base abnormalities produced by K depletion in the rat.

MECHANISM OF ADAPTATION TO RESPIRATORY ACIDOSIS IN THE TURTLE BLADDER. D. Kniaz, R. Mola*, Z. Talor and J.A.L. Arruda, Dept. of Medicine, Univ. of Illinois and WSVAMC, Chicago, IL.

The effect of in vivo respiratory acidosis (RA) for 4 and 48 hrs was examined in the turtle bladder by placing turtles in hypercapnic chambers (blood pH: 4hr 7.21 ± 0.01 , 48hr 7.12 ± 0.03 vs 7.43 ± 0.02 as compared to controls). One % CO_2 increased H^+ secretion more in bladders from turtles with RA for 48 hrs as compared to controls (Δ increase in H^+ current at 5 min 12.0 ± 0.6 vs $7.7 \pm 1.3 \mu\text{A}$, $p < 0.025$). The number of acid secreting cells was studied by fluorescence microscopy utilizing four different fluorescent probes for the identification of carbonic anhydrase cells: acridine orange (AO), rhodamine 123 (Rhod), 6-carboxyfluorescein (6CF) and the potential sensitive dye 3,3'DiOC2. Total cell counts were determined by the nuclear stain ethidium bromide. Results expressed as % of total cells/field (n=6-11, * = significant).

	Control	4 hrs RA	48 hrs RA
AO	11 ± 3	15 ± 2	$20 \pm 3^*$
Rhod	10 ± 1	$17 \pm 4^*$	$20 \pm 4^*$
6CF	5 ± 1	$13 \pm 3^*$	$15 \pm 2^*$
DiOC2	11 ± 1	12 ± 5	$18 \pm 1^*$

In vitro, 1% CO_2 increased H^+ secretion and the number of 6-CF cells, both of which could be blocked with SITS pretreatment. Thus, the adaptation to RA is associated with an increase in the number of acid secreting cells. Moreover, the increase noted as early as 4 hrs RA suggests cell transformation rather than proliferation.

BICARBONATE SECRETION (JsHCO₃): ROLE OF HCO_3 , PH, AND PCO_2 . J.D. Koethe,* R.L. Stephenson,* and G.M. Feldman. Dept. of Med., VA Med. Ctr., Univ. of Penna., Phila., PA.

We have reported that increasing bathing solution HCO_3 concentration increases JsHCO₃ in rat distal colon as has been reported for the cortical collecting duct. The mechanism may involve the HCO_3 ion itself or result from concomitant changes in pH. To evaluate these possibilities, we measured JsHCO₃ in rat distal colon while pH, PCO_2 , and $[\text{HCO}_3]$ were varied. Tissue surfaces were bathed with identical modified Ringer's solution, transepithelial voltage was clamped to zero, and JsHCO₃ was calculated from changes in mucosal pH and is reported as ueq/h/cm² +SEM.

Varying $[\text{HCO}_3]$ (5, 25 and 50 mM) while CO_2 tension was constant (5.6%), altered JsHCO₃; -0.1 ± 0.1 , 1.1 ± 0.1 and 1.4 ± 0.2 , respectively. The role of CO_2 tension was evaluated at constant $[\text{HCO}_3]$ (25 mM); altering CO_2 from 2.8% to 9.9% did not alter JsHCO₃ (1.1 ± 0.2 and 1.1 ± 0.2 , respectively). Next, we maintained pH constant at 7.4 by varying CO_2 and $[\text{HCO}_3]$ simultaneously; JsHCO₃ increased from 0.1 ± 0.1 to 1.4 ± 0.1 as $[\text{HCO}_3]$ was increased from 10 to 40 mM. In the absence of CO_2 and HCO_3 (pH 7.4), there was no net base secretion and net H^+ secretion was 0.3 ± 0.1 .

We conclude that HCO_3 concentration is the major determinant of JsHCO₃. In addition, JsHCO₃ follows saturation kinetics and has a K_m of 33 mM. The failure of bathing solution pH and CO_2 to markedly influence JsHCO₃ suggests that intracellular pH is not a major regulator of transepithelial HCO_3 movement.

ELECTRONEUTRAL BICARBONATE SECRETION: COUPLING TO APICAL AND BASOLATERAL CHLORIDE TRANSPORT. Orly F. Kohn*, Peter P. Mitchell*, and Philip R. Steinmetz, Univ. of Conn. Health Ctr, Div Nephrol., Farmington, Connecticut

To define the transport steps involved in electroneutral HCO_3^- secretion (JHCO_3^-) by the beta carbonic anhydrase cells of turtle urinary bladder we 1) examined the dependence of electroneutral JHCO_3^- on luminal Cl concentration and 2) inhibited the exit of Cl across the basolateral membrane by serosal addition of 50 μM diphenylamine-2-carboxylate (DPC), a blocker of Cl channels. Total JHCO_3^- was measured by pH stat titration in gluconate Ringer's at luminal pH 6.8 after inhibition of hydrogen ion secretion with serosal SITS. Electrogenic JHCO_3^- was measured as short circuit current. A Cl⁻ dependent, neutral JHCO_3^- was defined which had an apparent Km for Cl of $3.5 \pm 0.5 \text{ mM}$ (30x that of the alpha cell exchanger) and was about 70% inhibitable by acetazolamide. In the presence of ambient Cl serosal DPC caused a reversible decrement in JHCO_3^- of $0.43 \pm 0.11 \mu\text{mol/hr}$, whereas DPC failed to inhibit JHCO_3^- in Cl-free gluconate Ringer's ($\Delta 0.07 \pm 0.09$). We conclude that neutral JHCO_3^- is coupled to Cl transport at an apical antiporter (which differs from the Cl- HCO_3^- exchanger of the alpha cell) and requires a basolateral Cl channel in series for the disposal of Cl taken up by the antiporter.

REGULATION OF CELL pH (pH_i) BY AMBIENT HCO_3^- , PCO_2 AND pH IN THE RABBIT PROXIMAL CONVOLUTED TUBULE (PCT). R. Krapf*, R.J. Alpern, C.A. Berry and F.C. Rector, Jr. UCSF, San Francisco, CA.

To examine the relative roles of ambient HCO_3^- , PCO_2 and pH in regulation of pH_i , pH_i was measured microfluorometrically in the rabbit PCT using BCECF.

For the same changes in external pH (pH_e), changes in HCO_3^- and PCO_2 affected pH_i similarly (PCO_2 : $\Delta\text{pH}_i/\Delta\text{pH}_e=0.64$, $[\text{HCO}_3^-]$: $\Delta\text{pH}_i/\Delta\text{pH}_e=0.67$, n.s.). Isohydic changes in $[\text{HCO}_3^-]$ and PCO_2 did not change steady state pH_i significantly. Changes in peritubular $[\text{HCO}_3^-]$ elicited larger changes in pH_i than changes in luminal $[\text{HCO}_3^-]$. Bath SITS inhibited ΔpH_i in response to changes in bath $[\text{HCO}_3^-]$ but enhanced ΔpH_i in response to changes in luminal $[\text{HCO}_3^-]$. An acute change in PCO_2 produced a rapid ΔpH_i followed by a partial recovery to steady-state pH_i (pH_i defense). This pH_i defense to acute decreases and increases of PCO_2 was sodium-dependent and chloride-independent. 1 mM bath SITS inhibited the pH_i defense against increases and decreases in PCO_2 ; whereas, 1 mM luminal amiloride only inhibited pH_i defense when PCO_2 was increased.

Conclusions: 1) For a given change in ambient pH, changes in $[\text{HCO}_3^-]$ and PCO_2 produced quantitatively similar changes in steady state pH_i , 2) the rate of the basolateral $\text{Na}/(\text{HCO}_3)_3$ cotransporter is a more important determinant of pH_i than is the rate of the luminal Na/H antiporter in the rabbit PCT and 3) cell pH defense against acute changes in PCO_2 depends on the basolateral $\text{Na}/(\text{HCO}_3)_3$ cotransporter (acid and alkaline loads) and the luminal Na/H antiporter (acid loads).

ROLE OF $\text{Cl}^-/\text{HCO}_3^-$ EXCHANGE IN THE REGULATION OF STEADY STATE INTRACELLULAR pH (pH_i) IN INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS. J.A. Kraut and E.P. Nord. Divisions of Nephrology, Wadsworth VA Med Ctr. and CHS, Department of Medicine, UCLA School of Medicine, Los Angeles, CA.

The role of base entry and exit processes in the regulation of pH_i of IMCD cells was examined by monitoring changes in pH_i with the pH sensitive fluorescent probe, BCECF in monolayers of a primary culture of rat IMCD cells. In cells bathed in 25 mM $\text{HCO}_3^-/5\% \text{ CO}_2$ at pH 7.4, steady state pH_i was 7.33 ± 0.02 (n=13). Acute removal of Cl^- from the bathing solution resulted in a rapid rise in pH_i to 7.54 ± 0.03 (n=13) with return to baseline pH_i on readdition of Cl^- . This increment in pH_i was independent of extracellular Na^+ and was inhibited by 50 μM DIDS. In the presence of Cl^- , acute removal of $\text{HCO}_3^-/\text{CO}_2$ and replacement by HEPES (pH 7.4) resulted in a rapid increase in pH_i followed by a fall to a new steady state level of 7.49 ± 0.02 (n=17). When extracellular pH (pH_o) was decreased from 7.4 (25 mM $\text{HCO}_3^-/5\% \text{ CO}_2$) to 6.7 (5 mM $\text{HCO}_3^-/5\% \text{ CO}_2$), pH_i fell from 7.33 ± 0.01 to 6.99 ± 0.08 (n=5) and returned to baseline when pH_o was changed back to 7.4. **Conclusion:** A Na^+ -independent DIDS inhibitable $\text{Cl}^-/\text{HCO}_3^-$ exchange process is present in the plasma membrane of IMCD cells, which contributes importantly to the regulation of steady state pH_i .

APICAL AND BASOLATERAL Na^+/H^+ ANTIPORTERS REGULATE INTRACELLULAR pH (pH_i) IN THE RABBIT THIN DESCENDING LIMB (TDL). I. Kurtz. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

The present study was designed to investigate whether the TDL possesses Na^+ -dependent H^+ transport pathways. TDLs (deep nephrons) were dissected from the inner stripe of the outer medulla, and perfused (L) and bathed (B) in the nominal absence of HCO_3^- , organic anions and SO_4^{2-} , pH 7.4. Steady state pH_i measured with BCECF was 7.14 ± 0.09 (n=8). Removal of Na^+ (L) decreased pH_i by 0.52 ± 0.05 pH units, $p < 0.001$. The decrease was inhibited 65% by 1 mM amiloride (L). When Na^+ was removed from (B), pH_i decreased by 0.38 ± 0.03 pH units, $p < 0.001$. The decrease was inhibited 80% by 1 mM amiloride (B). When pH_i was acutely decreased ≈ 1.2 units following a transient exposure to NH_4Cl (L,B), pH_i failed to recover in the absence of Na^+ (L,B). 140 mM Na^+ added to (L) caused pH_i to recover at an initial rate of 0.45 ± 0.14 pH/min. 1 mM amiloride (L) inhibited the pH_i recovery, 0.02 ± 0.02 pH/min. In separate experiments, when 140 mM Na^+ was added to (B), pH_i increased 0.30 ± 0.04 pH/min. 1 mM amiloride (B) inhibited the pH_i recovery, 0.03 ± 0.009 pH/min.

Conclusion: The rabbit thin descending limb (inner stripe segment) of deep nephrons possesses apical and basolateral Na^+/H^+ antiporters which regulate pH_i .

METABOLIC ACIDOSIS SELECTIVELY STIMULATES H-ATPase IN RAT NEPHRON. N.A. Kurtzman and S. Sabatini, Texas Tech Univ. Health Sciences Center, Lubbock, TX.

H-ATPase activity is present along virtually the entire rat nephron. Enzyme activity increases (V_{max} doubles) in cortical collecting tubule, but not in medullary collecting tubule, after acid loading. We studied the effect of NH_4Cl loading (3d) on H-ATPase activity in other segments of rat nephron. In the S_1 segment of the proximal tubule metabolic acidosis decreased H-ATPase activity (438 ± 41 vs 269 ± 58 pmols/mm/hr, control vs acidotic, respectively, $N=14$, $P<0.05$). H-ATPase activity markedly increased in medullary thick ascending limb (241 ± 14 vs 471 ± 83 pmols/mm/hr, control vs acidotic, respectively, $N=18$, $P<0.02$). In papillary collecting duct, before entry into the ducts of Bellini, H-ATPase activity, under control conditions, was 330 ± 93 pmols/mm/hr, $N=8$; after entry into the ducts of Bellini H-ATPase activity was ≈ 4 fold lower (60 ± 9 pmols/mm/hr, $N=8$, $P<0.01$). Papillary collecting duct segments microdissected after entry into the ducts of Bellini did not respond to metabolic acidosis. These results are consistent with active proton secretion in thick ascending limb. In collecting tubule, active proton secretion occurs up to, but not including, the ducts of Bellini. Acid loading stimulates proton secretion in thick ascending limb and cortical collecting tubule. In the proximal tubule the adaptation to acidosis does not appear to involve active proton secretion.

FLOW RATE IS A DETERMINANT OF BICARBONATE REABSORPTION IN PERFUSED COLLECTING TUBULES. M E Laski, Texas Tech University Health Science Center, Lubbock, Tx.

Current models of acidification predict that flow rate (V) may modify total CO_2 flux (JTCO₂) in the cortical and outer medullary collecting tubules (CCTs & MCTs), but most studies in these segments have used only slow V. To examine the effect of V on JTCO₂, CCTs and MCTs from the inner stripe were perfused with standard artificial solutions (KRB with 25 mM HCO_3 , bubbled with 6.7% CO_2 mixtures) at a variety of flow rates; at least two rates were used with each tubule. Bath was identical to perfusate.

CCTs which initially secreted bicarbonate showed no consistent effect of flow rate. In CCTs which initially reabsorbed bicarbonate, JTCO₂ increased with V as V was raised from 1 to 6 nl/min; no further increase in JTCO₂ was seen as V increased from 6 to 12 nl/min. Average JTCO₂ for $V < 6$ nl/min was 8.2 pmol/mm/min, and for $V > 6$ nl/min average JTCO₂ was 18.6 pmol/mm/min. Analyzing JTCO₂ vs V by linear regression gives $p < 0.001$. JTCO₂ also increased with flow rates in MCTs taken from the inner stripe and did not appear to reach a maximum. Analysis of JTCO₂ vs V in the MCT by linear regression again yielded $p < 0.001$.

Conclusions: 1) V is a determinant of JTCO₂ in the CCT and the MCT, possibly because high V disturbs unstirred layers along the tubule. 2) Slow V, although allowing the tubule to generate greater total CO_2 gradients, may lead to underestimation of acidifying capacity. 3) The variation in JTCO₂ in studies from different laboratories may be primarily due to V used.

RESPONSE OF IN VIVO RAT DISTAL TUBULE BICARBONATE REABSORPTION (JtCO₂) TO FEEDING AND $NaHCO_3$ GAVAGE. David Z. Levine, Michelle Iacovitti,* Lorena Nash,* and David Vandorpe.* Univ. of Ottawa, Dept. of Medicine, Ottawa, Ontario, Canada.

We have previously shown that overnight fasting enhances rat distal tubule JtCO₂ *in vivo* (Clin. Res. 35:551A, 1987). Since feeding normal rat chow (22% protein) leads to a higher urine pH than in fasted rats ($6.66 \pm .09$ vs $6.26 \pm .06$) we evaluated the possibility that $NaHCO_3$ gavage could simulate the effects of feeding. Male Sprague Dawley rats were individually caged and when fed overnight gained 12 ± 2 vs -19 ± 1 gm in fasted rats. Fasted rats gavaged with $NaHCO_3$ (GAV) had urine pH = $7.41 \pm .05$ but weight loss persisted. Distal tubules were perfused with a 28 mM HCO_3 solution at two flow rates.

	No.Rats/ No.Tubules	JtCO ₂ (pMol.min ⁻¹ .mm ⁻¹) 8 nl/min	26 nl/min
Fed	7/10	+6 ± 4	-54 ± 12
Fasted	7/11	+50 ± 6*	+84 ± 15*
Fasted + GAV	8/10	+12 ± 8	-27 ± 14

* $p < 0.01$ vs fed rats value

In fasted and fasted + GAV rats JtCO₂ was also significantly correlated with urine pH. Since the positive JtCO₂ in fasted rats changed to negative values after alkali gavage, we infer that bicarbonate secretory fluxes at high flow seen after feeding do not depend on feeding-induced hormone release and may be attributed to an alkali residue diet.

STIMULATION OF EARLY PROXIMAL HCO_3 REABSORPTION IN CHRONIC METABOLIC ACIDOSIS. David A. Maddox and F. John Gennari, U. of Vermont, Burlington, VT.

We and others have shown that the early proximal tubule (first 1-2 mm) is the major site of HCO_3 reabsorption in the rat. Little is known, however, about the factors affecting reabsorption in this segment. Chronic metabolic acidosis (CMAcid) enhances Na^+/H^+ antiporter activity in renal brush border vesicles and increases HCO_3 reabsorptive capacity in perfused mid-late proximal tubules. In this study, we examined whether CMAcid alters early proximal HCO_3 reabsorption. CMAcid was induced in Munich-Wistar rats by 4-7 days of NH_4Cl feeding (mean $[HCO_3] = 19.1 \pm 1.0$ mM). Absolute (APR HCO_3) and fractional (FPR HCO_3) proximal HCO_3 reabsorption were measured at varying distances from Bowman's space, and the results compared with normal rats (N1) at comparable filtered loads (FL HCO_3). Shown below are results at 1-2 mm (early proximal) and 4.5-6.5 mm (late proximal) in rats with FL HCO_3 , values between 600-1100 pmols/min (mean=890±25 pmol/min in CMAcid, 905±28 pmol/min in N1).

	APR HCO_3	FPR HCO_3 , mm	APR HCO_3	FPR HCO_3 , mm
Normal	443(7)	0.50	821(9)	.90
	+40	+0.04	+34	+0.01
CMAcid	696(10)	0.78	853(6)	.96
	+35	+0.02	+28	+0.01
$p =$	<.001	<.001	NS	<.005

Thus, at FL HCO_3 levels comparable to N1 rats, CMAcid stimulates bicarbonate reabsorption in the early proximal tubule. As a result, proximal HCO_3 reabsorption is shifted towards the glomerulus.

AMMONIA PRODUCTION BY ISOLATED PERFUSED MOUSE PROXIMAL TUBULES. EFFECT OF BATH AND LUMINAL POTASSIUM CONCENTRATION. G. T. Nagami and P. Lee*. Medical & Research Svcs, VAMC West Los Angeles and UCLA Sch of Med. Los Angeles, CA.

The state of potassium balance influences renal total ammonia production (TAP) and excretion. We examined the effect of altered bath and luminal potassium concentration, $[K^+]$, on TAP in isolated mid-portions of mouse proximal tubules perfused in vitro. Tubules were bathed in Krebs-Ringer bicarbonate buffer (KRB) containing 0.5 mM L-glutamine and 1.0 mM acetate, pH 7.4. Tubules were cannulated and perfused with KRB at flow rates of 20 nl/min. In proximal tubules perfused with control 5 mM K^+ KRB, the rate of TAP was higher in tubules bathed in 2 mM K^+ KRB than in tubules bathed in control 5 mM K^+ KRB (24.6 ± 0.8 vs. 19.6 ± 0.6 pmol/min per mm, $p < 0.01$) while the rate of TAP was lower in tubules bathed in 8 mM K^+ KRB buffer (16.1 ± 0.6 pmol/min per mm) than in control tubules, $p < 0.05$. In contrast, TAP rates did not significantly differ among groups of tubules bathed in 5 mM K^+ KRB and perfused with various KRB buffers containing 0, 5 or 10 mM K^+ even though mean luminal fluid $[K^+]$ differed among the groups (2.0 vs. 5.0 vs. 8.2 mM). Although altered luminal $[K^+]$ had no effect on TAP, net secretion of total ammonia into the luminal fluid was 20% higher in tubules perfused with 10 mM K^+ KRB and 25% lower in tubules perfused with 0 mM K^+ KRB than in tubules perfused with 5 mM K^+ KRB. Conclusions: 1) luminal $[K^+]$ affects net total ammonia secretion into the luminal fluid of the mouse proximal tubule but has no effect on TAP; and 2) it is the peritubular $[K^+]$ which regulates TAP.

INTRACELLULAR pH IN SINGLE LLC-PK₁ CELLS: COMPARISON OF PARENTAL CELLS TO MUTANTS OVEREXPRESSING OR DEFICIENT IN Na-H EXCHANGE ACTIVITY. N.L. Nakhoul*, N. Agarwal*, C.W. Slayman* and W.F. Boron. Yale Univ. Sch. of Med., New Haven, CT.

The purpose of this study was to compare pH_i regulation in parental LLC-PK₁ (clone 4) cells, and two mutants derived from this parental line, PKE20 and PKE5. Amiloride-sensitive ^{22}Na uptake is increased in PKE20 (Haggerty et al., *Kidney Int.* 31:168, 1987), but reduced in PKE5 (Agarwal et al., *Am. J. Physiol.* 251:C825, 1986), suggesting that PKE20 overexpresses and PKE5 underexpresses the Na-H exchanger. In this study we measured the absorbance spectrum of 4',5'-dimethyl-5-(and-6)-carboxyfluorescein to continuously monitor pH_i in single cells attached to cover slips, and incubated in nominally HCO_3^- -free solutions. The steady-state pH_i of parent cells was 6.98 ± 0.05 (n=11), whereas that of PKE20 was 7.18 ± 0.07 (n=8, $P < 0.05$), and that of PKE5 was 6.94 ± 0.04 (n=9, NS). We assessed Na-H exchange activity by measuring pH_i recovery rate from acid loads applied by prepulsing with NH_4^+ . In the parent cells, the pH_i recovery rate at $pH_i = 6.8$ was $26 \pm 6 \times 10^{-4}$ pH/sec. Given a measured intracellular buffering power (β) of 7.1 mM/pH, the Na-dependent H^+ flux (J_H) was $18.5 \mu M/sec$ in the parent cells. In PKE20 cells, the pH_i recovery rate at $pH_i = 6.8$ was $46 \pm 8 \times 10^{-4}$ pH/sec (n=6), β was 10.7 mM/pH, and J_H was $49.2 \mu M/sec$, more than 2.5 greater than in the parent cells. In the PKE5 cells, the pH_i recovery rate was only $4.1 \pm 1.6 \times 10^{-4}$ pH/sec (n=7) at $pH_i = 6.75$, β was 12.9 mM/pH, and J_H was only $5.3 \mu M/sec$, less than 30% the value in parental cells. 100 μM EIPA substantially reduced the rate of pH_i recovery in parent and PKE20 cells. We conclude that the PKE20 mutants have a higher-than-normal steady-state pH_i and a greater Na-dependent pH_i recovery rate from acid loads, whereas the PKE5 mutants have a greatly reduced pH_i recovery rate from acid loads.

K DEPLETION: THE STIMULUS TO RENAL Na/H ANTIporter ACTIVITY (AA) IN METABOLIC ACIDOSIS (MA)? TE Northrup*, E Perticucci*, SG Garella, JJ Cohen Michael Reese Hosp. U of Chicago, Chicago, IL

We have shown that MA, but not respiratory acidosis (RA) stimulates AA, casting doubt on the mediating role of blood pH (Clin Res 35:554a, 1987). Because MA but not RA causes Na and K depletion, we reasoned that deficits of one or both ions might mediate the increased AA in MA. To test this hypothesis, 5 groups of rats were studied: control (C), MA, Na + K depletion (NKD), K depletion (KD), and Na depletion (ND). MA rats had free access to 1.5% NH_4Cl drinking water; all others drank deionized H_2O . C and MA rats were fed normal chow; ND, KD and NKD rats were fed diets free of Na, K or Na and K respectively. ND rats were also treated with furosemide for the first 2 days of the study. Five rats from each group were studied 7 days after onset of treatment. AA (V_{max}) in BBM was measured using Acridine Orange.

Group	V_{max} (% Cont)	Hct.	$[K^+]_{mm}$
C	100	43+1	4.5+0.2
MA	138+9*	43+2	4.6+0.2
NKD	120+7*	45+1*	4.0+0.1*
KD	131+7*	44+1	3.5+0.2*
ND	87+4*	47+1*	4.1+0.1*

Data are Mean + SEM, * $p < 0.05$ vs Cont

As can be seen, AA was augmented by Na + K depletion (NKD) and by K depletion alone (KD), the latter to a degree similar to that seen in MA. Na depletion alone (ND) suppressed AA. Taken together with our previous findings dissociating blood pH *per se* from augmented AA, these data suggest that K depletion could be responsible for the changes in AA observed in chronic MA.

CHRONIC METABOLIC ACIDOSIS (CMA) CAUSES AN ADAPTATION IN THE BASOLATERAL MEMBRANE (BLM) $Na(HCO_3)_3$ SYMPORTER IN THE RAT PROXIMAL CONVOLUTED TUBULE (PCT). PA Preisig and RJ Alpern, Dept. Med., UTHSCD, Dallas, TX.

CMA has been shown to increase HCO_3^- absorption in the PCT (AJP 249:F62-68, 1985). To elucidate the transport mechanisms responsible for this, we examined the effect of CMA on the intrinsic properties of the BLM $Na(HCO_3)_3$ symporter. Cell pH_i (pH_i) was measured in vivo in the doubly microperfused rat PCT using the pH-sensitive dye BCECF. CMA was induced by adding 20 $\mu moles$ HCl/kg BW to diet x 3-5 days. CMA rats had a significantly lower plasma pH (7.31 ± 0.01 vs 7.38 ± 0.01 , $p < 0.001$) and $[HCO_3^-]$ (20.3 ± 0.9 vs 23.2 ± 0.9 , $p < 0.05$) than control diet rats (CON).

In vivo calibration of BCECF using the nigericin technique showed similar pH_i sensitivity in CMA and CON. Lowering capillary $[HCO_3^-]$ from 25 to 10 mM caused a cell acidification which occurred at a faster rate in CMA than CON (7.3 ± 0.7 vs 3.7 ± 0.4 pH units/min, $p < 0.001$) and was larger in magnitude ($\Delta pH_i = 0.34 \pm 0.01$ vs 0.29 ± 0.01 , $p < 0.01$). Lowering capillary $[Na]$ from 147 to 50 mM also caused a faster decrease in pH_i in CMA than CON (4.9 ± 0.2 vs 3.4 ± 0.4 pH units/min, $p < 0.005$), although ΔpH_i were similar (0.28 ± 0.02 vs 0.24 ± 0.02 , NS). To rule out an enhanced rate of change of pH_i due to decreased buffer capacity (β), β were measured by NH_4^+/NH_3 addition and were similar in CMA and CON (76.8 ± 12.6 vs 74.4 ± 9.1 mmol/liter·pH unit, NS).

In conclusion, CMA causes an adaptation in the BLM $Na(HCO_3)_3$ symporter which can be demonstrated in vivo in the absence of contact with native luminal and capillary fluids.

CHARACTERISTICS AND POLARIZED DISTRIBUTION OF THE Na^+/H^+ ANTIPORT SYSTEM IN A CLONE OF MDCK CELLS WITH HIGH TRANSEPIITHELIAL ELECTRICAL RESISTANCE. Carlos A. Rabito and Salvador Viniestra. Nuclear Medicine Div., Dept. of Radiology, Mass. Gen. Hospital, Harvard University, Boston, MA.

The Na^+/H^+ antiport system present in the apical membrane of the renal proximal tubular cells has been proposed as the exit step in the transport of H^+ across the proximal tubule. Similar systems are also involved in cellular functions such as intracellular pH regulation, control of cell division and cell volume regulation. In a previous study, we demonstrated the presence of a Na^+/H^+ antiport system in the apical membrane of the clone LLC-PK_{1A}, a cell line with differentiated characteristics of renal proximal tubular cells (Vinegra and Rabito Fed Proc 45, 510a, 1986). In the present study, we analyzed the characteristics and distribution of the Na^+/H^+ antiport system in confluent monolayers of the clone 4 of MDCK cells with transepithelial electrical resistance in the order of 5.000 cm^2 (Rabito et al J Cell Biol 103, 71a 1986). The activity of the Na^+/H^+ antiport system was determined by measuring the ^{22}Na influx at 1.43 mM after imposing an outwardly oriented H^+ gradient (pH 6.0, pH 7.4). No significant differences in the affinity for Na^+ or the inhibitory effect of amiloride were observed between LLC-PK_{1A} and MDCK cells. Contrary to LLC-PK_{1A} cells, however, the activity of the Na^+/H^+ antiport in MDCK cells reach maximum activity when the cells are in active proliferation. Unidirectional ^{22}Na influx measurement from the apical and basolateral side indicated that the Na^+/H^+ antiport system is localized in the basolateral side of MDCK monolayers. These results are consistent with the idea that the Na^+/H^+ antiport system involved in transepithelial H^+ transport is different from the system observed in other cellular systems.

Na-HCO₃ COTRANSPORT OF BASOLATERAL RENAL MEMBRANE IS ENHANCED IN CHRONIC HYPERCAPNIA. O.S. Ruiz*, J.A.L. Arruda and Z. Tavor, Dept. of Medicine, Univ. of Illinois and WSVAMC, Chicago, IL.

Na-HCO₃ cotransport is present in basolateral (BL) renal membranes. In chronic hypercapnia, HCO₃ reabsorption is enhanced through an adaptive increase in the V_{max} of the Na-H antiporter. This increase in Na-H antiporter should be associated with an increase in Na-HCO₃ cotransport. We measured HCO₃ dependent ^{22}Na uptake in BL membranes at 3 seconds and the Na-H antiporter (acridine orange) in luminal membranes prepared from rabbits exposed to 10% CO₂ for 48 hours and from control rabbits. Hypercapnic rabbits had significantly higher pCO₂ than controls (79 ± 0.3 vs. 38 ± 0.8 mmHg). Plasma HCO₃ was higher (31.3 ± 2.15 vs. 23.5 ± 1.4 meq/l) and blood pH was lower (7.22 ± 0.03 vs. 7.40 ± 0.02). HCO₃ dependent ^{22}Na uptake in BL membranes was linear with Na concentration in the medium reaching a maximum (V_{max}) at 30 mM. The V_{max} of the HCO₃ dependent ^{22}Na uptake was higher in membranes from hypercapnic rabbits as compared to control (2.6 ± 0.1 vs. 1.5 ± 0.5 nmoles/mg protein, $p < 0.025$). Likewise, the V_{max} of Na-H antiporter was increased in hypercapnia (884 ± 7.6 vs. 503 ± 44 FU/300 μg protein/min, $p < 0.01$). The V_{max}'s of both the Na-HCO₃ cotransport and the Na-H antiporter were linearly related to blood HCO₃ and H^+ concentrations. Thus, hypercapnia leads to a parallel adaptive increase in both Na-HCO₃ cotransport and Na-H antiporter.

IN VITRO METABOLIC ACIDOSIS (MA) STIMULATES ENDOCYTIC ACTIVITY OF CORTICAL COLLECTING DUCT (CCD) INTERCALATED CELLS (IC). Lisa M. Satlin and George J. Schwartz. Dept. of Peds., Albert Einstein Coll. Med., Bronx, N.Y.

In vivo MA in rabbits results in a 4-fold increase in number of endocytic IC (E+ IC), a 30% reduction in number of IC binding peanut lectin (PNA+ IC), and no change in total number of IC identified with 6-carboxyfluorescein (Clin. Res. 35:637A, 1987). This response occurs 5-20 h after acid loading and can be inhibited by administration of Actinomycin D. We have now examined this model in vitro by perfusing CCD for 3 to 5 h in a culture medium containing DMEM + 3% FCS. We counted E+ IC and PNA+ IC in the same 200 μm length of CCD before and after this incubation. Incubation for 5 h at pH 7.4 resulted in no significant change in number of E+ IC (6 ± 3 to $8 \pm 2/\text{mm}$) and PNA+ IC (144 ± 8 to $138 \pm 9/\text{mm}$). After 5 h of in vitro MA (pH 7.0), the number of E+ IC increased (3 ± 2 to $34 \pm 4/\text{mm}$; $p < 0.001$) and PNA+ IC decreased (136 ± 9 to $127 \pm 9/\text{mm}$; $p < 0.05$). Up to 10% of E+ IC had small lectin caps. Earlier, by 3 h of MA, 2 of 3 CCD showed 50 and 55 E+ IC/mm, but 90% of these E+ IC had lectin caps. Thus, these studies indicate that in vitro MA increases the number of E+ IC, in part, at the expense of PNA+ IC. We speculate that MA results in a reversal in functional polarity of some IC from HCO₃⁻ secretion to H^+ secretion. These cellular changes, evident at 3 h, are mediated by complex intracellular processes, including transcription, translation, and cytokinesis.

ACETAZOLAMIDE (ACTZ) EFFECT ON KINETICS OF ANION EXCHANGE INHIBITOR BINDING TO KIDNEY MEDULLARY COLLECTING DUCT (MCD) CELLS. J. L. Seifter, A. Janoshazi*, P. Silva and A. K. Solomon*, Biophysical Lab. and Dept. of Medicine, Harvard Med. Schl., Boston, MA.

A membrane protein with immunoreactive properties similar to red cell band 3 has been found on the basolateral face of outer MCD cells in rabbit kidney (Schuster et al (1986) *Kidney Int.* 29:376). Cl⁻/HCO₃⁻ exchange in isolated MCD cells is inhibited by the stilbene inhibitor, DIDS, and also by ACTZ (Zeidel et al (1986) *J. Clin. Inv.* 77:1682 and *Kidney Int.* 29: 379). We have probed the DIDS site with a fluorescent analog, DBDS (4,4'-dibenzamido-2,2'-disulfonic stilbene), and find that DBDS binding affinities and kinetics are virtually identical with those for red cell band 3 (Seifter et al (1987) *J. Gen. Physiol.* 90: 35a). In preliminary experiments, we find that 1 mM ACTZ decreases the rate of the conformational change that follows DBDS binding to kidney 'band 3' by about 25%. In related preliminary experiments, we have measured Cl⁻/HCO₃⁻ exchange in MCD cells using the fluorescent dye, SPQ (6-methoxy-N-(3-sulfopropyl)quinilium); the exchange follows an exponential time course with $\tau \approx 0.13$ s at $[\text{Cl}^-] = 125$ mM. 1 mM ACTZ inhibits Cl⁻ flux by $\approx 50\%$ or more. These experiments suggest that ACTZ binding leads to a conformational change in the DBDS site and that both sites are on the same or contiguous proteins.

BENEFICIAL EFFECT OF CARBICARB AND DELETERIOUS EFFECT OF BICARBONATE IN THE TREATMENT OF SYSTEMIC ACIDOSIS IN THE RAT. J.I. Shapiro, R. Kucera,* N. Kendig,* G. Filley,* and L. Chan. Univ. Colorado Sch. Med., Denver, CO.

Sodium Carbicarb (C) has qualities which may make it superior to sodium bicarbonate (B) for the treatment of life-threatening systemic acidosis. We compared the effects of bolus administration of B and C in anesthetized and paralyzed rats (n=11) made acidemic by controlled hemorrhage and hypoventilation. ^{31}P nuclear magnetic resonance spectra were acquired every 2.5 min to measure intracellular brain pH (ICpH) based on the chemical shift of inorganic phosphate. Arterial blood samples were drawn to assess changes in systemic pH, pCO_2 , and lactate. Blood pressure was monitored continuously. There were no differences in baseline parameters. No significant differences in blood pressure and arterial lactate concentration developed between B and C treated rats in the 15 min following treatment. B-induced increases in systemic pH ($.13 \pm .03$ at 2 min, $p < .01$) as well as increases in pCO_2 (9 ± 2 mmHg at 2 min, $p < .01$) which persisted for 15 min. ICpH fell progressively in B treated rats ($-.03 \pm .02$ at 2 min to $-.10 \pm .03$ at 15 min, $p < .01$). In contrast, C-induced rises in arterial pH ($.13 \pm .03$ at 2 min, $p < .01$) without changes in pCO_2 which persisted for 15 min and caused a sustained increase in ICpH ($.08 \pm .02$ at 2 min and $.06 \pm .04$ at 15 min, $p < .01$). C corrects systemic and intracellular brain acidosis where B worsens intracellular brain acidosis. These data suggest that C may potentially be a superior alkalinizing agent than B for administration in the setting of life-threatening systemic acidosis.

IONIC MECHANISM OF $\text{Na}^+:\text{HCO}_3^-$ COTRANSPORT IN RENAL BASOLATERAL MEMBRANE VESICLES (BLMV). M. Soleimani* and P.S. Aronson. Yale Univ. Sch. of Med., Depts. of Med. and Physiol., New Haven, CT

The exit of HCO_3^- across the basolateral membrane of the proximal tubule cell occurs via the electrogenic cotransport of three equivalents of base per Na^+ . We have used BLMV isolated from rabbit renal cortex to identify the ionic species transported via this pathway. Media of varying pH and pCO_2 were employed to evaluate the independent effects of HCO_3^- and CO_3^{2-} on ^{22}Na transport.

Na uptake was stimulated when $[\text{CO}_3^{2-}]$ was increased at constant $[\text{HCO}_3^-]$, indicating the existence of a transport site for CO_3^{2-} . In the presence of HCO_3^- , Na influx was stimulated more than 3-fold by an inward sulfite gradient. Sulfite-stimulated Na influx was DIDS-sensitive, confirming that it occurred via the $\text{Na}^+:\text{HCO}_3^-$ cotransport system. Increasing $[\text{CO}_3^{2-}]$ at constant $[\text{HCO}_3^-]$ reduced the stimulation of Na influx by sulfite, suggesting competition between sulfite and CO_3^{2-} at a common divalent anion site. Additional divalent anions that were tested, such as SO_4^{2-} , oxalate $^{2-}$ and HPO_4^{2-} , did not interact at this site. Sulfite stimulation of Na influx was HCO_3^- -dependent and was increased as a function of $[\text{HCO}_3^-]$, indicating the presence of a separate HCO_3^- site.

Lastly, we tested whether Na^+ interacts via ion pair formation with CO_3^{2-} or binds to a distinct site. Li^+ , which has higher affinity than Na^+ for ion pair formation with CO_3^{2-} , was found to have >5-fold lower affinity than Na^+ for the $\text{Na}^+:\text{HCO}_3^-$ cotransport system, arguing against ion pair formation. Moreover, when its inhibition was studied as a function of $[\text{Na}^+]$, harmaline was found to be a competitive inhibitor of Na influx, indicating the existence of a distinct cation site.

In conclusion, our data are compatible with a model in which base transport across the basolateral membrane of the proximal tubule cell takes place via 1:1:1 cotransport of CO_3^{2-} , HCO_3^- , and Na^+ on distinct sites.

IONIC AMMONIUM (NH_4^+) EXIT FROM THE RAT PROXIMAL CONVOLUTED TUBULE (PCT) IN VIVO. E.E. Simon, C. Merli, B. Fry, K. Hering-Smith, and L.L. Hamm. Jewish Hosp. and Washington Univ., St. Louis, MO.

Recent studies have suggested that NH_4^+ flux ($J_{\text{NH}_4^+}$) may contribute significantly to total ammonia flux along the PCT. The purpose of the present studies was to directly examine the contribution of $J_{\text{NH}_4^+}$ and J_{NH_3} to ammonia exit from the PCT utilizing *in vivo* microperfusion in the rat. Solutions containing 10 mM NH_4Cl were perfused at 50 nl/min with the following results:

Soln	Perfusate [HCO_3^-] (mM)	Log Mean Luminal [HCO_3^-] (mM)	J_{Ammonia} (pmoles/min/mm)
1	40	32.7 ± 1.0	$254 \pm 25^*$
2	25	18.6 ± 1.4	174 ± 18
3	5	6.0 ± 0.6	152 ± 11

* $p < 0.005$ vs Solution 3, $p < 0.02$ vs Solution 2

J_{Ammonia} though almost 2X higher from Solution 1 than 3 should have been ~5X higher if all loss were via NH_3 (based on the mean luminal $[\text{HCO}_3^-]$ s). Assuming that the efflux of NH_3 and NH_4^+ depended only on their respective concentrations and that their apparent permeabilities were independent of luminal pH, J_{NH_3} and $J_{\text{NH}_4^+}$ could be estimated. J_{NH_3} was consistent with previous studies (apparent permeability 0.031 cm/sec). However, the efflux of NH_4^+ was ~10X higher than that predicted from prior NH_4^+ permeability measurements in the rabbit proximal tubule.

Conclusion: NH_4^+ loss from the rat PCT is significantly higher than previously estimated. Such NH_4^+ flux could contribute importantly to PCT total ammonia concentrations *in vivo*.

ENHANCEMENT OF Na-H ANTIPORTER IN CHRONIC HYPERCAPNIA IS MEDIATED BY INCREASED NUMBER OF ANTIPORTERS. Z. Talor, P. Pahlavan*, G.C. Mejicano*, E. Cragoe, Jr.*, D. Kniaz and J.A.L. Arruda. Dept. of Medicine, U of I and WSVAMC, Chicago, IL and Merck Sharp and Dohme Res. Lab., West Point, PA.

Chronic hypercapnia is associated with an adaptive increase in V_{max} of Na-H antiporter in brush border membrane vesicles (BBMV). This increase in V_{max} could be mediated by increased number/increased turnover of antiporters and/or by a decrease in intracellular pH. To clarify the possible mechanism we measured ^3H -methyl-isobutyl-amiloride (MIA) to BBMV from control and hypercapnic rabbits. Scatchard analysis showed that the maximal number of binding sites (B_{max}) was higher in hypercapnia than in controls (6.1 vs. 2.9 nmoles/mg protein, $p < 0.01$), suggesting increased number of antiporters. Furthermore, B_{max} for ^3H -phorbol-12,13-dibutyrate (a protein kinase C activator) was also significantly higher in hypercapnia than in controls (31.7 vs. 23.9 pmoles/mg protein, $p < 0.01$). Actinomycin D administration prevented the increase in V_{max} of the Na-H antiporter in hypercapnia (615 ± 34 vs. 908 ± 15 fluorescence units/300 μg protein/min, $P < 0.025$). Intracellular pH measured by the fluorescent probe BCECF was not different in proximal tubules isolated from control and hypercapnic rabbits (7.11 ± 0.04 vs. 7.14 ± 0.06). These data demonstrate that the adaptive increase in V_{max} of the Na-H antiporter in chronic hypercapnia is mediated by increased synthesis of antiporters but is independent of steady-state intracellular pH.

EXCRETION OF NH_4HCO_3 BY THE ALLIGATOR KIDNEY: STRUCTURE/FUNCTION CORRELATES. S Ventura*, TE Northrup*, G Schneider*, JJ Cohen, S Garella, Michael Reese Hosp, U of Chicago, and Loyola U, Chicago IL.

Alligators cannot synthesize urea; to excrete most of their nitrogen load, they utilize urinary ammonia. Such a strategy would lead to inexorable alkalization of body fluids if, as in mammals, urinary NH_3 obligated equivalent net proton excretion (e.g., NH_4Cl); disruption of acid-base equilibrium is avoided by excretion of the neutral salt NH_4HCO_3 . Indeed, HCO_3^- excretion in this species routinely exceeds HCO_3^- filtration, prima facie evidence of net tubular secretion. Moreover, acetazolamide inhibits rather than promotes HCO_3^- excretion.

Reasoning that these distinctly non-mammalian characteristics probably belie qualitative differences in renal functional anatomy, we studied the histochemical distribution of carbonic anhydrase (CA) in the alligator kidney and Na/H antiporter activity (AA) in BBM vesicles (Acridine Orange). CA was undetectable in proximal tubule but was abundant in distal tubule cells where intense linear staining of basolateral membranes, moderate diffuse staining of cytoplasm and no staining of luminal membranes was evident. We found no evidence for Na/H AA in BBM; H^+ efflux was linearly related to external [Na] or [Li] without evidence of saturation kinetics and was not inhibited by amiloride. The absence of both proximal CA and Na/H AA in the alligator, taken together with the low GFR known to characterize this species, leads us to conclude that the distal rather than the proximal tubule (as in mammals) is the major site of HCO_3^- transport.

IDENTIFICATION OF A $\text{Cl}^-/\text{HCO}_3^-$ TRANSPORTER IN THE BASOLATERAL MEMBRANE OF INTERCALATED CELLS (IC) IN THE COLLECTING DUCT OF MAN. JW Verlander*, KM Madsen, BP Croker, Jr., PS Low*, DP Allen* and C.C. Tisher. University of Florida, Gainesville, FL and Purdue University, West Lafayette, IN.

Band 3 protein, a major anion transporter that catalyzes $\text{Cl}^-/\text{HCO}_3^-$ exchange across cell membranes, was identified in the human collecting duct using a rabbit polyclonal antibody directed against the 43kDa fragment of the cytoplasmic domain of human RBC band 3. Tissue from 8 human kidneys removed for surgical disease was fixed in phosphate buffered formalin or 1% glutaraldehyde and processed for immunocytochemistry. For light microscopy paraffin sections were exposed to the primary antibody followed by the avidin-biotin complex procedure. For electron microscopy Lowicryl sections from both cortex and medulla were stained with the primary antibody followed by colloidal gold-conjugated anti-rabbit IgG. There was heavy basolateral staining of all IC in the outer medullary collecting duct (OMCD) and the majority of IC in the connecting segment (CNT) but only in one population of IC in the cortical collecting duct (CCD). A second population of IC in the CCD was negative as were principal cells. Heavy basolateral staining was also observed in many cells throughout the inner medullary collecting duct (IMCD). We conclude that a $\text{Cl}^-/\text{HCO}_3^-$ transporter is present in the basolateral plasma membrane of IC in the OMCD and CNT and in one type of IC in the CCD. In addition, cells with an apparent $\text{Cl}^-/\text{HCO}_3^-$ transporter are located throughout the IMCD. These findings provide the initial evidence for the presence of two types of IC in the CCD of man and for a $\text{Cl}^-/\text{HCO}_3^-$ transporter in the deep IMCD.

PROTON TRANSLOCATING ATPase OF THE RAT INNER MEDULLARY COLLECTING DUCT. SM Wall*, JA Kraut, and S Muallem*. Med and Resch Services, VA Med Ctr, West LA and Cedars-Sinai Med Ctr and Dept of Med, UCLA School of Medicine, Los Angeles, CA.

A Cl and N-ethyl maleimide (NEM) sensitive H-ATPase has been detected within intracellular vesicles and on the luminal membrane of the outer medullary collecting duct (OMCD). To examine if a similar proton pump is present in the inner medulla (IMCD), freshly isolated tubules from the IMCD of the rat were stained with acridine orange (AO), a weak base which accumulates in acidic spaces. The IMCD tubules showed punctate staining consistent with acidic vesicles. To evaluate H-ATPase activity, AO quenching was measured and expressed per mg of protein. The H-ATPase in the IMCD was inhibited by NEM and removal of chloride. To further characterize the H-ATPase in the intracellular vesicles, plasma membranes were selectively permeabilized by incubation with the detergent saponin. A dose-response curve for saponin was constructed for each experiment. Addition of ATP led to quenching of AO fluorescence which was Cl sensitive and inhibited by NEM, consistent with the presence of a cytoplasmic H-ATPase. To evaluate a possible proton pump on the plasma membrane, cells were loaded with the pH sensitive dye, BCECF and pHi changes were measured. When acidified cells were placed into Na free media, NEM sensitive alkalization occurred.

These data indicate that an H-ATPase is present in the rat IMCD similar to that described in the OMCD, and presumably contributes to urinary acidification.

TRYPSIN STIMULATION OF RENAL Na^+/H^+ EXCHANGER. E.J. Weinman, W. Dubinsky*, and S. Shenolikar*. Univ. of Texas Medical School, Division of Nephrology, Houston, Texas.

Studies were performed in renal brush border membranes (BBM) from the rabbit and in artificial proteoliposomes prepared from detergent extracts of BBM proteins to examine the effect of limited trypsin digestion on Na^+/H^+ exchange activity. Incubation of BBM with trypsin (25-50 $\mu\text{g}/\text{ml}$ for 10 min) resulted in a significant increase in the amiloride sensitive component of proton gradient stimulated 22Na^+ uptake at 2 sec ($61.8 \pm 13.5\%$) and at 10 sec ($35.8 \pm 7.0\%$). Amiloride insensitive 22Na^+ uptake, equilibrium sodium concentration, sodium dependent and independent glucose uptake, and equilibrium glucose concentration were not affected by trypsin. Trypsin significantly increased sodium dependent efflux of protons as determined by acridine orange fluorescence by $46.5 \pm 8\%$.

Incubation of solubilized BBM proteins with trypsin significantly increased proton gradient stimulated, amiloride inhibitable sodium uptake when these proteins were reconstituted into artificial phospholipid vesicles from $6.3 \pm 0.4 \text{ nmol} \cdot \text{mg protein}^{-1} \cdot 2 \text{ min}^{-1}$ to 9.3 ± 0.6 . Incubation of preformed proteoliposomes with trypsin also increased Na^+/H^+ exchange activity by $30 \pm 8\%$ ($p < 0.05$).

These studies indicated that trypsin digestion stimulates the activity of the renal Na^+/H^+ exchanger. The results are consistent with the possibility that the renal Na^+/H^+ exchanger has an inhibitory subunit and that this subunit is inactivated by trypsin.

IMPORTANCE OF THE DISTAL NEPHRON IN MAINTENANCE OF CHLORIDE-DEplete CHRONIC METABOLIC ALKALOSIS (CMA) IN RATS. D.E. Wesson and H. Babino. V.A. Med. Ctr., Baylor College of Medicine, Houston, Tx.

The role of the distal nephron in maintenance of Cl-deplete CMA is unknown. Free-flow micropuncture was done in Munich-Wistar rats given furosemide and a low electrolyte diet 4-5 wks before study. Thirteen control (Con) and 12 experimental (CMA) rats were supplemented with NaCl + KCl and NaHCO₃ + KHCO₃, respectively. CMA rats had greater plasma total CO₂ (TCO₂) (36.2±1.3 vs 23.7±1.2 mEq/L, p<0.001) but both groups had plasma K⁺ similar to rats fed standard chow. Micropuncture was done in euvolemic rats receiving I.V. D5W and in a separate group receiving isotonic NaHCO₃, then isotonic NaCl each at 1% bw/hr without volume expansion. During D5W, both groups had similar early distal (ED) TCO₂ deliveries and distal segment (DS) absolute TCO₂ reabsorption despite a lower CMA late distal (LD) tubular fluid to plasma TCO₂ ratio (TF/P) (0.07±0.01 vs 0.17±0.03, p<0.02), indicating a less favorable TCO₂ reabsorption gradient during CMA. NaHCO₃ infusion yielded greater ED TCO₂ deliveries for both groups but only CMA rats responded with greater DS TCO₂ reabsorption (73±5.6 pmol/min, p<0.02), unchanged LD (TF/P) (0.21±0.04, p<0.001) and reduced plasma TCO₂ (34.5±1.9 mEq/L, p<0.02) compared to its respective D5W group. Subsequent NaCl infusion into CMA rats yielded reduced DS TCO₂ reabsorption (44±5.1 pmol/min, p<0.05), greater LD (TF/P) (0.21±0.04, p<0.001) and reduced plasma TCO₂ (34.5±1.9 mEq/L, p<0.02) compared to its respective NaHCO₃ group. The data indicate Cl-responsive stimulated distal nephron TCO₂ reabsorption during Cl-deplete CMA which correlates directly with increasing and decreasing metabolic alkalosis.

GLOMERULAR ADAPTATION AFTER UNILATERAL NEPHRECTOMY IN INFANCY. Gianni Celsi, Lars Larsson, Istvan Seri, Virginia Savin, and Anita Aperia. Dept. of Ped., Karolinska Institute, Stockholm, Sweden and Dept. of Med., Univ. of Kansas Medical Ctr., Kansas City, Kansas.

To study the glomerular determinants of increased nephron filtration rate following renal ablation, we assessed glomerular filtering area, ultrafiltration pressure and hydraulic conductivity (Lp) in rats uninephrectomized (Nx) or sham-operated (S) at 5 days of age. Rats were fed normal protein diet and studied 8 weeks after surgery. Results are mean ± SEM. SNGFR (nl/min) was 80.7 ± 4.6 in Nx and 43.5 ± 3.2 in S rats. The filtering area (10⁴/μm²) of the glomerular basement membrane, measured from electron micrographs was increased in Nx rats (23.3 ± 3.7 vs 9.9 ± 0.01). The ultrafiltration pressure (P_{UF}; mmHg) by stop flow technique was significantly elevated in Nx rats (28.3 ± 1.0 vs 23.2 ± 1.1). Lp (μl/min·mmHg·cm²) of single isolated glomeruli, was significantly reduced in Nx rats (1.52 ± 0.11 vs 2.35 ± 0.17). Albumin excretion (mg/h), in urine samples from anesthetized bladder-catheterized rats, was significantly higher in Nx rats (2.18 ± 0.42 vs 0.06 ± 0.04). The incidence of focal glomerular sclerosis (FGS) was very low in both Nx and S 8-week-old rats. In 6-month-old rats the incidence of FGS was significantly higher in Nx than in S rats. The increase in filtering area and in P_{UF} both contribute to increased GFR following ablation of renal tissue in early life. The decrease in hydraulic conductivity is interpreted as an early sign of glomerular capillary damage.

RENAL PHYSIOLOGY— HEMODYNAMICS

RESPONSES OF THE JUXTAMEDULLARY NEPHRON MICROVASCULATURE TO CHANGES IN PERFUSION PRESSURE. Pamela K. Carmines, Peter J. Veldkamp,* and L. Gabriel Navar. University of Alabama at Birmingham, Birmingham, AL.

Autoregulation of renal blood flow is generally thought to involve active adjustment of preglomerular resistance, presumably at the level of the afferent arterioles (AA); however, interlobular arteries (ILA) have recently been implicated as important effector elements in outer cortical autoregulation. The purpose of this study was to evaluate directly the role of ILA and AA in the deep nephron response to changes in perfusion pressure (PP). Experiments were performed utilizing the *in vitro* blood perfused juxtamedullary nephron technique, which provides direct access to the microvasculature of the normal rat kidney. Videometric microscopy was utilized to determine the ability of ILA and AA to respond actively to changes in PP. At a PP of 114±4 mmHg, ILA inside diameter averaged 52±12 μm and AA diameter was 16±2 μm. When PP was varied within the range of 70-180 mmHg, ILA diameter changed -0.07±0.01 μm/mmHg ΔPP. AA diameter was more responsive to changes in PP (-0.16±0.03 μm/mmHg). In other studies, intravascular pressure was measured by direct puncture during alterations in PP. In nephrovascular units exhibiting glomerular capillary pressure autoregulation, AA pressure was variably regulated, changing 0.54±0.36 mmHg/mmHg ΔPP; however, ILA pressure exhibited no evidence of regulation, changing 0.92±0.08 mmHg/mmHg ΔPP. Therefore, although both ILA and AA adjust their diameters in response to PP alterations, adjustments in AA caliber represent the primary effector system responsible for autoregulatory resistance changes in juxtamedullary nephrons.

TIME COURSE OF PROXIMAL TUBULE RESPONSE TO ACUTE ARTERIAL HYPERTENSION IN THE RAT. Chung-Lin Chou and Donald J. Marsh. USC Sch. of Med., Dept. of Physiol. & Biophys., Los Angeles, California.

Acute hypertension was previously shown to inhibit reabsorption of fluid from proximal tubules perfused *in vivo*. We sought to determine whether the inhibition also occurred in intact tubules receiving native glomerular filtrate. We used a recently developed videodensitometric method for measurement of tubular flow rate that does not disturb flow to the macula densa. Hypertension was induced by increasing total peripheral resistance. Acute hypertension produced a 2 - 3 fold increase of urine flow rate. Whole kidney glomerular filtration rate and renal blood flow were fully autoregulated. End proximal fluid velocity had increased by 16% as early as 1.5 - 2 min following onset of hypertension, and increased over 25 - 30 min to reach values 50% greater than controls. Tubular diameter did not change significantly during this time. These results confirm that hypertension increases the fluid load to the loop of Henle by an effect on the proximal tubule. This increase in fluid load could signal the macula densa and contribute to autoregulation. It could also provide a significant fraction of the increased fluid and salt excretion of pressure natriuresis.

FAILURE OF INTRARENAL DOPAMINE (DA) INFUSION TO INDUCE NATRIURESIS IN DOGS WITH LOW CARDIAC OUTPUT. S.Y. Chou, D.I. Baumstein and J.G. Porush. Division of Nephrology & Hypertension, Brookdale Hospital Medical Center, Brooklyn, New York.

DA induces natriuresis in cardiac failure, an effect attributed to its ability to improve cardiac function, its action on renal DA receptors, or both. In the present study, we examined the effects of intrarenal DA infusion (into the left renal artery at 1 µg/min/kg) on renal hemodynamics, sodium excretion and vasoactive hormonal systems in 7 anesthetized chronic caval dogs with low cardiac output and 8 normal dogs. In normal dogs, DA infusion induced a significant increase in urine flow (from 0.8±0.1 to 1.6±0.3 ml/min), Na excretion (from 88±17 to 211±39 µeq/min), renal blood flow (RBF) (from 2.8±0.1 to 4.0±0.4 ml/min/g), and GFR (from 34±4 to 42±4 ml/min. In caval dogs DA infusion significantly increased RBF (from 2.7±0.3 to 3.3±0.3 ml/min/g) but failed to significantly alter urine flow (0.5±0.1 ml/min), Na excretion (5±1 µeq/min) or GFR (28±3 ml/min). In both normal and caval dogs intrarenal DA infusion did not alter arterial pressure or cardiac output. The baseline renal output of norepinephrine (NE), renin and PGE₂ was 5-, 12-, and 4-fold greater, respectively, in caval dogs than in normal dogs. The renal vasodilation induced by DA in normal or caval dogs was not associated with suppressed NE or renin, nor enhanced PGE₂ production in the kidney. Thus, in caval dogs intrarenal DA infusion caused renal vasodilation but failed to induce diuresis or natriuresis, suggesting that the renal effects of DA alone are insufficient to cause diuresis and natriuresis in cardiac failure.

GLOMERULAR HEMODYNAMICS (GH) RESPONSE TO PUROMYCIN AMINONUCLEOSIDE (PAN) BEFORE THE APPEARANCE OF PROTEINURIA. FB. Gabbai*, N. Bobadilla*, E. Tapia*, C. Calleja*, L. Romero*, J. Herrera-Acosta, Dept. of Nephrology and Pathology, Inst. Nac. Cardiologia, Mexico City.

Administration of PAN is associated with glomerular sclerosis (GS) after the nephrotic stage. Although glomerular hypertension has been implicated in this process, some studies do not support this possibility. To evaluate the possible role of hemodynamic factors in inducing glomerular lesions, we analyzed the changes in GH induced by PAN 3 days after its IP (15mg/100mg of body weight) administration, before the onset of proteinuria in 6 Wistar rats. Mean blood pressure (MBP, mmHg), glomerular filtration rate (GFR, ml/min), single nephron GFR (SNGFR, nl/min), transcapillary hydrostatic pressure gradient (ΔP, mmHg), glomerular ultrafiltration coefficient (LpA nl/sec.mmHg) were determined in PAN rats and 6 normal euvoletic controls. Mean values are shown. *p < 0.05.

	MBP	GFR	SNGFR	ΔP	LpA
Control	104	1.2	29	33	.045
PAN	113	0.4*	12*	43*	.010*

Before the appearance of proteinuria, PAN rats showed significant decreases in GFR, SNGFR and LpA with concomitant increases in ΔP. Glomerular histology showed only fusion of the glomerular epithelial foot processes. These results suggest that PAN administration is associated with significant increases in P which may participate in the development of GS later in this disease.

PARADOXICAL HEMODYNAMIC EFFECTS OF INSULIN (I) IN DIABETIC AND NONDIABETIC PERFUSED KIDNEYS. AJ Cohen and DM McCarthy*. Univ of Mass Med School, Worcester, MA.

Experimental diabetes is characterized by alterations of the renal circulation that may be influenced by I therapy. I produces vasodilation in the isolated perfused kidney (IPK), (Cohen et al. KI, 1985; 29:382). We examined the effect of I on renovascular resistance (RVR, mmHg/ml/min/g) and GFR (ml/min/g) in IPKs from streptozotocin-diabetic (STZ) and vehicle-injected, nondiabetic (ND) rats. All kidneys were perfused at fixed arterial pressure, with either control media (C) or media containing I (100 U/ml), for two basal 10 minute collection periods followed by two periods with angiotensin II (AII) infusion (100ng/min). Basal and AII RVRs were averaged and shown below:

IPK	Nondiabetic			STZ-diabetic		
	BASAL	AII	%	BASAL	AII	%
C(4)	3.2±.2	10.3±1.0	207±13	3.4±.2	9.1±.2	176±16
I(4)	2.9±.2	7.4±.6	152±13	3.4±.1	11.9±.8*	254±27*
p, I vs C	NS	<.05	<.025	NS	<.02	<.025

* p<.01, STZ vs ND
I caused slight vasodilation and significant blunting of AII-induced vasoconstriction in ND, as shown previously. By contrast, in STZ, I failed to produce basal vasodilation and significantly enhanced AII-vasoconstriction. Basal GFR was identical in ND and STZ perfused with C. However, STZ-IPKs perfused with I displayed reduced GFR, compared with ND, both in the basal state (.41±.13 vs .75±.06, p<.05) and during AII (.10±.04 vs .28±.03, p<.01).

We conclude that I attenuates the renovascular effects of AII in IPKs from ND, but enhances AII-induced renal vasoconstriction in STZ. These paradoxical responses in the IPK indicate a direct action of I on the renal circulation which is independent of I's effect on metabolic control.

PLASMA CATECHOLAMINES IN RENAL FAILURE AT DIFFERENT VASCULAR SITES. E. Heiddreder, K. Schaffers, K. Bausewein, U. Gilge, A. Heidland (intr. by J.D. Kopple). Nephrol. Dept., Med. University Clinic, Wuerzburg, FRG.

Baseline plasma norepinephrine (NE) values are usually elevated in chronic renal failure prior to initiation of hemodialysis. The major object of the present study was to clarify whether the overflow of NE from sympathetic clefts into the circulation is uniform for the different organs in acute renal failure (ARF) and chronic renal failure (CRF). A total of 56 persons was examined: 16 control persons, 19 pts. with non-traumatic ARF and 21 pts. with CRF. Almost all pts. had been catheterized via the femoral vein for subsequent hemodialysis. Blood samples were taken from the hepatic vein, the right renal vein, the iliac vein and the femoral artery. The catecholamines were evaluated by HPLC method. The highest values of NE were measured in renal venous blood, especially in ARF, the lowest values were found in hepatic venous blood. The epinephrine and dopamine levels did not differ significantly in the different groups. Plasma-renin-activity values evidenced a strong correlation to NE levels, particularly in renal venous blood. Plasma cortisol was not changed in any group. The calculation of net release rate of NE and its hepatic extraction revealed a significant increase of renal release of NE in ARF, in CRF, however, no difference to control group was observed. The hepatic net extraction was increased both in ARF and CRF. These results indicate an increment of sympathetic nervous system activity in ARF, in CRF, however, presumably uremia per se affects NE metabolism.

THE EFFECT OF DIFFERENT SOURCES OF DIETARY PROTEIN ON RENAL FUNCTION IN HEALTHY SUBJECTS. P.S.Kontessis, R.A.Dodds, S.J.Jones, G.C.Vibertti. Introduced by F.Pugliese. Unit for Metabolic Medicine, UMDS, Guy's Campus, London, England. Healthy vegetarian subjects have different levels of renal function than omnivores. It is however unknown whether this is related only to the quantity or also to the quality of the protein ingested. We investigated, in a randomised, cross-over study the effect of 3 weeks vegetable protein diet (VPD) or animal protein diet (APD) on renal function in 6 healthy, omnivore subjects. The diets were isocaloric, with similar levels of macronutrients; protein intake on VPD was 75 ± 16 g/day and 75 ± 19 g/day on APD. VPD was supplemented with calcium and phosphate to achieve the same level as the APD. Excretion of urea, sodium and potassium, fasting plasma urea, body weight and mean blood pressure were unchanged on the two diets. Mean (SD) glomerular filtration rate (inulin clearance) was consistently higher 113.4 (13.5) on APD than VPD 102.4 (12) ml/min/ $1.73m^2$ ($p < 0.01$). Renal plasma flow (PAH clearance) fell on VPD to 572.6 (96.7) from 633.6 (118.3) ml/min/ $1.73m^2$ on APD ($p < 0.05$). Source of dietary protein has a significant influence, independently of quantity, on renal function in normal subjects.

MODULATION OF THE PRESSURE-NATRIURESIS RELATIONSHIP BY ANGIOTENSIN. D.L. Mattson*, H. Raff* and R.J. Roman. Dept. of Physiol., Med. Coll. of WI, Milwaukee, WI.

The exact mechanism by which angiotensin (AII) alters renal function and participates in the long-term control of arterial pressure is unclear. This may reflect direct tubular or vascular effects of AII or secondary effects on the neural and humoral control of renal function. The present study examined the influence of AII on the acute pressure-natriuretic response and renal hemodynamics in rats. Neural and humoral control of the kidney was fixed by renal denervation and infusion of norepinephrine, ADH, cortisol, and aldosterone. Plasma AII levels in the control rats averaged 48 ± 5 pg/ml. Na excretion increased from 3 to 17 uEq/min/g kwt as renal perfusion pressure (RPP) was varied from 100 to 160 mmHg (N=8). GFR and RBF were unaltered by changes in RPP. Captopril (2mg/kg, iv) lowered AII levels to 18 ± 2 pg/ml but did not alter the pressure-natriuresis relationship, GFR, or RBF (N=9). AII infusion (20 ng/kg/min) increased plasma levels to 232 ± 42 pg/ml, shifted the pressure-natriuresis relationship to the right by 14 mmHg, and lowered RBF and GFR by 30% (N=9). These results indicate that elevated levels of AII shift the pressure-natriuretic response to higher pressures due to renal hemodynamic effects; however, lowering AII levels with captopril had no effect on this relationship. This suggests that the effects of captopril on the chronic pressure-natriuretic relationship may not be due to blockade of the renal effects of circulating AII.

SEX DIFFERENCES IN BAROREFLEX SENSITIVITY.

Richard H. Merrill, A-R.A. Abdel-Rahman* and W. R. Wooles.* Depts. of Medicine and Pharmacology, East Carolina University School of Medicine, Greenville NC.

In a previous study we noted that there was a significant difference in baroreflex sensitivity (BS) between males and females which we initially thought was due to a lower heart rate in the male subjects. We also showed that this difference was only apparent when BS was evaluated by the bolus (ramp) method and was not present when evaluated by the steady-state method. BS was defined as the ratio of Δ heart period (HP = reciprocal of heart rate)/ Δ MAP induced by bolus injection of phenylephrine. We extended these studies by the addition of a third group of female subjects, who had a heart rate comparable to that of male subjects. Group I were females with HP < 800 ms; group II were female with HP > 1000 ms; group III were males with HP > 1000 ms. Resting MAP and age were comparable in all groups.

	Group I	Group II	Group III
HP (ms)	776.3 \pm 27	1003.9 \pm 46	1012.0 \pm 43
BS (mg/mmHg)	21.7 \pm 3.7	23.5 \pm 3.6	46.1 \pm 5.6

The data clearly show there is a marked difference in baroreflex control of heart rate between males and females which is not explicable by differences in resting heart rate. Because the difference was apparent only with the ramp method it suggests the vagal component of the reflex may be more active in males than females.

ENHANCEMENT OF TUBULOGLOMERULAR FEEDBACK RESPONSES DURING PERITUBULAR CAPILLARY INFUSIONS OF ANGIOTENSINS I AND II. Kenneth D. Mitchell* and L. Gabriel Navar. Univ. of Alabama at Birmingham, Birmingham, Alabama.

Experiments were performed in pentobarbital-anesthetized rats to determine if increases in intrarenal generation of angiotensin II (ANG II) can enhance the sensitivity of the tubuloglomerular feedback (TGF) mechanism. Stop-flow pressure (SFP) feedback responses to step increases in late proximal perfusion rate were obtained during control conditions and during simultaneous peritubular capillary infusion of either angiotensin I (ANG I) or ANG II. Infusion of $10^{-7}M$ ANG II, at a rate (18 ± 1 nl/min, n=19) which did not affect resting SFP, enhanced the magnitude of SFP feedback responses both at a low perfusion rate of 10 nl/min (2.9 ± 0.9 vs 0.3 ± 0.2 mmHg) and at a perfusion rate (30 nl/min) which elicited a maximal feedback response (13.0 ± 1.0 vs 10.1 ± 0.7 mmHg). Similarly, infusion of $10^{-5}M$ ANG I, at a rate (15 ± 1 nl/min, n=13) which did not affect resting SFP, enhanced the magnitude of SFP feedback responses at both low (4.5 ± 1.0 vs 0.1 ± 0.1 mmHg) and high (13.5 ± 1.6 vs 9.8 ± 0.8 mmHg) perfusion rates. With a higher ANG I infusion rate (20 nl/min), control SFP decreased from 39.2 ± 0.6 to 12.0 ± 2.8 mmHg (n=18). These effects were blocked when the ANG II receptor antagonist, saralasin ($10^{-5}M$), was added to the infusate. These findings indicate that ANG II, either added or formed de novo beyond the glomerular circulation, can enhance the sensitivity of the TGF mechanism.

MATHEMATICAL MODEL OF ASCENDING MYOGENIC RESPONSES (MR) TO TUBULOGLOMERULAR FEEDBACK (TGF) INDUCED CHANGES IN AFFERENT VASCULAR RESISTANCE. L.C. Moore and A. Rich*. Dept. of Physiology and Biophysics, SUNY at Stony Brook, NY 11794.

Experimental data suggest that autoregulatory adjustments in afferent vascular resistance in rats are mediated by both TGF and a distributed intrinsic myogenic mechanism that responds to changes in intravascular pressure (IVP). If TGF acts on the distal portion of the preglomerular vasculature, then any TGF-induced vasoconstriction should raise upstream IVP and, thereby, trigger a myogenic response in the more proximal vascular segments. As this MR is driven by an increase in downstream resistance rather than a change in arterial blood pressure, we refer to this as an ascending MR (AMR). We modelled the renal vasculature with a resistance network where the resistances proximal to the site of TGF action vary with IVP. The magnitude of the AMR (AR_u) is $AR_u = KAR_u R_a / R_c$, where AR_u is the TGF-mediated increment in afferent resistance, R_a/R_c is the ratio of TGF-independent afferent and efferent resistances prior to the AMR, and K is a coefficient that expresses the efficacy of myogenic regulation of IVP. This analysis suggests that a full AMR is similar in magnitude to R_a and requires parallel TGF excitation in all nephrons. Hence, the effects of TGF excitation on whole kidney hemodynamics may be much greater than the effects of TGF excitation in a single nephron. Moreover, a significant fraction of the intrinsic myogenic autoregulatory response to increased BP may be induced by the TGF mechanism.

ARTERIAL PRESSURE AND LOOP FLOW AS DETERMINANTS OF THE TUBULOGLOMERULAR FEEDBACK RESPONSE AND MYOGENIC AUTOREGULATION. Jurgen Schnermann, The University of Michigan, Department of Physiology, Ann Arbor, Michigan.

The present experiments had two goals: 1) to assess the effect of a fixed loop of Henle flow rate (Vlp) on the stop flow pressure (SFP) response to changes of arterial blood pressure (AP), and 2) to assess the effect of AP on the response of SFP to changes in Vlp. Results: 1) SFP in response to AP changes between 130 and 75 mm Hg at three rates of Vlp changed as follows (means in mm Hg \pm S.E.):

AP (mm Hg)	125-130	110-115	85-90	<80
Vlp 0	45.6 \pm 1.8	38.2 \pm 1.3	33.0 \pm 1.1	29.1 \pm 2.7
Vlp 20	36.4 \pm 2.3	33.2 \pm 1.5	32.2 \pm 2.2	29.4 \pm 1.7
Vlp 45	28.6 \pm 3.1	30.5 \pm 2.4	31.0 \pm 1.2	25.9 \pm 2.8

During infusion of dopamine the difference in the dependence of SFP on AP between Vlp 0 and Vlp 45 disappeared: SFP was highly pressure dependent at all Vlp. 2) At an AP of 118.8 \pm 1.27 mm Hg SFP fell from 43.6 \pm 1.5 to 30.4 \pm 1.8 mm Hg when Vlp was increased from 0 to 45 nl/min. At an AP of 98.8 \pm 0.5 mm Hg it fell from 38.9 \pm 1.8 to 30.8 \pm 1.9 mm Hg and at an AP of 78.8 \pm 1.7 mm Hg it fell from 33.5 \pm 1.36 to 30.4 \pm 1.7 mm Hg.

Conclusions: 1) TGF-independent, probably myogenic mechanisms can produce complete autoregulation of SFP when Vlp is high, 2) by affecting vascular tone TGF determines the efficiency of myogenic autoregulation, 3) vasodilatation by dopamine blunts TGF-independent autoregulation and TGF-mediated vasoconstriction, 4) AP importantly modulates TGF reactivity probably by changing myogenic vascular tone.

CHLOROTHIAZIDE DECREASES GLOMERULAR FILTRATION RATE VIA TUBULOGLOMERULAR (TG) FEEDBACK. Mark D. Okusa* and Fred S. Wright. Yale University School of Medicine and VA Medical Center, New Haven, CT.

Systemic administration of thiazide diuretics have been shown to decrease glomerular filtration rate (GFR). To define the mechanism by which thiazide diuretics decrease GFR, clearance and free-flow micropuncture experiments were performed during iv infusion of control (C) solution (125 mM Na, 25 mM NaHCO₃ and 4mM K) followed by chlorothiazide (CTZ; 0.25mg/kg/min). GFR and fluid flow rate in the proximal (V prox) and distal (V dist) tubule were measured and single nephron glomerular filtration rate (SNGFR) determined by sampling both proximal fluid and distal fluid.

	C	CTZ	
GFR (ml/min)	1.01	0.77	p<.001
SNGFR proximal (nl/min)	33.1	32.5	NS
V proximal (nl/min)	16.3	15.1	NS
SNGFR distal (nl/min)	30.0	26.0	p<.01
V distal (nl/min)	7.4	9.1	NS

CTZ decreased whole kidney GFR significantly. CTZ did not affect SNGFR measured in the proximal tubule, however, SNGFR was decreased significantly when distal fluid was sampled. This indicates that the reduction in GFR during CTZ is feedback mediated. Although there was a tendency for distal flow rate to be higher with CTZ consistent with a decrease in fluid reabsorption in the loop of Henle this difference did not reach statistical significance. In conclusion: 1) CTZ produces a decrease in whole kidney GFR, 2) this decrease in GFR is the result of a more active TG feedback mechanism, and 3) the precise mechanism by which CTZ produces this effect on TG feedback is not known at present.

TUBULOGLOMERULAR FEEDBACK IN DIABETIC RATS. FD Sency, Jr, Ronald Salmond*. Univ Texas HSC, Southwestern Medical School, Dallas, TX.

To determine whether changes in tubuloglomerular feedback (TGF) function contribute to increased GFR in early diabetes, adult male Sprague-Dawley rats were anesthetized and prepared for micropuncture 2-3 months after induction of diabetes by streptozotocin. TGF was assessed by comparing measurements of single nephron (SN) GFR in distal (D) and proximal (P) segments from each nephron studied:

	SNGFR P	SNGFR D	P-D SNGFR
	nl/min		
Diabetic rats (DR)	58.6 \pm 1.9	50.2 \pm 1.3	8.3 \pm 1.2
Control rats (CR)	52.7 \pm 1.5	45.8 \pm 1.6	6.9 \pm 1.3
P value	0.02	0.04	NS

Tubule fluid flow rates (V) in late proximal (LP) and early distal (ED) nephron segments, absolute fluid reabsorption rate in surface proximal tubules (ARP) and fractional fluid reabsorption in proximal tubules (FRP) were:

	VLP	VED	ARP	FRP
	nl/min			
DR	31.5 \pm 1.7	16.1 \pm 1.2	18.4 \pm 1.1	37.1 \pm 2.3
CR	24.1 \pm 1.1	11.6 \pm 0.8	21.0 \pm 1.2	46.4 \pm 1.7
P value	0.001	0.003	NS	0.004

These results show that TGF continues to operate in diabetic rats. However, TGF activity fails to increase significantly even though the diabetic state substantially increases flow through the loop of Henle by increasing GFR and limiting proximal fluid reabsorption. We conclude that in the diabetic state, the increase in GFR is directly attributable to mechanisms independent of TGF while a resetting of the TGF mechanism permits GFR to rise.

THE EFFECT OF ADENOSINE ANALOGUES ON TUBULOGLOMERULAR FEEDBACK RESPONSES. Hidehisa Soejima* and Jurgen Schnermann, The University of Michigan, Department of Physiology, Ann Arbor, Michigan.

Adenosine (A) has been proposed to mediate tubuloglomerular feedback (TGF) responses. We therefore assessed the effect of luminal application of A analogues on maximum TGF responses of stop flow pressure (SFPmax). During orthograde perfusion A1-agonists (10 μ M) increased SFPmax from 6.3 \pm 0.34 mm Hg to 12.6 \pm 0.8 mm Hg (N-cyclopentyl-A, CPA), 12.2 \pm 1.96 mm Hg (N-cyclohexyl-A, CHA), and 10.0 \pm 0.8 mm Hg (R-PIA). A and the A2-agonist NECA did not modify SFPmax (5.9 \pm 0.51 mm Hg) at 10 μ M. At higher concentrations (100 μ M or 1 mM) all analogues as well as A blunted or reversed responses. Augmented TGF responses in the presence of A1-agonists were essentially flow-independent. While furosemide (0.1 mM) blocked SFP responses to control and 10 μ M NECA solutions (1.1 \pm 0.58 and 0.8 \pm 0.94 mm Hg), the response to 10 μ M CPA or CHA was maintained (SFPmax 8.7 \pm 1.25 and 11.8 \pm 1.26 mm Hg). In the presence of 1 μ M CPA SFPmax was 16.3 \pm 1.42 mm Hg with an isotonic mannitol solution (0.64 \pm 0.18 mm Hg in the absence of CPA). IBMX (0.25 mM) partially blocked the SFP response to 10 μ M CPA (in mm Hg: 5.6 \pm 0.52 control, 11.3 \pm 1.42 CPA, 8.2 \pm 1.4 CPA + IBMX). Intravenous infusion of angiotensin II (0.03 μ g/kg min) enhanced the response to 1 μ M CPA from 12.4 \pm 0.77 to 18.5 \pm 1.55 mm Hg. A-analogues exert profound effects on TGF reactivity which bypass the luminal signal step. If A is involved in TGF mediation its effect must be limited to activation of A1-receptors.

INFLUENCE OF CALCIUM CHANNEL BLOCKADE ON THE RENAL VASOCONSTRICTIVE ACTIONS OF PLATELET ACTIVATING FACTOR. Charles E. Thomas* and L. Gabriel Navar. University of Alabama at Birmingham, Birmingham, Alabama.

Platelet activating factor (PAF) is known to reduce renal blood flow (RBF) and glomerular filtration rate (GFR). To examine the mechanism by which this occurs, whole kidney and superficial cortical responses to intrarenal arterial infusion of PAF (25 ng/min·kg) were studied in anesthetized dogs. In 11 dogs, PAF infusion decreased both GFR (from 0.81 \pm 0.04 to 0.61 \pm 0.08 ml/min·g) and RBF (from 4.34 \pm 0.38 to 3.23 \pm 0.31 ml/min·g). Similarly, superficial cortical blood flow (SCBF), as measured by laser doppler techniques, was reduced from 15.8 \pm 0.8 to 13.9 \pm 0.9 units by PAF treatment. Autoregulatory efficiency in response to reduced renal arterial pressure was impaired during PAF. In 5 other dogs, servo-null measurements of stop-flow pressure were used to estimate the glomerular pressure (GP) response to PAF. PAF infusion decreased GP from 62.5 \pm 0.4 to 56.4 \pm 2.6 mmHg (P<0.05), indicating that the vasoconstriction occurs predominantly at pre-glomerular sites. To assess the role of Ca²⁺ entry in these responses, PAF and diltiazem (0.5 mg/min) were administered simultaneously. Neither GFR (0.82 \pm 0.06 vs. 0.82 \pm 0.02 ml/min·g), RBF (4.34 \pm 0.37 vs. 4.22 \pm 0.45 ml/min·g), nor SCBF (16.8 \pm 1.3 vs. 15.6 \pm 1.1 units) was significantly altered by this maneuver. We conclude that PAF reduces RBF and GFR through a preglomerular vasoconstriction. This action of PAF is dependent upon Ca²⁺ entry via slow channels and interferes with autoregulatory vasodilator responses to decreases in renal arterial pressure.

INTERACTIONS OF 4-6 DAY AND ACUTE TREATMENTS WITH ANGIOTENSIN II (AII) AND PROSTAGLANDINS (PG) INHIBITORS ON GLOMERULAR HEMODYNAMICS. B.J. Tucker* and R.C. Blantz, Univ. of Calif., San Diego and VA Medical Center, La Jolla, California.

AII and PG not only antagonize their respective renal hemodynamic actions, but also stimulate production of each other, modifying the net result of the renal vascular response. Also, acute treatment of inhibitors of either AII or PG may evoke a different response than longer term protocols due to renal adaptive mechanisms. Glomerular hemodynamics measurements were performed in 4 groups of Munich-Wistar rats (n=6 each); euvolesmia (C), dual 4-6 day treatment with Enalapril (MK421) and Meclofenamate (MEC), 4-6 days with MK421 followed by acute MEC in the second period, and 4-6 days of MEC followed by acute infusion of MK421. Mean arterial pressure (MAP), nephron filtration rate (SNGFR), nephron plasma flow (SNPF), glomerular hydrostatic pressure gradient (Δ P), and the ultrafiltration coefficient (LpA) were measured. (\dagger =P<0.05 to C, $*$ =P<0.05 to group 2, \ddagger =P 0.05 to respective first period)

	MAP (mmHg)	Δ P	SNGFR (nl/min)	SNPF (nl/min/mmHg)	LpA
1) C	115 \pm 3	34 \pm 1	33 \pm 2	111 \pm 7	0.09 \pm .02
2) MK421+MEC	84 \pm 4 \dagger	26 \pm 1 \dagger	24 \pm 2 \dagger	90 \pm 7 \dagger	0.08 \pm .01
3) MK421	90 \pm 2 \dagger	30 \pm 1 $\dagger*$	32 \pm 2 $\dagger*$	120 \pm 11 $\dagger*$	0.06 \pm .01
3) +MEC	84 \pm 1 \ddagger	31 \pm 1 $\dagger*$	29 \pm 2	114 \pm 9 $\dagger*$	0.05 \pm .01
4) MEC	106 \pm 4 $\dagger*$	31 \pm 1 $\dagger*$	25 \pm 1 \dagger	88 \pm 4 \dagger	0.05 \pm .01
4) +MK421	90 \pm 6 $\dagger\ddagger$	30 \pm 1 $\dagger*$	29 \pm 1 $\dagger\ddagger$	113 \pm 10 $\dagger\ddagger$	0.06 \pm .02

Blocking both AII and PG for 4-6 days did alter glomerular hemodynamics from C. 4-6 day treatment resulted in differing glomerular hemodynamic responses compared to acute infusions indicating acute infusions may not be accurate predictors for responses to long term treatments of MK421 and MEC.

MODULATION OF TUBULOGLOMERULAR FEEDBACK: INTERACTION BETWEEN ANGIOTENSIN AND THROMBOXANE. W.J. Welch and C.S. Wilcox, Div. of Nephrology and Htn., Univ. of Florida, Gainesville, FL.

We showed that blockade of thromboxane (Tx) synthesis or receptors blunted the single nephron tubuloglomerular feedback response (TGF). However, release of renin and angiotensin II (AII) were increased which might therefore have offset the effects of Tx antagonists on TGF. To study the interaction between Tx and AII in the rat, we measured TGF during two protocols. TGF was assessed in paired studies from changes in proximal tubule stop-flow pressure during increases in orthograde perfusion of the loop of Henle from 0 to 40 nl/min, before and after administration of drugs in maximal doses. In protocol 1, TGF responses were reduced by 45 \pm 3% by an angiotensin converting enzyme inhibitor (CGS-14824A; 50 mg/kg; n=7) or by 35 \pm 3% by a Tx receptor antagonist (CGS-13080; 50 mg/kg; n=7) but the combination of both drugs (n=7) led to a greater (p<0.01) inhibition of 62 \pm 3%. In protocol 2, TGF responses were reduced by 22 \pm 3% by an AII receptor antagonist (saralasin; 10 μ g/kg/min; n=8), by 37 \pm 3% by a Tx receptor antagonist (SQ-29,548; 8 mg/kg; n=7), but, during ongoing AII blockade with saralasin, blockade of TGF responses by SQ-29,548 was potentiated (p<0.01) to 64 \pm 5% (n=15). Conclusions: 1) The TGF response is modulated separately by the AII and Tx systems; 2) Tx antagonists release AII which conceals the full blocking effects of these drugs on the TGF response. Thus, these two hormone systems can interact strongly in the regulation of renal hemodynamics.

GLOMERULAR POLYANION NEUTRALIZATION IN THE DOG DOES NOT ALTER CAPILLARY PERMEABILITY.

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The mechanisms whereby glomerular polyanions (GPA) maintain normal capillary function are not entirely known. To determine the effects of GPA neutralization, polycations - protamine sulfate, polyethyleneimine or lysozyme - were infused into the renal artery of anesthetized dogs (N=20). Permeability to sulfated (S) and neutral (N) ³H-Dextran (³H-Dext) was examined during polycation perfusion using the multiple indicator dilution (MID) method. A .3 cc bolus of ¹²⁵I-albumin (plasma reference), ¹⁴C-inulin and S or N³H-Dext was injected into the left renal artery followed by rapid serial sampling of urine output. During each polycation perfusion, urinary N³H-Dext/¹⁴C-inulin recovery, a measure of glomerular size permeability, remained unchanged. However, S³H-Dext/¹⁴C-inulin decreased which was reversed by injecting unlabeled sulfated dextran 1 minute after the MID bolus. S³H-Dext intraglomerular binding indicated that the polycations were bound to the GPA. Polycation perfusion consistently decreased the renal plasma flow (RPF) and ¹⁴C-inulin recovery (filtration fraction [FF]), but did not increase microproteinuria. To conclude, acute GPA neutralization does not alter glomerular permeability to neutral solutes nor plasma protein, but does decrease RPF and FF. In glomerulonephritis, immunoglobulin association with the intact GPA may reduce the GFR but not be the etiology of proteinuria.

UNMASKING PROMINENT α_2 ADRENOCEPTOR MEDIATED RENAL VASOCONSTRICTION IN CONSCIOUS RATS. DW Wolff, FA Gesek, and JW Strandhoy. Wake Forest University Medical Center, Winston-Salem, NC.

Renal vascular reactivity to intrarenal arterial boluses of NE, phenylephrine (PHE, α_1 agonist (AG)) and guanabenz (GBZ, α_2 AG) was assessed in 7 tethered Wistar rats (361 \pm 14 g). After \geq 4 days recovery, dose-response curves (DRC's) were obtained for each AG before and after the cumulative addition of propranolol (PROP, β antagonist (ANTG)), corynanthine (CRN, α_1 ANTG) and idazoxan (IDX, α_2 ANTG). AG dosages producing 15% and 75% peak decreases in RBF (ED₁₅, ED₇₅) were estimated in ng by regression through \geq 3 pts. Rats were normotensive (96.4 \pm 3.0 mmHg, 336 \pm 11 bpm), and neither AG nor ANTG caused systemic effects.

	+ β ANTG	+ β, α_1 ANTG	+ $\beta, \alpha_1, \alpha_2$ ANTG
NE			
ED ₁₅	.771 \pm .109	1.26 \pm .200*	4.44 \pm .510**
ED ₇₅	10.9 \pm 1.06	28.4 \pm 4.83**	51.9 \pm 6.12**
PHE			
ED ₁₅	22.1 \pm 2.81	59.4 \pm 6.45**	76.9 \pm 10.4
ED ₇₅	135 \pm 13.4	393 \pm 54.9**	542 \pm 84.7
GBZ			
ED ₁₅	127 \pm 26.5	168 \pm 59.1	852 \pm 211**

* P < .05, ** P < .01 by repeated measures ANOVA. PROP had no DRC effects. The NE ED₁₅ DRC region resembled a GBZ DRC (shallow slope, comparatively CRN insensitive, IDX sensitive) whereas the ED₇₅ portion was similar to a PHE DRC (steeper slope, CRN sensitive). In anesthetized rats, high sympathetic tone masks the α_2 adrenoceptor mediated renal vasoconstriction which predominates at the lower physiological NE dosages in conscious rats.

UNCOUPLING OF THE AUTOREGULATION OF RENAL BLOOD FLOW (RBF) AND GLOMERULAR FILTRATION RATE (GFR) IN IMMATURE RATS: ROLE OF THE RENIN-ANGIOTENSIN SYSTEM (RAS). A. Yared & T. Yoshioka. Department of Pediatrics, Vanderbilt University, Nashville, TN.

To understand the high susceptibility of immature mammals to acute prerenal failure, we examined the autoregulation (AR) of RBF and GFR in young (Y, 6 week-old) and adult (A) Munich-Wistar rats. RBF and GFR were measured in Y (n=4) and A (n=8) rats, before and after \approx 30% reduction in renal perfusion pressure (RPP) by aortic constriction. Both Y and A demonstrated efficient RBF-AR: change in RBF from baseline value, Δ RBF, was 13 \pm 5% in Y, and 12 \pm 4% in A. However, while GFR was well preserved in A (Δ GFR -13 \pm 4%), it fell dramatically in Y (Δ GFR -84 \pm 10%, p < 0.001 vs A). Coupling of RBF and GFR-AR is known to be mediated, in normal adult rats, by angiotensin II (A-II)-induced efferent arteriolar constriction, hence maintenance of glomerular capillary hydraulic pressure (PGC). We therefore measured single nephron (SN)GFR and PGC in separate groups of Y (n=4) and A (n=8), before and after \approx 30% reduction in RPP. While in A, SNGFR was well preserved (-13 \pm 3%) and PGC decreased only minimally (-7 \pm 2%), this maneuver led in Y to a marked decrease in SNGFR (-48 \pm 7%, p < 0.001), ascribed largely to a profound fall in PGC (-37 \pm 2%, p < 0.001).

To assess the role of the RAS in the failure of Y rats to maintain PGC during reduced RPP, we examined the responsiveness of Y (n=5) and A (n=8) to exogenous AII infusion. The dose of AII required to similarly increase PGC by 30% was several-fold higher in Y than A (0.39 vs. 0.05 μ g/kg/min). Measurement of baseline plasma renin activity (PRA) was 4.1 \pm 0.7 ng/ml/hr in Y (n=6), and 6.4 \pm 0.7 ng/ml/hr in A (n=6, NS). Water deprivation to induce identical changes in body weight (-16%) and hematocrit (+26%), led to an increase in PRA to 41.0 \pm 2.9 in A (n=6), and only to 18.7 \pm 4.3 in Y (n=6, p < 0.005).

These studies demonstrate that: 1) Uncoupling of RBF and GFR autoregulation occurs in young animals in the face of a reduction in RPP. 2) The inability to maintain GFR in Y, observed both at whole kidney and SN levels, is due to failure to maintain PGC when RPP is reduced. 3) This inefficient AR of GFR in Y can be attributed to both limited ability to activate the RAS and low efferent arteriolar responsiveness to AII.

RENAL PHYSIOLOGY—
Na, K, & Cl

CHRONIC RENAL DENERVATION AND THE ACUTELY REDUCED KIDNEY. M.E.M. Allison*, N.G. Moss*, C.A. Staley*, J.T. Adkinson*, D.R. Kiser* [Intr. by C.W. Gottschalk]. Dept. of Med., Univ. of N. Carolina, Chapel Hill, NC.

A possible role for renal nerves in the remarkable compensatory changes in renal function found after acute reduction in renal mass [Allison et al., Kid. Int., 3, 354-363, 1973] was studied in uninephrectomized male Wistar rats. Animals were denervated [DNX, n=7] or sham denervated [SDNX, n=8] 20-49 days after uninephrectomy. Seven-14 days later they were studied by clearance methods before and for 2 hrs after infarction of 40% of renal mass. Complete DNX was verified by the absence of renal catecholamine content.

Before partial infarction anesthetized DNX compared to SDNX rats had significantly lower BP [95.1 \pm 4 SEM vs 117 \pm 6.2mmHg], plasma renin activity [PRA], [4.8 \pm 0.8 vs 12.5 \pm 3.3ngA1/ml/hr], urine flow rate [V], [3.73 \pm 0.2 vs 4.68 \pm 0.3ul/min/gmkwt]. However, there was no difference in GFR or electrolyte excretion between the two groups.

After partial infarction PRA rose significantly in both groups after 1-2 hrs [P<0.05], although the only significant rise in BP was in the DNX group [P<0.05]. There was no significant difference between the 2 groups in the magnitude of the compensatory rise in V and electrolyte excretion from the surviving nephrons.

We conclude that in the anesthetized, uninephrectomized rat kidney the lack of afferent/efferent renal nerve activity does not change the compensatory adaptive response of surviving nephrons to acute reduction in renal mass.

ONTOGENIC INCREASE OF Na⁺-H⁺ EXCHANGE INDUCES INCREASE Na-K ATPase IN RAT PROXIMAL CONVOLUTED TUBULE (RPCT). Anita Aperia, Yutaka Fukuda* and Claude Lechene. Karolinska Institute, Stockholm, and Harvard Medical School, Boston.

We have shown that in short term culture of rat proximal tubular cells, developmental increase of Na influx via increased Na-H exchange precedes that of Na-K pump activity. To examine whether increased Na-H exchange serves as an inducer of the Na-K pump we have determined Na-K ATPase activity at V_{max} conditions in PCT from 16-20 day-old rats, the period of developmental increase of the enzyme. The Na-H exchanger was either stimulated by chronic metabolic acidosis (MA) induced by NH₄Cl feeding for 4 days, or inhibited by chronic amiloride treatment (AM) .013 µg/gbw/h given 4 days IP with mini-pumps. In control and vehicle-infused rats, Na-K ATPase (pMol Pi/mm tubule/hour) increased from 481 ± 78 in 16 day-old rats to 1274 ± 88 in 20 day-old rats (m ± SE) (n= 5 to 8 rats). The development of Na-K ATPase was significantly stimulated in 20 day-old MA rats (1717 ± 109) and significantly inhibited in 20 day-old AM rats (858 ± 75). There was no effect of MA and AM on body weight and GFR. There was no significant effect of AM on kidney weight, cortical DNA content, PCT ouabain-insensitive ATPase, or medullary thick ascending limb (TAL) Na-K ATPase, and no acute effect of AM (10 µg/gbw) on PCT Na-K ATPase. This indicates that inhibition of developmental increase of Na-K ATPase in 20 day-old rats was not due to a direct effect of AM on the enzyme. Na-K ATPase in TAL of 16-20 day-old rats was not stimulated by chronic MA. Four days of MA or AM treatment had no significant effect on PCT Na-K ATPase in adult rats. The results strongly suggest that developmental increase in Na-H exchange activity is a determinant of the developmental increase of Na-K ATPase in PCT. The ontogenic increase of Na-K ATPase is thus likely secondary to increased Na influx, although the role of a possible alkaline shift of intracellular pH may not be excluded.

PHORBOL ESTERS AND DIOCTANOYLGLYCEROL INHIBIT TRANSPORT IN THE RABBIT PROXIMAL CONVOLUTED TUBULE (PCT). Michel Baum and Steven R. Hays. Southwestern Medical School, Dept. of Pediatrics and Medicine, Dallas, TX.

The present in vitro microperfusion study examined the effect of protein kinase C activation on transport in the PCT. PCT were perfused with an ultrafiltrate-like solution and bathed in a serum-like albumin solution. During the experimental period the effect of protein kinase C activation on volume absorption (J) by phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-dibutyrate (PDB) and L-α-dioctanoylglycerol (DiC8) was examined.

Protocol	n	J (nl/mm·min)		%Inhibition
		Control	Experimental	
5x10 ⁻⁹ M PMA	5	0.82±0.13	0.81±0.12	--
5x10 ⁻⁸ M PMA	5	1.06±0.10	0.77±0.07*	27%
5x10 ⁻⁷ M PMA	6	0.76±0.14	0.48±0.08*	37%
5x10 ⁻⁷ M PDB	5	0.72±0.08	0.53±0.08*	26%
10 ⁻⁴ M DiC8	10	0.96±0.08	0.71±0.08*	26%

(*p<0.01)

The inactive phorbol ester, 5x10⁻⁸M 4-α phorbol, had no effect on volume absorption (0.95±0.14 vs 0.96±0.14 nl/mm min).

The effect of protein kinase C activation on solute specific transport was also examined. Both 5x10⁻⁷M phorbol 12-myristate 13-acetate and 10⁻⁴M L-α-dioctanoylglycerol significantly inhibited glucose, bicarbonate and chloride transport in the PCT. These data demonstrate that protein kinase C activation plays an important role in the modulation of proximal tubular transport.

DISSOCIATION OF THE NATRIURESIS FOLLOWING EXTRACELLULAR FLUID VOLUME EXPANSION WITH 0.9% NaCl FROM URINARY DOPAMINE EXCRETION IN ANESTHETIZED RATS. AS Bass, MB Murphy*. U of Chicago, Chicago IL

The correlation between urinary sodium (UNaV) and dopamine (UDAV) excretions in response to infused or dietary NaCl, and suppression of UNaV by dopa decarboxylase inhibition has suggested that dopamine may be involved in the regulation of UNaV (Lee, 1986). The present study tested this relationship further in 3 groups of anesthetized Sprague-Dawley (SD) rats (n=6 in each) pretreated during the 24 hrs prior to the experiment with: 0.9% NaCl 25 ml/kg ip, every 8-12 hrs (Grp I); carbidopa 100 mg/kg ip, every 8 hrs (Grp II); or difluoromethyl dopa 100 mg/kg ip, every 12 hrs (Grp III). After 2 30-min urine collections (C), rats were subjected to extracellular fluid volume expansion with 0.9% NaCl (VE) equal to 10% body weight at 1 ml/min, followed by an infusion of 0.9% NaCl at 200 µl/min. Two 30-min urine collections were made 60 min after VE (E). Results:

	Grp I		Grp II		Grp III	
	UNaV+	UDAV++	UNaV	UNaV	UNaV	UNaV
C	0.6±0.15	1.7±0.15	0.6±0.10	0.6±0.13	0.6±0.13	0.6±0.13
E	8.9±0.46	1.4±0.12	7.9±0.54	7.9±0.79	7.9±0.79	7.9±0.79

µEq/min/g kidney weight; ++ng/min/g kidney weight
In Grp I the increase in UNaV produced by VE was not associated with an increase in UDAV. In Grp II and III, dopa decarboxylase inhibition, confirmed by inhibition of the pressor response to L-dopa 20 mg/kg iv, at the end of the experiment, did not affect the increase in UNaV produced by VE. Changes in blood pressure were the same in the 3 groups. These data demonstrate that in anesthetized SD rats, the natriuresis induced by VE is independent of endogenous dopamine production.

THIAZIDE DIURETIC RECEPTOR IN RAT KIDNEY IDENTIFIED BY ³H-METOLAZONE BINDING. Kevin Beaumont,* Duke A. Vaughn,* and Darrell D. Fanestil. Univ. of Calif., San Diego, Dept. of Med., La Jolla, CA.

Benzothiadiazine (thiazide) and related diuretic drugs are thought to act by blocking a NaCl cotransporter in the distal tubule. The thiazide-sensitive transporter has been studied by physiological methods but has not yet been directly labeled. We now report that ³H-metolazone (Zaroxolyn), a quinazolinone diuretic with a thiazide-like mechanism of action, binds to a site in rat kidney membranes with characteristics of the thiazide-sensitive transporter. ³H-Metolazone (11 Ci/mmol) was obtained by reduction of a precursor compound. Kidney membranes were incubated with ³H-metolazone in a tris-phosphate buffer and collected by filtration. Acetazolamide was used to block binding to carbonic anhydrase. Scatchard plots indicated the presence of high affinity (K_d=4.27 nM; B_{max} = 0.717 pmol/mg pro) and low affinity (K_d=289 nM; B_{max} = 3.92 pmol/mg pro) binding sites in kidney membranes. Twelve thiazide and thiazide-like diuretics competed for the high affinity site with K_is that ranged from 0.43 nM (methylclothiazide) to 3.7 µM (chlorothiazide) and that correlated significantly (p < 0.01) with average daily dose. Kidney contained by far the greatest density of high affinity sites, with little or no binding present in 11 other tissues. Low affinity binding was found in many tissues and was not inhibited by most thiazides tested. High affinity binding was strongly inhibited by Cl⁻, Br⁻, and I⁻ but not by F⁻, CH₃COO⁻, SO₄⁼, Na⁺, K⁺ or Ca⁺⁺. These properties of the high affinity ³H-metolazone binding site are consistent with its identity as the thiazide-sensitive transporter.

STIMULATION OF Na⁺ TRANSPORT BY NOVIOBIOCIN IN CULTURED KIDNEY A6 CELLS. R.I.M. Bindels*, J.A. Schafer and M.C. Reif. NRTC, Depts. of Physiol. & Biophys., and Med., Univ. of Alabama at Birmingham, Birmingham, Alabama.

The action of the antibiotic novobiocin on short-circuit current (I_{sc}, μA/cm²) and transepithelial conductance (G, mS/cm²) of A6 cells was studied. All experiments were performed with cells grown on Millipore filter cups for a period of 2 to 4 weeks in defined, serum-free medium. Addition of novobiocin to the apical side resulted in a sustained and reversible stimulation of both I_{sc} and G that was amiloride-sensitive. A dose-response curve of novobiocin indicated a half-maximal effect at 3·10⁻⁴ M, but at high concentrations (>10⁻² M) novobiocin decreased rather than increased the I_{sc} in 4 out of 6 experiments. All subsequent experiments were performed at a novobiocin concentration of 10⁻³ M, at which 71±9% of the maximal response was reached. The novobiocin-induced increase in I_{sc} and G was additive to the effects of aldosterone and of arginine vasotocin (AVT). Two differences between the action of novobiocin and AVT were found: 1) A6 cells respond to novobiocin with an increase in I_{sc} (from 13.9±0.3 to 16.9±0.3, n=5, p<0.001) accompanied by a minor increase in G (from 0.34±0.02 to 0.36±0.02, p<0.001), whereas upon addition of 10⁻⁷ M AVT the maximal stimulation of I_{sc} (from 12±1 to 16±2, n=11, p<0.001) was accompanied by a much larger increase in G (from 0.26±0.02 to 0.40±0.05, p<0.001). 2) The AVT-induced increase in I_{sc} was preceded by the major increase in G, while addition of novobiocin increased both the I_{sc} and G simultaneously. A dose-response curve of amiloride obtained in the presence of AVT showed that amiloride completely inhibits I_{sc}, indicating that the I_{sc} is due to trans-cellular Na⁺ transport. Pretreatment of the A6 cells with aldosterone for 24 h shifted the dose-response curve to the right, as expressed in a doubling of the K_i value (from 1.8·10⁻⁷ to 3.8·10⁻⁷ M). In contrast, novobiocin had no effect on the dose-response curve of amiloride.

We conclude that novobiocin, AVT and aldosterone all stimulate transepithelial Na⁺ transport in A6 cells, but through different mechanisms of action.

INHIBITORY EFFECT OF A NEW CHLORIDE (Cl) CHANNEL BLOCKER ON Cl TRANSPORT IN RABBIT RENAL MICROVILLUS MEMBRANE VESICLES (MMV's). Mark R. Boelkins* and Lawrence P. Karniski. Univ. of Iowa and VA Med. Ctr., Dept. of Med., Iowa City, IA.

5-Nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), a structural analog of diphenylamine carboxylic acid (DPC), is reportedly the most potent of a new class of Cl-channel blockers. We investigated NPPB's effect on Cl transport in MMV's isolated from rabbit renal cortex. NPPB was a potent inhibitor of two separate anion exchangers that mediate Cl transport across the proximal tubule luminal membrane: formate/Cl exchange (I₅₀ 15 μM) and Cl(formate)/oxalate exchange (I₅₀ 40-50 μM). ³⁶Cl uptake in the presence of an inside-positive membrane potential (K_o>K_i+Val) was also inhibited by NPPB (I₅₀ 100 μM), suggesting Cl conductance may involve more than simple diffusion. Inhibitory effects on all 3 Cl pathways were rapidly reversible. Relatively high concentrations (150 μM) of the agent had no appreciable effect on other transport systems studied, including Na-H exchange, Na-glucose cotransport, and Na-sulfate cotransport. Kinetic effects of NPPB on the initial rate of Cl(formate)/oxalate exchange were also examined. Plots of V vs V/[oxalate] and 1/V vs 1/[oxalate] were linear; both demonstrated a reduction in V_{max} with increasing [NPPB] but not a significant change in the K_m for oxalate (100 μM). This indicates that binding of oxalate and NPPB to the exchanger are not mutually exclusive. We conclude that NPPB is a selective and apparently noncompetitive inhibitor of Cl transport systems in rabbit MMV's and is the most potent inhibitor of formate/Cl exchange demonstrated to date.

^{99m}TECHNETIUM-PERTECHNETATE UPTAKE IN LLC-PK1 CELLS IS INHIBITED BY FUROSEMIDE. H.A. Bock*, I. Herrera*, and M.D. Lifschitz. UTHSCSA and VA Hospital, San Antonio, Texas

Although the Na/K/2Cl transporter was at one time felt to be confined to the thick ascending limb, current evidence suggests a more generalized presence in transporting epithelia. Since a recent study in rat parotid cells (JCI 79:1310, 1987) demonstrated uptake of ^{99m}Technetium-Perchnetate (^{99m}TcO₄) by the Na/K/2Cl transporter, we evaluated the possibility that ^{99m}TcO₄ was transported by the same mechanism in LLC-PK1 cells, a cell line of renal origin. The presence of the Na/K/2Cl transporter in this cell line has previously been demonstrated. Studies were performed in confluent cells grown in Dulbecco's modified Eagle medium (DMEM). ^{99m}TcO₄ was added in HEPES buffered DMEM and uptake was measured at 22°C. ^{99m}TcO₄ uptake was inhibited by furosemide 10⁻³ to 10⁻⁵ M in a dose-dependent manner. Time course studies revealed an increase in specific (furosemide-inhibitable) ^{99m}TcO₄ uptake from 0 to 5 minutes. Specific ^{99m}TcO₄ uptake was completely abolished by exposure to 40°C. If ^{99m}TcO₄ were transported on a Cl transporter, removal of medium Cl should enhance ^{99m}TcO₄ uptake. Indeed, when gluconate was substituted for Cl in the medium, specific ^{99m}TcO₄ uptake increased by 24%. We conclude: 1) ^{99m}TcO₄ is taken up by LLC-PK1 cells by a transport process which normally involves Cl and is inhibited by furosemide. 2) This mechanism most likely represents the Na/K/2Cl transporter. 3) ^{99m}TcO₄ appears to be a valuable tool for studying and quantitating Na/K/2Cl transport in-vitro and -possibly - also in-vivo.

GLUCOCORTICOIDS (G) REGULATE ELECTRONEUTRAL SODIUM ABSORPTION (abs). L. Bressler and C. Bastl. Temple Univ. Health Sci. Ctr, Phila, PA.

Massive doses of G stimulate electrogenic sodium (Na) abs in distal rat colon, similar to aldosterone's effect. The pathway mediated by physiologic doses of G has not been investigated. Chronically adrenalectomized rats (ADX) were treated with 2.5 μg/100g body weight of dexamethasone (DEX) two hours prior to in vitro flux measurement in distal colon and rectum under short circuit conditions. This dose is predicted from plasma levels to occupy less than 25% of aldosterone receptors. Compared to ADX acute DEX did not increase short-circuit current (I_{sc}), transmural PD or conductance in either segment while aldosterone did. Amiloride 10⁻⁶ M did not affect these parameters in colons of DEX treated rats. DEX acutely stimulated mucosal to serosal (m+s) flux and net Na and Cl abs:

μEq/cm ² /hr	RECTUM		DISTAL COLON	
	ADX	DEX	ADX	DEX
Na (m+s)	5.7±0.5	7.6±0.6*	8.5±0.7	13±1*
Na net abs	1.5±0.8*	3.5±0.5*	3.9±0.5	6.9±1*
Cl(m+s)	11±1	13±0.8	14±0.7	18±1*
Cl net abs	1.7±1.2*	3.4±0.8	3.5±0.6	6.4±1*

* Sig > than ADX, + not dif. than zero

Serosal to mucosal flux was unaltered by DEX. I_{sc} was 50% less than the value of net Na abs (p<0.05) and similar to residual flux. Twice daily treatment with this dose of DEX also did not produce an amiloride sensitive I_{sc}. Na and Cl (m+s) and net abs were similar to acute DEX in both segments and similar to values in intact rats. Thus, DEX restores colonic NaCl abs. The most rapid and sensitive effect of G is stimulation of electroneutral NaCl abs.

SIMULTANEOUS MEASUREMENTS OF CELL [Ca] AND TRANSEPI-
 THELIAL Na TRANSPORT IN CULTURED TOAD BLADDER
 CELLS SHOW THAT VASOPRESSIN CAUSES PARALLEL
 INCREASES IN [Ca] AND Na TRANSPORT H.Chase and
 S.Wong* Columbia U. NY, NY

Calcium may serve as a second messenger for
 those hormones which stimulate epithelial ion
 transport. To elucidate calcium's involvement in
 the natriuretic action of vasopressin in tight
 epithelia we simultaneously measured transepi-
 thelial Na transport and intracellular free [Ca]
 ([Ca_i]) in cultured toad bladder (TB) cells.

TB cells were grown to a high resistance
 (>1000ohms.cm²) on collagen coated nucleopore
 filters. [Ca_i] was measured by loading the cells
 with the Ca-sensitive dye fura-2, exciting the
 sample at 350 or 380nm, capturing the image with
 a SIT camera and measuring light intensity with a
 video analyzer. [Ca_i] was calculated using the
 ratio of the emitted light intensity of the
 sample when excited at 350 and 380nm, as well as
 after adding ionomycin and EGTA to obtain maximum
 and minimum signals. We used a voltage clamp to
 record the short circuit current (I_{sc}), a measure
 of transepithelial sodium transport.

Vasopressin significantly increased I_{sc} and
 [Ca_i]; I_{sc} rose from 11 ± 3 to 25 ± 6 microamps and
 [Ca_i] increased from 62 ± 5 to 127 ± 12nM (n=4).
 Not only were the increases in I_{sc} and [Ca_i]
 comparable, 150 ± 37% and 110 ± 21% respectively,
 but the time courses of the rises were identical.

The fact that [Ca_i] increased pari passu with
 the rise in I_{sc} suggests that the increase in
 [Ca_i] may play a role in the hormone's natriuretic
 action. These experiments also demonstrate the
 utility of making simultaneous measurements of
 ion transport and [Ca_i].

SODIUM-DEPENDENT CHLORIDE TRANSPORT IN BASOLATERAL
 MEMBRANE VESICLES (BLMV) ISOLATED FROM RABBIT
 PROXIMAL TUBULE. Pei-Yuan Chen* and A.S. Verkman.
 Dept. Medicine, Taipei Medical College, Taiwan
 & Cardiovascular Research Institute, University
 of California, San Francisco, CA.

Cl transport across renal cortical BLMV was
 examined using the entrapped Cl-sensitive fluores-
 cent indicator, 6-methoxy-N-[3-sulfopropyl] quino-
 linium (SPQ). Initial rates of chloride influx
 (J_{Cl}) were determined from the measured time
 course of SPQ fluorescence in BLMV following
 inwardly directed gradients of Cl and of other
 ions and/or pH. For a 50 mM inwardly directed
 Cl gradient in BLMV which were voltage and pH
 clamped (7.0) using K/valinomycin and nigericin,
 J_{Cl} was 0.80 ± 0.14 nmol/s/mg vesicle protein
 (mean ± SD, n=8 separate preparations). In the
 absence of Na and CO₂/HCO₃ in voltage clamped
 BLMV, J_{Cl} increased 56 ± 5% in response to a
 1.9 pH unit inwardly directed H gradient; the
 increase was further enhanced by 40 ± 3% in
 the presence of Cl/HCO₃, and inhibited 30 ±
 8% by 100 μM H₂DIDS. Na gradients did not increase
 J_{Cl} in the absence of CO₂/HCO₃, however an out-
 wardly directed Na gradient in the presence
 of CO₂/HCO₃ increased J_{Cl} by 31 ± 8% with a
 Na K_D of 7 ± 2 mM. The Na effect required CO₂/HCO₃
 but not K. These results indicate the presence
 of Cl/OH and Cl/HCO₃ exchange, and coupled
 Cl/Na(HCO₃) transport. There was no significant
 effect of K gradients in the presence or absence
 of valinomycin, suggesting absence of K/Cl co-
 transport and Cl conductance under experimental
 conditions. The Na-dependent Cl/HCO₃ exchanger
 may provide an important route for Cl exit across
 the proximal tubule basolateral membrane.

RAPID ADAPTIVE RESPONSE TO ALTERED AMINO ACID
 UPTAKE: INCORPORATION OF PRE-FORMED TRANSPORTER
 FOR Na⁺ - TAURINE ACCUMULATION INTO RAT RENAL
 BRUSH BORDER MEMBRANE VESICLES (BBMV). Russell W.
 Chesney, Kent Jolly, Chris Iwahashi, Department
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 Davis, Ca 95616

The renal adaptive response to varied intake
 of sulfur amino acids is demonstrated by an
 increase in the initial rate Na⁺ - taurine (T)
 symport by rat renal BBMV after 8-14 days of a
 low methionine diet (LTD). A high taurine diet
 (3%) (HTD) reduces Na⁺ - T symport. Fasting for
 3 days, which depletes renal tubule cell T
 content, also enhances Na⁺ - T symport both
 initially (15s) and throughout the overshoot
 (Chesney et al J Clin Invest 76:2213, 1985).
 This study examines the possibility that a rapid
 onset adaptive response is expressed in BBMV with
 the increased Na⁺ T symport reflecting
 incorporation of pre-formed symporter into
 membranes, rather than new synthesis.

Rats fed the LTD for 14 days were placed on
 HTD for 18 hours; Na⁺ - T symport activity fell
 by 40%. Fasting for 4 hours restored LTD
 levels of Na⁺ - T symport activity (92 p moles/mg
 prot/15s). Colchicine (0.6 mg) was injected
 prior to fasting in a group of rats, since it
 will block the incorporation ("import") of pre-
 formed symporter into the membrane. Colchicine
 alone did not inhibit Na⁺ - T symport.
 Colchicine injected animals had a pattern of BBMV
 uptake similar to that found in animals switched
 to the HTD for 18 hours. A reduction in initial
 rate Na⁺ - T symport of 16.5% was evident, p <
 .02. These results indicate that the nephron can
 respond rapidly to changes in the intake of amino
 acids, conserving T in periods of nutrient lack
 within 4 hours and excreting excess T in 12-18
 hours in periods of surfeit. It is unlikely this
 rapid response involves new protein synthesis.
 This rapid response is expressed at the brush
 border surface and the use of colchicine
 indicates that the increase in Na⁺ - T symport
 activity is due to incorporation ("import") of
 transporter into the BBM rather than de novo synthesis.

STIMULATION OF CORTICAL COLLECTING TUBULE (CCT)
 NA-K ATPASE ACTIVITY BY ACUTE INHIBITION OF RENAL
 PROSTAGLANDIN (PG) SYNTHESIS IN THE ADRENALECTOM-
 IZED (ADX) RABBIT. H.R. Cordova*, J.P. Kokko and
 D. Marver. UTHSCD, Dept of Med, Dallas, TX.

Inhibition of renal PG synthesis with chronic
 Indo therapy results in a rise of CCT Na-K ATPase
 in the ADX rabbit (Cordova, et al., ASN-Abstract,
 1986). To explore the time course of this stimu-
 lation and the resultant change in urinary elec-
 trolyte composition, we gave Indo to ADX rabbits
 twice (0 and 3 hrs) during a 6 hr period. CCT's
 were dissected, freeze dried and enzyme activi-
 ties monitored. This dose of Indo (5mg/kg x2)
 decreased PG excretion rate (ng/6hrs): control
 (n=4); 416±121; Indo(n=5); 97±25, p<0.05. More-
 over, to rule out mineralocorticoid-like effect
 of Indo, spiro lactone (SC-26304)(1.5mg/kg) was
 given 30' and 15' before each Indo dose to an-
 other group of rabbits. Results as follows:

Grp	n	Rx	U _{Na} V Na-K ATPase Mg ATPase		
			meq·10/6h	moles P _i /kg	dry wt/hr
1	7	Vehicle	102.3 ± 8.4	0.94 ± 0.29	7.76 ± 0.68
2	9	Indo	67.1 ± 11.2*	2.2 ± 0.39*	7.19 ± 0.86
3	6	Indo+SC-26304	51.5 ± 8.1†	2.46 ± 0.57*	8.59 ± 0.96

*p<0.05 vs Group 1; †p<0.002 vs Group 1.

As a control, in another group of rabbits aldost-
 erone (Aldo) (10μg/kg iv x2) (Group 4) or Aldo+SC
 26304 (Group 5) was given in a similar way and
 the resultant CCT Na-K ATPase was: Group 4 (n=3);
 6.73±0.47 and Group 5 (n=5); 2.45±0.26 (p<0.002).
 We conclude that acute inhibition of renal PG
 synthesis results in stimulation of CCT Na-K
 ATPase and a decrease in urinary Na excretion
 rates which were not blocked by spiro lactone.

ROLE OF RENAL NERVES IN CONGESTIVE HEART FAILURE. G.F. DiBona and L.L. Sawin*. Dept. Int. Med., Univ. Ia. Col. Med. & VAMC, Iowa City, IA.

In congestive heart failure (CHF), the natriuretic response to both endogenous and exogenous atrial natriuretic peptide (ANP) is attenuated; similarly, the natriuretic response to po or iv NaCl loading, which further increases plasma ANP concentrations, is also attenuated. We examined the role of the renal nerves in conscious rats with CHF due to left coronary artery ligation - myocardial infarction (LVEDP>15mmHg). Conscious 4 wk. CHF rats with chronic renal denervation (RDNX) or without (SHAM) were given po or iv 0.9% NaCl loads.

	% Na Load Excreted/2 hrs			P vs. CHF-SHAM
	N	PO	IV	
Control (C)	7	68±5	72±8	<.01
CHF-SHAM	12	13±4	16±4	
CHF-RDNX	12	47±11	44±9	<.01

To confirm elevated renal sympathetic nerve activity (RSNA), direct measurements of RSNA were made in conscious CHF rats during iv 0.9% NaCl (5% bwt/30 min). Basal RSNA was higher (67±9%) in CHF than C. There was a greater decrease in RSNA in C (-41±4%) than in CHF (2 wk: -27±4%, 3 wk: -16±3%) despite similar increments in right atrial pressure.

Conclusion: Increased renal sympathetic nerve activity attenuates the natriuretic response to NaCl loading in conscious rats with congestive heart failure.

ISO-OSMOTIC CELL SWELLING INDUCED BY PROPIONATE. G.M. Feldman, F.N. Ziyadeh, G.W. Booz,* A. Kleinzeller.* Dept. of Med. & Physiol., U. of Pa., Thos. Jefferson U., VAMC, Phila., PA., & Mt. Desert Island Bio. Lab., Salsbury Cove, ME.

Cell Volume is usually maintained in iso-osmotic medium unless Na-K-ATPase activity is altered. An exception is cell swelling in iso-osmotic medium containing salts of weak acids. We studied this form of volume regulation in slices from shark rectal gland, a Cl transporting epithelium analogous to thick ascending limb. Substituting propionate (PROP) for Cl under iso-osmotic and iso-hydric conditions increased tissue H₂O from 3.2±0.1 to 4.2±0.1 kg H₂O/kg dry weight (DW). Cation content also increased (K by 80% and Na by 36%) while apparent cell concentrations were unaltered. These changes persisted while in PROP and reversed upon return to Cl medium. Na-K-ATPase function was normal as assessed by 22-Na efflux and 86-Rb uptake. Efflux of 86-Rb, however, was decreased from 0.79±0.07 to 0.30±0.02 mEq/kg DW/min, suggesting that PROP reduces K egress. Ouabain addition did not alter the degree of PROP swelling, but, as expected, did increase Na and decrease K cell content and concentrations. To evaluate Na entry, amiloride (1 mM) was added to PROP. It inhibited swelling, reducing H₂O, Na and K accumulation by 48%, 80% and 34%, respectively, suggesting a role for Na/H exchange in PROP swelling. Thus, in PROP induced iso-osmotic cell swelling propionic acid diffuses into cells and H exits in exchange for Na while K efflux is reduced. PROP swelling does not diminish Na-K-ATPase activity and is not inhibited by ouabain. Cell acidification may drive Na/H exchange and reduce K efflux.

MECHANISM OF EARLY RENAL POTASSIUM ADAPTATION IN THE RAT. Y. Fujii*, S.K. Mujais, and A.I. Katz., U of Chicago and Northwestern U, Chicago, Illinois

When fully developed, renal K adaptation is characterized by an increased density of Na:K pumps in the cortical collecting tubule (CCT). However, even before this adaptation occurs, rats fed a high K diet for only 1-2 days excrete all the ingested K, in part by increasing the transporting capacity of existing pump units (Clin. Res. 35:662A, 1987). To further evaluate the short-term response of the CCT pump to an increased K load we measured its ATP-hydrolytic activity (Na-K-ATPase) and K-transporting capacity (ouabain-sensitive ⁸⁶Rb uptake) after an acute KCl infusion (5µg/100g/min x 60 min). In nonadapted rats, in which U_V increased from 0.9 to 3.0 µEq/min, ⁸⁶Rb uptake was higher than in controls receiving vehicle only (31.4 ± 2.1 vs 19.2 ± 1.9 SE pmols/mm/min, p < 0.001), while Na-K-ATPase hydrolytic activity was similar (1055±30 vs 1102±42 pmols/mm/h). In K-adapted animals (7 days on high K diet), Na-K-ATPase (3080 ± 261 pmols/mm/h) and basal ⁸⁶Rb uptake (39.4 ± 1.7 pmols/mm/min) were markedly higher than in non-adapted rats; nevertheless, the acute K load led to a further increase in ⁸⁶Rb uptake (to 53.6 ± 2.9 pmols/mm/min), as U_V rose from 3.7 to 10.8 µEq/min. Preliminary experiments using the isolated perfused kidney show a significant increment in ⁸⁶Rb uptake by CCT following an acute K load (10 µEq/min), and suggest that the increased pumping activity can occur independently of systemic alterations and hormonal stimuli. These results suggest that the immediate response of CCT Na-K-ATPase to a K load consists of increased K transport by enhanced turnover rate of existing pump units, and that this process is probably an intrinsic property of the pump.

Na⁺ PUMP AND K⁺ LEAK PATHWAYS IN THE BASOLATERAL MEMBRANE OF THE THIN DESCENDING LIMB OF HENLE'S LOOP: ROLE IN VOLUME REGULATION. William B. Guggino and Anibal G. Lopes*. Dept. of Physiol., Johns Hopkins Univ., Baltimore, M.D.

The thin descending limb of Henle's loop (TDL) is normally exposed to large variations in extracellular fluid (ECF) osmolality. To determine the transport properties of the basolateral membrane and the mechanisms responsible for volume regulation in hyposmolality, rabbit TDL were perfused in vitro. Transport properties and volume regulation were assessed with video and optical techniques (J.Gen.Physiol 86:31-58). Raising bath K⁺ from 5 to 90 mM, increased cell volume by 69±25% (n=8). K⁺ induced swelling was abolished in Cl⁻ free solutions. 10⁻⁴M ouabain in the bath caused a small, 30±16% (n=13), increase in cell volume. Reducing ECF osmolality to 200 mosmol caused cells to swell rapidly to 46±7% (n=6) above the control volume (CV) in 300 mosmol. This swelling was close to that predicted (50%) from the osmolality. The initial swelling was followed by a regulatory volume decrease (RVD) to 9±5% above CV. Prior addition of 9mM Ba²⁺ to the bath inhibited RVD. The final volume in Ba²⁺ after 15 min. at 200 mosmol was 24±10% (n=6) above CV. RVD was abolished by prior addition of ouabain to the bath. The final volume in ouabain after 15 min in 200 mosmol was 65±21% (n=3) above CV. In conclusion: 1. the basolateral membrane of the TDL has both a K⁺ permeability and a ouabain sensitive Na⁺ pump, 2. TDL cells regulate volume in hyposmotic solutions, and 3. both Na⁺ pump and K⁺ leak pathways participate in cell volume regulation.

DOSE-DEPENDENT INHIBITION OF Na^+ ABSORPTION AND K^+ SECRETION BY ACTIVATORS OF PROTEIN KINASE C (PK-C) IN THE RABBIT CORTICAL COLLECTING TUBULE (CCT). S.R. Hays, M. Baum and J.P. Kokko, UTHSCD, Dallas, Tx.

Previously, we reported that $2.5 \times 10^{-8}\text{M}$ phorbol 12-myristate 13-acetate (PMA) significantly inhibits V_T , net J_{Na} and net J_{K} in the rabbit CCT (Kid. Int. 31:435A). To examine the specificity of this effect, in vitro microperfusion studies were performed to examine 1) the dose-dependency of the PMA effect; 2) the effect of L- α -1,2-dioctanoylglycerol (L- α -1,2-DOG), another activator of PK-C; and 3) the effect of a PK-C inhibitor (H-7) on the PMA effect. As shown in the table, PMA and L- α -1,2-DOG inhibit V_T , J_{Na} and J_{K} in the rabbit CCT in a dose-dependent manner ($p < 0.05$).

Protocol	% Inhibition of Control		
	J_{Na}	J_{K}	V_T
$2.5 \times 10^{-7}\text{M}$ PMA	88.9*	85.1*	87.6*
$2.5 \times 10^{-8}\text{M}$ PMA	82.1*	66.1*	65.7*
$1.6 \times 10^{-9}\text{M}$ PMA	34.6	29.6	43.7
$1.6 \times 10^{-10}\text{M}$ PMA	-1.0	4.3	9.7
$7.5 \times 10^{-5}\text{M}$ L- α -1,2-DOG	39.5*	44.6*	54.8*
$5.0 \times 10^{-5}\text{M}$ L- α -1,2-DOG	38.8	39.2	33.4
$5.0 \times 10^{-6}\text{M}$ L- α -1,2-DOG	8.4	14.8	8.8

$7.0 \times 10^{-6}\text{M}$ H-7 significantly decreased the percent inhibition produced by $2.5 \times 10^{-8}\text{M}$ PMA on J_{Na} , J_{K} and V_T . In addition, this inhibition produced by PMA was not due to endogenous PGE_2 production because $5.0 \times 10^{-5}\text{M}$ indomethacin did not alter the effect of $2.5 \times 10^{-8}\text{M}$ PMA. These results demonstrate that activation of PK-C inhibits Na^+ absorption and K^+ secretion in the rabbit CCT.

SEPARATE ACTIONS OF 2-CL-ADENOSINE (ADO) ON cAMP PRODUCTION AND Na^+ TRANSPORT BY RAT PAPILLARY COLLECTING DUCT (PCD) CELLS IN CULTURE. Russell F. Husted and John B. Stokes. Univ. of Iowa, Dept. of Medicine, Iowa City, IA.

The PCD plays an important role in determining the final Na^+ concentration in the urine and receptors for ADO have been found in the papilla. To investigate whether ADO receptors are present on PCD cells we used primary cultures grown on filter-bottom cups. Cells obtained from Sprague-Dawley rats were grown for 5 days in a serum-free medium (Dulbecco's/Ham's F12) supplemented with insulin, transferrin, triiodothyronine, hydrocortisone, and albumin. ADO-stimulated cAMP formation (measured in the presence of aspirin and RA-233 to inhibit prostaglandin production and cAMP phosphodiesterase activity) displayed typical dose-response characteristics with maximal response 5 min after ADO exposure. Thus, ADO receptors are present on PCD cells. However, exposure to ADO for 10 min had no effect on Na^+ uptake across the apical membrane. Likewise, exposure of the cells to ADO or cGMP for 1 hr had no effect on Na^+ uptake while 8-Br-cAMP caused a small increase (0.53 ± 0.05 vs. control: 0.43 ± 0.03 nmol/cm²/min). Exposure of the cells to 8-Br-cAMP, cGMP, or inosine for 24 hr had no effect on Na^+ uptake. However, exposure of the cells to 0.1 mM ADO for 24 hr resulted in a 44% inhibition of Na^+ uptake. The disparate effects of ADO on cAMP production and Na^+ transport indicates that ADO has two separate actions on these cells. The failure to reproduce the ADO-mediated reduction in Na^+ transport by cAMP or cGMP suggests that another second messenger is responsible for this effect.

LOW SODIUM ENVIRONMENT INDUCES ADAPTIVE HYPERTROPHY OF AMBYSTOMA DILUTING SEGMENT.

David J. Hirsch, Nikolas S. Morgunov*, and Ian Mobbs*. Dalhousie University Depts. of Anatomy, Medicine and Physiology/Biophysics, Halifax, Nova Scotia, Canada.

Three *Ambystoma tigrinum* were maintained for 2 weeks in distilled water (D), $[\text{Na}] = 0$ mEq/L. and six in pond water (P), $[\text{Na}] = 1.2$ mEq/L. Serum sodium, osmolarity, and total inulin space were similar in both groups, but diluting segments in group D showed evidence of increased transport capacity. Morphometric studies revealed diluting segment hypertrophy: mean tubule diameter of group D was 58 ± 3 μm versus 47 ± 2 μm in P; mean cell height of D was 21 ± 0.8 μm vs. 18 ± 0.7 μm ; apparent tubule wall volume of D 2436 ± 225 $\mu\text{m}^3/\mu\text{m}$ length vs. P 1625 ± 149 $\mu\text{m}^3/\mu\text{m}$ length in P (N=6 tubules per group, $p < 0.02$ for all comparisons). In association with tubule size changes, both Na-K-ATPase activity and ouabain-binding increased in group D isolated diluting segments compared to P:

	Na-K-ATPase nM ADP/min/mm	ouabain binding fmol/mm
P	21.7 ± 4.3 (8)	9.1 ± 0.9 (3)
D	40.2 ± 6.9 (7)*	20.7 ± 2.3 (6)**

*, $p < 0.05$ ***, $p < 0.02$

In proximal segments all parameters were similar in both groups. We conclude that in response to sodium depletion, homeostasis is maintained partially by adaptive changes in transport capacity of the diluting segment.

DA-1 RECEPTORS IDENTIFIED IN RAT RENAL HOMOGENATES, SLICES, AND MICRODISSECTED NEPHRONS WITH ¹²⁵I-SCH23982. Shohei Kinoshita*, Mark Canada*, and Robin A. Felder*, Intr. by Pedro A. Jose. The University of Virginia Medical Center, The Dept of Pathology, Charlottesville, VA.

We have identified dopamine-1 (D-1) receptors in rat proximal convoluted tubule (PCT) using a novel dopaminergic ligand ¹²⁵I-SCH-23982 (SCH) which has been described as the ligand of choice to identify D-1 receptors in the brain. We have extended these studies using SCH to quantitate dopamine receptors in rat renal cortical homogenates and renal slices and microdissected rat nephrons by quantitative autoradiography. Brain striatum was used as control. Binding of SCH to homogenates yielded a dissociation constant (K_d) of 12.2 ± 1.9 nM and a maximum receptor density (B_{max}) of 1.03 ± 0.15 (one log higher than that found in rat brain). Autoradiograms of rat renal slices showed heterogeneous SCH binding sites renal cortex but not in medulla. The rank order potency in homogenates and renal slices was similar to that found in brain. Autoradiograms of microdissected nephrons confirmed our previous report of specific binding sites in PCT; specific binding sites were also noted in pars recta, distal convoluted, and cortical collecting tubule but not in the cortical thick ascending limb.

Conclusion: SCH identifies D-1 receptors in specific segments of the nephron in cortex but not in medulla. The nephron D-1 receptor is comparable to that noted in striatum but with lower affinities.

RENAL NERVES REGULATE SODIUM-HYDROGEN ANTIPORTER ACTIVITY. Y. Kon and T. Homma*. Department of Pediatrics, Vanderbilt University, Nashville, TN.

Renal nerves have been shown by us and others to have important modulating influences on the vasomotor tone of glomerular microcirculation and reabsorptive capacity of the renal tubules in physiologic as well as pathophysiologic conditions, in particular, extracellular fluid volume depletion. Since previous micropuncture and microperfusion experiments suggest that renal nerves exert profound influences on proximal tubule reabsorption, we examined the Na/H antiporter activity in proximal tubule brush border membrane vesicles of unilaterally denervated kidneys and compared it with antiporter activity in contralateral innervated kidneys of normal euvolemic rats (n=12) and also in animals unilaterally denervated then deprived of water for 48 hrs (ECFD) (n=22). In the presence of an outwardly directed pH gradient ^{22}Na uptake at 5 sec in the denervated kidneys of euvolemic rats was 0.744 ± 0.059 nmol/mg protein versus 1.531 ± 0.097 in the contralateral innervated kidneys ($p < 0.0005$). Determination at 10 sec showed the same finding of significantly lower ^{22}Na uptake in denervated kidneys. In ECFD animals, antiporter activity was also significantly less in denervated kidneys compared with innervated kidneys in 5 and 10 sec determinations, on average by 33% and 27%, respectively. Kinetic analysis revealed that changes in the initial uptake were due to changes in V_{max} and not K_m . Of note was the finding of higher antiporter activity in denervated kidneys of ECFD when compared with denervated kidneys of euvolemic animals (1.081 ± 0.058 nmol/mg protein versus 0.744 ± 0.059 , $p < 0.005$). Since angiotensin II (AII) is increased in ECFD and acts to increase tubule reabsorption *in vivo*, we evaluated whether AII contributes to the enhanced uptake in denervated kidneys of ECFD by treating another group of ECFD unilaterally denervated rats with enalapril (3mg/kg/day)(ECFD+E)(n=16). Antiporter activity was again significantly lower in denervated vs contralateral kidneys within the ECFD+E group. By contrast, enalapril did not significantly affect ^{22}Na uptake in denervated or non-denervated kidneys of ECFD. These results suggest that renal sympathetic nerve fibers are local regulators of Na/H antiporter activity by modulating V_{max} , and this effect is independent of AII.

PASSIVE K^+ TRANSPORT IN RABBIT INNER MEDULLARY COLLECTING DUCT (IMCD) CELLS IS VIA BARIUM (Ba^{++})-SENSITIVE PATHWAYS. BC Kone,* ML Zeidel, and SR Gullans, Harvard Medical School, Boston, MA.

The pathways that mediate net K^+ transport in the rabbit IMCD are unknown. In other nephron segments, including the cortical collecting duct, Ba^{++} -sensitive and -insensitive K^+ fluxes have been observed. To identify these pathways in suspensions of IMCD cells, we measured net K^+ fluxes with an extracellular K^+ electrode. The electrode output was amplified (120X), filtered, and converted to a digital signal for computer analysis of initial rates. This system resolved changes of 3-5 nM K^+ . IMCD cells were prepared in isotonic, nonbicarbonate Ringer's solution by enzymatic digestion and density gradient centrifugation. Ba^{++} (5mM), by blocking K^+ conductive pathways, allows quantitation of K^+ transport by the Na^+ , K^+ -ATPase. Ouabain (10^{-4}M) inhibits the Na^+ , K^+ -ATPase, allowing measurement of K^+ transport through passive permeability pathways. Ba^{++} caused a net K^+ influx of 61 ± 6 nmol/mg/min, which was equivalent to the ouabain-induced efflux of 59 ± 5 nmol/mg/min (n=7). In the presence of Ba^{++} and ouabain, no net flux was observed (n=4). Furosemide (10^{-3}M), an inhibitor of Na/K/2Cl and KCl cotransport, was without effect, alone or with Ba^{++} . In conclusion, in the rabbit IMCD under isotonic conditions, conductive K^+ transport occurs primarily through Ba^{++} -sensitive pathways; Na/K/2Cl cotransport does not significantly contribute to net K^+ transport.

DECREASE IN RENAL Na-K-ATPase ACTIVITY IN BB DIABETIC RATS. Kathleen Laborde*, Laurence Bussieres*, Michele Dechaux*, and Charles Sachs. Necker Enfants-Malades Univ., Dept. of Physiol., Paris, France.

Decrease Na-K-ATPase activity in peripheral nerve and myocardial tissue has been reported in diabetic rats. To test whether this phenomenon could apply to renal Na-K-ATPase also, the enzyme activity was measured in diabetic (BB) or non diabetic (C) BB rats. The enzyme activity has been determined by quantitative cytochemistry on kidney slices in proximal convoluted tubule (PCT), medullary ascending limb of Henle (MALH) and distal convoluted tubule (DCT). Results show:

	Na-K-ATPase activity (pmol Pi/mm μm^2 /h)	
	BB	C
PCT	$0.33 \pm 0.16^{***}$	0.66 ± 0.04
MALH	1.17 ± 0.12	1.32 ± 0.02
DCT	$1.23 \pm 0.57^{***}$	1.47 ± 0.04

The possible role of Insulin (I') was studied by adding the hormone *in vitro* at various concentrations on BB kidney slices:

	Na-K-ATPase activity (pmol Pi/mm μm^2 /h)	
	BB+ I' 13 μU /ml	BB+ I' 43 μU /ml
PCT	0.29 ± 0.08	0.32 ± 0.10
MALH	$1.59 \pm 0.13^{*\#}$	$1.59 \pm 0.10^{*\#}$
DCT	1.50 ± 0.09	$1.71 \pm 0.03^{*\#}$

Renal Mg-ATPase activity was identical in diabetic and non diabetic BB rats, and was insensitive to I' addition.

In conclusion: renal Na-K-ATPase activity depression observed in diabetic BB is not modified by I' in PCT but is restored in MALH and DCT, suggesting that in these segments the I' deprivation is responsible of the observed decrease, whereas the decrease in PCT could be secondary to other metabolic or hormonal factors.

* vs C. ** $p < 0.01$; *** $p < 0.001$.

vs BB. $\#$ $p < 0.01$; $\#\#$ $p < 0.001$.

DIRECT MEASUREMENT OF THE BASOLATERAL MEMBRANE POTENTIAL (V_{bl}) FROM THE CELLS OF THE MACULA Densa (MD) AND THE THICK ASCENDING LIMB (TAL). J.Y. Lapointe*, P.D. Bell and J. Cardinal. G.R.T.M., Univ. of Montreal, Montreal, Canada and Dept. of Physiol. & Biophys., Univ. of Alabama at Birmingham, Birmingham, Alabama.

At the present time, little is known on the electrophysiology of the MD cells and on their sensitivity to changes in luminal fluid composition. To address this issue, TAL containing the MD segments and the attached glomeruli were isolated from rabbit kidneys and studied with microelectrodes in bicarbonate free Ringer solutions. In the presence of an hypotonic perfusate ($\text{NaCl} = 45$ mM) mimicking the normal luminal solution, the V_{bl} of MD cells averaged -30 ± 2.8 mV (mean \pm SEM, n = 25) a value significantly different ($p < 0.001$) from the mean V_{bl} of TAL cells (-64 ± 6.2 mV, n = 6). When the luminal NaCl concentration was increased to 150 mM, the MD cells depolarized ($p < 0.01$, n = 13) by an average of 9.0 ± 2.2 mV whereas the TAL cells did not change their potential. In this isotonic perfusate, the V_{bl} of MD cells did not change significantly following the luminal application of furosemide 50 μM (n = 8) while the TAL cells hyperpolarized by an average of 19.7 ± 5.7 mV (n = 6). Finally, neither the MD cells (n = 6) nor the TAL cells (n = 3) changed their V_{bl} significantly when the osmolarity of the hypotonic solution was restored with mannitol. These first electrophysiological results from MD cells show clearly the electrical difference between the MD and the TAL cells and suggest that the MD cells respond to the luminal NaCl concentration by a furosemide insensitive mechanism.

INTRACELLULAR K^+ AND Ca^{++} ACTIVITIES ($[K_i]$, $[Ca_i]$) AND TRANSEPITHELIAL SODIUM TRANSPORT IN THE RABBIT PROXIMAL CONVOLUTED TUBULE (PCT). R. Laprade, J.Y. Lapointe*, P.D. Bell, and J. Cardinal. Membrane Transport Research Group, Univ. of Montreal and Dept. of Physiol. & Biophys., Univ. of Alabama in Birmingham.

$[K_i]$ was measured by two methods: 1) with double-barrelled K^+ selective microelectrodes and 2) by determination of the K^+ equilibrium potential (E_K) using variations in the basolateral membrane potential (ψ_{BL}) induced by Ba^{++} during various transepithelial current injection. In control conditions, using the first method, values of $[K_i]$, E_K and ψ_{BL} were 41 mM, -63 and -36 mV ($n = 19$) while with the second method, these values were respectively, 71 mM, -78 and -48 mV ($n = 13$), thus confirming that E_K is superior to ψ_{BL} by ≈ 30 mV. On the other hand, with the first method, inhibition of active transepithelial Na transport by luminal replacement of 110 mM Na and Na-cotransported solutes, produced a ψ_{BL} transitory hyperpolarization of 21 mV with a sustained increase in $[K_i]$ of 13 mM resulting in no change in the driving force for K^+ efflux (\bar{u}_K) in the steady state. Since, in these conditions, the K^+ efflux is necessarily reduced, secondary to the lower K uptake through the Na-K-ATPase, the K^+ permeability of the basolateral membrane has to be decreased proportionally. This is probably not related to a reduction in the activity of putative Ca^{++} activated K channels, since measurements of $[Ca_i]$ with Fura-2 yielded a control activity of 102 nM and an average 13% increase ($n = 6$, $p < 0.01$) during Na transport inhibition, instead of the expected decrease.

EFFECTS OF Ca ON Na-FLUX ACROSS RAT RENAL LUMINAL MEMBRANE Jiann-Trzuo Lin, Erich Heinz and Erich E. Windhager, Cornell Univ. Med. College, Dept. of Physiol. & Biophys., New York, NY.

Effects of calcium on the sodium-flux across renal luminal membranes were investigated in studies of the amiloride-sensitive and the amiloride-insensitive Na-uptake by brush-border membrane vesicles. Vesicles were freshly prepared from rat kidney cortex with the Mg/EGTA method. In the presence of ionomycin and an outwardly directed $[H^+]$ gradient, $[Ca]$ at 0.1 mM inhibited the amiloride-sensitive Na-uptake by 40% but increased the amiloride-insensitive Na-uptake by 60%. The inhibition of Na-uptake was approximately halved when Ca was present only inside the brush border membrane vesicles, suggesting that Ca affects the Na/H exchange from both sides of the membrane. Neomycin, an inhibitor of phospholipase C, that attenuates the degradation of phosphatidylinositol, abolished the increase of the amiloride-insensitive but not the reduction of the amiloride-sensitive Na-uptake. This finding excludes the possibility that elevation of the intravesicular $[Na^+]_i$ via a Ca-induced increase in the membrane permeability to Na was responsible for the reduction of Na-uptake. These results suggest that Ca has two independent but opposing effects on Na-flux across brush-border membranes, a decrease in the rate of Na^+/H^+ exchange and an increase in P_{Na} . Whereas the Ca-induced increase in P_{Na} may be due to enhanced degradation of phosphatidylinositol the mechanism of inhibition of the Na^+/H^+ exchange by Ca remains to be elucidated.

CAMP REGULATES CHLORIDE CONDUCTANCE IN RAT RENAL BRUSH BORDER MEMBRANES. M.S. Lipkowitz and R.G. Abramson. Mt Sinai School of Medicine, NY, NY.

Recent data suggest that intracellular messengers regulate membrane conductive pathways. The effect of cAMP on membrane conductance was therefore evaluated in brush border membrane vesicles (BBMV) prepared by Mg precipitation. Cortical homogenate containing 0.2 mM PMSF (a protease inhibitor) was incubated for 30' at 37°C with or without 5 mM cAMP and 1 mM IBMX (a phosphodiesterase inhibitor). BBMV were then isolated, without additives, at 0°C. Intravesicular $[KCl]$ and conductances (G) relative to GK were determined using the potential sensitive fluorescent probe diS-C3-(5) in vesicles preloaded in 100 mM KCl for 3 h at 22°C. 3H-glucose uptake was evaluated in unloaded BBMV in media containing 100 mM NaCl.

GCl/GK was 67% greater than control (1.14 ± 0.06 vs 0.69 ± 0.06 $p < 0.005$) in BBMV prepared from cAMP/IBMX exposed homogenates. GNa/GK, GRb/GK, and Ggluconate/GK did not change. This selective cAMP-induced increment in GCl/GK resulted in an increased GCl/GNa. Since the latter would induce a more electronegative vesicle interior in the presence of an inwardly directed NaCl gradient, electrogenic Na-glucose cotransport should be stimulated. Indeed, at 1' Na-dependent glucose uptake was significantly increased by cAMP (568 ± 41 vs 398 ± 28 pmol/mg protein, $p < 0.05$); at equilibrium ($120'$), uptake was identical to the control.

These findings indicate that cAMP can regulate GCl in brush border membranes, and in this manner affect membrane potential. Thus these data suggest that, in addition to the previously reported phosphorylation of transport proteins, cAMP mediated hormones may affect proximal tubule solute transport by regulating transmembrane driving forces.

CRITICAL ROLE FOR ANGIOTENSIN II (A_{II}) IN PROXIMAL GLOMERULO-TUBULAR BALANCE. Fu-Ying Liu & Martin G. Cogan, Dept. Med. & CVRI, UCSF, CA.

A_{II} inhibition decreases bicarbonate transport in the early more than late proximal convoluted tubule (PCT) while suppressing chloride transport in both early and late PCT. Under free-flow conditions, we hypothesized the overall inhibitory effect of A_{II} blockade would be on chloride reabsorption; the late PCT could compensate for altered early PCT bicarbonate transport. Free-flow micropuncture was performed in 7 Munich-Wistar rats in control (CON) hydropenia and following 1 μ g/kg/min saralasin (SAR) i.v. SAR slightly increased SNGFR (30 ± 1 to 32 ± 1 nl/min). Filtered 1st mm-PCT End-PCT Urine

		peq/min			
HCO ₃ Deli-	CON	789±18	400±15	76±4	6±2
	very SAR	874±19*	526±19*	78±3	7±3
Cl ⁻ Deli-	CON	3443±66	3102±76	2014±41	371±34
	very SAR	3695±57*	3501±88*	2248±29*	740±83*

* $p < 0.05$ compared with control

Despite increased filtered load, SAR inhibited bicarbonate and chloride reabsorption in the early (1st mm) PCT (387 ± 22 to $348 \pm 23^*$ and 341 ± 37 to $194 \pm 69^*$ peq/mm \cdot min). Late PCT bicarbonate reabsorption increased appropriately, so end-PCT bicarbonate delivery was normal. In contrast, late PCT chloride reabsorption did not compensate for the enhanced delivery during SAR. The entire increment in filtered chloride load (≈ 250 peq/min) was transmitted to the end-PCT and to the distal tubule (delivery: 340 ± 22 to 611 ± 25 peq/min, $p < 0.001$), and a chloruresis ensued.

In conclusion, despite inhibition of early PCT acidification, the overall impact of A_{II} blockade is to markedly attenuate normal load-dependence of PCT sodium chloride reabsorption.

VOLTAGE-DEPENDENT CHLORIDE CHANNEL IN THE APICAL MEMBRANE OF INTERCALATED CELLS IN CULTURE. D. Light*, G. Fejes-Toth*, A. Naray-Fejes-Toth*, F. McCann*, T. Keller* and B. Stanton. Dept. of Physiol., Dartmouth Med. Sch., Hanover, N.H. and Henry Ford Hosp., Detroit, MI.

Patch clamp studies were conducted to characterize the conductive properties of intercalated cells (ICC) in culture. Cortical collecting ducts (CCD) from rabbit were isolated with a monoclonal antibody (McAb). Using selective culture conditions we obtained a homogeneous population (>95%) of cells from CCD that are homologous to ICC. ICC grown on Millipore filters bound a McAb specific for ICC (97% of cells) but not a McAb specific for principal cells. These ICC also bound peanut lectin and secreted acid via an electrogenic mechanism. Cl channels in the apical membrane were rarely active in the cell-attached configuration: activity increased after patch excision. In inside-out patches, the conductance of the Cl channel was 324 ± 18 pS (n=12), and $P_{Cl}:P_{Na} = 10:1$ (n=3). The channel was voltage dependent and exhibited subconductance states. The percent open time (POT) was 7% at +60 mV, 81% at +10 mV, 86% at -10 mV and 33% at -60 mV (voltage referenced to bath, n=8). The Cl channel blockers DPC (0.1 mM) and DIDS (0.5 mM) inhibited channel activity. The channel was Ca sensitive. A decrease of Ca in the bath from 1 mM to 1 μ M decreased the percent open time from 71% to 9% (+30 mV, n=3). In cell-attached patches, the Ca ionophore A23187 (2 μ M), in the presence of dB-cAMP (0.1 mM), activated Cl channels (n=2). dB-cAMP alone had no effect (n=6). Conclusion: the Cl channel in the apical membrane of ICC in culture may be regulated by intracellular Ca.

MODULATION OF Cl CHANNEL BLOCKER EFFECT IN RABBIT CORTICAL COLLECTING TUBULE (CCT) BY HCO_3^-/CO_2 . K. Matsuzaki*, V.L. Schuster, and J.B. Stokes, Univ. of Iowa, Dept. of Int. Med., Iowa City, IA.

Cl channel blockers are useful tools for studying Cl transport. In CCT, ^{36}Cl absorption occurs via an intercalated cell apical anion exchanger in series with a basolateral Cl conductance (g_{Cl}). Cl channel blockers added to the basolateral membrane in the absence of exogenous HCO_3^-/CO_2 (HEPES buffer) can completely inhibit transcellular ^{36}Cl flux (K_{Cl}). In the present studies we have found an interaction between channel blockers and HCO_3^-/CO_2 . Diphenylamine-2-carboxylate (DPC), dichloro-DPC (DCDPC), 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) and isobutyrate at concentrations sufficient to reduce K_{Cl} by 69-88% in HEPES bath reduced K_{Cl} by only 17-25% in 25 mM $HCO_3^-/6\% CO_2$ bath. In the presence of .01 mM DCDPC, isohydric increments of bath $[HCO_3^-]$ progressively reversed DCDPC blockade of K_{Cl} ; the apparent K_i for HCO_3^- was 2.1 mM. To more directly implicate an interaction at the Cl channel, we assessed blocker effects on the principal cell g_{Cl} by comparing changes in Rb tracer flux $2(K_{Rb})$ vs. transepithelial conductance ($G_{T, mS/cm^2}$). Bath DCDPC (.01 mM) reduced $G_{T, mS/cm^2}$ by 6.6 ± 1.5 in HEPES, but only 2.6 ± 0.6 in 25 mM HCO_3^- (p<.05); reductions by DPC (.1 mM) were smaller but qualitatively similar. Neither blocker changed K_{Rb} , indicating that the $\Delta G_{T, mS/cm^2}$ probably owes to effects on g_{Cl} . We conclude that the ability of Cl channel blockers to inhibit CCT intercalated and principal cell basolateral Cl conductances is impaired by HCO_3^-/CO_2 . The results suggest a regulatory site on the Cl channel which is sensitive to HCO_3^- , CO_2 , or cell pH.

REGULATORY ROLES OF INTRACELLULAR Ca^{2+} AND cAMP ON Na^+ TRANSPORT MECHANISMS IN THE EPITHELIAL CELL LINE, LLC-PK₁. Matthias Mohrmann*, Horacio F. Cantiello* and Dennis A. Ausiello. Mass. Gen. Hospital, Boston, Massachusetts.

The regulatory effects of Ca^{2+}_i , cAMP_i, and pH_i were investigated on two major amiloride inhibitable transport mechanisms for Na^+ in the tubular epithelial cell line LLC-PK₁, the Na^+/H^+ antiporter and the Na^+ channel. $^{22}Na^+$ influx and BCECF techniques were used to follow Na^+ uptake and intracellular pH changes in acidified and non-acidified cells. An increase in Ca^{2+}_i by A23187 reduced the $^{22}Na^+$ uptake in non-acidified cells via the Na^+ channel from $1,828 \pm 168$ (n=21) to $1,075 \pm 128$ (n=12) nmoles Na^+/mg DNA·min. In contrast, addition of A23187 stimulated the rate of amiloride-sensitive intracellular alkalization by 70% in preacidified cells exposed to an outwardly directed H^+ gradient ($0.11 \pm .02$ (8) vs. $0.18 \pm .04$ (8) pH units/min). Addition of AVP, IBMX or 8Br-cAMP decreased the rate of intracellular alkalization and Na^+ uptake in acidified cells by 70% with no effect on the Na^+ entry via the channel. Furthermore, cAMP blunted the Ca^{2+} stimulation of the Na^+/H^+ antiporter. An increase in $[H^+]_o$ decreased Na^+ uptake via the Na^+ channel with no effect on the Na^+/H^+ antiporter. Thus an increase in Ca^{2+}_i induces a dual effect, an increase in the activity of the Na^+/H^+ antiporter and a decrease in the activity of the Na^+ channel. In contrast, an increase in cAMP reduces the basal activity and blocks the Ca^{2+} -stimulated effect on the Na^+/H^+ antiporter. These data imply a regulatory system for Ca^{2+} , cAMP and pH by which epithelial cells may control their functional activity for Na^+ absorption and/or H^+ secretion.

FLURAZEPAM (FLZP) ANTAGONIZES THE RESPONSE OF THE MOUSE MEDULLARY THICK ASCENDING LIMB (mTALH) TO PERITUBULAR HYPERTONICITY. D.A. Molony, University of Texas Medical School, Houston, Texas.

Benzodiazepine receptors have been specifically identified along the mTALH (Am.J.Physiol.247:F718, 1984). In order to probe the effects of benzodiazepine receptor agonists on the transport properties of the mTALH, I studied the interplay of two agents in modulating transcellular conductance ($G_c, ms \cdot cm^{-2}$) and the spontaneous transepithelial voltage (V_e, mV) in isolated mouse mTALH segments. These were: peritubular hypertonicity which I have previously shown abolishes V_e and inhibits G_c via a direct inhibition of the basolateral membrane Cl^- channels (Clin. Res.35:553A, 1987); and the benzodiazepine receptor agonist FLZP (10^{-12}) which augments the conductance of brain Cl^- channels. The results of paired observations for V_e and G_c in mTALH segments studied in solutions made symmetrically hypertonic with the addition of 600 mM urea are as follows (mean value \pm SEM, n=9):

	-FLZP	+FLZP	P
V_e	1.6 ± 0.4	2.1 ± 0.4	<0.05
G_c	64.7 ± 3.7	75.7 ± 4.7	<0.001

Thus, under hypertonic conditions, FLZP increased V_e and G_c by 32% and 17%, respectively. I conclude that FLZP restores, at least partially, the fall in basolateral membrane Cl^- conductance that is mediated by peritubular hypertonicity.

CALCIUM (Ca) INCREASES SODIUM (Na) TRANSPORT BY RABBIT RENAL BRUSH BORDER MEMBRANE (BBM). G. Morduchowicz* and N. Yanagawa. Nephrology Div., Sepulveda VAMC, UCLA Sch. of Med., Los Angeles, CA. Intracellular Ca (Cai) has been proposed to regulate transepithelial Na transport by modulating apical membrane Na influx. In tight epithelia, Cai was shown to suppress apical membrane Na transport. To examine if Cai exerts similar effect in leaky epithelia, we have studied the effect of Ca on Na transport by renal BBM. BBM was isolated from rabbit renal proximal tubules (PT) preincubated for 30 min. in medium containing 0 or 1 mM Ca. BBM Na uptake was measured by rapid filtration method. In contrast to tight epithelia, the presence of Ca during PT preincubation enhanced BBM Na uptake (2.7 ± 0.2 vs. 1.6 ± 0.1 nmol/mg/30 sec., $n=7$, $P<0.01$). The higher BBM Na uptake was proton-gradient dependent and abolished by amiloride (1 mM), suggesting the involvement of Na/H antiport system. The higher BBM Na uptake was blunted by verapamil and enhanced by ionomycin during PT preincubation, confirming the effect of Cai. The stimulatory effect of Ca on BBM Na uptake was not affected by calmodulin inhibitor (trifluoperazine, 0.1 mM) during PT preincubation, but was blunted when PT were depleted of protein kinase C by 24 hr. preincubation with phorbol ester (TPA, 1 μ M). Thus, in PT, Cai enhances BBM Na/H antiport possibly through activation of protein kinase C.

CHLORIDE TRANSPORT MECHANISMS LINKED TO SODIUM IN AMBYSTOMA KIDNEY PROXIMAL TUBULE. Solange Abdunour Nakhoul* and Emile L. Boulpaep, Yale Univ. Sch. Med., Dept. of Cellular and Molecular Physiology, New Haven, CT.

Modes of Cl transport which are dependent on Na were examined in isolated perfused proximal tubules, using determinations of basolateral (V_1), luminal (V_2) and transepithelial (V_3) potential differences as well as of intracellular chloride activity (aCl_i). In control substrate-Ringer aCl_i is 13.0 ± 0.7 mM ($n=33$). When organic substrates are removed from the lumen aCl_i falls by 1.5 ± 0.6 mM ($n=23$) and V_2 hyperpolarizes by 11.6 ± 1.7 mV ($n=17$). When all Na is removed from the lumen aCl_i falls by 4.2 ± 0.6 mM ($n=19$) and V_2 hyperpolarizes by 27.3 ± 2.2 mV ($n=14$). Subsequent Na removal from bath increases aCl_i by 2.9 ± 1.0 mM ($n=9$) and V_1 depolarizes by 18.9 ± 1.2 mV ($n=9$). This latter finding suggests the operation of a basolateral Na/HCO₃-Cl/H antiporter together with an electrogenic Na/(HCO₃)_n symporter. The luminal effect of Na removal on aCl_i is apparently not caused by apical Na/Cl or Na/K/2Cl symport, since bumetanide in the lumen does not significantly affect steady-state aCl_i . Indirect dependence of aCl_i on luminal Na entry could result from a fall in aNa_i and subsequent contralateral activation of a basolateral Na/HCO₃-Cl/H antiporter. However, after addition of SITS to the bath, luminal removal of organic substrates still reduces aCl_i by 1.4 ± 0.5 mM ($n=6$) and luminal removal of all Na still reduces aCl_i by 3.5 ± 0.6 mM ($n=4$). It is concluded that the linkage of Cl transport to luminal Na is indirect, either through the effect of membrane potential on Cl channels or through the effect of changes of intracellular pH on Cl-Base⁻ antiport.

REGULATION OF RENAL OXYGEN CONSUMPTION BY PERITUBULAR PROTEIN IN THE ISOLATED PERFUSED RAT KIDNEY. K.A. Olynyk* and A.D. Baines, Institute of Medical Sciences, University of Toronto, Toronto, Ontario.

Does peritubular protein regulate proximal sodium reabsorption (TNa) by altering either passive or active transport processes? To distinguish between these two mechanisms, the effect of perfusate albumin concentration on TNa and oxygen consumption (QO₂) was examined in isolated perfused rat kidneys. Proximal TNa was estimated from Li clearance. Lowering perfusate albumin concentration (6.6 ± 0.2 to 4.0 ± 0.3 g/dL) at constant arterial pressure increased GFR (0.77 ± 0.14 to 1.01 ± 0.21 mL/min.g), decreased proximal TNa (70 ± 16 to 33 ± 14 μ mol/min.g) and increased distal TNa (36 ± 14 to 69 ± 19 μ mol/min.g) so that total TNa remained unchanged (106 ± 19 to 103 ± 16 μ mol/min.g). Whole kidney QO₂ was also unchanged (8.3 ± 1.6 to 7.7 ± 1.2 μ mol/min.g). Distal tubular anaerobic metabolism, estimated from lactate production, did not increase despite increased distal delivery of Na, and the renal response to lowering perfusate protein was unchanged when pyruvate was used as a perfusion substrate and gluconeogenesis inhibited by mercaptopicolinate. Micropuncture measurements of net proximal transtubular reabsorptive pressures ($\Delta\pi-\Delta P$) showed no change with dilution of perfusate protein concentration even though calculated reabsorption decreased 50%. The results indicate that a reduction of peritubular protein decreases Na-K-ATPase activity in the proximal tubule. Whole kidney QO₂ was conserved as increased distal TNa compensated for reduced proximal TNa. We conclude that peritubular protein stimulates oxygen-consuming transport processes in the proximal tubule of the isolated perfused rat kidney.

ALDOSTERONE AND PCO₂ ENHANCE VANADATE-SENSITIVE RB ABSORPTION IN RAT DISTAL COLON. Ronald D. Perrone and David E. McBride.* New England Medical Center, Boston, MA.

Recent studies of rabbit colon show the existence of a vanadate-sensitive K-dependent proton pump which is similar to the gastric H-K-ATPase. The existence of such a mechanism for colonic K absorption in the rat has not been determined. To this purpose, we attempted to detect pH-linked mechanisms for K absorption in voltage-clamped segments of rat distal colon using ⁸⁶Rb as a marker for K. We found that Rb absorption in Na Ringer increased directly with the *in vitro* pCO₂ level (1%-10%) in aldosterone-stimulated but not in control rats. Rb fluxes in Na-free Ringer were markedly augmented by 10% pCO₂ in both control and aldosterone-stimulated rat colon. Rb absorption was inhibited (50% decrease) by orthovanadate, SCH28080, and mucosal ouabain, but there was no effect of omeprazole, furosemide, or bumetanide. Barium applied to the serosa was also effective in inhibiting Rb absorption suggesting that Rb exit from the cell was conductive. These findings are consistent with the presence of a transport ATPase in series with conductive Rb exit. This ATPase is activated by pretreatment with aldosterone and increased *in vitro* pCO₂, and is inhibited by orthovanadate, SCH28080, and mucosal ouabain. The constellation of findings is not classic for either Na-K-ATPase or H-K-ATPase and indicates that there may be a novel colonic ATPase.

CHRONIC HYPERCALCEMIA INHIBITS THICK ASCENDING LIMB NaCl REABSORPTION IN VIVO. Linda N. Peterson, Departments of Pediatrics and Physiology, University of Ottawa, Ottawa, Ontario, Canada.

Chronic hypercalcemia (HC) induced by dietary supplementation of dihydroxycholesterol (DHT) is associated with a nephrogenic concentrating defect. The purpose of the present study is to assess the effects of HC on thick ascending limb (TAL) NaCl reabsorption in vivo using microstop-flow conductivity technique. Feeding DHT (.417 mg/100 g diet) for 3-5 days was associated with an increase in plasma calcium from 2.17 ± 0.047 (n=11) control to 2.65 ± 0.060 (n=9) HC (p<.001), no change in GFR (1.09 ± 0.035 ml/min.gkw⁻¹, Control, vs 1.04 ± 0.043 ml/min.gkw⁻¹, HC) and a decrease in maximal urine osmolality (2914 ± 77.2 , Control, vs 2259 ± 110.9 , HC, mOsm/kg H₂O p<.001). NaCl transport by the TAL was assessed by measuring the conductivity of tubular fluid emerging from the loop of Henle following 3,5,7,10,15,20,30,45 and 60 second intervals of stop-flow in perfused nephrons. After all stop-flow periods in HC rats, TAL[NaCl] did not decrease to the same extent as in controls. For example, after 30 seconds of stop-flow TAL[NaCl] was 25% greater in the HC group. Linear regression analysis of the ln [NaCl] vs time for times 0,3,5,7,10 and 15 seconds was performed on data collected in individual nephrons. The calculated intercepts were not significantly different, however, the slope of the line (α) was significantly reduced by 25% in the HC nephrons ($\alpha = .060 \pm .0034$, Control (n=14) vs $.045 \pm .0023$, HC (n=9) p<.005). These data provide evidence that TAL NaCl reabsorption is impaired in HC which would be expected to contribute to the presence of a renal concentrating defect.

MUZOLIMINE BLOCKS BASOLATERAL K⁺ CONDUCTANCE IN THE AMPHIBIAN LATE DISTAL TUBULE. G. Planelles*, B. Meilhac*, J. Teulon*, S.R. Thomas*, A. Edelman* and T. Anagnostopoulos, Hôpital Necker, Paris, France.

Muzolimine (Mu) is a diuretic which inhibits NaCl absorption in the thick ascending limb of Henle's loop. We studied the effects of Mu at a more distal site, the late distal tubule of Necturus, in vivo, by electrophysiological techniques. This segment displays large basolateral membrane (BM) potential (V_B), -70 to -85 mV, and lumen negative transepithelial potential (V_{TE}), -14 ± 1 mV, n = 25. Its BM is highly selective to K⁺, since a rise of peritubular K⁺ ([K⁺]_{pt}) from 3 to 9 mM results in 22 ± 2 mV (n = 6) V_B depolarization; V_{TE} depolarizes by 10 ± 2 mV due to circular current flow. Moreover, peritubular perfusion with BaCl₂, 2 mM, results in 33 ± 4 mV V_B depolarization, n = 4. Introduction of peritubular Mu, 1 mM, elicited a 26 ± 4 mV (n = 8) depolarization. Raising [K⁺]_{pt} from 3 to 9 mM during Mu exposure produced only 2 ± 1 mV V_B depolarization, n = 4, suggesting that Mu blocks BM K⁺ conductance (g_K). As a further check, when Mu was added to a Ba-containing solution, at the peak of the plateau Ba²⁺ depolarization, there was no further change in V_B (n = 4), suggesting that Mu affects V_B by a mechanism similar to that of Ba²⁺. Finally, we found that peritubular perfusion with Mu, 1 mM, increased luminal Cl⁻ activity (double-barreled Cl⁻ microelectrodes) by 10 ± 1 mM, n = 8, suggesting that Mu interferes with NaCl absorption. A connection between this luminal NaCl accumulation and the block of g_K remains to be established. We hypothesize that the BM Na-K-pump/g_K-leak system is affected when either of its components is altered, e.g. reduction of g_K by Mu appears to alter the Na-K pump rate.

POSSIBLE MEDIATORS OF THE Na⁺/K⁺-ATPase RESPONSE TO CHRONIC INHIBITION. B. Rayson & S. Kongsamut Dept. of Physiol. Cornell U. Med. Coll. NY, NY 10021

When rat outer medullary kidney tubular cells are subjected to chronic Na⁺/K⁺-ATPase inhibition (10⁻⁴M ouabain) the rate of Na⁺/K⁺-ATPase synthesis is increased and its degradation rate reduced. The resultant increase in active sites restores the intracellular electrolyte milieu to approximately pre-insult conditions. Both Na⁺/Ca²⁺ and Na⁺/H⁺ exchange mechanisms are demonstrable in the cells. Thus the potential response mediators include Na⁺, K⁺, H⁺ and Ca²⁺ ions. We measured free cytosolic Ca²⁺ levels ([Ca_i²⁺]) and intracellular pH during the response using Fura-2 (Tsien et al, Cell Calcium 6:145, 1985) and BCECF (Montrose & Murer, J. Membr. Biol. 93:33, 1986). The basal [Ca_i²⁺] was 210 ± 16 nM (n=9, mean±SE). The basal intracellular pH was 6.98 ± 0.03 (n=3, mean±SE). The levels of each parameter during the response are indicated below ($\bar{x} \pm SE(n)$). [Ca_i²⁺] was elevated after 6 hrs' ouabain treatment but was restored to levels not different from controls after 18 hrs. No change in pH was detectable over the course of the response.

6 hr: Ctl.	Ouab. 18hr: Ctl.	Ouab.
[Ca _i ²⁺] 163 ± 22	$272 \pm 32(6)$	256 ± 58
(nM)		$243 \pm 48(6)$
	<0.05	>0.95
pH 7.09 ± 0.10	$7.02 \pm 0.05(3)$	7.07 ± 0.04
	>0.2	$7.03 \pm 0.04(4)$
		>0.6

(Paired t test. Controls not different: ANOVA).

Changes in [Na_i⁺] and [K_i⁺] have previously been proposed as mediators of the response described. Our data suggest that maintained changes in intracellular pH do not contribute to the response. [Ca_i²⁺] however, either alone or in conjunction with [Na_i⁺] and/or [K_i⁺] may regulate the density of Na⁺/K⁺-ATPase in kidney cells.

ANOMALOUS RECTIFICATION OF POTASSIUM CHANNELS IN THE PAPILLARY SURFACE EPITHELIUM. W. B. Reeves and T. E. Andreoli. University of Texas Medical School, Houston, Texas.

We studied the properties of basolateral membranes of rabbit papillary surface epithelial (PSE) cells using intracellular microelectrodes and epithelial voltage-clamp techniques. The basolateral membrane voltage (V_{b1}, mV) was -57.2 ± 1.1 mV. The fractional resistance of the apical membrane (F_a) was 0.94 ± 0.01 , i.e., the basolateral membrane was the major conductive element of the cell. The primary conductive species of the basolateral membrane was potassium, as evidenced by a 33.9 ± 2.3 mV depolarization of V_{b1} with a 10-fold increase in basolateral [K], and by a 25.2 ± 1.4 mV depolarization of V_{b1} with 5 mM Ba⁺⁺ in the basolateral solution. The cellular resistance was measured by injecting current via intracellular microelectrodes. The input resistance was 14.1 ± 2.3 megohms. The specific resistances of the basolateral and apical membranes were 204 ohm cm² and 3203 ohm cm², respectively. Thus the transepithelial resistance of the PSE (100 ohm cm²) is determined by the paracellular pathway. The I-V relation obtained during intracellular current injection demonstrated anomalous rectification; but with 5 mM Ba⁺⁺ in basolateral solutions, the I-V relations became linear. Thus basolateral K⁺ channels may account for the anomalous rectification of basolateral membranes. In the mTALH, the apical membrane potassium channels may also demonstrate anomalous rectification (Am J Physiol. 252:F177, 1987). Thus basolateral membrane K⁺ channels of the PSE may be analogous to the apical membrane K⁺ channels of the mTALH.

SINGLE CHANNEL RECORDINGS AT THE BASOLATERAL SIDE OF ISOLATED SALAMANDER PROXIMAL TUBULE CELLS. Etienne C. Reverdin* and Emile L. Boulpaep, Yale University School of Medicine, Department of Cellular and Molecular Physiology, New Haven, CT.

Single tubule cells were isolated from *Ambystoma tigrinum* kidneys by enzymatic digestion and ultracentrifugation in Percoll gradients. A homogeneous population of proximal tubule cells with bipolar shape was documented by electronmicroscopy. Membrane potential measured with conventional microelectrodes ranged from -30 to -68 mV. Using the patch-clamp technique, 16 cell-attached patches were obtained on the basolateral larger curvature of the cells. The pipet solution contained [in mM]: 92.5 K⁺, 1 Mg⁺⁺, 1 Ca⁺⁺, 6 Na⁺, 96.5 Cl⁻, 13.4 Hepes (pH = 7.4) and the bath solution (Substrate-Ringer): 98.55 Na⁺, 2.5 K⁺, 1 Mg⁺⁺, 1.8 Ca⁺⁺, 102.7 Cl⁻, 0.5 H₂PO₄⁻, 13.4 Hepes (pH = 7.5), 2.2 Glucose, 1.8 Lactate, 0.5 Glutamine, 0.5 D,L-Alanine, 0.05 L-Glutamate, 0.2 L-Lysine. Without knowing the true cell potential, all patch potentials are indicated as relative changes of cytoplasmic potential versus pipet potential. We observed an inward rectifying, voltage dependent chloride-channel with a reversal potential (E_{rev}) of -40 mV. E_{Cl} = -41.2 mV and E_K = +7.3 mV, assuming a_{Cl}⁻ = 15.3 mM and a_K⁺ = 54.7 mM [Sackin and Boulpaep (1983)]. The plot of the open probability versus patch potential is sigmoid with 0.5 open probability at a depolarized potential of +30 mV. The data for voltage dependence could be fitted with the Boltzman equation (z = 0.78). The chord conductance between -40 and +20 mV patch potential was 50 pS. With Substrate-Ringer solution in the pipet and the bath a low conductance potassium-channel of 20 pS and 23 pS was obtained in two patches without signs of rectification or voltage dependence of the channel open probability. E_{rev} was 0 mV compared with an E_K of -85.9 mV. A similar channel of 36.5 pS was recorded when cyclamate was substituted for chloride in bath and pipet. E_{rev} was also 0 mV leading to the conclusion that this channel was identical to the other two K-channels. These observations are the first direct measurements of single channel currents at the basolateral membrane of isolated cells from proximal tubules.

STRETCH-ACTIVATED POTASSIUM CHANNELS IN THE BASOLATERAL MEMBRANE OF PROXIMAL TUBULE. Henry Sackin. Dept. of Physiol., Cornell Univ. Med. College, New York, N.Y.

A short open-time, voltage-gated potassium (K) channel (previously identified in the basolateral membrane of *Necturus* proximal tubule) is also activated by membrane stretch. Application of between 12 and 20 cm H₂O negative pressure to the patch-pipet reversibly increases mean number of open basolateral K channels (NP) by a factor of 5.3±2 in cell-attached patches (n = 4) and a factor of 13.7±5 in excised patches (n = 8). This stretch-activation does not alter channel selectivity or conductance and depends on neither the direction of K current nor the orientation of the patch ("inside-out" vs. "outside-out"). The increase in NP occurs within seconds after applying negative pressure to the patch, and is proportional to applied negative pressure. Stretch-activation of the basolateral potassium channel may play an important role in proximal tubule cell volume regulation. For example, if swelling stretches the basolateral membrane, the resulting increase in mean number of open K channels could restore cell volume by loss of K (with an accompanying anion) followed by osmotic exit of water.

HORMONE EFFECTS ON SODIUM CHLORIDE PERMEABILITY OF RAT INNER MEDULLARY COLLECTING DUCT. J.M. Sands, H. Nonoguchi*, and M.A. Knepper, NHLBI, NIH, Bethesda, MD.

A low NaCl permeability in the inner medullary collecting duct (IMCD) is thought to be important for renal NaCl conservation. It has been proposed that regulation of NaCl excretion occurs in part by hormonal effects on NaCl permeability in the IMCD. To measure NaCl permeability in isolated perfused terminal IMCDs, a bath-to-lumen NaCl concentration gradient was imposed and the resulting fluxes of Na and Cl were measured. Apparent permeabilities (P_{Cl} and P_{Na}) were calculated from measured fluxes, concentrations, and voltages using the Goldman-Hodgkin-Katz equation. Atrial natriuretic factor (rat ANF 1-28, 100 nM) significantly decreased P_{Cl} from 2.20±0.44 to 1.84±0.39 x10⁻⁵ cm/s, but did not alter P_{Na} (1.11±0.25 x10⁻⁵ cm/s). Addition of vehicle alone did not affect P_{Cl}. ANF also decreased P_{Cl} in IMCDs from deoxycorticosterone (DOC)-treated rats (1.14±0.01 to 0.98±0.02 x10⁻⁵ cm/s). Vasopressin (AVP, 10nM) did not affect P_{Cl}. The transepithelial resistance (R_t) was 39.3±9.5 ohm·cm² in IMCDs from control rats, and was increased to 62.3±6.6 ohm·cm² in tubules from DOC-treated rats. However, neither ANF or AVP affected R_t in either group.

CONCLUSIONS: 1) The results do not support the hypothesis that atrial natriuretic factor causes natriuresis by increasing the NaCl permeability of the terminal IMCD. 2) Instead, ANF significantly decreased the chloride permeability. 3) Vasopressin had no demonstrable effect on NaCl permeability. 4) Mineralocorticoid induced antinatriuresis may be due in part reduced terminal IMCD NaCl permeability.

KCL COTRANSPORT ACROSS BASOLATERAL MEMBRANE OF RABBIT PROXIMAL STRAIGHT TUBULES. Sei Sasaki, Kenichi Ishibashi*, Naoki Yoshiyama*, and Tatsuo Shigaï*. Dept. of Internal Medicine, Tokyo Medical & Dental Univ. Tokyo, Japan

Mechanisms of the transcellular Cl transport in mammalian renal proximal tubules are poorly understood. To determine whether KCl cotransport exists in the basolateral membrane of proximal tubule, isolated rabbit proximal straight tubules (S2 segment) were perfused in vitro, and intracellular activities of Cl and K (Cl_i and K_i) were measured by double barreled ion-selective microelectrode. Cl_i did not change when basolateral membrane voltage (V_{bl}) was altered (by +15mV) by application of a direct current (+1100nA) through perfusion pipette. The Cl_i changes in response to bath Cl elimination were not affected by current application as well, indicating that basolateral Cl transport is electroneutral. An increase in bath K from 5 to 20 mM reversibly increased Cl_i from 21.2 to 32.9 mM (n=5, p < 0.05), and decreased V_{bl} by 17 mV. This response of Cl_i to a change in bath K was also observed when luminal Cl was removed, or ambient Na was totally removed. K_i decreased from 60.0 to 54.6 mM (n=6, p < 0.05), and V_{bl} slightly decreased by 2 mV when bath Cl was totally replaced with gluconate.

These data demonstrate the existence of an electroneutral Na independent KCl cotransport in basolateral membrane of rabbit proximal tubules. Calculated electrochemical driving force is favorable for the movement of KCl from the cell to the peritubular fluid.

VASOPRESSIN (AVP) ENHANCES VOLTAGE-DEPENDENT POTASSIUM SECRETION. W. Schlueter,* K. Jacksack,* C. Gutterman,* DC Batlle. Northw. Univ. and Lakeside VA, Chicago, Illinois.

It has been suggested that AVP increases potassium secretion by increasing its conductance in the collecting tubule. Because AVP is known to enhance sodium transport in reptilian epithelia and in the cortical collecting tubule, we reasoned that its kaliuretic effect could be secondary to a voltage-dependent effect. If this were the case the kaliuresis should be obliterated by amiloride, an agent that decreases sodium transport in the cortical collecting tubule. This study was designed to examine whether the kaliuretic effect of AVP is voltage-dependent and cAMP mediated. The infusion of AVP (6mU/hr) resulted in a marked decrease in urine flow and an increase in urine osmolality and potassium excretion. At comparable urine flows, potassium excretion after cAMP was comparable to that seen after the administration of AVP. The data thereby suggest that the effect of AVP on potassium excretion is mediated by cAMP. In amiloride pre-treated rats the administration of AVP failed to increase potassium excretion, and the increase in urine osmolality was blunted. We conclude that the kaliuretic effect of AVP is secondary to enhancement of sodium transport in the CCT and, like its effect on H₂O transport, is cAMP mediated.

INHIBITION OF NA-K-ATPASE-DEPENDENT O₂ CONSUMPTION BY L-DOPA (D) IN RAT RENAL CORTICAL TUBULE CELL SUSPENSION (RCTCS) IS DEPENDENT ON INTRACELLULAR SODIUM CONCENTRATION (Na_i⁺).

I Serl,* BC Kone,* SR Gullans, A Aperia, BM Brenner and BJ Ballermann. Harvard Med. Sch., Boston MA and Karolinska Institute, Stockholm. Dopamine (DA), generated locally from D, inhibits Na-K-ATPase (NaK) in permeabilized rat proximal tubules under V_{max} conditions for Na⁺ (AJP, 252:F39,1987). To examine whether DA inhibits NaK in intact cortical tubule cells we studied the effect of D on ouabain-sensitive O₂ consumption (OS-QO₂) in rat RCTCS. At 140 mM extracellular Na⁺ (Na_e⁺), D inhibited OS-QO₂ in a dose-dependent manner, with a K₁ of 5x10⁻⁷ M and a maximal inhibition of 14±1% (p<0.02) at 10⁻⁵ M. Carbidopa (10⁻⁴ M), an inhibitor of the conversion of D to DA, eliminated the effect of D on OS-QO₂. D had no effect on ouabain-insensitive QO₂ or on CCCP-uncoupled respiration. Thus, locally formed DA only affects NaK-dependent QO₂. The cationophore nystatin (N) alone stimulated OS-QO₂ by 38±2% (p<0.001 vs C), while with D (10⁻⁴ M) stimulation was significantly (p<0.01) reduced to 11±2% (p<0.02 vs C) indicating that D directly inhibits maximal NaK and not Na⁺ entry. The D-induced inhibition of NaK in RCTCS was confirmed by ⁸⁶Rb-uptake studies (13±2%, p<0.05). The dependence of the D-induced NaK inhibition on Na_i⁺ was also studied using 20 and 40 mM Na_e⁺ in the presence of N. Inhibition of OS-QO₂ was significantly (p<0.05) greater at 40 mM (21±2%, p<0.01 vs C) than at 20 mM (16±3%, p<0.05 vs C) Na_e⁺. Conclusions: DA, formed from D, inhibits NaK in rat RCTCS at physiological Na_i⁺, and the magnitude of inhibition is dependent on Na_i⁺.

REGULATION OF K⁺ SECRETION IN CULTURED CORTICAL COLLECTING TUBULE (CCT) CELLS: EFFECTS OF AVP AND LUMINAL pH. P. Schmiedlin-Ren*, A. Náray-Fejes-Tóth* and G. Fejes-Tóth* (intr. by O.A. Carretero) Hypert. Res. Div., Henry Ford Hosp., Detroit, MI

Immunodissected CCT cells, when grown on permeable support, retain many differentiated transport functions. We used this model to study the regulation of K⁺ secretion by luminal pH and by arginine-vasopressin (AVP). CCT cells were cultured in a defined medium (2% Ultrosor G in RPMI 1640) and the luminal pH was altered between 5.2 and 7.7. A positive correlation was observed between the rate of K⁺ secretion and luminal pH.

lum. pH (n=5)	5.2	5.7	6.2	6.7
K ⁺ secr. (neq/cm ² /h)	72±8	116±33	334±95	430±71

K⁺ secretion did not change significantly at a luminal pH >6.7. The effect of Ba²⁺ on K⁺ secretion was also pH dependent (63% inhibition at pH 6.7 and only 14% at pH 5.2). The effect of AVP was studied on cells grown in medium without Ultrosor G, to avoid possible influences of its hormone content. AVP (10⁻⁸ M for 24 h) increased K⁺ secretion by 208%. This was accompanied by a 62% increase in Na⁺ reabsorption. Transepithelial voltage (V) at 24 h was -0.57 mV in control cultures and -11.8 mV with AVP treatment. The effect of AVP on K⁺ secretion and V persisted for at least 72 h after its removal. AVP also caused a transient rise in intracellular Ca²⁺ (iCa) from 54 to 183 nM, and a 4.2 fold increase in cAMP levels. However, the effect of AVP on K⁺ secretion could be mimicked by 8-Br-cAMP without altering iCa. We conclude that luminal acidification reduces K⁺ secretion by inhibiting a Ba²⁺-sensitive apical K⁺ conductance, and that AVP stimulates K⁺ secretion mainly through a cAMP-dependent mechanism.

SITE AND MECHANISM OF ACTION OF TRICHLORMETHIAZIDE (TCM) IN THE DISTAL NEPHRON SEGMENTS. T. Shimizu^{1,2}, K. Yoshitomi¹, M. Nakamura², M. Imai¹ (Intr. by K. Kurokawa) Dept. of Pharmacol. National Cardiovasc. Center¹, 565 Osaka, and Shionogi Co. Ltd, & Shionogi Research Laboratories², 553 Osaka Japan

In vitro microperfusion technique was used to determine the site and mechanism of action of TCM in the distal convoluted tubule (DCT), the connecting tubule (CNT), and the cortical collecting tubule (CCT) of the rabbit kidney. Addition of 10⁻⁴ M TCM to the lumen reversibly decreased unidirectional fluxes of ²²Na and ³⁶Cl across the CNT (J_{Na}, 523±49 to 345±42 pmol mm⁻¹min⁻¹; J_{Cl}, 913±123 to 657±74 pmol mm⁻¹min⁻¹) without affecting the transmural voltage (V_T). Basolateral addition of TCM was without effect on J_{Cl} in CNT. TCM did not change J_{Cl} across DCT and CCT. In CNT, amiloride at 10⁻⁵ M decreased both J_{Na} and V_T. In the presence of 10⁻⁵ M amiloride, however, TCM showed further inhibitory effect on J_{Na} without changes in V_T. To characterize further the nature of electroneutral Cl⁻ transport in CNT, the effect of TCM on J_{Cl} was examined in the absence of Na⁺ or HCO₃⁻. When Na⁺ was removed from the lumen, J_{Cl} was decreased but TCM did not cause further inhibitory effect. Elimination of HCO₃⁻ from the entire system decreased J_{Cl}. TCM caused further inhibition of J_{Cl} in the absence of HCO₃⁻. The possible contribution of double exchanger of Na⁺/H⁺ and Cl⁻/HCO₃⁻ was also excluded by the observation that TCM decreased J_{Cl} in the presence of 10⁻³ M amiloride. From these data we conclude: 1) TCM inhibits NaCl transport in CNT but not in DCT and CCT. 2) TCM inhibits the Na⁺-Cl⁻ cotransport system located in the luminal membrane of CNT.

EXTRARENAL K ADAPTATION (EKA): THE ROLE OF ALDOSTERONE (aldo). A. Spital, C. Braggins,* and R. Sterns. Univ. of Roch. Sch. of Med., Roch., NY.

An acute K load given after nephrectomy (Nx) increases plasma K (PK) less in rats previously fed a high K diet (HK) than in controls (C). This EKA occurs because adrenally intact (ADI) HK rats paradoxically become more K depleted than C when fasted before Nx (K.I. 30:532, 1986). We studied the role of aldo in EKA. In ADI rats, plasma aldo was higher in HK vs C before (377 ± 106 vs 56 ± 18 ng/dl) and after fasting 40 hrs (79 ± 12 vs 12 ± 7). With adrenalectomy (ADX) and basal aldo and corticosterone replacement during HK or C diets, HK rats became only slightly more K depleted than C when fasted for 40 hrs before Nx (PK = 3.95 ± 1.2 vs 3.96 ± 0.9 mEq/L; muscle K = 430 ± 4 vs 444 ± 3 μ Eq/gm FFDS); after Nx and I.P. KCl (2.5 mmol/kg), the increases in PK (Δ PK) did not differ in HK and C (2.11 ± 1.2 vs 2.49 ± 2.0 mEq/L)--i.e., EKA did not occur. With acute ADX and basal steroid replacement at the start of fasting, HK became more K depleted vs C (PK = 3.49 ± 2.4 vs 4.25 ± 0.8 ; muscle K = 456 ± 2 vs 486 ± 5)--but less so than in ADI rats--and EKA did not occur (Δ PK = 1.96 ± 3.2 vs 2.11 ± 5.2 mEq/L). Without fasting before Nx, EKA did not occur in ADI rats or in ADX rats given high-dose DOCA (7.5 mg/day). We conclude: high aldo levels are important for EKA, but not because of a direct effect on tissue K uptake; more likely, the hormone increases urinary K losses when dietary K is withdrawn, magnifying paradoxical K depletion, and thereby indirectly enhances tissue uptake of an acute K load.

ELECTROLYTE, UREA, AND WATER TRANSPORT IN A TWO NEPHRON CENTRAL CORE MODEL OF THE MEDULLA.

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A one nephron model (Stephenson et al, Am. J. Physiol. In press) has been extended to include both short-looped and long-looped nephrons. Variables are volume flow, Na^+ , K^+ , Cl^- , urea, hydrostatic pressure, and electric potential. The ratio of short to long-looped nephrons is one of the parameters of the model. Also permeabilities can be varied along the thin ascending (tAL) and descending limbs (DL). With rabbit permeability data, concentrations in excess of 1000 mosm/l can be generated in the papilla with no active transport, if urea permeabilities are less than 10^{-5} cm/sec. As they are increased to 10^{-4} cm/sec, the inner medullary gradient flattens completely. If the hydraulic permeability of lower DL is decreased significant gradients can be obtained with urea permeabilities of the order of 5×10^{-5} cm/sec. This gives an alternative mode of concentration without active transport, in which electrolytes diffuse out of both limbs of Henle's loop and mix with urea and water entering from the collecting duct. Urea diffuses into the lower loop and diffuses out of upper tAL. However, when water, electrolyte, and urea permeabilities are increased to the values reported for hamster (Imai et al, Kidney Int. 31:565-579, 1987) the inner medullary gradient is flat. This remains true even if active transport in tAL is postulated; the large back leak inhibits the development of any significant hypotonicity of tAL relative to core.

CYCLIC AMP-STIMULATED APICAL MEMBRANE AMPLIFICATION IN TURTLE BLADDER β CELLS: ACTIVATION OF CHLORIDE CHANNELS? David L. Stetson. The Ohio State Univ., Dept. Zoology, Columbus, Ohio.

Transepithelial HCO_3^- secretion in turtle urinary bladder is a Cl^- -dependent process that is stimulated and becomes electrogenic with increases in intracellular cyclic AMP (cAMP): cAMP appears to activate an apical Cl^- channel. This study examines the morphological effects of cAMP stimulation on turtle bladder epithelial cells by thin-section and freeze-fracture electron microscopy. Turtle bladders were stimulated in vitro with 8-bromo-cAMP and isobutyl methylxanthine (1×10^{-5} and 5×10^{-5} M respectively) or forskolin (5×10^{-5} M) and fixed for electron microscopy 5-15 min later. Thin-section samples were stained with 0.1% ruthenium red (RR) added to the fixatives to label mucosubstances on the external surfaces of plasma membranes, thus labelling compartments continuous with the extracellular space. In all stimulated tissues, frequent profiles of RR-stained membrane in the form of tubular invaginations were seen to extend from the apical membrane into the cytoplasm of β carbonic anhydrase-rich cells. These tubular membrane profiles formed a network that extended at least 5 μ m into the cytoplasm. By freeze-fracture, these invaginations appeared as pits or ice-filled craters that extended into the cell from the apical surface. No change was seen in other epithelial cell types. I conclude that activation of cAMP-dependent chloride channels may involve insertion of membrane into the apical surface from cytoplasmic compartments.

ADH IS REQUIRED FOR RAPID HYPERTONIC CELL VOLUME REGULATION IN THE PERFUSED RAT INNER MEDULLARY COLLECTING DUCT (IMCD). Adam M. Sun* and Steven C. Hebert. Brigham & Women's Hospital and Harvard Medical School, Boston, MA.

Electrophysiologic and computer-assisted video imaging techniques were used to assess the cell volume regulatory increase (VRI) responses of the rat IMCD to hypertonic media. IMCD segments were isolated from the mid-inner medullary region of barrier-raised Sprague-Dawley rats and perfused in vitro at 37°C. The transepithelial voltage and resistance were -0.7 ± 0.2 mV and 125.4 ± 30 ohm cm^2 , respectively (n=4), and unaffected by ADH. Symmetrical increases in perfusate and bath osmolality from 290 to 340 mOsm/kg H_2O using sucrose resulted in equal and rapid reductions in cell volume either with or without peritubular bath ADH (82 vs 88%, respectively, NS). However, cell volume increased rapidly to the isotonic control value only in tubules pre-incubated in ADH (100 uU/ml). The rates of VRI were: (-ADH) 0.007 ± 0.004 nL/min cm^2 or -0.2 ± 0.1 %/min (n=3); and (+ADH) 1.040 ± 0.235 nL/min cm^2 or 23.5 ± 4.6 %/min (n=6) [$p < 0.02$ -ADH vs +ADH]. An overshoot in cell volume was observed upon return to the isotonic media only in the ADH exposed tubules exhibiting a hypertonic VRI response. These studies indicate that the rat IMCD is capable of a rapid VRI response only in the presence of ADH. When taken together with our previous studies of hypertonic cell volume regulation in the mouse medullary thick ascending limb of Henle, the present results suggest that ADH may play a general role in supporting hypertonic VRI in medullary cells.

PHORBOL-ESTER IS BOUND SPECIFICALLY TO RENAL LUMINAL MEMBRANES AND STIMULATES THE Na-H ANTIporter. Z. Tabor, G.C. Mejicano*, J. Handoko, F.T. Kear* and J.A.L. Arruda, Dept. of Medicine, Univ. of Illinois and WSVA Medical Center, Chicago, IL.

Protein kinase C stimulates the Na-H antiporter in many systems. To evaluate the role of protein kinase C in the regulation of the Na-H antiporter in renal luminal membranes we characterized binding of ^3H -phorbol-12,13-dibutyrate (PE) (an activator of protein kinase C) to highly purified rabbit luminal membranes and measured its effect on the kinetics of the Na-H antiporter. There was 95% specific binding of ^3H -PE to luminal membranes and this binding was time, temperature and pH dependent with optimal binding at 4°C and pH 7.4. The ^3H -PE bound to the luminal membrane was displaced by increased concentrations of the unlabeled PE with half maximal displacement (K_{d50}) at concentration of 10^{-6} M. Scatchard analysis of the binding revealed K_d of 0.9 μM and B_{max} of 23.9 pmoles/mg protein. The effect of phorbol-12,13-dibutyrate on the kinetics of the Na-H antiporter was assessed by acridine orange quenching in control and in the presence of PE (10^{-7} - 10^{-5} M). PE stimulated the V_{max} of the Na-H antiporter by $2.6\% \pm 0.8$ at 10^{-7} M, by $25.5\% \pm 2.3$ at 10^{-6} M and by $37.8\% \pm 2.4$ at 10^{-5} M. Thus, there is a correlation between PE binding and PE stimulation of the Na-H antiporter. These data demonstrate that protein kinase C plays a role in the stimulation of the Na-H antiporter in renal luminal membranes.

CELL VOLUME REGULATION IN LATE PROXIMAL TUBULE OF NECTURUS KIDNEY. G. A. Tanner, J.-D. Horisberger*, and G. Giebisch. Dept. Physiol., Yale Univ., New Haven, Connecticut.

This study was designed to investigate cell volume regulation, and its ionic basis, in the Necturus proximal tubule. Isolated, late proximal tubules were perfused in vitro and subjected to a decrease in bath and lumen osmolality from 202 to 151 mosm/kg H_2O , produced by removing 50 mM sucrose. In 9 control tubules, cell volume, measured photographically, increased by 18 ± 5 (SD) %. In response to the same hypotonic challenge, cell volume increased by $30 \pm 11\%$ ($n=7$) with 0.5 mM bath SITS, by $30 \pm 9\%$ ($n=7$) with 2 mM bath Ba^{++} , by $21 \pm 6\%$ ($n=5$) with 0.1 mM bath bumetanide, and by $18 \pm 6\%$ ($n=9$) in the absence of bicarbonate (replaced by HEPES, pH 7.5). SITS and Ba^{++} significantly ($P < 0.01$) increased swelling in response to the hypotonic challenge, but bumetanide or removal of bicarbonate did not. In 4 tubules, 0.01 mM bath 5-nitro-2-(3-phenylpropylamino)-benzoic acid, a chloride channel blocker, had no significant effect on the swelling induced by a hypotonic challenge. A greater hypotonic challenge (osmolality decreased from 202 to 122 mosm/kg H_2O), produced by removing 80 mM sucrose, increased cell volume by $42 \pm 9\%$ ($n=6$). These results suggest that the Necturus late proximal tubule shows a modest volume regulatory response when confronted with an acute hypotonic challenge. This response probably involves loss of cell potassium (inhibited by Ba^{++}) and an accompanying anion (efflux inhibited by SITS). The identity of the anion is uncertain.

K⁺ AND Cl⁻ CHANNEL CURRENTS IN THE BASOLATERAL MEMBRANE OF RABBIT DISTAL CONVOLUTED TUBULE (DCT) J. Taniguchi, K. Yoshitomi and M. Imai. (intr. by S. Sasaki) National Cardiovascular Center, Department of Pharmacology, 565 Osaka, Japan.

To identify conductive pathways in the basolateral membrane of rabbit DCT, we recorded its membrane voltage, V_m , and single channel current, i_s , in isolated DCT. V_m was -74.9 ± 1.4 mV ($n=24$) and was depolarized by 44.0 ± 1.4 mV ($n=23$) with an increase of basolateral K^+ concentration, $[\text{K}^+]_o$, from 5 to 50 meq/l. With a reduction of $[\text{Cl}^-]_o$ from 160 to 5 meq/l, its depolarization was 4.8 ± 1.3 mV ($n=5$). These data suggest large K^+ and relatively small Cl^- conductance in this membrane.

When we clamped this membrane using patch pipette filled with 130 mM KCl, 5.4 mM CaCl₂, and K-HEPES (pH 7.4), we found 3 types of inward i_s (48.7 ± 1.4 n=9, 60.6 ± 1.4 n=7 and 76.6 pS n=2) and an outward rectified i_s (10.9 ± 0.7 pS n=6). All the inward i_s should pass through K^+ channels, since their extrapolated zero current voltage, V_o , (0 to -10 mV) was near to equilibrium voltage of K^+ . Both 48.7 and 60.6 pS K^+ channels were blocked by 0.1 mM BaCl₂ in the pipette. Open probability was ~ 0.5 at intrinsic V_m and did not show clear voltage dependence in both channels. The 76.6 pS K^+ channel with low incidence showed rapid flickering during burst period and was insensitive to Ba^{++} . An extrapolated V_o of outward rectified i_s was -71.0 ± 4.7 mV ($n=6$) and it was not blocked by 1 mM BaCl₂ in the pipette. Therefore this i_s might be Cl^- channel current.

We concluded there are 3 types of K^+ channel and a Cl^- channel in the basolateral membrane of rabbit DCT.

ATP-, ADP- AND AMP-INHIBITION OF A Ca⁺⁺-ACTIVATED NON-SELECTIVE CATION CHANNEL FROM THE BASOLATERAL MEMBRANE OF THE CORTICAL THICK ASCENDING LIMB OF HENLE'S LOOP (CAL). J. Teulon*, M. Paulais* and T. Anagnostopoulos. Hôpital Necker, Paris, France.

We have previously shown that the basolateral membrane of CAL tubules from the mouse contains a Ca^{++} -activated non-selective cation channel (NSCC) with a unit conductance of about 27 pS (Kidney Int 31: 442, 1987). We now report that several adenine nucleotides inhibit the NSCC when applied on the cytoplasmic side of the cell membrane. Standard patch-clamp techniques were applied to inside-out patches, excised from basolateral membranes of microdissected, collagenase-treated, segments of CAL from the mouse. The open probability of the channel (P_o) was successively determined in control (Ringer's solutions bathing both sides of the membrane) and several test conditions (bath Ringer's solution supplemented with a nucleotide). $[\text{Ca}^{++}]$ and $[\text{Mg}^{++}]$ were kept constant at 1 and 1.2 mM, respectively, by taking into account the stability constants of ATP and ADP for H^+ , Ca^{++} and Mg^{++} ; $[\text{Ca}^{++}]$ was checked with a calcium electrode. ATP concentrations of 1, 0.5, 0.1, $5 \cdot 10^{-2}$, 10^{-2} and $5 \cdot 10^{-3}$ mM respectively reduced P_o (% of control) to 6.5 ± 6.8 (S.D.), 5.7 ± 7.0 , 13.7 ± 18.3 , 58.3 ± 8.9 , 77.0 ± 2.4 , 92.4 ± 5.2 ($n = 2$ to 6). 50 % reduction of P_o was achieved with an ATP concentration of $6 \cdot 10^{-2}$ mM. ADP and AMP were at least as effective as ATP: 1 mM of either compound decreased P_o by 95 to 98 %. The inhibition seems specific to the adenine base since 1 mM GTP reduced P_o only by 35 %. We conclude that 1) similarly to some K^+ -channels in cardiac muscle cells and pancreatic B-cells, the NSCC of the CAL is inhibited by ATP and 2) contrary to these K^+ -channels, the NSCC is also blocked by ADP and AMP.

MULTIPLE PATHWAYS FOR POTASSIUM TRANSPORT ACROSS BASOLATERAL MEMBRANE OF RABBIT DISTAL CONVOLUTED TUBULE CELL. Heino Velázquez, David H. Ellison, and Fred S. Wright. Yale University School of Medicine and VA Medical Center, New Haven CT.

We have shown previously that the rabbit distal convoluted tubule (DCT) has a very low luminal membrane conductance; fractional resistance > 0.98 (KI 29: 409, 1986). We sought to investigate mechanisms of K exit from the cell. DCT segments dissected from rabbit kidneys were perfused in vitro. We used electrophysiologic methods to measure basolateral membrane voltage (V_{b1}) and intracellular K ion activity (aK^i); measurements were unpaired in different cells. We used rapid changes in bath [K] and addition of 1mM Ba to the bath to assess presence of basolateral membrane K conductance and intracellular location of K electrode. V_{b1} (mV), voltage sensed by the K selective electrode (V_K , mV), aK^i (mV), and the calculated equilibrium voltage for K (E_K , mV) were:

Solution	V_{b1}	V_K	aK^i	E_K	$P(V_{b1} vs E_K)$
Control	-80	-23	50	-75	NS
20 K	-46	8.8			
1 Ba	-41	19.6			

Raising bath [K] from 4 to 20 mM depolarized V_{b1} by 34 mV indicating presence of basolateral K conductance; apparent transference number is 0.8. Barium reversed polarity of V_K indicating intracellular location of K electrode. Intracellular K activity is 50 mM; calculated E_K is not above V_{b1} . We conclude: 1) Electrochemical driving force for K is not necessarily from cell to peritubular bath; 2) Conductive basolateral K flux may be into cell; 3) Because luminal membrane lacks significant K conductance, cell may have additional neutral K exit mechanisms to balance cell K uptake.

COMPARISON OF NA CL EXCRETION DURING HYPERTONIC NA CL INFUSION IN MALE AND FEMALE RATS. B.M. Wall, J.T. Crofton, L. Share. Introduced by C.R. Cooke, University of TN, Memphis, TN

To determine whether or not sexual dimorphism in the renal excretion of NaCl loads exists in rats, clearance studies were performed in conscious, chronically instrumented age-matched male (n=10) and randomly cycling female rats (n=26) during both isotonic (0.15M) and hypertonic (2.5M) NaCl infusions (0.1ml/kg/min). During the isotonic infusion mean arterial pressure (MAP), hematocrit (Hct), inulin clearance (C_{In}), osmolar clearance (C_{osm}), free water clearance (C_{H2O}), and absolute and fractional sodium excretion (FE_{Na}), did not differ between the sexes. During the hypertonic infusion MAP, plasma sodium concentration (P_{Na}), and plasma osmolality ($Posm$) increased, while Hct and total protein concentration decreased in both sexes. Urine flow rate, C_{In} , C_{osm} , and absolute and FE_{Na} increased similarly in both sexes. The percentage of the infused 2.5M NaCl load that was excreted in the urine also did not differ.

	ΔHct (%)	ΔMAP (mmHg)	$\Delta Posm$ (mOsm/kg)	Na Excreted* (%)
Male	-1.5±0.4	37±3	28±1.5	94.5±6
Female	-1.5±0.7	35±2	25±1.4	84.1±6

	C_{In}		FE_{Na}	
	(0.15M NaCl)	(2.5M NaCl)	(0.15M NaCl)	(2.5M NaCl)
*2.5M NaCl infusion	-----ml/min/100gBW----- %-----			
Male	1.06±0.1	1.38±0.1	0.20±0.03	5.6±0.4
Female	1.17±0.1	1.62±0.1	0.21±0.03	4.6±0.2†

† $p < 0.05$ compared to Male
Thus, with the exception of slightly lower FE_{Na} during 2.5M NaCl infusion in female rats, no significant sex-related differences in renal sodium handling could be demonstrated in these studies.

MECHANISM OF ALDOSTERONE-INDUCED INCREASE OF K CONDUCTANCE IN EARLY DISTAL RENAL TUBULE CELLS (EDC) OF FROGS. Wenhui Wang,* Robert M. Henderson,* and Gerhard Glöbisch. Yale Univ., Med. School, Dept. of Physiol., New Haven, Connecticut.

Aldosterone increases both intracellular pH in fused early distal tubule cells of frog kidney and the K^+ conductance (g_K) of the luminal membrane (Oberleithner, H. et al., 1987 Proc. Natl. Acad. Sci. USA 84, 1464-1468) The present study is designed to test whether intracellular pH could be a signal to increase g_K . Experiment were performed in isolated EDC of frogs using the whole-cell perfusion-recording technique (cell pH constant at 7.40) to study Ba^{++} inhibitable g_K in the physiological range of cell membrane potentials. Two cell types with high and low g_K exist. Aldosterone (1 μM , 20 hrs) increases the g_K of the cell type with high g_K from 4.2 ± 0.7 (n=9) to 12.5 ± 0.7 nS (n=10), but has no effect on the low g_K cell (control 0.7 ± 0.2 , n=7, experimental value 0.6 ± 0.2 , n=7). Incubation of cells with 0.1mM amiloride and 1 μM aldosterone results in a lower g_K (6.5 ± 0.8 nS; n=9). IF 0.1 mM ouabain is used together with 0.1 mM amiloride and 1 μM aldosterone, g_K is 3.2 ± 0.6 nS (n=8), the aldosterone-induced increase of g_K being totally abolished. The results suggest that increased g_K of EDC by aldosterone results from an increase in the number of K^+ channels recruited into the cell membrane as a result of the rise of cell pH and the elevated activity of the Na^+/K^+ pump.

EVIDENCE FOR REVERSIBLE Cl-DEPENDENT K FLUX ACROSS THE RABBIT CORTICAL COLLECTING TUBULE (CCT). C.S. Wingo and Scott Straub*, VA Medical Center and Univ. of FL, Div. of Nephrology, Gainesville, FL

We have demonstrated active Cl and K secretion by the CCT. The aim of these studies was to determine whether a component of Cl and K transport is tightly coupled. Such coupling has been observed in the rat distal nephron but K transport in the rabbit CCT is believed to be strictly conductive. Each series examines the effect of basolateral Cl removal with gluconate substitution on K flux [J_K , ($pmol \cdot mm^{-1} \cdot min^{-1}$) (-) = secretion]. First, in 12 K-loaded rabbits J_K decreased from -12.7 ± 2.2 to -8.28 ± 1.2 ($p < 0.01$) during active Na transport, while V_T and ^{22}Na efflux ($^{10}J_{Na}$) were unchanged. Second, we used one of two Na-free perfusates with 10 μM amiloride to inhibit V_T -dependent K transport and perfusate [Cl] as below.

Perfusate:	[Cl]=0 mM (N=6)	[Cl]=112 mM (N=6)		
Bath [Cl]	V_T (mV)	J_K	V_T (mV)	J_K
112 mM	6.44±1.5	0.09±0.84	5.4±2.0	-0.19±0.97
0 mM	10.3±1.5*	0.64±0.97	8.0±2.3*	2.03±0.57*

* $p < 0.05$ bath [Cl]=112 mM vs bath [Cl]=0 mM
Bath Cl removal increased V_T significantly regardless of luminal [Cl], but this same maneuver had significantly different effects on J_K depending on the luminal [Cl]. Bath Cl removal specifically stimulated K reabsorption only in the presence of a lumen-to-bath Cl gradient. Conclusions: The CCT possesses a Cl-dependent K secretory mechanism that can be influenced by the Cl gradient independent of V_T or $^{10}J_{Na}$ and can be reversed by reversal of the ambient Cl gradient. These data imply a tight coupling between a component of K and Cl fluxes, consistent with the presence of KCl co-transport in the rabbit CCT.

ROLE OF INCREASED RENAL ARTERY PRESSURE (RAP) IN CONTROL OF SODIUM EXCRETION DURING ACTH HYPERTENSION. Lori L. Woods, H. Leland Mizelle*, and John E. Hall. Univ. Miss. Med. Ctr., Dept. of Physiol., Jackson, MS

The finding that chronic ACTH hypertension is associated with increased total peripheral resistance rather than increased extracellular fluid volume has been interpreted as evidence that the hypertension is caused by an effect of ACTH to constrict the peripheral vasculature. However, ACTH may also have a primary effect to cause sodium and water retention that is overridden by its vasoconstrictor effects, which tend to cause pressure natriuresis and diuresis. The purpose of this study was to test the hypothesis that, in the presence of high circulating catecholamines, ACTH decreases renal excretory capability and that its natriuretic effects are caused by increased RAP. In 6 chronically instrumented conscious dogs, norepinephrine (NE, 0.4 ug/kg/min) alone for 5 days caused a small rise in arterial pressure (AP) from 102±6 to 115±8 mmHg. An infusion of ACTH (600 ug/day) for 7 days, superimposed upon the NE, caused a further rise in AP to a plateau of 143±9 mmHg after 5 days, while sodium excretion ($U_{Na}V$) rose from 75±3 to 94±7 mEq/day for 7 days of infusion. Glomerular filtration rate (GFR) rose from 46.7±2.8 to 69.6±3.4 ml/min. Water intake increased from 424±208 to 4727±1061 ml/day after 7 days and urine volume (UO) rose from 1100±233 to 5384±990 ml/day. In contrast, when ACTH infusion was repeated while RAP was prevented from increasing using a servo-controlled aortic occluder, $U_{Na}V$ fell from 78±3 to 47±6 mEq/day and AP rose from 108±5 to 138±5 mmHg after 7 days and did not plateau. GFR increased from 47.5±3.2 to 69.4±4.6 ml/min. Water intake rose to 2483±606 ml/day by the 2nd day and then plateaued, while UO increased from 1294±187 to 2809±565 ml/day after 2 days and then remained relatively constant. Thus, in dogs receiving a background infusion of NE, ACTH causes moderate hypertension and natriuresis. However, when RAP is not allowed to rise, ACTH causes sodium retention and severe systemic hypertension, indicating that the natriuretic effects of ACTH are caused by increased RAP and that the natriuresis blunts the chronic hypertensive effects of ACTH.

SEGMENTAL ANALYSIS OF TUBULAR Na HANDLING AND ATRIAL NATRIURETIC FACTOR (ANF) IN MINERALOCORTICOID ESCAPE (ME). B. Yee,* N. Miller,* L. Ganousis,* A. Alfrey, R. Schrier, and J. Durr.* Univ. of Colo. and VAMC, Denver, Colorado.

ME was induced by Florinef (F) 0.3 mg bid in 6 subjects. Daily plasma ANF, 24 h endogenous lithium clearance (C_{Li}), Na balance and response to water immersion (WI) before and after ME were obtained. F led to a prompt decrease in fractional distal Na excretion (FDENa) from 3.3±.4 to 1.2±.2% (mean±SEM) as assessed by C_{Li} , a positive Na balance, and gradual rise in blood volume (ΔBV) of 16.3%, ANF from 9.5±1.4 to 35.5±5.5 pg/ml and distal delivery of Na (DDNa) from 4.7±.5 to 6.9±.5 mEq/min to reach a new Na balance, all $p < .005$. ANF correlated with DDNa ($r = .95$) and ΔBV ($r = .96$) and inversely with aldosterone and renin ($r = .90$ and $.91$). At ME, a < 50 mEq Na breakfast (B) stimulated ANF and WI led to magnified ANF and DDNa responses, while distal Na reabsorption (DRNa) rose further: p at least $< .01$ vs first value*; ME vs control (C), all values $p < .01$.

	npo	B(0')	60'	120'	180'
ANF(C)	8.2	11.9	35.3*	33.7*	34.4*
ANF(ME)	32.2	66.7*	138.5*	130.1*	101.6*
DDNa(C)	mEq/min	3.6	4.8*	5.4*	5.0*
DRNa(C)	mEq/min	3.5	4.6*	5.1*	4.6*
DDNa(ME)	mEq/min	5.9	10.0*	10.5*	9.0*
DRNa(ME)	mEq/min	5.8	9.7*	9.9*	8.4*

In conclusion, ME is mediated by a decrease in proximal Na reabsorption that correlates with ΔBV and ANF. These changes are magnified during ME by WI-induced acute volume expansion. ANF seems to have no effect on decreasing DRNa.

FUNCTIONAL CELLULAR HETEROGENEITY IN THE RABBIT CONNECTING TUBULE. Koji Yoshitomi, Toshikatsu Shimizu and Masashi Imai. (Intr. by Kiyoshi Kurokawa) National Cardiovascular Center, Dept. of Pharmacology, 565 Osaka, Japan

Cellular heterogeneity was examined in the rabbit connecting tubule (CNT) perfused *in vitro* with microelectrode technique. CNT segments were identified according to their granular appearance with bifurcation in the labyrinth. In 31 tubules, random measurements of the conductive properties across the basolateral membrane revealed three cell population: 1) Intercalated (IC) cells having the basolateral membrane potential (V_b) of -24.2 ± 1.8 mV ($n=41$) and $\Delta V_b(Cl)$ of $+47.3 \pm 1.6$ mV ($n=33$) when the bath Cl^- was reduced from 140 to 5 mM. 2) CNT cells having V_b of -75.2 ± 1.6 mV ($n=35$), $\Delta V_b(K)$ of $+38.8 \pm 1.7$ mV ($n=22$) when the bath K^+ was increased from 5 to 50 mM and $\Delta V_b(Cl)$ of $+6.3 \pm 0.9$ mV ($n=27$) with bath Cl^- reduction. 3) Principal (P) cells having V_b of -75.3 ± 1.3 mV ($n=18$), $\Delta V_b(K)$ of $+1.8 \pm 0.6$ mV ($n=8$) with increase in bath K^+ and $\Delta V_b(Cl)$ of $+18.7 \pm 1.5$ mV ($n=12$) with bath Cl^- reduction. None of the tubules showed the combination of CNT cells and P cells. Therefore, 17 tubules were identified as CNT segment which consisted of CNT and IC cells, 7 tubules as initial collecting tubule (CCT) with P and IC cells, and remaining 7 tubules remained unidentified because we observed only IC cells in these tubules.

We conclude: 1) CNT cells are characterized by the presence of a large K^+ conductance and a small Cl^- conductance in the basolateral membrane. 2) Both CNT and initial CCT are present in the labyrinth.

Na^+/H^+ EXCHANGE IS INCREASED IN RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV) FROM NEONATAL RAT. Israel Zelikovic, Elizabeth Stejskal, Peter Lohstroh, Russell W. Chesney, University of California-Davis Medical Center, Department of Pediatrics, Sacramento, CA 95817

Little is known about the ontogeny of the Na^+/H^+ exchanger present in the luminal membrane of the renal tubular epithelium. We studied Na^+/H^+ antiport activity in renal BBMV prepared from the renal cortex of adult, 21 day and 7 day old rats. The time course of ^{22}Na uptake into vesicles was determined using a "rapid-reaction" millipore filtration technique. At all age groups the imposition of an outwardly directed $[H^+]$ gradient ($pHi = 5.5$ $pHo = 7.5$) resulted in an overshoot of $1mM Na^+$ uptake as compared to the accumulation in the absence of an $[H^+]$ gradient ($pHi = pHo = 7.5$). The peak of the overshoot (60 sec) was 1.4 - 1.7 higher than the equilibrium value (2 hr). $1mM Na^+$ accumulation was similar in vesicles from adult and 21 day old rats at all points during the time course. However, significantly higher initial rate (3 sec) $1mM Na^+$ uptake (1.854 ± 0.136 vs 1.484 ± 0.082 nMol/mg protein, $p < 0.05$) and peak values (4.961 ± 0.222 vs 3.649 ± 0.259 nMol/mg protein; $p < 0.001$) were found in 7 day old rats when compared to adult rats. External amiloride ($0.5mM$) significantly blunted the overshoot of Na^+ uptake at all ages and resulted in 42-55% inhibition of initial rate (3 sec) uptake and 42-60% inhibition of the peak accumulation.

We conclude that an enhanced, amiloride sensitive, Na^+/H^+ exchange activity exists in the proximal tubular luminal membrane of the neonatal rat. We postulate that this increased Na^+/H^+ exchanger may result in a rapid dissipation of the Na^+ gradient necessary for Na^+ - amino acid cotransport, thereby contributing to the aminoaciduria characteristic of early life.

BENZODIAZEPINES AND THICK ASCENDING LIMB: INHIBITION OF TRANSPORT. FN Ziyadeh and ZS Agus. Dept. of Med., Univ. of Penn., Philadelphia, Pa.

The novel peripheral benzodiazepine (BZ) receptor is present in several non-neuronal tissues including the kidney where it is localized to the thick ascending limb (TAL) and distal convoluted tubule. We have previously shown that a selective peripheral BZ agonist, Ro5-4864, inhibits ouabain-sensitive oxygen consumption (qO_2) dose-dependently, implying inhibition of active NaCl transport in suspensions of rabbit medullary TAL. To test the specificity of this response, we evaluated the effects of a CNS-active BZ analogue on TAL and the effects of Ro5-4864 in non-TAL tubules in separate fractions from Percoll gradients of tubule suspensions obtained following collagenase-hyaluronidase treatment of rabbit medullary tissue.

Maximal doses of Ro5-4864 (500 μ M) inhibited TAL qO_2 (basal $qO_2 = 26.2 \pm 1.3$ nmol O_2 /mg protein/min, n=43) by $44 \pm 5\%$, n=9 comparable to 1 mM ouabain ($47 \pm 3\%$, n=9). In contrast, 500 μ M Ro15-1788, a structurally related BZ but with CNS selectivity, did not significantly alter qO_2 compared to vehicle addition in paired TAL tubules (n=8). On the other hand, in medullary tubule suspensions derived from fractions not enriched in TAL segments, 500 μ M Ro5-4864 reduced qO_2 by only $27 \pm 4\%$, not statistically different from the reduction ($17 \pm 3\%$) with vehicle in paired tubules (n=6).

In conclusion, the selective peripheral BZ-receptor agonist and not a CNS active analogue inhibits ouabain-sensitive (transport-related) qO_2 in medullary TAL in a dose-dependent fashion. In the renal medulla, this effect appears to be confined to TAL perhaps via interaction with peripheral BZ receptors localized to this nephron segment. We propose that this receptor may be involved in modulating TAL transport function.

RENAL PHYSIOLOGY— OTHER SOLUTES AND WATER

DEVELOPMENT OF BRUSH BORDER (BB) MEMBRANE GLUCOSE TRANSPORT AND ENZYME ACTIVITY. J.C. Beck, M.S. Lipkowitz, and R.G. Abramson. Mt. Sinai School of Medicine, NY, NY.

The developmental mechanism for glucose transport was studied in renal brush border membrane vesicles (BBMV) prepared by Mg precipitation from fetal (F) and adult (A) rabbits. The kinetics of the Na⁺-dependent glucose transporter (assayed in 0.05 - 1 mM glucose) and specific binding of [³H]-phlorizin were studied under voltage-clamped conditions. Neither the Km for glucose [0.26±0.03 mM (A); 0.36±0.07 mM (F)] nor the Kd for phlorizin [0.57±0.09 (A); 0.41±0.08 μ M (F)] were significantly different in adult and fetal BBMV. However, the Vmax of the adult glucose transporter was 2.2X that of the fetal carrier [9.5±1.8 vs 4.4±0.4 nmoles/min/mg protein] and the number of binding sites in the adult was 2.6X that in the fetus (156±22 vs 59.2±6.7 pmoles/mg protein). Of note, specific activities of 4 enzymes in adult and fetal homogenates also show developmental changes: while leucine-aminopeptidase was only slightly greater in the adult (240±9 vs 184±16 nmoles/min/mg protein), alkaline phosphatase, maltase, and gamma-glutamyltransferase activities increased 2.5, 4, and 5.5 fold, respectively, with maturation.

Since the developmental increase in Vmax of the glucose transporter and the increase in phlorizin binding sites are of the same magnitude, the rise in Vmax cannot be due to increased turnover of transporters. Rather, these findings imply that additional transporters insert in the BB membrane with maturation. The differential increment in the specific activity of each enzyme further suggests that mature expression of membrane proteins (enzymes and transporters) occurs at different stages of development of renal proximal tubule cells.

MONITORING OF OSMOLYTE LEVELS IN PIG PAPILLARY COLLECTING TUBULES. Y. Boulanger, P. Vinay, A. Tejedor, J. Noel and P. Legault, Université de Montréal, Montreal, Canada.

Papillary collecting ducts are surrounded by a hyperosmolar environment *in vivo* and molecules such as glycerophosphorylcholine (GPC) and sorbitol have been proposed as intracellular osmolytes capable of maintaining a high intracellular osmolality. In order to examine the osmolality-induced variations in these osmolyte concentrations, ³¹P and ¹H NMR as well as biochemical assays have been used. Isolated pig papillary collecting ducts (5 g) were inserted in dialysis microfibers and perfused by recirculating around the fibers Krebs buffer containing 5 mM glucose under normal osmolar (300 mOsm) and hyperosmolar (600 mOsm) conditions induced by adding 300 mOsm of either mannitol or NaCl in the perfusate. NMR spectra and enzymatic assays performed at the beginning and at the end of the experiment (3 hrs at each osmolality) showed a 35% decrease in ATP (from 3.0 to 1.8 mM) but no significant change in GPC (1.5 mM), sorbitol (7 mM), betaine and inositol. The same results were obtained under aerobic and anaerobic conditions, supporting O₂ consumption measurements which have shown that these tubules possess a practically anaerobic metabolism. Our results therefore suggest that the osmolytes observed do not respond rapidly to osmotic changes and are probably not responsible for the fine regulation of intracellular osmolality.

MECHANISMS INVOLVED IN THE RESISTANCE TO VASOPRESSIN INDUCED BY K⁺ DEPLETION. C.P. Carvounis, G. Carvounis, C. Bernstein*, Dept. Medicine, VAMC and SUNY HSC, Syracuse, NY.

K⁺ depletion induces resistance to Vasopressin both *in vivo* and *in vitro*. We evaluated various proposed mechanisms in the toad bladder. The possibility that prostaglandins are responsible was found untenable on grounds of substantial Vasopressin resistance of bladder bathed in K⁺-free bath even when PGE₂ production was rendered undetectable by incubation with Naproxen 10⁻⁴M (26 ± 3 vs 68 ± 5 ul/min, n=5, p<0.001).

We also found that the decreased cell K⁺ is not by itself responsible, for the following reasons: a) Incubation for 1 hr with Ouabain 10⁻⁷M had no effect on Vasopressin stimulated water flow (24.5 ± 2.4 vs 24.6 ± 3.2, n = 5) despite a profound decrease in cell K⁺. b) K⁺-free bath decreased substantially the response to Vasopressin when compared to paired hemi-bladders treated with Ouabain 10⁻³M (39.8 ± 5.8 vs 69.7 ± 6.7, n=7, p<0.005) despite similar decreases in cell K⁺. c) Lysine increases Vasopressin stimulated water flow even though it decreases cell K⁺.

Of the proposed mechanisms, an increase in intracellular Ca⁺⁺ appears to be the most likely to account for Vasopressin resistance with K⁺ depletion for the following reasons: a) There is no additive effect with agents known to increase cell Ca⁺⁺ such as elimination of serosal Na⁺; b) Vasopressin resistance is both prevented and/or reversed when the pH of both control and OK⁺ is increased. High pH is thought to decrease cell Ca⁺⁺; c) Vasopressin resistance was substantially lower with low Ca⁺⁺ Ringer's.

STRUCTURAL HETEROGENEITY ALONG THE RAT INNER MEDULLARY COLLECTING DUCT (IMCD). W.L. Clapp, K.M. Madsen, J.W. Verlander* and C.C. Tisher. Div. of Nephrology, Hypertension & Transplantation, University of Florida, Gainesville, FL.

There is physiologic evidence for axial heterogeneity along the IMCD with regard to urea and osmotic water permeability. To determine if structural heterogeneity also exists, a qualitative and quantitative morphologic analysis of the IMCD cells was performed in the IMCD₁ (outer), IMCD₂ (middle) and IMCD₃ (inner) segments. Kidneys of five male rats were fixed by *in vivo* perfusion with glutaraldehyde for scanning (SEM) and transmission (TEM) electron microscopy. The IMCD₁ consisted of principal and intercalated cells, whereas only one cell type, the IMCD cell, was present in the IMCD₂. In contrast, the IMCD₃ contained both principal and IMCD cells. By TEM, IMCD cells had more microvilli, but less basal infoldings than principal cells. Numerous free ribosomes were present in the cytoplasm and prominent coated pits were observed on the basal membrane. The lateral intercellular space was most prominent in the IMCD₂ and IMCD₃. SEM revealed numerous small microvilli, but no cilium on the luminal surface of IMCD cells. Morphometric analysis revealed that the S_V of apical and basal plasma membranes decreased from IMCD₁ to IMCD₃. However, because of an overall increase in cell size there were no major differences in absolute areas of apical or basal membranes. We conclude that major anatomical differences exist between the principal cells of the IMCD₁ and IMCD cells in IMCD₂ and IMCD₃. The former resemble principal cells in the outer medullary collecting duct, while IMCD cells appear to represent a distinct and separate cell type.

AMINO ACID TRANSPORT BY JUXTAMEDULLARY NEPHRONS IN VIVO: DISTAL REABSORPTION AND POSSIBLE RECYCLING. William H. Dantzer and Stefan Silbernagel*. Dept. Physiol., Univ. Ariz., Tucson, AZ and Physiol. Inst. Univ. Wurzburg, Wurzburg, FRG.

Previous studies suggested that amino acid reabsorption by juxtamedullary nephrons, including not only proximal tubules but also loops of Henle and distal tubules, must differ from reabsorption by superficial nephrons. To examine amino acid transport by juxtamedullary nephrons directly *in vivo et situ*, we performed free-flow micropuncture on Henle's loops, collecting ducts, and vasa recta and continuous microinfusion on Henle's loops in exposed rat papillae. Fractional deliveries (FDs) of six neutral amino acids, two acidic amino acids, and taurine to tips of Henle's loops of juxtamedullary nephrons could be substantially below those to early distal loops of superficial cortical nephrons, indicating greater reabsorption could occur prior to tips of Henle's loops in juxtamedullary than in superficial cortical nephrons. FDs of amino acids to tips of Henle's loops were usually much greater than to collecting ducts at the same level, suggesting reabsorption distal to the tips of Henle's loops. This was confirmed by continuous microinfusion of ascending limb of Henle's loop showing 30% reabsorption of infused glycine and glutamine distal to the tip of Henle's loop. Distal site of reabsorption is unknown, but previous studies appear to eliminate collecting ducts and failure of furosemide to block reabsorption suggests it is not involved with inorganic ion reabsorption in thick ascending limb. Reabsorption distal to the tip of Henle's loop may involve passive movement out of thin ascending limb and recycling into vasa recta and descending limb. Recycling is supported by high FDs to the tip of Henle's loop (sometimes > 1.0) for some amino acids (e.g., aspartate and serine), higher amino acid concentrations in ascending than in descending vasa recta at the same level, and high mean amino acid concentrations in vasa recta.

ADAPTATION TO TAURINE (T) DEPRIVATION IN PHOSPHATE (P) DEPLETED RATS. Shermine Dabbagh, Marilyn Epley*, Warren Diven*, Demetrius Ellis. Children's Hospital of Pgh, Univ. of Pgh., Pgh, PA.

Taurinuria has been described to occur in P depletion. The abnormality is manifest at the brush border membrane (BBM) by decreased affinity of the symport and blunting of the peak of the "overshoot" of uptake. To study the effect of T deprivation on the renal adaptive response, 40-day-old rats were placed on the following diets: 1) Normal T-normal P (P⁺T⁺); 2) Normal T-low P (P⁻T⁺); 3) Low P-low T (P⁻T⁻); and 4) Normal P-low T (P⁺T⁻). All diets are vitamin D replete and contained 1.2% Ca.

Diet	Urine T	V _{max}	K _m
P ⁺ T ⁺ (control)	4.1±0.3	101±6.5	30±2.4
P ⁻ T ⁺	9.6±1.4*	158±44.7	286±39.8*
P ⁻ T ⁻	0.2±0.05*	504±108.5*	199±36.1*
P ⁺ T ⁻	0.2±0.03*	421±51.6*	37±6.2

T deprivation caused avid renal reabsorption of T associated with lower plasma and renal cortical tissue T concentrations when compared to control (Table*; p<0.001). T uptake by BBM vesicles, prepared from the above animals, was blunted by 47.6 ± 10.35% at the peak of the "overshoot" in P⁻T⁺ and P⁻T⁻, and increased by 185% in P⁺T⁻ (p<0.001). K_m was increased by 9.47- and 6.58-fold in P⁻T⁺ and P⁻T⁻, respectively (Table*; p<0.001) and remained unchanged in P⁺T⁻. There was a 4-fold increase in V_{max} in all T depleted diets while vesicle size remained unchanged.

Thus, adaptation to T deprivation occurs in P depletion in spite of decreased affinity of the symport. The adaptive response may involve stoichiometrical changes in the T-Na⁺ carrier. (Note: *statistically significant at p<0.001).

RENAL MEDULLARY SOLUTE LOSS DURING ADH INHIBITION AND OSMOTIC DIURESIS. C.L. Davis, U of Tx HSCD and Dallas VAMC, DART, TX.

The contribution of flow rate (V) to the maintenance of the medullary solute gradient is unknown. To investigate this problem male SD rats were infused with NS (control n=7), clonidine (Cl) (as an ADH inhibitor, n=7, 2 µg/kg/h), or mannitol (M) (as an osmotic agent, n=7 200 µl 25%). One kidney was then removed at 5 min (control), 15 min Cl, or 5 min M for tissue analysis. There was no change in BP. U_{osm} reached a minimum 15 min after Cl (1550 to 522) and 5 min after M (1385 to 817). V increased from 8 to 71 µl/min with Cl and from 7 to 35 µl/min with M; control V remained unchanged at 7 µl/min. Papillary tip osmolality fell from 1084 to 548 with Cl (p<0.001) and to 676 with M (p<0.001); osmols per mg solute free dry tissue fell from 6.72 to 2.96 with Cl and to 4.63 with M (both p<0.005). No group showed an increase in tissue water content. Thus ADH inhibition results in a rapid gradient loss (15 min). A purely osmotic agent M sharply increased V (5 x control) but did not eliminate the medullary gradient. Thus transport processes influence the medullary gradient more than V.

LOCALIZATION OF DOPAMINE (DA) RECEPTORS ON BASOLATERAL PLASMA MEMBRANE VESICLES (BLMV) FROM RABBIT PROXIMAL TUBULES. S. Demassieux,* A. Cardot,* and S. Carrière. Department of Medicine, Univ. of Montreal and Maisonneuve-Rosemont Hospital Research Center, Montreal.

The natriuretic properties of DA attributed to changes in renal hemodynamics or receptor-mediated inhibition of tubular Na⁺ reabsorption involving Na⁺-K⁺ ATPase. We have therefore attempted to localize DA receptors in membranes isolated from rabbit proximal tubules (PT) using binding studies. Kidneys were perfused with Hank's buffer supplemented with vitamins, aminoacids and calf serum and containing 0.05% iron oxide. The cortex was sliced and incubated at 37°C for 45 min with 0.15% collagenase. The glomeruli were removed with a magnetic bar and a pure preparation of PT was obtained after a centrifugation using a discontinuous Percoll gradient. Brush border membrane vesicles (BBMV) and BLMV, identified with Alkaline phosphatase and Na⁺-K⁺ ATPase as marker enzymes, were purified using a discontinuous (20, 32.5 and 45%) sucrose gradient centrifugation. DA receptor binding assays were performed at 25°C with ³H-SCH 23390 as DA₁ receptor antagonist, using the filtration technique through 0.45µ Millipore filters (HA type). Non-specific binding was evaluated with Haloperidol. DA₁ receptor were found to be located on BLMV (B_{max} = 933 fmoles/mg proteins) with a K_D of 3.76 nM. DA₁ receptor density is far less in BBMV (B_{max} = 131 fmoles/mg of proteins) probably due to BLMV contamination as shown by some remaining ATPase activity in the BBMV fraction.

AMILORIDE-SENSITIVE ACTIVE UREA TRANSPORT IN BUFO MARINUS SKIN. G. Dytko* and L.B. Kinter, Smith Kline & French Labs., Dept. of Pharmacology, Swedeland, PA, USA.

Active urea transport takes place across the skin of the euryhaline toad via an amiloride-sensitive, Na-insensitive pathway (Rapoport et al., *Kidney Int.* 31:450, 1987). We have examined the kinetics of urea transport in the skin and urinary bladder (UB) of tap water (TW) and salt-adapted (150 mM, HS) toads (Bufo marinus). In skins and UBs from TW toads, urea influx (J_i) was 4.74 ± .50 and 35.4 ± 6.6 and efflux was 2.37 ± .58 and 26.4 ± 4.0 nM/cm²/hr; these became 22.7 ± 6.5 and 90.8 ± 19.4 and 1.2 ± 0.2 and 93.2 ± 22.7, respectively, following HS adaptation. Transepithelial Na conductance in TW skins was 0.451 mS/cm² and decreased to 0.187 mS/cm² in HS skins (p<.001). Conductance did not change in UB. Phloretin (Phl), an inhibitor of J_{urea} in UB, had no effect on J_i in HS-adapted skins. Vasopressin (VP), which stimulates J_{urea} in UB, had no effect in HS-adapted skins. Amiloride (Am, 10⁻⁴M) inhibited J_i 50% in the presence of Na (p<.001). Summary (± SEM) of 4 paired experiments in HS toad skin:

	Cont.	Phl	Cont.	VP	Cont.	Am
J _i	27±2	24±1	25±2	25±2	17±3	9±2
SCC (% control)	106±2	---	530±150	---	---	0±1

Thus, preferential urea influx is stimulated by HS adaptation in Bufo marinus skin, but not in urinary bladder. J_i urea is not linked to changes in SCC, and is predominately via an amiloride-sensitive Na-independent pathway. The AM-sensitive J_i urea mechanism is distinguished from the AM-sensitive Na conductance, and may represent a separate transport protein.

EFFECT OF MILD CHARGE MODIFICATION OF ALBUMIN ON ITS RENAL EXCRETION IN THE RAT. D. de Zeeuw*, R. Tomasini*, M. Haas*, R. Remie*, J.J. Weening*, G.K van der Hem (intr. by L.W. Statius van Eps). Univ. of Groningen and Leiden, the Netherlands.

Cationization of albumin (pI>8) increases its glomerular sieving. Since endogenous albumin is present in two differently charged configurations: major part pI 4.6, minor part pI 6.0, we investigated the effect of mild chemical charge modification of endogenous rat albumin (RA) to pI 6.5 on its renal excretion in 8 conscious Wistar rats (with jugular vein catheter) in normal and proteinuric (adriamycin: 2mg/kg) state. With a 7 day interval fractional clearance of I-125 nRA and I-125 mRA (FnRA and FmRA) was measured by a continuous infusion method (reaching steady state plasma levels). Urine was collected by spontaneous voiding over 3 periods of ± 1 h. During normal state mean FnRA (1000 x) was .09 (range .05 - .18), whereas mRA was cleared 12x faster in each rat (FmRA: 1.07, .7 - 1.34). FnRA/FmRA ratio was .09. In proteinuric state FnRA increased ± 40x (4.21, 1.0 - 7.5), and about equalled FmRA in each rat (3.6, 1.1 - 7.1). FnRA/FmRA now was 1.4. The small change in FnRA/FmRA from control to proteinuric state may indicate a functional charge defect, but may also be explained by a small size defect which will contribute more to the clearance of nRA that is highly restricted under normal conditions. In conclusion, a slight charge modification of endogenous albumin (within the physiological range) leads to a drastic increase in renal clearance, which may have implications for understanding the causes of proteinuria with no obvious renal pathology.

CHOLINE DEHYDROGENASE, RESPONSIBLE FOR BETAINE SYNTHESIS, IS PRESENT IN INNER MEDULLA OF LONG EVANS RATS. Eric B. Grossman* and Steven C. Hebert, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Betaine has recently been implicated as an osmoregulatory substance in the inner medulla of the rat and rabbit (JBC 261:5872, 1986). In order to determine whether betaine synthesis occurs within the inner medulla, we assayed the inner medulla of Long Evans rats for the presence of choline dehydrogenase. This mitochondrial enzyme is the first and rate-limiting step in the conversion of choline to betaine and, specifically, it catalyzes the oxidation of choline to betaine aldehyde which, in turn, is rapidly oxidized enzymatically to betaine. A mitochondrial preparation of inner medulla was incubated at 37°C for one hour with ³H-choline in the presence of the electron acceptor phenazine methosulfate. All ³H-betaine aldehyde present was then chemically oxidized to ³H-betaine with H₂O₂ and NaOH. ³H-betaine was separated from choline and quantitatively recovered over an LiOH-equilibrated cation exchange column. Kinetic studies revealed a K_m of 8.2 mM and a V_{max} of 21.7 nmol/mg protein/min. Enzyme activity was 15.9±3.6 nmol/mg protein/min (n=3), a value comparable to that found in mitochondrial preparations of liver and whole kidney. Thus, the inner medulla contains the mitochondrial enzyme capable of converting choline to betaine, and therefore betaine accumulation may be the result of *in situ* synthesis. Variation in inner medullary betaine may result from modulation of choline dehydrogenase activity.

DEHYDRATION INCREASES ORGANIC OSMOLYTES IN THE RENAL INNER MEDULLA. SR Gullans, JD Blumenfeld,* JA Balschi,* RM Brenner,* CW Heilig,* and SC Hebert. Brigham and Women's Hospital and Harvard Medical Sch. Boston, MA.

The regulation of osmotically active organic solutes (osmolytes) in the inner medulla (IM) during antidiuresis is largely undefined. Perchloric acid extracts of IM were prepared from normally hydrated control rats (Wistar-Kyoto) and from rats deprived of water for 3 days. Betaine, glycerophosphorylcholine (GPC), inositol, sorbitol, amino acids (AA), and urea were measured using either H-1 NMR spectroscopy or biochemical assays. Water deprivation increased urine osmolality from 1503 ± 68 to 3748 ± 142 mosm/kg and IM urea content from 2036 ± 230 to 4689 ± 501 nmol/mg protein. Dehydration also significantly increased all the osmolytes except the AA. (mean \pm SEM, in nmol/mg protein; * $p < 0.05$).

	Sorbitol	Inositol	Betaine	GPC	AA
Control (n=9)	64 \pm 9	103 \pm 16	133 \pm 16	265 \pm 32	344 \pm 43
Dehydr. (n=13)	131* \pm 23	194* \pm 32	258* \pm 46	553* \pm 43	353 \pm 51

Although dehydrated rats had higher total osmolyte content, the relative quantities of betaine and sorbitol varied widely among animals. For example, in dehydrated rats the IM ratios of betaine/GPC and of sorbitol/GPC varied from 0.1 to 1.0 and from 0.1 to 0.4, respectively. In conclusion, dehydration increases IM osmolytes. In addition, the observed variability of betaine and sorbitol changes indicates that osmolytes can be independently modulated by, as yet, undefined factors.

POTENTIAL NEW MODEL TO STUDY ADAPTATION OF TUBULE TRANSPORT. L.L. Hamm, G. Schreiner, and D. Kohan*. Departments of Medicine and Pathology, Washington University School of Medicine, St. Louis, MO

The purpose of these studies was to develop an in vitro model to study chronic adaptations in transepithelial transport in intact nephron segments. Rabbit proximal convoluted tubules were dissected freehand and transferred to standard cell culture media. After 15-20 hours of in vitro incubation at 37° C single tubules were again transferred and studied by standard microperfusion techniques. The viability of these tubules and the feasibility of this method were indicated by ouabain inhibitable transport: mean J_v decreased from 0.25 to 0.11 nl/mm/min, $J_{glucose}$ decreased from 18 to 6 pmol/mm/min and transepithelial voltage decreased from -1.3 mV to -0.1 mV with ouabain. (All significant, n=6.) Integrity of the tubules was also indicated by the absence of inulin leaks from the perfusate to bath. The lower transport in these tubules compared to freshly perfused tubules may be a consequence of the absence of transepithelial transport during incubation (collapsed lumens during incubation). Despite this limitation, these methods should allow the study of adaptive processes which require several hours to develop, e.g. hormonal effects requiring new protein synthesis.

EFFECT OF ALTERED RENAL HEMODYNAMICS ON ALBUMIN EXCRETION BY THE NON-DIABETIC RAT KIDNEY. Valerie L. Johnson and Eduardo Perelstein. *Cornell Univ. Med. College, Dept. of Pediatrics, New York, N.Y.

Concomitant elevations of glomerular plasma flow rates and mean glomerular capillary hydraulic pressures have been implicated in hyperfiltration (HF) and the pathogenesis of diabetic glomerulopathy rather than metabolic factors. In the present study the non-diabetic isolated perfused kidney (IPK) was used to dissociate the effects of HF from the metabolic alterations of diabetes. HF of the IPK was induced with hyperglycemic perfusion media. Two collection periods preceded the glucose infusion followed by two collection periods with hyperglycemia. Acute hyperglycemia (408 ± 33 mg%, n=5) resulted in increases in GFR ($.71 \pm .03$ to $.93 \pm .05$ ml/min, $p < 0.05$), decreases in total renal resistance (R_T) ($3.56 \pm .16$ to $3.2 \pm .1$ mmHg x g kidney wt x min/ml, $p < 0.05$), and albuminuria (287 ± 64 to 367 ± 109 ug/min, $p < 0.05$). A control group observed over the same perfusion time without glucose infusion demonstrated no alteration in these parameters. Infusion of mannitol (n=5) to produce an osmotic diuresis equivalent to that of hyperglycemia did not alter GFR ($.66 \pm .06$ to $.62 \pm .06$ ml/min, $p > 0.05$) or albumin excretion (205 ± 27 to 205 ± 30 ug/min, $p > 0.05$). R_T increased ($3.25 \pm .28$ to $3.71 \pm .20$ mmHg x g kidney wt x min/ml). These experimental studies suggest that hyperglycemia per se is a factor in initiating the renal hemodynamic changes of diabetes since the fractional excretion rate of albumin (U_{alb}/GFR) was not altered with hyperglycemia (413 ± 93 vs 448 ± 142 ug/ml, $p > 0.05$) the observed albuminuria in these studies is most likely a result of the increased filtered load of albumin consequent to the increased GFR.

EFFECT OF ADH ON BUTANOL PERMEABILITY IN TOAD BLADDER. W.A. Kachadorian, J. Muller,* and V.A. DiScala. NIA, NIH, Baltimore, MD, FDA, Bethesda, MD, and Winthrop-Univ. Hosp., Mineola, NY

n-butanol permeability (Pbutanol) was measured for fully stretched paired toad bladders in the absence of an osmotic gradient before and for 40 min of ADH stimulation at either room temperature (RT) (25°C) or in the cold (10°C). In the absence of ADH Pbutanol was $1952 \pm 30 \times 10^{-7}$ cm/s at RT and $578 \pm 41 \times 10^{-7}$ cm/s in the cold; Q10 was 2.3 ± 0.1 . Pbutanol remained near pre-stimulation level for the first 20 min of ADH action at RT, then fell precipitously, reaching $819 \pm 117 \times 10^{-7}$ cm/s by the 30-40th min of treatment. In contrast, Pbutanol during stimulation in the cold steadily increased above baseline, achieving a value of $919 \pm 47 \times 10^{-7}$ cm/s by the 20-30th min, then plateaued. This latter finding correlates with the increase expected in luminal membrane area due to aggregate fusion. Our results suggest that Pbutanol can under certain conditions be affected by factors in addition to unstirred layer thickness. Absent a special transport mechanism for butanol, we believe the striking fall in Pbutanol during later stages of ADH action at RT may be due to a metabolically dependent alteration in membrane lipid organization. The physiologic significance of this time-dependent event remains to be established. Potentially it may have to do with mechanism(s) through which hormonally stimulated transmembrane movement of water and/or solutes is attenuated with time.

MICRONEPHROCALCINOSIS IN CYSTIC FIBROSIS (CF).

Katz, SM, Falkner, B, Moscola, R*, & Krueger, L*. Hahnemann University, Dept. of Pathology, Philadelphia, PA.

The Cystic Fibrosis (CF) phenotype is defined by ion abnormalities in sweat duct and airway epithelium. To describe lesions consistent with aberrant renal ion transport, we performed histopathologic and electron microscopic examination on 30 CF autopsy kidneys. The age ranged from newborn to 36 years; 20 were female, 10 were male. Histopathologic examination uncovered no major secondary renal disorder. Ca⁺⁺ stains disclosed focal microscopic tubular and peritubular deposits of Ca⁺⁺ in 27 of 30 cases (90%). No deposit was birefringent, and by histochemistry, none exhibited oxalate or urate. Electron microscopy of 3 CF cases showed electron-dense flocculent deposits, morphologically indistinguishable from Ca⁺⁺, within otherwise normal renal tubular mitochondria. By contrast, a control group of 10 cadaver kidneys harvested for renal transplantation, and a control group of 10 childhood fatalities from chronic illness unrelated to CF, each lacked Ca⁺⁺ deposits. The presence of Ca⁺⁺ in a stillborn and two neonatal CF patients supports the hypothesis that the renal deposits are not secondary to longstanding chronic infection, ischemia or disease progression. Micronephrocalcinosis in CF may indicate that the kidney mirrors the cellular ionic transport defects previously shown for sweat gland and airway epithelium. Ca⁺⁺ is an intracellular mediator of several biological cascades which regulate ionic function. The CF Ca⁺⁺ abnormality will aid in the elucidation of basic renal transport processes, and, alternately, renal physiology may be seminal to the elucidation of the CF defect.

RENAL PROTON NMR IMAGING: EFFECTS OF DIURETICS.

L.B. Kinter, G.A. Holland*, R.E. Lenkinski*, & S.K. Sarkar*, Smith Kline & French Labs., Dept. of Pharmacology, Swedeland, PA, USA and University of Pennsylvania, Phila., PA.

Proton NMR imaging sharply delineates tissues based upon their intrinsic T₁ and T₂ relaxation times. Renal T₂ images show a characteristic cortico-papillary (C-P) intensity gradient with T₂ relaxation times shortest in the cortex and longest in the papilla. We studied the effects of 2 diuretics, furosemide (F, 60 mg/kg) and the vasopressin antagonist desGlyd(CH₂)₅D-Tyr(Et)VAVP (SK&F 101926, 500 µg/kg), on C-P T₂ gradients in anesthetized 48 hr hydropenic rats using a GE 1.5 T unit. Sequential transverse image slices (3 mm) were collected at T = 0 min (basal) and repeated at 15 and 30 min post-drug or vehicle. C-P signal intensities were expressed as a ratio of that of nearby skeletal muscle (*, p<.05).

	C	P	C	P	C	P
Control	5.4 *	8.4	5.2 *	8.6	6.0 *	8.6
Furosemide	3.5 *	4.9	4.7	5.1	6.3	6.1
SK&F 101926	4.4 *	6.5	4.2 *	5.6	4.4 *	6.0

The results demonstrate the C-P T₂ gradient in 48 hr hydropenic rats. F dissipates the gradient in 15 min as cortical T₂ relaxation times increase to papillary levels. SK&F 101926 has little effect on T₂ gradients. Furosemide increases cortical water proton mobility most likely by increasing solute delivery to distal nephron segments, thereby limiting cortical water reabsorption. SK&F 101926 primarily affects water reabsorption in medullary tubule segments. We propose that renal C-P T₂ gradients are predominately an index of luminal water content.

FLUORESCENCE MEASUREMENT OF TRANSEPITHELIAL WATER PERMEABILITY IN PERFUSED COLLECTING TUBULES.

M. Kuwahara*, C.A. Berry and A.S. Verkman. Cardiovascular Research Institute, UCSF, CA.

Transepithelial osmotic (P_f) and diffusional (P_d) water permeability in isolated perfused kidney tubules is usually measured from the change in concentrations of ³H-inulin (P_f) and ³H₂O (P_d) in luminal perfusate fluids. New fluorescence methods have been developed to measure continuously P_f and P_d in perfused tubules. Isolated rabbit cortical collecting tubules (CCT) (1.4-1.8 mm length) were perfused with impermeable fluorescent indicators at 2-50 nl/min; luminal fluorescence was monitored over a 0.05 mm tubule length by epifluorescence microscopy at 200X with photodiode array detection. For P_f studies, CCT were perfused with a volume-sensitive fluorophore (fluorescein sulfonate) having concentration dependent self-quenching, and a volume-insensitive fluorophore (pyrene tetrasulfonic acid) as a reference for lumen volume. The bright fluorescence of luminal fluorophores and the continuous luminal flow eliminates photobleaching and photodynamic tubule injury. A computer based data acquisition system was implemented to measure fluorescence vs lumen perfusion rate. Results were validated in CCT perfused with 290mOsm buffer and bathed in 290 or 390mOsm buffer + 250 µU/ml vasopressin. At 22°C, P_f measured by fluorescence (120±15 x 10⁻⁴cm/s) agreed well with P_f measured by ³H-inulin (110±30 x 10⁻⁴cm/s). P_d measurement was based on the sensitivity of luminal tryptamine fluorescence to H₂O/D₂O composition where lumen/bath were perfused with D₂O/H₂O buffers. These fluorescence methods provide accurate and continuous measures of CCT water transport to facilitate physiological studies of vasopressin action.

INCREASE IN BRAIN AND KIDNEY INOSITOL DURING CHRONIC HYPERNATREMIA: ONE LESS IDIOGENIC OSMOL?

J.W. Lohr*, J. McReynolds*, M. Acara, Dept. of Med. Biophys. and Pharmacol., SUNYAB, Buffalo, NY.

During chronic hypernatremia, increases in brain electrolytes do not account for the entire increase in brain osmolality. This suggests that there is intracellular generation of undetermined solutes termed "idiogenic osmoles". We performed experiments to determine if there is an increase in the intracellular levels of the polyols inositol (I) and sorbitol (S) in the brain and kidney during acute and chronic hypernatremia. Rats were made acutely (2 hr) and chronically (72 hr) hypernatremic by intraperitoneal injection of NaCl and water restriction. After decapitation, extracts of brain and kidney were analyzed by gas chromatography-mass spectrometry for determination of inositol and sorbitol content. Data is expressed as mean ± SEM.

	N	Plasma Osmol. mOsm/kg	BRAIN		KIDNEY	
			I	S	I	S
			µmol/g wet weight			
Acute	5	367	10.5	0.06	7.9	0.50
Hypernatremia		±2	±0.5	±0.1	±0.8	±0.2
Chronic	5	375	17.4*	0.03	21.1*	0.67
Hypernatremia		±6	±0.9	±0.04	±1.9	±0.16
Control	6	301	11.4	0.07	7.7	0.29
		±1	±0.3	±0.05	±0.8	±0.06

* p < .01 compared to control.

Thus, inositol levels are increased in brain and kidney during chronic, but not acute, hypernatremia. This accumulation of inositol suggests that it may play a significant role as an intracellular osmolyte in brain as well as kidney in chronic hyperosmolar states.

CALCIUM DEPENDENCY OF HYPOTONIC VOLUME REGULATION IN PROXIMAL TUBULE. Nael A. McCarty*, Jan M. Reid*, and Roger G. O'Neil* (intr. by A.M. Kahn). U. of Texas Health Science Center, Houston, Texas.

The Ca^{++} -dependency of hypotonic regulatory volume decrease (RVD) was studied in isolated nonperfused proximal straight tubule (PST) of rabbit and proximal convoluted tubule (PCT) of killifish using video microscopy techniques. In hypotonic media (HYPO) with 1.0 or 1.5 mM Ca^{++} , cells were observed to swell, then regulate their volume to within $17.9 \pm 2.1\%$ (S.E.M., n=15) of original volume (V_0) for rabbit PST and $8.4 \pm 1.5\%$ (n=31) for fish PCT. RVD was diminished in 100 μM Ca^{++} HYPO and abolished in 10 μM Ca^{++} HYPO. Tubules exposed to low Ca^{++} HYPO (10 μM Ca^{++}) and returned to control media shrank to a volume close to V_0 . However, tubules swollen in low Ca^{++} HYPO and returned to normal Ca^{++} HYPO did not RVD, indicating a short "time window" in which high extracellular Ca^{++} is needed for RVD. To determine a role of changes in intracellular Ca^{++} (Ca_i^{++}) isolated PST were loaded with the calcium indicator, Fura-2 (Fura-2/AM incubation, 2-10 μM , 20' 37°C), and the fluorescent emission ratio (511 nm) determined with excitation at 350 and 380 nm. Preliminary data (n=3) show that Ca_i^{++} may rise transiently from near 75 to 170 nM in rabbit PST upon exposure to normal Ca^{++} HYPO, but not upon exposure to low Ca^{++} HYPO. Furthermore, in the presence of the calmodulin antagonist, trifluoperazine (1 or 10 μM), RVD is abolished in PST (n=11). It is concluded that RVD in proximal tubule is a Ca^{++} -dependent process which may involve a Ca^{++} /calmodulin-regulated pathway.

DO RABBIT CORTICAL COLLECTING TUBULES (CCT) SHOW VOLUME REGULATORY INCREASE (VRI)? E. Natke, Jr., Dept. of Med./Nephro., Winthrop-Univ. Hospital, Mineola, N.Y., (intr. by Vincent A. DiScala)

We have previously reported that non-perfused CCT lack a rapid VRI across the basolateral membrane in response to hypertonic NaCl solutions. We call this Type I VRI. Other cell types exhibit VRI only after solute-depletion in hypotonic media. We call this Type II VRI. We studied the response of non-perfused CCT to solute depletion. CCT's were immersed in isotonic HCO_3^- -buffer, suspended between a pair of glass micropipets, and maintained at 37°C. Continuous measurements of tubular diameter were made with an image splitting eyepiece. The total volume of the CCT was calculated by $\pi D^2 L/4$. When the media osmolality was lowered to 150 mOsm, CCT's swelled to $145 \pm 4\%$ of control volume (n=6). After this initial swelling, CCT's showed typical Volume Regulatory Decrease (VRD) by a 64% return to control volume in 10 min. When CCT were returned to isotonic media at 30 min, they shrank to $78 \pm 3\%$ of control volume and began to show Type II VRI by swelling back to control volume (0.4% per minute). A separate group (n=7) were perfused (lumen open) with isotonic solution and subjected to a hypertonic challenge to investigate if Type I VRI in CCT requires a "functional" lumen. To correct for the luminal volume both the outer and the luminal diameter was measured with the image splitting eyepiece. When media osmolality was increased to 450 mOsm, at 1 min CCT shrank to $86 \pm 3\%$ of control. At 30 min the relative volume did not change ($p < 0.2$). These experiments show that CCT's: 1) Lack a rapid Type I VRI 2) but show Type II VRI after solute depletion in hypotonic.

ORGANIC CATION TRANSPORT BY CULTURED RENAL CELLS. T. Dwight McKinney and K. Vincent Speeg Jr.* VA Hospital and Univ Tx Hlth Sci Ctr, San Antonio, Texas.

Organic cations undergo active proximal tubular transport. To determine if mediated transport occurs in a cultured renal epithelium with characteristics of proximal tubules, uptake of the prototypic organic cation ^3H -tetraethylammonium (TEA) across the apical cell membrane was measured in confluent LLCPK₁ cells. Uptake at 4 °C was 9% of that observed at 22 °C. Subsequent studies were conducted at 22 °C. Two minute uptake over a concentration range of 0.06-300 μM was saturable with V_{max} of 800-1000 fmol/ μg DNA and K_m of 77-105 μM . Other organic cations inhibited TEA uptake. With a TEA concentration of 60 nM, 10 μM procainamide and, 25 μM verapamil, and 1 mM cimetidine and quinidine caused 25, 47, 59, 91 and 92% inhibition, respectively. Uptake at 2-30 minutes was 50% greater with an external pH of 8 vs. 7 and was 225% greater at pH 8 vs. 6 regardless of whether preincubation pH was 6, 7 or 8. These results indicate that LLCPK₁ cells possess an active apical transport system for organic cations that is temperature and pH dependent, saturable and inhibited by other organic cations. These cells should serve as a useful model for further investigation of this transport system.

PHOSPHATE (P_i) TRANSPORT KINETICS DURING DEVELOPMENT. Richard Neiberger*, Mario Barac-Nieto and Adrian Spitzer. A. Einstein Coll. Med., Dept. Pediatrics, Bronx, NY.

We have demonstrated that, at any filtered load below T_m , the newborn guinea pig (GP) reabsorbs at least 3-fold more P_i per g kidney than the adult. In order to determine the underlying mechanism, we examined the kinetics of P_i transport in brush border membrane (BBM) vesicles prepared from kidneys of 3-14 and >57 day old GP. Animals were fed either a standard (C) diet (100 mg P_i /kg BW per day) or a diet high (E) in P_i (400 mg P_i /kg BW per day) for 4-7 days prior to the measurements. Uptake of P_i was measured by a rapid filtration technique. The results revealed a higher V_{max} ($\mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{s}^{-1}$) in the BBM of the newborn than in those of the adult whether the animals were on C (665 \pm 63 vs 145 \pm 3) or E (413 \pm 58 vs 41 \pm 11). Supplementation with P_i depressed the V_{max} relatively less in the newborn (by 38%) than in the adult (by 72%) despite the fact that the serum P_i concentration (mg/dl) increased by 83% in the newborn (from 5.9 \pm 1.1 to 10.8 \pm 1.5) and by only 22% in the adult (from 5.4 \pm 0.9 to 6.2 \pm 2.2) animals. The K_m values were not affected by age or P_i supplementation. Thus, the high P_i reabsorptive capacity observed in newborn is due to a high V_{max} of the P_i -cotransporter in the BBM of the proximal tubule. The V_{max} is less affected by changes in the diet and serum concentration of P_i in the newborn than in the adult.

THE EFFECT OF URETERAL EXCISION ON THE MAGNITUDE AND COMPOSITION OF THE CORTICOMEDULLARY (CM) INTERSTITIAL GRADIENT IN THE RAT. Thomas L. Pallone* and Rex L. Jamison, Depts. of Med., Stanford Univ., Stanford, CA and Univ. of Rochester, Rochester, NY.

The role of an intact ureter in preserving maximal urinary osmolality (Uosm) is not understood. To examine the importance of partial versus total excision of the ureter, the left kidney of the hydropenic Munich-Wistar rat was prepared for micropuncture. Uosm was measured and the left ureter either completely excised-Group I (Gp I) (N=10) or partially excised 1/2mm proximal to the papillary tip-Group II (Gp II) (N=10). Within 10 min. of excision-Period 1 (Per 1)-, a descending vas rectum (DVR) was sampled for osmolality (VROsm), sodium [Na], and potassium [K] concentration. After 45 min-Period 2 (Per 2)-another DVR was sampled and Uosm again measured, this time from collecting duct urine. The results (means only):

Gp	Per	Uosm (mOsm)	VROsm (mOsm)	[Na] (mEq/l)	[Urea] (mM)	[Urea] VROsm
I	1	2063	1742	339	1024	0.59
	2	736**	860**	211**	411**	0.46**
II	1	2038	1830	348	1094	0.59
	2	1551*	1504*	347	775**	0.49**
		++	+	++	+	

[Urea], calculated from VROsm - (1.94[Na]+2[K]

*P < .05 **P < .01 Per 1 vs. Per 2

†P < .05 ††P < .01 Gp I vs. Gp II

The results indicate that total ureteral excision alters both the magnitude of the osmotic gradient and the composition of medullary interstitial solute, causing a greater fall in [Urea] than in [Na]. The presence of the remnant ureter moderates the fall in osmotic gradient by preventing the decline in [Na].

REFLECTION COEFFICIENTS OF PROXIMAL TUBULE VESICLES DETERMINED BY SELF-QUENCHING OF AN ENTRAPPED FLUOROPHORE. D. Pearce*, P.-Y. Chen*, and A.S. Verkman. CVRI, UCSF, CA and Taipei Medical College, Taiwan.

Determination of cell or vesicle membrane reflection coefficients (σ) requires accurate measurement of volume flow early (<100 ms) after vesicles are subjected to an osmotic gradient. We have developed a new technique to accurately measure: (1) osmotic water permeability (P_f), (2) solute permeability (P_s) and (3) σ , based on the self-quenching of fluorescein sulfonate (FS), the best of a series of dyes screened for self-quenching, brightness and vesicle loading/trapping. To validate the method, rabbit renal brush border membrane vesicles were loaded with 1-10 mM FS for 12 h at 4°C and washed 3X to remove extravascular FS. FS leakage occurred over >6 h at 4°C and >30 min at 23°C. Fluorescence intensity was calibrated against vesicle volume from the time course of fluorescence decrease (ex 450, em>505) following a series of inward osmotic gradients in a stopped-flow apparatus. For vesicles containing 50 mM sucrose, 10 mM Hepes/Tris, pH 7.4 at 23°C, P_f was 0.005±0.001 cm/s, independent of osmotic gradient size (25-300 mM inward sucrose gradient), and inhibited 67% by 0.5 mM HgCl₂. For urea, P_s was 2x10⁻⁶ cm/s and σ .95±.04 based on fluorescence time course analysis and on the extravascular [urea] required to obtain zero initial volume flow (null method). These results demonstrate the usefulness of the FS-quenching method for determination of P_f , P_s and σ . More importantly, interpretation of FS data in terms of vesicle volume changes is not confounded by changes in refractive index or vesicle aggregation, which limit the usefulness of light scattering for σ and P_s determination.

EFFECT OF INTRACEREBROVENTRICULAR MANASSANTIN ON SOME RENAL FUNCTIONS OF RATS. K. V. Rao and V. N. Puri. Univ. of Florida Med. Ctr., Dept. of Medicinal Chemistry, Gainesville, FL.

Long term neuroleptic treatment of psychiatric patients is invariably associated with disturbances in water and mineral metabolism. Manassantin, a neutral nonnitrogenous atypical neuroleptic is being designed and developed in this institution for its potential as a novel neuroleptic agent for treatment of psychiatric ailments. In this study effect of intracerebroventricular (ICV) Manassantin on some renal functions of male Sprague Dawley rats (200-280 g) is reported. ICV Manassantin (10, 32, 100 µg) produced alterations in some of the renal functions of conscious rats. 10, 32 µg dose of drug produced increased in urine volume while effect was not statistically different with 100 µg treatment. Manassantin (32 µg ICV) produced increase in urine volume from 1222 + 20 g in Control to 2732 + 151 µL/hr/100 g BW in drug treated group, effect being statistically significant (P<0.01). Increase in urine output was associated with decrease in urinary osmolality (P<0.01) while urinary protein excretion was increased from 1977 + 535 µg/hr/100 g BW in control to 3026 + 596 in the drug treated group (P<0.01), urinary creatinine excretion with this tested dose was increased (P<0.01). Results of this investigation indicate that dose response curve by ICV Manassantin on some renal functions is dome bell shape and the diuretic response with 32 µg ICV dose may be due to inhibition of release of vasopressin from central hypothalamo-hypophyseal sites.

THE RELATIONSHIP OF ACTIVE UREA TRANSPORT TO PROTON GRADIENTS IN TOAD SKIN. J. Rapoport*, C. Chaimovitz*, Z. Noeh*, A. Abuful*, and R.M. Hays. Soroka Med. Center, Beersheva, Israel, and Albert Einstein Coll. of Med., Bronx, NY.

Active urea transport takes place inwardly across the skin of Bufo viridis, achieving influx/efflux ratios of 50 to 1 following 10 days of saline adaptation. Previous studies have shown that urea transport is not coupled to that of sodium, since it proceeds in the absence of sodium, and is unaffected by ouabain. We now report studies with a series of metabolic inhibitors, designed to determine the relationship of urea transport to proton gradients in the skin. The protonophore CCCP (10⁻⁴M), inhibited urea influx (J_i) by 85% (p<0.05); nigericin (25µM), a proton-potassium exchanger, inhibited J_i by 39% (p<0.01). Acidification of the epithelium by bubbling with 5% CO₂ caused an 80% inhibition of J_i (p<0.0005). Alkalinization of the epithelium by means of the stilbene DIDS (10⁻⁴M) caused a 30% increase in J_i (p<0.025). The electrochemical proton gradient may be at least partially produced by a proton-translocating ATPase, since J_i was inhibited 44% by 10⁻³M DCCD (p<0.025) and 90% by 10⁻³M N-ethylmaleimide (p<0.025). Our studies show a central role for electrochemical proton gradients in active urea transport by the toad skin.

CO-EXISTENCE OF Na^+ -DEPENDENT GLUCOSE AND FRUCTOSE TRANSPORT SYSTEM IN A CLONE OF THE PROXIMAL TUBULAR CELL LINE LLC-PK₁. Juan Riveras and Carlos A. Rabito. Nuclear Medicine Division, Department of Radiology, Massachusetts General Hospital, Harvard University, Boston, MA.

The definition and characterization of the sugar transport systems and their assignment to a particular cell type in the proximal tubule have been severely hindered by the cellular heterogeneity and the immutable cell composition of the conventional preparations. In the present study we analyzed the presence of a Na^+ -dependent fructose transport by a clone of the renal epithelial cell line LLC-PK₁ which has a well-characterized Na^+ dependent glucose transport system (LasHeras and Rabito Fed Proc 45, 510a, 1986). The transport of D-Fructose was measured at 37°C using ¹⁴C labelled D-fructose under conditions approaching initial entry rate. The influx of D-fructose in confluent monolayers of LLC-PK_{1A} increases with the incubation time. The absence of Na^+ produced a complete inhibition of fructose influx. Similar inhibition was observed in presence of 10⁻⁴ ouabain. D-fructose influx is a saturable process with a Km of 0.16 mM and a V^{max} of 6.29 nM.h⁻¹.mg DNA. Phloretin but not phlorizin inhibit the Na^+ -dependent fructose transport. The presence of an excess of the non metabolizable glucose analog 1-0-methyl-2-D-glucopyranoside do not inhibit the fructose transport. These results suggest that the transport of D-fructose and D-glucose occur through two different transport systems. The coexistence of D-glucose and D-fructose transport system in a single cell strain is consistent with the concept that at least part of the functional heterogeneity of the renal proximal tubule represent the functional heterogeneity of each cell type present in this nephron segment.

CRITICAL ROLE OF SHORT-CHAIN FATTY ACIDS IN ISO-VOLUMETRIC REGULATION (IVR) OF PROXIMAL S₂ SEGMENTS IN HYPEROSMOTIC MEDIA. L. Rome*, C. Lechene, V. Savin and J. Grantham. Univ. of KS Med. Ctr., K.C., KS; Harvard Med. Sch., Boston, MA.

When extracellular fluid osmolality is increased above 295 mOsm by the addition of NaCl (<2 mOsm/min), non-perfused rabbit proximal S₂ segments maintain cell volume constant (IVR) in medium containing acetate and glucose as major energy sources. Above 360 mOsm cells shrink. Addition of citrate, lactate and alanine did not enhance IVR but in medium also containing butyrate and/or valerate, tubules maintain IVR to 460 mOsm. Fatty acid addition stabilized cell electrolyte concentrations in isotonic media over 80 min. Butyrate dose response indicated a K_{0.5} of 260 uM for the enhanced effect on IVR. Analysis of cell Cl, K and Na with respect to cell P by wave length dispersive EPA in single tubule segments subjected to hyperosmotic gradient conditions are shown.

	OSM	Cl/P	K/P	Na/P
Control (N=6)	295	.13±.01	.87±.03	.15±.02
Hyper (N=6)	400	.41±.05	.64±.06	.37±.06

Hyperosmotic IVR was accompanied by a net influx of Cl and Na, and a net efflux of K. The Cl increase accounts for IVR if one-half of the accumulated solutes are Cl ions. Changes in cell cation content indicate that osmolyte not detected by EPA may accumulate. Alternatively, the magnitude of changes in cell ionic content may be related to volume equilibrium of cytoplasmic gel. We conclude that short-chain fatty acids better maintain normal cell function and strikingly increase the magnitude of IVR in S₂ segments. Fatty acids may be rate-limiting for cell volume maintenance in hyperosmotic states.

DUAL AFFINITY TRANSPORT OF PYROGLUTAMYL-HISTIDINE IN RENAL BRUSH BORDER MEMBRANE VESICLES. HA Skopicki*, K Fisher, D Zikos, G Flouret*, and DR Peterson*. Univ. of Health Sciences/The Chicago Med. School and VA Med. Center. N. Chicago, IL and Northwestern Univ. Med. School, Chicago, IL.

The kidney plays an important role in the conservation of a variety of organic solutes. Both low and high affinity carriers exist for the transport of glucose and various amino acids. We have previously shown that a high affinity, low capacity system (Km=9.3 X 10⁻⁶ M, Vmax=6.1 X 10⁻¹⁸ mol/mg/min) exists for the transport of the N-terminal degradative dipeptide of luteinizing-hormone releasing hormone, pyroglutamyl-histidine (pGlu-His). Using New Zealand white rabbits, a renal brush border membrane vesicle preparation was used to discern if a dual affinity system exists for the transport of the dipeptide, pGlu-His. In addition to the high affinity transport of pGlu-His, which has been shown to be sodium independent, hydrogen ion stimulated and uninhibited by a variety of small peptides, a second carrier was found. Transport of pGlu-His by this carrier was of lower affinity, higher capacity (Km=6.3 X 10⁻⁴ M, Vmax=2.2 X 10⁻⁹ mol/mg/min), and inhibited by the dipeptides glycyl-proline, glycyl-sarcosine, carnosine and the tripeptide, pyroglutamyl-histidyl-proline. We conclude that both high and low affinity carriers are present in the luminal membrane of renal proximal tubular cells for the transport of the dipeptide, pGlu-His, as has been observed for certain amino acids and glucose. Furthermore, it appears that the low affinity carrier is non-specific, in contrast to the specific, high affinity transporter.

ARGINYL AND HISTIDYL GROUPS ARE ESSENTIAL FOR ORGANIC ANION EXCHANGE IN RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV). Paul P. Sokol, Peter D. Holohan and Charles R. Ross. SUNY-Health Science Ctr., Syracuse, NY.

The functional groups required for anion exchange in BBMV were identified. The effects of arginyl- (phenylglyoxal (PG) and 2,3-butanedione (BD)) and histidyl-modifying (diethylpyrocarbonate (DEPC)) reagents on the transport of p-amino-[³H]hippurate (PAH), a prototypic organic anion substrate, were evaluated. These studies were conducted employing a OH⁻ gradient since the mechanism for transport involves the exchange of an organic substrate (PAH) for an inorganic one (OH⁻ or Cl⁻). PG, BD and DEPC inactivated the specifically-mediated PAH transport with IC₅₀ values of 710uM, 1780uM and 160uM, respectively. The rates of PAH inactivation by DEPC and PG suggest multiple pseudo-first order reaction kinetics: more than 1 residue is inactivated. The presence of substrate (5mM PAH) affected the rate of DEPC inactivation but not the rate of PG inactivation. We conclude, that a histidyl, but not an arginyl residue is present at the active site.

These findings were amalgamated with our previous results showing essential sulfhydryl groups (Sokol et al BBA 862, 335, 1986) into a model whereby the cationic binding site for the anionic substrate consists of a histidyl residue stabilized by a neighboring thiolate side chain. The arginyl residues are distal to the active site and function to guide the substrate to the histidyl binding site.

REGULATORY CELL VOLUME DECREASE (RVD) IN RABBIT CCT. K. Strange. Wright State Univ. Sch. Med., Dept. of Physiol. & Biophys., Dayton, OH.

In most mammalian cells studied to date, RVD induced by osmotic swelling is mediated largely by passive KCl efflux via KCl cotransport, K⁺ and Cl⁻ channels, or K⁺/H⁺ and Cl⁻/HCO₃⁻ exchange mechanisms. RVD was studied in rabbit CCT by DIC video microscopy. Principal (PC) and intercalated (IC) cells swell 40.3 ± 2.4% and 34.9 ± 2.8% (N = 10-11 CCT, 14 cells), respectively, when bath osmolality is reduced to 190 mOsm and then return to within 5.5 ± 1.7% and 6.0 ± 1.9%, respectively, of control volume. Initial rates of RVD were -3.88 ± 0.35%/min for PC and -3.22 ± 0.34%/min for IC. Following return to isotonic media, PC and IC shrink -27.1 ± 1.7% and -25.1 ± 2.6%, respectively, below control volume. RVD is blocked by reducing bath temperature to 22°C but is unaltered by bilateral application of (in mM) 2 Ba²⁺, 2 anthracene-9-COOH, 0.5 SITS, 0.02 bumetanide, bilateral HCO₃⁻ removal or bath addition of 0.1 ouabain (0 Na lumen) [P > 0.4, N = 4-8, 4-13]. Bilateral elevation of K⁺ from 5 mM to 52 mM results in K⁺ influx and causes PC and IC to swell 99.8 ± 9.9% and 53.4 ± 6.0%, respectively, in hypotonic saline, but has no effect on rates or magnitudes of RVD in either cell type (P > 0.4; N = 8-9, 12-13). Cl⁻ or K⁺-depleted CCT (1 hour bilateral perfusion with Cl⁻ or K⁺-free saline) exhibited normal rates and magnitudes of RVD in Cl⁻ or K⁺-free hypotonic saline (P > 0.4; N = 6, 8-11). These results argue against conventional KCl loss mechanisms as being the primary means of RVD in CCT. The putative role of organic compounds in CCT volume regulation is under study. NIH grant AM37317.

FATTY ACID TRANSPORT BY RENAL BASOLATERAL MEMBRANES: EVIDENCE FOR ANION EXCHANGE. Mary Ellen Trimble, SUNY Health Sci. Ctr., Dept. of Physiol., Syracuse, NY.

Long chain fatty acids (LCFA) are a significant energy source for the kidney and understanding their mode of entry into cells is important. Using a rapid filtration technique, we previously reported pH gradient-stimulated transport of the LCFA, palmitate, in rat renal basolateral membrane vesicles (BLMV) (KI 31:451, '87). The present studies were done to further characterize this transport. Initial (15 sec) binding of palmitate is approx. 54% (lysis method) to 58% (osmotic method) of total uptake. In the presence of an inwardly directed proton gradient (pH 6_o/7.4_i) total initial (15 sec) uptake of 0.18 μM ¹⁴C-palmitate was 81 ± 1 pmoles/mg protein. Preincubation of BLMV with 1 mM DIDS (4,4'-diisothiocyanato-2,2'-disulfonic acid) reduced total uptake to 52 ± 5 pmoles/mg (N=3 preps, p<.005). Assuming 55% binding, this represents 87% inhibition of transport. In the absence of a pH gradient (6_o/6_i), total uptake was 63 ± 3 pmoles/mg protein and was not affected by DIDS. In countertransport experiments, BLMV were treated with valinomycin and gramicidin or FCCP and preloaded with furosemide, sulfate or bicarbonate (5-10 mM). This caused trans-stimulation of palmitate uptake which, tested at 5 mM furosemide, was inhibitable by DIDS. Results show that pH gradient-stimulated transport of LCFA into BLMV is inhibited by DIDS, suggesting specific interaction with a membrane protein. Trans-stimulation of LCFA uptake by other anions, also DIDS-inhibitable, suggests the transport may be mediated by an anion-exchanger.

UREA FLUX IN THE RENAL PELVIS AND URETER. Bryan Walser,* Yoram Yagil* and Rex L. Jamison. Division of Nephrology, Stanford University Medical Center, Stanford, California.

We previously reported that in the rat, approximately 7% of the urea present in the urine emerging from the terminal collecting ducts is reabsorbed by the time it reaches the distal end of the ureter (Clin. Res. 35:176A, 1987), which is consistent with urea reflux in the renal pelvis. To rule out urea reabsorption along the ureter as an alternative explanation for the decrease in urea flow, rats were anesthetized and the left ureter exposed. The ureter was cannulated proximally near the uretero-pelvic junction and distally near the bladder and perfused with rat urine of known composition labeled with ¹⁴C urea and tritiated inulin at 6.26 ± 0.10 μl/min. In 9 rats the ratio of urea concentration in fluid after to that before perfusion through the ureter (distal/proximal) corrected for water movement was 0.93 ± 0.02 (p < 0.01). This corresponds to a 7% decrease in urinary urea flow along the ureter. Radioactive urea was detected in urine from the contralateral kidney. Potassium and creatinine were also reabsorbed (3.4 ± 0.9% (p < 0.01) and 3.5 ± 1.2% (p < 0.05), respectively) while sodium was added to the perfusate (9.2 ± 2.3% (p < 0.01)). These data suggest that the difference in urinary urea flow between the proximal and distal end of the ureter is accounted for primarily by reabsorption through the ureteral epithelium rather than by urea reflux in the renal pelvis.

RENAL HANDLING OF MURAMYL-DIPEPTIDE: EVIDENCE FOR INTACT LUMINAL UPTAKE IN THE PROXIMAL NEPHRON. D. Zikos, K. Fisher, and D. R. Peterson*, Univ. of Health Sciences/The Chicago Medical School, Dept. of Physiol. and Biophys., and Medicine, N. Chicago, IL. and Veterans Administration Medical Center, N. Chicago, IL.

The recognition that muramyl dipeptide (MDP) possesses important biological activities, in conjunction with evidence that the kidney is implicated in its metabolism, led us to study its renal handling. Since the available data established a role for the proximal tubule epithelium in the metabolism of small peptides, we microperfused rabbit proximal nephron segments in vitro with tritiated MDP. Radio-labeled material appeared in the bathing medium at an average transport rate of 2.13 pg/mm tubule length/min. 58% of the transported radiolabel was in the form of intact dipeptide, although no metabolites were found in the collected luminal fluid. The results suggest that MDP: 1) remains intact in the luminal fluid, escaping the hydrolytic action of brush border membrane peptidases, 2) is transported across the proximal tubular epithelium partly intact and partly in the form of metabolites. The data are consistent with a reabsorptive mechanism involving luminal uptake by the previously described dipeptide carrier, followed by intracellular degradation.

TRANSPLANTATION

ENDOTHELIAL CELL EXPRESSION OF A URINARY ANTIGEN FROM RENAL TRANSPLANT PATIENTS. Arlene C. Y. Ali* and Andrew D. Bajnes. Dept. of Clin. Biochemistry Univ. of Toronto, Toronto, Ontario, Canada.

Kidney transplant recipients excrete an acidic urinary protein (MAUP), not found in normal individuals. Monospecific polyclonal antibodies to MAUP bind exclusively to human capillary endothelium. Immunofluorescent and HRP staining indicates that MAUP differs from vimentin, HLA, and human Ia antigens. Anti-MAUP antibodies react with cells isolated from the endothelium of human umbilical veins (HUVE); 75% of cells were uniformly stained, while 25% of cells were more intensely stained in the perinuclear region. HUVE cells, grown to confluency, were injured by vigorous scraping or mild trypsinization, followed by multiple passages through an 18g needle. Uninjured control cells were suspended by mild trypsinization. Control and injured cells were examined for reactivity with anti-MAUP serum after various incubation times. At 0 min. all cells expressed 100% of control values. Expression of MAUP was most reduced after 10 min. (42%, scraped cells; 76%, trypsinized cells), and returned toward control levels by 40 min. (74%, scraped cells; 94%, trypsinized cells). The results indicate that (1) the MAUP antigen is specific to human capillary endothelium; (2) endothelial cells in culture express the MAUP antigen; and (3) expression of the MAUP antigen is decreased by cell injury; the decrease is proportional to the severity of cell injury; and cell recovery is accompanied by an increase in expression of the MAUP antigen toward control levels. Thus, in renal transplant patients urinary MAUP may be a sign of damage to donor endothelial cells during rejection episodes.

RENAL TUBULAR ACIDIFICATION STUDY IN CYCLOSPORIN MAINTAINED CARDIAC TRANSPLANT PATIENTS.

V.K. Bansal, L. Potempa*, M. Costanzo-Nordin*, S. Hecker*, G.A. Kozeny, J. Robinson*, and J.E. Hano. Loyola University Medical Center, Maywood, Ill.

Cyclosporin (CsA) is associated with nephrotoxicity, hypertension and hyperkalemia, the etiology of which is unclear. Because hyperkalemia and hypertension may be present with suppressed renin, it is possible that patients on CsA may be associated with tubular acidification defects. To test that hypothesis, we prospectively studied ten cardiac transplant patients, at least 3 months post-transplant with stable renal function and on same maintenance dose of CsA for a month prior to the study. The investigations included determination of Creatinine clearance (Ccr), Inulin clearance (Cin), Para-aminohippuric acid clearance (CpAH), Urinary pH, Urine to plasma pCO₂ difference after a standard bicarbonate loading test, and upright plasma renin and aldosterone levels. The mean, Ccr was 52.5 ml/min (range 30-98 ml/min), mean Cin 53.6 ml/min (range 30-85 ml/min with no statistical difference between two P > 0.1). Renal blood flow was decreased with a mean CpAH 314 ml/min (range 166-514 ml/min.) First morning urinary pH was greater than 5.5 in only 4 out of 10 patients; following standard bicarbonate test loading, the U-B pCO₂, was greater than 25 in 5 out of 10, but clearly abnormal only in one patient (10%). The plasma renin, aldosterone, and potassium levels were normal in all patients. No correlation was found between hypertension, renin-aldosterone level and CsA levels. We conclude that no definitive renal tubular acidification was associated with CsA therapy and no difference was found between Cin and Ccr.

FINE NEEDLE ASPIRATION BIOPSY AND CORE BIOPSY COMPARISON IN RENAL ALLOGRAFT RECIPIENTS. C. Boshkos*, D.R. Steinmuller, A. Fishleder*, A. Novick, S. Stream*, R. Cunningham, B. Dlugozs*, Dept. of Hypertension & Nephrology, Cleveland Clinic Foundation, Cleveland, Ohio.

Fine needle aspiration biopsy (FNAB) has been claimed to be a sensitive and specific test to differentiate renal allograft rejection, cyclosporine nephrotoxicity and acute tubular necrosis (ATN). We have performed simultaneous FNAB and core biopsy in 18 recipients of renal allografts treated with cyclosporine-based immunosuppressive regimens from January to May, 1987. Acute deterioration in renal function (>25% increase in serum creatinine) or a plateau in the serum creatinine above baseline triggered such biopsies once mechanical causes of renal dysfunction were excluded. FNAB scores were derived from the sum of differences in differential counts between peripheral blood and aspiration material multiplied by a weighting factor for each cell type. In 4 patients in whom their incremental score was <3, 1 had membranous glomerulopathy with interstitial fibrosis, 1 had no pathologic findings and the remaining 2 had mild acute cellular rejection with patchy ATN. The remaining 14 patients had incremental scores of >3. 12 of 14 had moderate-severe acute cellular rejection, several with evidence of intracapillary fibrous deposits, or ATN. The remaining 2 patients had chronic rejection and interstitial hemorrhage (presumably due to antibody mediated rejection). The magnitude of increase in the incremental score tended to correlate with the severity of rejection except in 2 patients in whom concurrent systemic CMV infection occurred. Here, the incremental score increase was temporally associated with viremia and a mononuclear shift of the differential. No antirejection treatment was used, with renal function spontaneously improving. No correlation was found between the likelihood of response to treatment of allograft rejection and the incremental score.

CYCLOSPORINE A & PREDNISONE VS. CYCLOSPORINE A & AZATHIOPRINE & PREDNISONE IN FIRST TIME CADAVERIC RENAL TRANSPLANTS. K. Brinker, T. Gonwa, *R. Dickerman, A. Hull, *J. Langley, D. Long, D. Nesser, *G. Trevino, R. Velez, and P. Vergne, Methodist Medical Center, Dallas, Texas.

We are prospectively comparing 2 immunosuppressive regimens in 1st cadaveric renal transplants (tx). Patients randomly receive cyclosporine (CsA) & prednisone (P) (Group A) or CsA & azathioprine (AZT) + P (Group B). We report on the 1st 114 pts. entered (A, 62 B, 52).

Group A & Group B have not differed as to age, sex, diabetics, DR matching or cold ischemia time. The table shows that graft survival, rejection incidence, & serum creatinine (Cr D/C) are not different between the 2 groups.

	Graft Surv. (12 mo)	1st Rej.	2nd Rej.	Cr (D/C)	Cr (3 mo)
Group A	77%	56%	23%	2.4±0.1	1.8±0.1
Group B	81%	48%	16%	2.9±0.4	2.1±0.2
p values	.652	.372	.357	.131	.224

Although D/C Hct (D/C Hct 30.3±.6 vs 28.1±.8, p.026), and D/C CsA blood levels (623±5 vs 466±35, p.014) were higher in Group A, these differences were gone 3 mos. post tx.

Further, the incidence of infection (A 27%, B 23%, p.662), hypertension (A 80%, B 83%, p.747), & 1st re-hospitalization (A 52%, B 44%, p.383) have not differed. The incidence of acute renal failure (ARF) has been higher in Group B than in Group A (56% vs. 35%, p.03). This is not explained by cold ischemia times & has been of mild degree.

To date we find no differences in 1st cadaver tx given a double vs. triple immunosuppressive regimen.

DIABETIC KIDNEY TRANSPLANT RECIPIENTS: IMPROVED PROGNOSIS AND REHABILITATION. J.Cheigh, K.Stenzel, R.Green*, N.Schechter*, R.Riggio, M.Suthanthiran, W.Stubenbord. The Rogosin Kidney Center, The New York Hospital/Cornell Medical College, New York, NY

Insulin dependent diabetic patients with end stage renal disease have a poor prognosis due to continued progression of retinopathy, neuropathy and angiopathy. To evaluate the long-term prognosis of these patients after kidney transplantation, we studied physical performance, nerve conduction time and patient and graft survival rates of 50 diabetic patients (19 from related and 31 from cadaver). Patient and graft survival rates of these patients were compared with that of 509 non diabetics (107 from related and 402 from cadaver) transplanted during the same period (1977-1988).

Survival at 5 years (%)

	Patient	Graft
Diabetic	80	Related 58; Cadaver 33
Non-diabetic	83	Related 64; Cadaver 34

Twenty one of 24 diabetic patients (89%) with functioning grafts (follow-up: mean 36 months) maintained a productive life with minimal physical disability. Physical performance by Karnofsky scale improved from 76% pre-transplant to 86% post-transplant ($p < .001$). All improved or maintained the same degree of visual acuity, however 2 required amputation of lower limbs. Nerve conduction time measured serially revealed minimal improvement. In conclusion, diabetic kidney transplant patients have comparable patient and graft survival rates with that of non-diabetic patients. While physical performance significantly improves after a successful kidney transplant, retinopathy, angiopathy and neuropathy may remain status quo but rarely worsen.

SUCCESSFUL CLINICAL COURSE AND OUTCOME OF PREGNANCY IN RENAL TRANSPLANT RECIPIENTS. C.Chinea, J.Cheigh, K.Stenzel, W.Stubenbord. The Rogosin Kidney Center The New York Hospital, New York, New York.

Pregnancy in the renal transplant recipient carries risks to mother, graft and infant but can have a successful outcome in a selected group of patients. We reviewed our experience of 21 pregnancies in 16 renal transplant recipients with a mean age of 27 years old and a mean time of 50 months (14-155 mos) after transplant when conception occurred. All patients were on Imuran and prednisone and mean creatinine was 1.2 mg/dl (.6-2.0). Our data showed that 42% had an appropriate increase in renal function in the first trimester. Six of twenty-one pregnancies (28%) developed deterioration in renal function, hypertension or an increase in proteinuria in the third trimester. Only one patient with hypertension and a creatinine of 2.0 prior to pregnancy had deterioration of renal function which progressed during the post partum year to eventual loss of the graft. There were no rejection episodes or infectious complications. Several patients with a history of urinary tract infections continued to have recurrences during pregnancy. Gestational age at the time of delivery ranged from 34 to 39 weeks and there were no neonatal complications. Approximately 38% of patients required a Caesarean section for deterioration of renal function, hypertension or other obstetrical reasons. We conclude from our data that in renal transplant recipients with normal blood pressure and a creatinine of 2.0 mg/dl or less, we can expect a successful pregnancy and delivery. The graft can respond to the pregnancy with an increase in function. The infant has an overall good prognosis.

RENAL TRANSPLANTATION IN BROWN NORWAY RATS. P. C. Churchill, M. C. Churchill, and A. K. Bidani. Wayne State Univ. Dept. of Physiol., Detroit, MI and Rush-Presbyterian St. Luke's Medical Center Dept. of Med., Chicago, Il.

In a previous study by Coffman et al. (Kidney Int. 30:20, 1986), Lewis rat kidneys were transplanted into littermates following unilateral nephrectomy. Seven days later, inulin and PAH clearances were markedly lower in the transplanted kidneys (0.6 and 1.9 ml/min/kg) than in the contralateral kidneys of the same rats (8 and 20 ml/min/kg) or than in the nontransplanted kidneys of control Lewis rats (5.3 and 13.9 ml/min/kg). Since rejection could be ruled out, the diminished function of the transplant was attributed to ischemia-induced acute renal failure. In the present study, Brown Norway rats were used as donors and recipients, and warm ischemia was avoided during surgery. The clearances of inulin and PAH (ml/min/kg) measured eight days later are shown below, along with values measured in the two native kidneys of control Brown Norway rats.

	C_{in}	C_{PAH}
transplant (n = 10)	3.1±0.3	9.9±0.9
contralateral native	3.9±0.2	10.8±0.6
native (n = 7)	4.2±0.3	11.5±1.0
native	4.5±0.1	12.1±0.8

The smaller differences in C_{in} and C_{PAH} between transplanted and native kidneys suggest that avoiding warm ischemia has a beneficial effect on transplant function.

EFFECTS OF CAPTOPRIL (C) ON POST-TRANSPLANT HYPERTENSION IN THE RAT. T.M. Coffman, S.I. Himmelstein*, C.F. Best*, and P.E. Klotman. Duke Univ. and Durham VA Medical Centers, Durham, N.C.

Hypertension complicating successful renal transplantation remains a significant clinical problem. In some patients, the presence of diseased native kidneys (NKs) appears to play an important role in the pathogenesis of hypertension, however, the mechanisms are unknown. To address this question, we developed an animal model of post-transplant hypertension in which the effects of NKs could be examined independent of the confounding variables of rejection and immunosuppression. To produce disease in NKs, PVG rats were subjected to 5/6 nephrectomy. Four weeks following renal ablation, animals were transplanted with kidneys from syngeneic PVG donors. Four weeks later, clearances of inulin (C_{in}) and PAH (C_{PAH}) were determined before and after administration of C (10 mg/kg).

	CONTROL	+CAPTOPRIL
Mean BP (mmHg)	147±6	122±8**
C_{in} (ml/min/kg)	3.08±0.47	3.99±0.20*
C_{PAH} (ml/min/kg)	8.24±1.37	13.48±0.98**

In hypertensive transplant recipients with NKs, captopril significantly lowered BP and increased GFR and C_{PAH} . A subgroup of hypertensive animals underwent native nephrectomy (NNx) at the time of transplantation. In the NNx group, BP was significantly lower 110±9 mmHg ($p < .005$) and C_{in} (5.64±0.45 ml/min/kg) and C_{PAH} (16.33±0.61 ml/min/kg) were significantly higher when compared to transplant recipients with NKs ($p < .005$). Captopril had no significant effect on C_{in} , C_{PAH} or BP in the NNx group. Thus, in this model, the presence of abnormal NKs is associated with hypertension and a reduction in renal transplant function, possibly mediated by angiotensin II. As has been suggested by others, captopril may be useful in determining a pathophysiologic role for native kidneys in post-transplant hypertension.

OUTCOME OF RENAL TRANSPLANTATION IN HEROIN ASSOCIATED NEPHROPATHY (HAN). Eugene E. Cunningham, SUNY at Buffalo, Department of Medicine, Buffalo, New York.

Fifteen patients with Heroin Associated Nephropathy (HAN) underwent renal transplantation at the Erie County Medical Center in Buffalo, New York beginning in January 1973 through December 1986. All patients were black males, 18-46 years of age at the time of their renal transplants. These 15 patients received a total of 20 renal grafts. All but one of the grafts was from a cadaver. Eleven of the 20 grafts (55%) survived for one year, while 6 grafts (32%) survived 2 years. Eleven of 20 transplants in (9 patients) were performed after 1982 with 10 of 11 grafts (91%) surviving one year. Rejection at 3 months was the major cause of graft failure in 9 of the 20 transplants. Thirteen of 15 patients (87%) were still alive 2 years after their initial or second transplant. Of the 2 deceased patients, one died of liver failure with a functioning graft more than one year after transplantation. The other patient died at less than 2 months post-transplant with a non-functional graft.

Transplantation of patients with HAN may be performed successfully with acute rejection, the major determinant of graft failure. There was no evidence of recurrence of the original disease at followup of two years, despite continued use of drugs in many patients. The improvement in transplant results seen after 1982 may be attributed to better patient compliance, use of pre-transplant blood transfusions and use of antithymocyte globulin (ATGAM) for treatment of rejection.

RENAL ALLOGRAFT INFLAMMATORY CELLS, BETA-2-MICROGLOBULIN (B2-MG) AND THROMBOXANE. Marie Foegh,* Kin Lim*, and Mohammad Alijani* (intr. by A. Haramati). Georgetown University Medical Center, Dept. of Surgery, Division of Transplantation, Washington D.C.

Fine needle aspiration biopsy (FNAB), urine immunoreactive-thromboxane B2 (i-TXB2) and serum B2-MG are used for diagnosis of kidney graft rejection. We investigated the correlation between these three parameters and the inflammatory cell most likely to be the source of i-TXB2. 505 FNABs from 75 consecutive patients undergoing 64 clinically diagnosed episodes of rejection were correlated with urine i-TXB2 and serum B2-MG obtained on the same day as FNAB. The correlation coefficient was 0.51 (p 0.004) and 0.55 (p 0.001) against urine i-TXB2 and serum B2-MG, respectively. Cells from FNAB's from 10 patients were stimulated in vitro with a calcium ionophore and TXB2 formation was analyzed. The correlation was determined between the number of different cell types in the FNAB. Only monocytes/macrophages (M0) correlated with i-TXB2 formed in vitro (R=0.90, p 0.006). The numerical score of the FNAB also correlated with i-TXB2 formation in vitro (R=0.82, p 0.03). Thus the inflammatory cells in the kidney allograft correlated positively with urine i-TXB2, in vitro formation of i-TXB2 and serum B2-MG. Our findings suggest that M0 may be a significant source of i-TXB2.

INFLUENCE OF INFUSION RATE ON CYCLOSPORINE NEPHROTOXICITY IN THE RAT. W.F. Finn, L.J. Hak,* A.J. McCormack,* and R.L. Clark, Departments of Medicine and Radiology and School of Pharmacy, University of North Carolina, Chapel Hill, N.C.

The pharmacokinetic profile of cyclosporine (CyA) given intraperitoneally (i.p.), resembles that of an intravenous (i.v.) injection. To determine if CyA associated toxicity could be reduced by slow infusion and avoidance of high peak plasma levels, the effects of daily doses of CyA given either i.p. 10 mg/kg (Group I; n=7) or i.v. by a four hour infusion, 20 mg/kg (Group II; n=8) were compared to vehicle treated controls (n=7).

Food consumption (g/day/100 g BW) and changes in body weight (g/day) were recorded daily. At one week, MAP (mm Hg), CIN (ml/min/100 g BW), RBF (ml/min/100 g BW) and RVR (mm Hg/ml/min/100 g BW) were determined. Results are means \pm SD. P < 0.05; ^a Group I vs vehicle; ^b Group II vs vehicle; ^c Group II vs Group I.

	VEHICLE	GROUP I	GROUP II
FOOD	6.7 \pm 1.0	5.3 \pm 0.6 ^a	4.3 \pm 1.0 ^{b,c}
Δ BW	3.0 \pm 3.8	0.9 \pm 4.4	-4.9 \pm 2.5 ^{b,c}
MAP	112 \pm 10	102 \pm 12	128 \pm 18 ^{b,c}
CIN	0.47 \pm 0.10	0.27 \pm 0.10 ^a	0.46 \pm 0.09 ^c
RBF	2.61 \pm 0.48	2.12 \pm 0.18 ^a	3.60 \pm 1.27 ^{b,c}
RVR	40.70 \pm 4.77	46.93 \pm 4.96 ^a	40.11 \pm 3.63 ^c

Microangiographic studies showed marked abnormalities in the intrarenal perfusion pattern in the rats injected for one week with CyA, 10 mg/kg. In the rats infused over four hours with CyA, 20 mg/kg the microangiographic pattern was normal.

These studies demonstrate that CyA nephrotoxicity may be minimized by prolonging the rate of administration and suggest that nephrotoxicity is in part related to peak plasma levels.

CYCLOSPORINE AUGMENTS THE RENAL VASOCONSTRICTOR RESPONSE TO NOREPINEPHRINE (NE). Michael D. Garr* and Mark S. Paller, University of Minnesota, Minneapolis, Minnesota.

The major obstacle to the use of the immunosuppressant drug cyclosporine (Cy) is renal dysfunction. Cy causes marked renal vasoconstriction and an associated decrease in GFR, effects which can be abolished in rats by renal denervation or α_1 -adrenergic blockade. In addition cyclosporine increases renal sympathetic nerve activity. We tested the possibility that Cy might also produce renal vasoconstriction through a post-synaptic effect to increase renal vascular reactivity to catecholamines. In anesthetized rats renal blood flow was measured by electromagnetic flowmeter and arterial pressure through a carotid artery cannula. To prevent any direct contribution of the renal nerves to changes in renal vascular resistance (RVR), denervation was accomplished by stripping the renal artery and applying 10% phenol. The renal blood flow (RBF) response to 3 graded intrarenal infusions of NE was tested after iv infusion of vehicle (10% lipid emulsion) and again after cyclosporine (20 mg/kg) in 6 animals (NE dose in ng/min; *p<0.05 vs vehicle).

NE dose:	RBF (ml/min)				RVR (mmHg/ml/min)				Δ RVR (%)			
	0	5	21	56	0	5	21	56	5	21	56	
Vehicle	9.0	8.6	8.2	7.7	14.0	14.5	15.7	16.9	3.4	12.0	20.4	
Cy	8.4	7.5*	6.5*	5.9*	15.2	17.0	20.3*	21.9*	10.4	30.9*	43.7*	

In summary, in animals in which direct vasoconstriction by Cy was prevented by renal denervation, renal vasoconstriction due to NE was augmented by Cy. Thus, Cy causes renal vasoconstriction not only by enhancing renal nerve activity, but also by augmenting sensitivity to NE. This may explain, in part, how Cy causes renal vasoconstriction and impaired renal function in denervated transplanted kidneys. A similar effect in the periphery could contribute to the development of hypertension.

A MODEL OF CHRONIC CYCLOSPORINE (CS) NEPHROTOXICITY IN THE SPRAGUE-DAWLEY (SD) RAT. D.M. Gillum*, L. Truong, W.N. Suki, Departments of Medicine and Pathology, Baylor College of Medicine, Houston, Texas. Lack of a suitable rodent model has hampered the study of chronic CS nephrotoxicity. Proximal tubule vacuolization and inclusions are consistently described in rat studies, but changes associated with chronic CS nephrotoxicity in humans (interstitial fibrosis, tubular atrophy, arteriolopathy) are infrequently reported. Using male SD rats we have administered CS in olive oil (o.o.) at 25 mg/kg/d intraperitoneally (i.p.) for 28 days. This protocol consistently results in a lesion of patchy interstitial fibrosis, tubular atrophy, interstitial inflammation, and marked juxtaglomerular apparatus (JGA) hypertrophy. Control animals were pair fed and received only o.o. i.p. Despite pair feeding CS treated animals gained only 10 ± 12 g, while controls gained 69 ± 18 g. Mild JGA hypertrophy was noted in some control animals but no other significant changes were identified. Eighteen of 20 rats given i.p. CS survived the protocol. GFR at the end of 28 days was 0.45 ± .18 ml/min in the CS group vs 1.20 ± .24 ml/min in the controls. We conclude the above protocol results in a reproducible lesion with many of the features of chronic CS nephrotoxicity in humans. The described model will permit additional studies of this daunting clinical problem to advance.

SINGLE KIDNEY RELATIVELY PROTECTED FROM CHRONIC CYCLOSPORINE (CS) DAMAGE. D.M. Gillum*, L. Truong, W.N. Suki, Department of Medicine, Baylor College of Medicine, Houston, Texas. Daily intraperitoneal (i.p.) injection of CS (25 mg/kg) in olive oil (o.o.) for 28 days in male Sprague-Dawley rats results in a renal lesion consisting of patchy interstitial fibrosis (IF), tubular atrophy, interstitial inflammation and marked JGA hypertrophy. This model was used to investigate the chronic effect of CS on the single kidney in a nontransplant model. Four groups of age matched rats were studied. Group I underwent sham nephrectomy (SNx) and received CS beginning 14 days after surgery. Groups II and III were uninephrectomized (UNx) and CS begun 14 days or immediately after surgery respectively. Group IV rats underwent SNx and received o.o. After 28 days single kidney GFR was measured. Foci of tubulointerstitial (TI) damage were counted and expressed per unit of surface area. JGA hypertrophy was expressed as a percentage of glomeruli with observable vascular poles.

	JGA Score	TI Score	GFR	Renal/ Body wt
I	57.78	122.4	220 ul/min	0.0033
II	49.62	34.84*	569 ul/min	0.0044*
III	45.13	30.35*	391 ul/min	0.0042*
IV	9.5+	2.44+	559 ul/min	0.0030

* p<.01, II, III vs. I; + p<.001, IV vs. I. We conclude that CS does not prevent compensatory hypertrophy of the remaining kidney, and that the single kidney is relatively protected from chronic CS damage. The mechanism of this protection is under study.

EXTRACORPOREAL REMOVAL OF HLA-ANTIBODIES (HLA-Ab) IN DIALYSIS PATIENTS BY IMMUNOSORPTION. R.M. Hakim, R. Watt*, P.A. Neighbour*, J.M. Lazarus, and E. Milford. Vanderbilt University Medical Center, Nashville, TN, E.I. DuPont, CR&D, Glenolden, PA, and Brigham & Women's Hospital, Boston, MA.

Approximately 60% of dialysis patients on the New England Organ Bank transplant waiting list have high levels of circulating Ab to HLA-antigens of potential donor kidneys. These antibodies are cytotoxic and their presence precludes engraftment with a given kidney.

In the first phase of this study, we investigated the clinical safety and feasibility of reducing the titre of these HLA-Ab by selective depletion of IgG using an immunosorption system. The system (Excorim KB, Sweden) consists of 2 columns of protein A bound to Sepharose with on-line regeneration of the columns during treatment. Patients were plasmapheresed using a continuous plasmapheresis system and their plasma perfused alternately over the 2 columns. Eight patients underwent 3 such procedures on consecutive days, and each procedure consisted of 2 plasma volume (PV) treatments. (Mean PV treated/patient = 14 litres).

By the end of the 3rd procedure, there was a 90% reduction in total IgG while levels of other proteins (e.g. albumin) did not vary by more than 15%, demonstrating the specificity of the system. The extent of specific HLA-Ab removal, determined by index cell cytotoxicity, was variable but the average was a 16 fold reduction in titre. Reduction in % panel reactivity was variable, but in 2 patients, panel reactivity decreased from >90% to 20%. There was no complement activation as demonstrated by C_{3a} levels and no adverse clinical or biochemical changes in any of these patients.

CHARACTERIZATION OF THE CD4+ SPECIFIC SUPPRESSOR CELL IN RATS WITH LONG-TERM UNRESPONSIVENESS TO ALLOGRAFTS. BM Hall, NW Pearce,* and SE Dorsch*. Royal Prince Alfred Hospital, Sydney, Australia.

In DA rats grafted with PVG hearts a 10-day course of cyclosporine induces indefinite survival and after 50 days develop specific unresponsiveness to the graft. A CD4+ subpopulation of suppressor T cells, identified by the monoclonal antibody (MoAb) W3/25, transfers specific unresponsiveness to irradiated DA rats and inhibits the capacity of naive cells to restore PVG graft rejection in these adoptive hosts (J Exp Med 1985:162, 1683-1694). In this study we have further characterized the CD4+ suppressor cell to be Ia+ (MoAb: MRC Ox6+) and Il-2 receptor positive (MoAb: MRC Ox39+). Using the MoAb MRC Ox22 it identified the alloreactive CD4+ subset from the cell that provides help for B cells which do not express MRC Ox22; the suppressor was MRC Ox22. Thymectomized hosts developed CD4+ suppressor, suggesting it is not generated from recently derived thymocytes. Analysis of the CD4+ cell from CSA 7, 20, 50 and 75 days after transplant, shows that suppression is not evident until 50 days. The site of action of suppressor cells was tested by mixing them with naive or sensitized CD4+ and CD8+ in the adoptive transfer assay. Effective suppression was demonstrated against naive CD4+ cells and naive and sensitized CD8+ cells. Sensitive CD4+ cells were not inhibited, however.

These studies further characterize the cell responsible for a specific suppressor mechanism, that may have relevance to development of tolerance to transplanted organs.

THE PRIMARY DETERMINANT OF ONE YEAR GRAFT SURVIVAL OF CADAVER KIDNEY TRANSPLANTS IS EARLY RENAL FUNCTION. P. F. Halloran, V. Farewell, M. April, D. Ludwin, R. Bear, U. of Alberta, Waterloo U., U. of Toronto, McMaster U., Canada

200 consecutive cadaver kidney transplants (Tx) performed in 3 units in 1984-86 with a protocol incorporating ALG, cyclosporine, azathioprine, and low dose prednisone, were analyzed for determinants of survival. Early function (EF) was assessed by falling s. creatinine, urine output >1L and absence of dialysis.

Group	N	Actuarial 12 m. graft (%)	Survival patient (%)
Overall	200	85	95
1° Tx.	167	86	94
previous Tx.	33	79	97
Diabetics	27	82	85
EF present	129	91	96
EF absent	71	75	92

Multivariate analysis of numerous factors affecting graft survival (e.g. HLA match, PRA, previous Tx) revealed that the only significant factor was early renal function ($p < 0.007$). There was a significant interaction between anastomosis time and early function.

Thus in our data poor early function is the major correlate of (and thus may contribute to) increased risk of graft loss. It follows that, with current immunosuppressive protocols, prevention of renal ischemic injury should be given high priority.

HIGH INCIDENCE OF CYTOMEGALOVIRUS (CMV) INFECTIONS IN RENAL ALLOGRAFT RECIPIENTS RECEIVING ANTI-OKT3 MONOCLONAL ANTIBODIES (OKT3-Ab). Ralph Hawkins*, Ellen Burgess, John Klassen*. University of Calgary, Calgary, Alberta, Canada.

A retrospective study of 54 renal allograft recipients who received renal transplants over a 24 month period was conducted to ascertain the incidence of CMV infection in patients who received OKT3-Ab for treatment of allograft rejection versus those who did not receive OKT3-Ab. The mean follow-up time was 12.6 months, range 2 - 25 months. All patients received prednisone and cyclosporine, with some patients in each group receiving azathioprine as well. Criteria of CMV infection included either a fourfold rise in complement fixation titres or histopathologic diagnosis on biopsy or autopsy specimens. Of the 11 patients who received OKT3-Ab, 5 (45%) had CMV infections and 2 (18%) died of disseminated CMV infection. Of the 3 survivors, 1 had pneumonitis, 1 had recurrent abdominal pain and fevers necessitating laparotomy, and 1 was asymptomatic. The pretransplant CMV titres were not predictive of subsequent CMV infection. One OKT3-Ab patient died of fulminant hepatitis B infection which activated from a carrier state subsequent to OKT3-Ab therapy. Of the 43 non-OKT3-Ab treated patients, only 1 developed CMV infection ($p < 0.001$, Fisher's exact test). In our patient population, a significantly greater incidence of CMV infection developed in patients treated with OKT3-Ab, with substantial mortality in excess of that in the published literature.

TUBULAR FUNCTION IN RENAL TRANSPLANTS TREATED WITH CYCLOSPORIN A. Peter F. Hoyer, Barry S. Oemar Johannes Brodehl, Gisela Offner. Children's Hospital, Medical School Hannover, F.R.G.

In order to evaluate whether cyclosporin A (CyA) has an effect on classical renal tubular function parameter i.e. the transport of phosphate, glucose and amino acids, short term inulin clearance studies were performed in 23 CyA treated children 5-6 weeks after transplantation. Data from 15 azathioprine (Aza) treated children served as controls. Mean age of the patients, cold ischemia times, number of rejection episodes were comparable in both groups. The inulin clearance exhibited significantly lower rates in the CyA than in the Aza group: 47 ± 16.5 ml/min/1.73 m² versus 83 ± 25 . The PAH clearance revealed 271 ± 110 for CyA versus 503 ± 181 ml/min/1.73 m² ($P < 0.001$). The filtration fractions were not different: 19.1 versus 17.1%. The tubular phosphate reabsorption per ml GFR was only slightly lower in the CyA group (0.76 ± 0.23 umol/ml versus 0.93 ± 0.29 , $P = 0.09$). The endogenous glucose clearance was equally elevated in both groups. Amino acid excretion rates and single amino acid clearance rates revealed no difference between CyA and Aza treatment. Most of the fractional amino acid clearance rates were elevated in transplanted compared with controls. Only the fractional clearance rates of arginine, glycine and serine were higher in the CyA than in the Aza group. No direct influence of CyA blood levels on single tubular transport systems was detectable. We conclude, that CyA treatment has no specific effect on tubular parameters.

DONOR SPECIFIC TRANSFUSIONS (DST) OR CYCLOSPORINE FOR LIVING RELATED DONOR KIDNEY TRANSPLANTATION? THE TRADE-OFF BETWEEN SENSITIZATION AND NEPHROTOXICITY. Robert L. Jayes, Jr.*, Andrew S. Levey. Tufts University, New England Medical Center, Boston, MA.

DST and cyclosporine are two strategies to improve first year graft survival in high MLC, one-haplotype matched living related kidney transplantation. However, each has disadvantages: The conventional strategy of DST may sensitize the recipient to donor antigens, precluding transplantation from that donor; cyclosporine may increase graft failure due to nephrotoxicity.

We used decision analysis to compare these two strategies. We assumed that the risk of sensitization by DST is 12%, that graft failure in the first year is equal in both strategies, but that the annual probability of graft failure in later years is 2.65% with DST vs. 3.30% with cyclosporine. Patients sensitized by DST and patients with graft failure undergo dialysis while awaiting cadaveric transplantation using cyclosporine. Outcomes were assessed as quality-adjusted years of survival. The analysis was deliberately biased in favor of DST, the conventional strategy.

Quality-adjusted life expectancy for a 40 year old patient is 17.95 years with DST vs. 17.82 years with cyclosporine. We consider this difference in survival to be small and unimportant. The difference remains below 1 year unless the risk of sensitization by DST exceeds 25% or the annual probability of graft failure with cyclosporine exceeds 4.05%.

We conclude that the current data on sensitization by DST and long-term cyclosporine nephrotoxicity indicate that DST and cyclosporine are equally efficacious strategies for recipients of high MLC, one-haplotype matched kidney transplants.

SAFE CONVERSION FROM CYCLOSPORINE TO AZATHIOPRINE WITH IMPROVED RENAL FUNCTION IN PEDIATRIC RENAL TRANSPLANTATION. Bruce Kaiser,* Stephen Lawless,* Stephen Dunn,* Martin S. Polinsky,* H. J. Baluarte. St. Christopher's Hospital for Children, Temple Univ. Sch. of Medicine Philadelphia, Pennsylvania.

Conversion (CON) from cyclosporine A (CyA) to Azathioprine (AZA) after renal transplant (Tx) may be necessitated by nephrotoxicity, financial reasons, patient request because of side effects or electively done in the hope of preventing nephrotoxicity. Because of some of these reasons we have converted 11 children (Group I = 8 males, \bar{x} age 10.3 \pm 4.3 yrs) who were at least 6 months (mos) post-Tx, receiving less than 0.5 mg/kg of prednisone and had a stable creatinine with no evidence of rejection for 3 mos prior to CON. Prior to CON, prednisone was increased to 1 mg/kg and AZA was started at 2 mg/kg. There were no rejections stimulated by this CON method. Group I was compared to 12 children (Group II = 5 males, \bar{x} age 14.8 \pm 3.0 yrs $p < .01$), who meet all criteria for CON but remained on CyA, for calculated creatinine clearance (C'cr). Schwartz, Pediatrics 58:259, 1976.

	Pre C'cr (ml/min/1.73m ²)		C'cr (ml/min/1.73m ²)	
post-Tx	3 mos	6 mos	9 mos	12 mos
Group I	63.4	67.6	76.6	72.8
(CON)	± 10.2	± 11.1	± 14.0	± 7.8
Group II	64.9	60.4	60.4	59.2
(No CON)	± 13.5	± 14.6	± 14.6	± 16.3

There was no difference in C'cr between I and II prior to CON however, post CON at both 9 and 12 mos Group I had a significantly greater C'cr $p < .02$. In addition Group I had significant improvement from 6 to 9 and 12 mos in C'cr $p < .05$, while Group II remained unchanged. Graft survival was similar in both Groups with only 1 child losing a graft (Group II). Safe CON from CyA to AZA is possible and results in an improvement of renal function.

LOW DOSE CYCLOSPORINE (50-100 ng/ml) FROM THE EARLY POST-OPERATIVE PERIOD YIELDS POTENT IMMUNOSUPPRESSION AFTER RENAL TRANSPLANTATION. K Kamel* HR Brady* M Harding* G Cooke* GA deVeber, and CJ Cardella. Renal Div. Toronto Western Hospital, University of Toronto, Ontario, Canada.

The lowest concentration of cyclosporine required for effective immunosuppression in the early post-transplant period has yet to be determined. We have recently demonstrated pharmacological synergism between cyclosporine (Cy) and other immunosuppressive agents in their inhibition of the *in vitro* MLC response, suggesting that Cy concentrations of 50-100 ng/ml yield potent immunosuppression (Brady et al KI 1987;31:445). We have now prospectively evaluated 2 immunosuppressive regimens using serum Cy concentrations of either 50 or 100 ng/ml from day 7 post-transplant in 94 primary recipients (Table 1). All patients received antithymocyte serum during the first 10 days post-operatively.

Table 1	Group1(n=24)	Group2(n=44)	Group3(n=26)
Cy (ng/ml)	100	50	0
Pred (mg/kg/day)	0.15	0.15	5- \rightarrow 0.15
Aza (mg/kg/day)	0	0.15	2-2.5
lyr graft surv. (%)	92	87	85
% free of rejection	63*	64*	19

after 1 year. * $p < 0.005$
The early use of low dose Cy was associated with a significant reduction in the incidence of rejection, a reduced steroid requirement, and produced no chronic nephrotoxicity. Cy requirements after transplantation may be lower than previously thought. Early low dose Cy yields effective immunosuppression, is steroid-sparing, and avoids chronic nephrotoxicity. Such regimens may obviate the need for Cy-azathioprine conversion.

ALTERATIONS IN CYCLOSPORINE (CSA) PHARMACOKINETICS (PK) AFTER RENAL TRANSPLANTATION. B.L. Kasiske, V.K. Rao, K.L. Heim-Duthoy, M. Rose, W. Awmi, Henn. Co. Med. Ctr., U. of M., Mpls, MN.

Although a number of factors may influence CSA PK, few studies have addressed whether changes in these factors explain rapid changes in CSA PK posttransplant. CSA PK were measured sequentially in 21 adult, cadaver renal transplant recipients 1, 3, and 12 weeks after oral CSA was begun. CSA was measured in whole blood using HPLC, and CSA PK were normalized for the dose of CSA. Results: the only CSA PK parameters that changed significantly over time were those reflecting increased CSA retention in blood, i.e., area under the curve (AUC), steady state concentration, and trough level (TL, ng/ml/mg). During the study period hematocrit (HCT), plasma lipids, e.g. cholesterol (CHOL), and serum total protein (TP) also increased. Differences in group means (\pm SD) were tested using ANOVA:

Week	HCT	CHOL	TP	AUC	TL
1	27 \pm 3	201 \pm 36	5.2 \pm 0.5	10.4 \pm 3.9	.12 \pm .06
3	31 \pm 4	233 \pm 52	5.5 \pm 0.5	10.8 \pm 4.1	.16 \pm .10
12	38 \pm 7	251 \pm 65	5.9 \pm 0.8	14.4 \pm 5.7	.24 \pm .13
p	.000	.017	.005	.036	.006

Some of the factors that correlated with the altered CSA PK were: AUC vs HCT ($r=.38, p=.005$), AUC vs CHOL ($r=.44, p=.001$), TL vs TP ($r=.49, p=.000$). CSA PK parameters that reflect elimination did not change. In addition, factors that could affect CSA elimination, e.g. renal and liver function, did not correlate significantly with CSA PK. Our data suggest that alterations in posttransplant CSA PK may, in large part, be due to changes in factors that increase the retention of CSA in the bloodstream.

PORTAL VENOUS BUT NOT I.V. INFUSION OF ALLOGENEIC MONONUCLEAR CELLS CAUSES SUPPRESSOR CELL INDUCTION AND PROLONGED HEART GRAFT SURVIVAL. Scott Kenick* and Robin P. Lowry, Royal Victoria Hospital, McGill University, Montreal, Canada.

Heterotopic heart graft survival is markedly or indefinitely prolonged in rats inoculated with donor strain mononuclear cells (MC) by the portal venous (p.v.) route (Transpl. Proceed. 19:478, 1987). Conversely i.v. inoculation of donor MC has virtually no effect on survival. Although we have documented that allogeneic MC infused p.v. undergo immunologically specific entrapment in the liver (Clin. Res. 35:458A, 1987) the role of the liver in tolerance induction is unclear. Accordingly we assessed specific alloreactivity in splenic and lymph node compartments of naive LEW and LEW inoculated with allogeneic (WF) MC by i.v./p.v. routes. Briefly, in three separate experiments, the proliferative response of splenic and lymph node cells of LEW sensitized by the i.v. route, to irradiated WF stimulators, significantly exceeded the proliferative responses of LEW sensitized by the p.v. route, the latter being equivalent to the response of control LEW (mixed lymphocyte reactions harvested days 3, 4 & 5). Reduced *in vitro* responsiveness of LEW sensitized by the p.v. route was due to the presence of suppressor cells. In coculture experiments, admixture of splenic MC of rats sensitized by the p.v. route suppressed the *in vitro* proliferative responses of MC of rats sensitized by the i.v. route, while the admixture of MC from naive LEW was without effect. We suggest that the dramatic prolongation of heart graft survival observed following p.v. but not i.v. infusion of allogeneic MC is linked to differential suppressor cell induction.

RENAL TRANSPLANTATION IN USA IS NOT DISTRIBUTED FAIRLY. C.M. Kjellstrand, Dept. Med. Karolinska Hospital, Stockholm, Sweden.

Chronic dialysis shows unequal distribution in Canada, USA and Europe. We studied if similar differences existed in USA for transplantation, by calculating the chance for 23,026 patients on chronic dialysis, of receiving a renal transplant 1983, based on age, sex and race. In a subsample of 3,453 patients, we studied the influence of all factors combined and of the presence of HLA-antibodies.

6,112 (27 %) were transplanted. Men had a 31 % chance of transplantation vs a 22 % chance for women, white patients had a 30 % chance vs a 20 % for black. Patients younger than age 20 had a 100 % transplant rate, those aged 21 - 45 years a 58 %, those 46 - 60 years a 23 % those above age 60 years a 3 % chance (all differences $p < 0.001$). When all factors were combined, black patients, independent of sex, aged 21 - 45 years, had only half the chance of transplantation of white, (men 36 %, women 51 % vs 79 % and 73 % ($p < 0.02$)), and women, irrespective of race aged 46 - 60 years had only half the chance of receiving a transplant of men (11 % black and 21 % white vs 36 % and 32 % ($p < 0.05$)). The inequality persisted even when correction had been done for the higher presence of antibodies in women.

As in chronic dialysis transplantation is not distributed fairly. Biological, cultural and sociological factors are partly responsible but misunderstanding and covert and overt prejudice probably also exist. Honest physicians not tolerate the latter factors but get rid of them.

CYCLOSPORIN A ENHANCES RENIN SECRETION FROM ISOLATED RAT RENAL JUXTAGLOMERULAR CELLS.

Karlwilhelm Kühn and Armin Kurtz (introduced by M. Lorenzen). Med.Hochschule, Dept.of Nephrology, Hannover,FRG, and Univ.Zürich, Dept.of Physiology, Zürich,Switzerland.

Stimulation of the renin-angiotensin system is a major side effect of the fungal immunosuppressant cyclosporin A(CyA). The aim of our investigation was to find out whether or not this effect of CyA results from a direct interaction with renal juxtaglomerular cells which are the site of renin synthesis and release. Using primary cultures from rat renal cortex containing more than 80% JG cells we found that CyA (0.01-10 μ g/ml) time and dose dependently caused a threefold stimulation of renin secretion. This stimulation of renin secretion was paralleled by a twofold increase of inactive renin within the cells, whilst the intracellular amount of active renin was not altered by CyA. In order to identify a possible second messenger that could mediate the effects of CyA on JG cells, the simultaneous effects of a single concentration of CyA (1 μ g/ml) on renin secretion, prostaglandin formation and intracellular cAMP levels were examined. However, we failed to detect an effect of CyA on prostaglandin formation and cAMP whilst renin secretion was significantly enhanced.

Our results indicate that CyA is capable to stimulate renin secretion and synthesis by a direct effect on renal juxtaglomerular cells. This action of CyA is not mediated by changes in cellular prostaglandin formation or intracellular cAMP levels.

LYMPHOCYTE RECOGNITION OF HLA-A2: TOWARDS AN UNDERSTANDING OF THE ALLOGENEIC RESPONSE IN TRANSPLANT REJECTION. Alan M. Krensky, Peter Parham*, Russ Salter*, Gary Schoolnik*, and Carol Clayberger*, Stanford University, Departments of Pediatrics, Cell Biology, Medicine, and Medical Microbiology, Stanford, CA.

The interaction of lymphocytes with HLA molecules is fundamental to understanding transplant rejection. We have used monoclonal antibodies, cytolytic T lymphocytes, and site specific mutants to define the regions of the HLA-A2 molecule recognized by B and T cells. As little as one amino acid change in the HLA-A2 sequence can result in gain or loss of immune recognition. Peptides as short as 8 amino acids can inhibit or induce T cell recognition. Inhibition of killing occurs when the peptide binds to the T cell, presumably the T cell receptor. Induction of killing occurs when peptide binds to the target cell, presumably the HLA molecule. The crystal structure of HLA-A2 has recently been solved and we can now draw precise structure-function correlations. Such studies are fundamental to understanding allogeneic recognition and may allow specific immunomanipulation of transplant rejection.

ELEVATED CICLOSPORIN METABOLITES IN DILTIAZEM TREATED KIDNEY TRANSPLANT RECIPIENTS? Ulrich Kunzendorf*, Gerd Walz*, Karl Wagner*, Hans-H. Neumayer, Gerd Offermann. *Free Univ. of Berlin, Dept. of Medicine, FRG.

The calcium channel-blocking agent diltiazem produces an unexplained rise in ciclosporin blood levels measured by radioimmunoassay (RIA) (Wagner and Neumayer, Lancet ii:523,1986). This prompted us to measure ciclosporin levels daily by RIA and high performance liquid chromatography (HPLC) in 21 patients during the first 30 days after kidney transplantation. Immunosuppression of all patients consisted of ciclosporin and low-dose steroids. In contrast to controls (n=14), 7 patients received an additional bolus of 0.28 mg/kg diltiazem before transplantation and 0.002 mg/kg/min diltiazem in a continuous infusion for 48 hrs after transplantation, followed by oral application of 120 mg daily. In spite of comparable ciclosporin intake, the blood trough levels measured by RIA were significantly elevated in patients treated with diltiazem ($p=0.001$). However, there was no significant difference in HPLC blood trough levels. The ratio of RIA/HPLC blood levels was significantly increased in the diltiazem treated group ($p=0.016$). While the RIA estimates a composite of ciclosporin plus cross-reacting metabolites, the HPLC is specific for ciclosporin. Therefore, the results may indicate elevated metabolite levels of ciclosporin by concomitant administration of diltiazem. It has recently been shown that ciclosporin metabolites have immunosuppressive properties in vitro (Freed, Rosano and Lempert, Transplantation 43:123-127, 1987).

PURE ANTI-T CELL THERAPY (OKT3) MAY FAIL TO REVERSE ACUTE REJECTION OF A CADAVERIC RENAL TRANSPLANT. A. Lazarovits*, and C. Shield*. Transplant Immunology Laboratory, Ottawa General Hospital and Univ. of Ottawa, Canada and St. Francis Regional Medical Center and Univ. of Kansas Wichita, USA. (intr. by D.Z. Levine)

Allograft rejection is the single largest impediment to successful transplantation. Therapy targeted to lymphocytes has been in practice for many years using polyclonal heteroantisera. While these products are useful, there have been problems with specificity, lot to lot variability, and supply. Therapy with monoclonal antibodies such as OKT3 may circumvent these problems, whilst allowing for refined specificity. OKT3 is highly effective at reversing acute renal allograft rejection. The few treatment failures were attributed to anti-mouse antibodies eliminating the OKT3, or to delay of therapy to such a late stage that rejection was irreversible. We present 2 cases which demonstrate that pure anti-T cell therapy with OKT3 may fail to reverse rejection in cadaveric renal transplants in spite of absent CD3 positive cells in the peripheral blood and the presence of excess OKT3 in the serum (as measured by indirect immunofluorescence and flow cytometry). The OKT3 used for therapy showed appropriate fluorescent profile and inhibited ^{51}Cr release in a lymphocytotoxicity assay. Thus the OKT3 used for therapy had not denatured. Both patients had their rejection reversed with methylprednisolone. These data imply that CD3 negative lymphocytes may contribute to the rejection phenomenon.

EFFECTS OF RENAL TRANSPLANTATION ON A PATIENT WITH MYELOFIBROSIS, AGAMMAGLOBULINEMIA, CIRCULATING ANTI-COAGULANT, AND ASA RESPONSIVE THROMBOCYTOPENIA. David C. Lowance, Marvin Cohn Spencer Brewer, William Osborne, and John Whelchel. Piedmont Hospital-Emory University Transplant Program, Atlanta, Georgia.

A 29 year old white female with known circulating anticoagulant presented with acute total bilateral renal infarction. For 18 months the patient was maintained on dialysis. Her course was complicated by the development of ASA responsive thrombocytopenia (plts. 5,000), myelofibrosis, agammaglobulinemia, and numerous infections. Unsuccessful therapies included exchange plasmapheresis, plasmapheresis, immunosuppression, anticoagulation, steroids, and androgens. Out of desperation a paternal renal transplant was performed. One year post transplant on CyA, prednisone, and ASA, the patient has normal Igs, WBC, RBC, and plts. Her serum creatinine is stable at 2.3mg%.

Renal transplantation and subsequent treatment served to reverse this patient's hematologic abnormalities and should be considered as possible therapy in selected similar cases since this type disorder has been uniformly fatal.

MURINE PLACENTAL LISTERIOSIS AS A MODEL FOR STUDYING ALLOGRAFT TOLERANCE. Christopher Y. Lu, *Raymond W. Redline, Colleen M. Shea, and Michael J. Lombardi. Depts. of Med. & Pathol., Brigham & Women's Hospital, Boston, MA

Survival of the fetus with its paternal antigens in the potentially hostile maternal environment is a natural model of allograft tolerance, and may have implications for transplantation nephrology. We have recently shown that fetal allograft tolerance may result from local immunosuppression in the placenta, the maternal-fetal interface (JCI 79:1234, '87). We now explore the nature of this local immunosuppression which enables *Listeria* to enjoy safe sanctuary at this site. The table summarizes the frequency of leukocytes in infected lesions at discrete sites of 13 placentas.

Leukocytes/ (mm ² x100)	PMN's	mac F4/80 ⁺	Ia ⁺	T-cells L3T4 ⁺
maternal liver	2	57	27	7
basal plate	112	1	8	2
allantoic plate	12	24	1	1

Activated macrophages and T cells, which are required for an effective anti-*Listeria* response in the maternal liver, are prevented from entering the basal plate of the placenta. This region is unique in that allogeneic fetal and maternal cells coexist. Such coexistence may result from local immunoregulation, and the immunologic price may be an increased local susceptibility to *Listeria*. The anti-*Listeria* response in the fetally derived allantoic plate of the placenta was also remarkable. Macrophages were present but were not activated as evidenced by the absence of cell-surface Ia.

TRIPLE DRUG THERAPY WITH CYCLOSPORINE, AZATHIOPRINE AND PREDNISONE (CAP) IMPROVES PEDIATRIC RENAL TRANSPLANT RESULTS.

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Since 1985, all patients ≤ 20 yrs of age have received triple drug therapy with CAP, random donor transfusions and rabbit anti-lymphocyte serum (RALS) in the first 1-2 post-operative weeks until adequate renal function permits C trough level of 125-200 ng/ml (whole blood HPLC). This group (CAP, n=32) is compared with 44 children receiving conventional Rx with P and A (PA), transplanted in 1982-84. The PA group received 19 infusions of RALS over nine post-operative weeks, but no C. All other aspects of management were identical, including Rx of acute rejection. Results were:

	PA	CAP
Patients (no)	44	32
Age (yr)	13.4	13.6
Males (%)	58	55
LRD (%)	59	63
First Tx (%)	69	73
Deaths (%)	7	0
Functioning grafts (%)		
6 mo	84	91
12 mo	77	91
24 mo	70	84
No rejection (%)	18	66

The decreased frequency of acute rejection with CAP therapy has resulted in improved graft survival, shortened initial hospitalization, and marked reduction in repeat hospitalization.

LONG-TERM PREDICTORS OF GRAFT SURVIVAL BY MULTI-VARIATE ANALYSIS FOR A SINGLE-CENTRE KIDNEY TRANSPLANT PROGRAM. J. Madrenas, S. Newman, J.R. McGregor, *T. Kovithavongs, *J.B. Dossetor, *P. Halloran. University of Alberta, Edmonton, Alberta, Canada.

To test the goodness of fit of Cox model for the analysis of a single-centre kidney transplant program, and the effect of cyclosporine use, we studied 287 patients (136 cadaveric allografts on AZA, 84 on CsA, 43 living-related on AZA, and 24 on CsA).

After establishing that type of donor and main immunosuppressive drug can be included in the model, and following a step-down procedure from the initially tested 14 covariates, we obtained the following set of significant variables to predict graft survival: main immunosuppressive drug ($p=0.001$), number of HLA A and B mismatches ($p<0.02$) (DR antigens were not considered), highest percentage of panel cell reactive antibodies ($p=0.009$), nephroangiosclerosis as primary renal disease ($p=0.04$), and type of donor ($p=0.01$) in the presence of an interaction term between type of donor and main immunosuppressive drug ($p=0.04$). Pretransplant blood transfusions, cold ischemia time, and donor ABO blood group were initially significant but they dropped out in the step-down procedure.

According to our results, multivariate analysis of renal transplants in single centres can be performed including type of donor and immunosuppressive regimen as covariates, and the presence of interaction between them. Cyclosporine use has not improved graft survival in LRD recipients, and it has decreased the relevance of HLA A and B mismatching.

RENOVASCULAR EFFECTS OF SIMULTANEOUSLY INFUSED ERYTHROMYCIN (ER) AND CYCLOSPORINE (CY) IN THE RAT. A.J. McCormack, R.G. Snipes and W.F. Finn, Univ. of North Carolina, Chapel Hill, N.C.

ER exacerbates CY nephrotoxicity. This has been attributed to ER's potential to reduce the hepatic microsomal metabolism and clearance of CY. ER may also be nephrotoxic. We tested the hypothesis that ER may have direct effects on the renal vasculature which are additive or synergistic with the effects of CY. 18 Sprague-Dawley rats were administered graded doses of i.v. ER (2.5, 5, 7.5 and 10 mg/kg BW/min over 10 min intervals) and CY (1, 2, 3 and 4 mg/kg BW/min over 10 min intervals) either alone or simultaneously. Results are % baseline (means \pm SEM); * $p < 0.05$ vs. ER and CY alone:

ER	CY	MAP			RBF			RVR		
		ER	CY	ER+CY	ER	CY	ER+CY	ER	CY	ER+CY
0	0	100	100	100	100	100	100	100	100	100
2.5	1	89	114	100	99	100	103	89	116	98
		± 3	± 5	± 5	± 1	± 3	± 5	± 3	± 8	± 4
5	2	81	120	109	89	91	63*	90	136	181*
		± 4	± 9	± 6	± 1	± 4	± 5	± 5	± 15	± 21
7.5	3	87	126	116	63	76	39*	147	177	340*
		± 4	± 10	± 4	± 6	± 6	± 7	± 23	± 27	± 55
10	4	95	131	Died	26	66	Died	517	232	Died
		± 3	± 9		± 6	± 8		± 132	± 55	

Despite different effects on MAP, each drug alone produced a striking decrease in RBF. This effect was more pronounced when the drugs were infused concomitantly. The reduction in RBF occurred in an additive manner as a direct consequence of increased RVR. These results demonstrate that CY-induced renal vasoconstriction is exacerbated by ER and suggest that the decline in renal function observed in patients coadministered these drugs may be due to additive renovascular toxicity.

LOW MOLECULAR WEIGHT HUMAN B CELL GROWTH FACTOR (BCGF) INDUCES T CELL PROLIFERATION AND IL-2 RECEPTOR EXPRESSION. Olivia Martinez, Marian Marra, and Marvin R. Garovoy. Immunogenetics and Transplantation Laboratory, University of California, San Francisco, CA.

Understanding the regulation of T cell activation is of critical importance in developing successful forms of immunotherapy. We have examined the effect of the lymphokine low molecular weight BCGF (distinct from IL-4) on T cell proliferation. Using a submitogenic dose (1-15 ng/ml) of anti-CD3 monoclonal antibody (specific for the T3 complex associated with the T cell receptor) which "primes", but by itself causes no T cell proliferation, we studied the effect of added BCGF vs. recombinant IL-2. In 15 experiments on 9 different individuals, the proliferative response of T cells cultured with submitogenic doses of anti-CD3 plus BCGF (25%) for 3 days ranged from 5829 cpm to 34142 cpm ($x=15479 \pm 7670$). When the proliferative activity was measured daily, the peak response occurred on day 4 and declined thru day 6. In contrast, the proliferative activity of T cells cultured with anti-CD3 plus IL-2 (10 u/ml) reached maximal levels on day 6. The subsets of responding T cells as analyzed by two color flow cytometry indicated that BCGF as well as IL-2 were able to induce IL-2 receptor (IL-2R) expression on CD4+ (helper) and CD8+ (suppressor/cytotoxic) cells. Neither IL-1 nor IFN-gamma were able to mimic the effect of BCGF on T cells. These results are the first indication that human BCGF can induce proliferation of human T cells and expression of IL-2R on both CD4+ and CD8+ T cell subsets. These data suggest the possibility of an alternative (non-IL-2 dependent) pathway for T cell activation.

MORPHOLOGICAL ANALYSIS OF GLOMERULAR LESIONS AFTER RENAL-TRANSPLANTATION (RTX) IMMUNOSUPPRESSED WITH CYCLOSPORINE (CyA). K. Morozumi, A. Yoshida, T. Saganuma, T. Fujinami, and H. Takagi*. Nagoya City Univ. and Univ. of Nagoya*, Dept. Med. and Surgery*, Nagoya, Japan.

After clinical application of CyA, alterations of glomerular lesions in RTX were evident. The aim of the present paper is to characterize and clarify the glomerular lesions immunosuppressed with CyA. One hundred and fifty-one renal biopsied specimens of 128 (LD 76, CD 52) recipients were studied by light, immunofluorescent and electron microscopy, and compared with 143 biopsied specimens of 137 recipients under conventional immunosuppressive methods.

Glomerular endothelial cells were well reserved and intra-glomerular coagulation was less frequent during acute rejection under CyA treatment. Expansion of subendothelial space that is a common finding associated with multiple layer capillary walls and mononuclear cells in conventional chronic rejection, was rare during CyA treatment. On the other hand, 22 cases of post-RTX glomerulonephritis (GN) were diagnosed and comprised 12 cases of proliferative GN including 9 IgA GN, 3 membranous GN, 1 crescentic GN, 1 lupus GN, 1 FGS, and 4 unclassified GN. One hour biopsy revealed 6 cases of Ig-A GN that were transplanted from donor. The incidence of GN is 17.2% and significantly higher than conventional group (6.6%). Glomerular alterations in recipients immunosuppressed with CyA were interestingly modified and available to study the pathogenesis of glomerular injury in rejection process and of glomerulonephritis.

EFFECT OF CYCLOSPORIN A ON POSTISCHEMIC ACUTE RENAL FAILURE IN CONSCIOUS DOGS: ROLE OF VASOACTIVE RENAL HORMONES.

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The possible detrimental effect of cyclosporin A (CyA) on ischemically damaged kidneys is still controversial. We therefore studied the effect of CyA on renal function in unilaterally nephrectomized conscious dogs subjected to 120 min of ischemia. In contrast to control group A (n=7), group B (n=8) had oral doses of 60 mg/kg/d CyA from 3 days prior to ischemia till day 7 after ischemia. Thus, CyA whole-blood trough levels of about 2000 ng/ml (2259±404 ng/ml) were achieved. The two groups showed no differences in weight loss (-9.8 vs -8.0%). Though the post-ischemic course of GFR was not influenced by chronic CyA loading until day 3 (-45 vs -44%), it further deteriorated in group B on day 7 (-56 vs -38%). In parallel with a distinct RBF reduction (-21 vs +2.5%), renal vascular resistance was markedly increased in group B (+63 vs +0.7%). This might be due to progressively stimulated plasma-renin activity in group B (14.6±5.9 vs 4.8±1.3 ngATI/ml/h) and a concomitant increase in thromboxane (TXB₂) formation, since urinary TXB₂-excretion was 6-fold higher than in controls (3.4±0.9 vs 0.6±0.2 ng/min, p<0.001). Urinary excretion of PGE₂ remained unaltered in both groups, while that of 6-keto PGF₁ was also 2-fold higher in group B, suggesting increased PGI₂ formation to counterbalance the raised renal vascular tone. Our data suggest that prolonged CyA administration promotes post-ischemic ARF only in the recovery phase, the initial phase remaining unaltered. This might be due to an imbalance of vasodilatory and vasoconstrictory autacoids.

CYCLOSPORINE-INDUCED NEPHROTOXICITY ON THE RAT IS PROTECTED BY THE GINKGOLIDE MIXTURE, BN 52063. E. Pirotzky, Ph. Colliez,* C. Guilmar, J. Schaefferbeke,* and P. Braquet (intr. by I. Salusky). Institut Henri Beaufour Research Labs., 72 avenue des Tropiques 91940 Les Ulis (France), *Laboratoire de Biologie Cellulaire, Université Paris 7, 75006 Paris (France).

Although cyclosporine is one of the most successful immunosuppressive agent for organ transplantation, its therapeutic use is limited because of nephrotoxicity. Spontaneous hypertensive rats (207.5 ± 3.9 g) were treated for 21 days with cyclosporine (25 mg/kg/day; p.o.), either alone or in association with a platelet-activating factor antagonist derived from the *Ginkgo biloba* leaves (BN 52063, 50 mg/g/day; p.o.). Administration of cyclosporine resulted in a slight but not significant decrease of glomerular filtration rate (GFR) from 1.06 ± 0.06 ml/min to 0.86 ± 0.05 ml/min at day 12 and from 1.15 ± 0.13 ml/min to 0.90 ± 0.1 ml/min at day 21 (means ± SD, n = 9). Cyclosporine treatment was also associated with significant increase in plasma urea (BUN) from 0.25 mg/ml ± 0.02 to 0.32 ± 0.03 mg/ml at day 12 and from 0.23 mg/ml ± 0.02 to 0.33 ± 0.04 mg/ml at day 21. In addition, a reduction in the body weight of cyclosporine-treated rats was observed. Rats treated with cyclosporine and receiving BN 52063 exhibited values of GFR, plasma BUN and body weight similar to those of the control animals. The concomitant administration of BN 52063 with cyclosporine did not modify the immunosuppressive effect against sheep erythrocytes nor the pharmacokinetic pattern of plasma cyclosporine. Morphologically, cyclosporine-induced nephrotoxicity appears to be characterized by focal interstitial fibrosis with tubular atrophy. The morphological alterations were markedly less modified in the group of BN 52063 protected animals. Thus, BN 52063 could be a useful tool in the elucidation of the pathogenesis and in the prevention of kidney alteration during cyclosporine treatment.

CYCLOSPORINE-VERAPAMIL INTERACTION. JC Peterson, J Brannigan,* T Pickard,* R Thompson,* DR Salomon. Div. of Nephrology, Hypertension & Transplantation, University of Florida, Gainesville, FL.

Cyclosporine (CyA) has greatly improved renal allograft survival and is now considered an integral part of the immunosuppressive regimen for transplant recipients. Major clinical concerns in the use of CyA have been raised concerning nephrotoxicity and, more recently, certain agents have been shown to potentiate the nephrotoxicity of CyA including erythromycin, ketoconazole, aminoglycosides, amphotericin, trimethoprim and oral contraceptives. A drug interaction has been reported with diltiazem resulting in elevated trough levels of CyA. We noted a similar temporal association of elevated CyA levels and renal dysfunction in a patient who had recently started verapamil (V). We therefore undertook a retrospective analysis of hypertensive transplant patients on CyA over the past two years. Linear regression analysis was performed on two groups of patients - 38 on CyA without V (controls) and 6 on CyA with V. Calculation of 95% confidence bands demonstrated a highly significant difference in the dose-level relationship between the control group and the V group. Further analysis confirmed a significant CyA-V drug interaction which occurs at low CyA doses and is more pronounced at high doses. The explanation of why the CyA-V interaction raises the CyA level is speculative, but may involve competition by both drugs for the P-450 liver enzyme system.

OUTCOME OF CONVERSION (CON) TO ALTERNATE DAY (QOD) PREDNISONE THERAPY (P) AFTER PEDIATRIC RENAL TRANSPLANTATION (T). Martin S. Polinsky,* H. Jorge Baluarte, and Bruce A. Kaiser.* Temple Univ. Med. Sch., Dept. of Pediatrics, St. Christopher's Hospital for Children, Phila., PA.

CON to a long-term QOD regimen of P following renal T in children (C) may help to minimize steroid-related complications and, in particular, may improve growth if renal function remains adequate. To evaluate the impact of CON to QOD P on renal function in cadaver and living-related transplant recipients (CAD and LR, respectively), the records of 101 C who received allografts between 3/78 and 3/85 were reviewed. Thirty-four CAD and 14 LR aged 2.5-18 yrs were identified, in whom CON to QOD P had been attempted, and for whom > 12 mos of follow-up was available post-CON. All patients were receiving azathioprine in addition to P. CON was initiated at 3±1.4 (X±SD) mos, when the mean daily P dose (mg/kg/day) was .84±.3 in CAD and .84±.35 in LR (p=ns); this was then tapered to a single QOD dose over 4.9±3.2 mos. On this regimen all 14 LR, but only 21 CAD (61.8%) underwent CON to QOD P for ≥ 12 mos without allograft loss or returning to long-term daily P (p<.02). A significant mean difference in calculated creatinine clearance (C'cr ml/min/1.73m²) pre- vs. post-CON was noted for CAD (83.1±22.5 vs. 59.8±33.4, p<.0001), but not for LR (83.3±12.5 vs. 69.9±23.1, p=ns). Successful CON was further defined as remaining on QOD P for ≥ 12 mos with a C'cr > 75% of pre-CON levels; by this criterion 19 CAD (55.9%) failed CON vs. only 3 LR (21.4%, p=.06). At 12 mos post-attempted CON the mean daily equivalent P dose for LR (.27±.13) was significantly lower than that for CAD (.52±.39, p<.025). In conclusion, in C receiving standard immunosuppression post-T, attempts at CON to QOD P while maintaining adequate levels of renal function, are more likely to succeed in LR than CAD.

THE IMPACT OF CADAVER DONOR AGE ON RENAL ALLOGRAFT SURVIVAL AND FUNCTION. K.V. Rao Univ. of Minnesota and Hennepin County Med. Ctr., Minneapolis, MN.

To assess the effect of donor age on transplant (Tx) outcome, we analyzed the graft survival data, short (1 month) and long term (latest) renal function of 592 consecutive patients(pts) who received cadaver kidney Tx's at our center between 1-1-70 and 12-31-86. Mean follow-up: 43.2 months (range 0-208 months). The results of graft recipients whose donors were in the ideal age group (21-40 yrs) were compared with those who received kidneys from younger donors (6-20 yrs) and older donors (41-61 yrs). Recipient's age, diabetic status, splenectomy, prior transfusions, AB and DR compatibility, PRA, preservation time, repeat Tx's, post Tx ATN and ALG Rx were not significantly different between control group (21-40yrs) and the study groups (6-20yrs and 41-61yrs). $P > .2$ however more pts in the older donor age group (22%) received cyclosporine Rx compared to control (10%) $P = .002$. Donor Recipients Graft survival(%) Graft loss P

Age(yrs)	(N)	(1yr)	(2yrs)	(5yrs)	(n)	(%)	Value
6-20	203	74	69	56	106	(52)	
21-40	262	75	70	54	125	(48)	.4
41-61	127	73	66	54	57	(45)	.5

Donor age(yrs) Mean Serum Creatinine (mg/dl)
(Range) Mean (1 month) P (Latest) P

Donor age(yrs)	Mean	Serum Creatinine (mg/dl)	P	(Latest)	P
6-20	(15.9)	2.0±1.9		2.8±2.6	
21-40	(29.2)	1.9±1.5	.7	2.8±2.9	.9
41-61	(47.8)	2.0±1.0	.8	2.5±2.0	.3

Results were similar when we compared the youngest (6-10yrs, N=17) and oldest (51-61, N=35) subgroups with the control group (21-40yrs).

Our data suggest that donor age has no significant effect on graft survival or renal function in recipients of cadaver kidney transplants.

RECIPIENTS WITH ACUTE RENAL FAILURE FOLLOWING TRANSPLANTATION OF A PERFUSED ALLOGRAFT HAVE DIMINISHED GRAFT SURVIVAL RATES. R. Riggio, W. Stubenbord,* J. Cheigh, M. Suthanthiran, L. Tapia, R. Haschenmeyer,* K. Stenzel and A. Rubin. Rogosin Institute, Dept. of Med., The New York Hospital-Cornell Med. Ctr., New York, New York.

Pulsatile Perfusion (PP) and Cold Storage (CS) are the methods used at our Center for the preservation of kidneys. We examined the impact preservation methods had on the incidence of postoperative ARF and allograft survival rates. The database consisted of cyclosporine treated (CYA) recipients and a matched number of recipients on Azathioprine therapy (AZA).

RESULTS:

A. Incidence of ARF following transplantation				
Database (#pts)	PP	CS	P	
CYA Group (127)	74%	69%	0.81	
AZA Group (127)	70%	63%	0.66	
B. Cumulative Survival Rate of Allografts (2 yrs)				
Database (pts)	PP	CS	P	
CYA Group:				
1) all recipients (127)	62%	84%	0.02	
2) ARF present (89)	53%	83%	0.01	
3) ARF absent (38)	86%	86%	0.94	
AZA Group:				
1) all recipients (127)	41%	46%	0.70	
2) ARF present (86)	34%	52%	0.18	
3) ARF absent (41)	61%	38%	0.14	

These results indicate that neither method of kidney preservation used is selectively responsible for the incidence of ARF. Further, transplantation of perfused kidneys results in decreased allograft survival if ARF is present.

POST TRANSPLANTATION GLUCOSE INTOLERANCE: INCREASED INCIDENCE IN CYCLOSPORINE (CyA) TREATED PATIENTS (pts). D.Roth, M. Milgrom,* V. Esquenazi,* J. Miller. U. of Miami, Miami, FL.

Glucose intolerance following renal transplant has been traditionally described as a steroid-induced effect. Following the introduction of CyA to our protocols in 1982, we have noted a marked increase in the incidence of hyperglycemia. We retrospectively reviewed the course of 444 consecutive renal transplants from January 1979-July 1987. Patients with type I diabetes mellitus were excluded. Of the remaining 342, 47 became diabetic (D) post transplant (overall incidence=14%). All pts. received similar doses of methylprednisolone (1 gm. qd x 3 plus 2 mg./kg./d.p.o. tapered to 0.20-0.25 mg/kg/d. by 3 mo.). CyA was used with steroids for cadaveric (C) and one-haplotype living-related recipients. Trough serum CyA levels by RIA were maintained < 200 ng/ml. Of 234 pts. receiving CyA, 38 (16%) became D, in contrast to 9/108 imuran treated pts (8%) ($p < .05$). There were no significant differences in age, histocompatibility and mean steroid dose between groups. Actuarial 4 yr. graft survival was 58% in CyA-D group and 36% in the imuran-D group, both significantly less than our 75% non-D 4 yr. graft survival. The onset of D in the CyA pts occurred earlier (30/38 within 6 weeks of transplant; range 3d-21 mos.) than in the imuran group (4/9 within 6 weeks; range 5d-91 mos.). Major histocompatibility antigens DR3 or DR4 differences were not significant between groups. In summary, this retrospective study supports other studies suggesting that CyA may increase the incidence of post transplant hyperglycemia.

ALLOGENEIC RAT T SUPPRESSOR INDUCER CELLS WITH IN VIVO AND IN VITRO ACTIVITY. D.M. Rothstein*, A. Frankel*, M. Sayegh*, C. Kwok*, E.L. Milford*, C.B. Carpenter. Immunogenetics, Brigham And Women's Hospital, Boston, MA.

The presence of allogeneic Ag-specific T suppressor inducer cells with *in vivo* activity has been demonstrated by Hutchinson et al (Transplantation 41:547) using a rat renal allograft model. We now demonstrate the presence of *in vivo* and *in vitro* T suppressor inducer activity in the W3/25⁺ (CD4⁺) subset of lymphocytes from WF strain rats receiving LEW strain heterotopic cardiac allografts 5 days previously. Adoptive transfer of 2-4 X 10⁷ of these immune day 5 W3/25⁺ splenocytes, or their sonicates 7 days prior to transplantation, prolongs allograft survival 2-3 fold in unmodified or irradiated hosts (200 to 300 rads). This prolongation is strain specific.

Graft:	LEW	LEW	BN
Irrad. (d=0)	-	+	+
Control	5.5±0.8 (6)*	7.1±0.9 (4)	14.3±5.9 (3)
d5W3/25 ⁺ spl	11.0±1.6 (4)	17.0±5.7 (4)	5.0 (1)
Sonicate	10.5±2.1 (2)	13.0±1.4 (2)	5.0 (1)

*Graft survival in days (n)

Analogous experiments *in vitro* using immune day 5 W3/25⁺ lymph node cells co-incubated with syngeneic naive OX8⁺ (CD8⁺) lymph node cells for 7 days, give rise only to non-specific suppression of a test MLR. This suppressor inducer activity can be maintained by selecting day 5 W3/25⁺ cells for OX22⁺ phenotype, but is decreased when OX22⁺ cells are depleted. Neither the naive OX8⁺, nor the day 5 W3/25⁺ lymph node cells, when incubated separately for 7 days with IL-2, suppress a test MLR. These results are consistent with the presence of CD4⁺OX22⁺ T suppressor inducer cells in both lymph node and spleen of allogeneically challenged rats.

SUPPRESSOR CELL INDUCTION AND INDUCER LYMPHOKINE: EFFECTS OF CYCLOSPORINE(CY) AND METHYLPREDNISOLONE (MP). D.R.Salomon, L.L.Pickard* and M.O.Downs* Div. of Nephrology, HTN and Transplantation, University of Florida, Gainesville, Florida.

We have developed an *in vitro* assay for suppressor cell(SC) induction in the rat MLR and investigated the function of a suppressor inducer lymphokine (SIL) required for the generation and maintenance of SC activity. SIL is produced by alloactivated helper T cells. By agarose and sephadex gel filtration SIL is a single peak with an apparent 50K MW. SIL is required to maintain SC activity in secondary culture. While mature SC activity is radiation resistant, the precursor cell responding to SIL is inactivated by 1000R. Both these findings suggest a ligand:receptor interaction. Quantitative analysis of SIL activity in 8 consecutive assays demonstrated a highly significant correlation with a quadratic model(mean $r=.93$). This result predicts a single ligand:receptor interaction is required for the expression of SIL activity.

The addition of either Cy or MP to the MLR potentially inhibits cell proliferation, CTL and IL2 generation. SC activity can be generated, however, indicating that SC induction is spared by these drugs. Nonetheless, we have also demonstrated that the amount of SC activity in 9 experiments was 40% less than in control MLR, indicating that these drugs inhibit SC amplification. Notably, both SIL and suppressor/inducer cell activities were greater in the Cy-modified MLR than the controls, thus SC amplification is not SIL-dependent. Further investigations of SC induction and amplification, the role of SIL and the effects of Cy and MP should enhance our working knowledge of suppressor cells and immunosuppressive drugs in transplantation.

SHORT-TERM CYCLOSPORIN A (CyA) TREATMENT DOES NOT LEAD TO URINARY MAGNESIUM (Mg) WASTING IN THE RAT. J.D. Scandling and D.B. Ornt. Dept. of Medicine, Univ. of Rochester, Rochester, NY.

CyA-induced urinary Mg wasting is the putative cause of profound hypomagnesemia in bone marrow transplant recipients treated with CyA.

To test this hypothesis, normal rats on normal diet (0.04 mmole/g Mg) were treated daily with CyA, 12.5 mg/kg, i.p. or D₅W i.p. for 7 days. This dosage regimen results in plasma CyA levels of about 2000 ng/ml by radioimmunoassay in our experience. The control group (n=8) was pair-fed to the CyA group (n=8) to insure similar Mg intake. Daily urinary Mg excretion did not differ between the groups at any point; mean daily urinary Mg excretion was lower in the CyA group, $.25 \pm .04$ vs $.30 \pm .06$ mEq/day, $p<.05$. Mean daily urinary sodium excretion was lower in the CyA group ($1.02 \pm .20$ vs $1.22 \pm .16$ mEq/day, $p<.05$). Mean daily urine flow (5.76 ± 1.02 vs 9.52 ± 2.06 ml/day) and mean daily urinary potassium excretion ($.99 \pm .04$ vs $1.11 \pm .07$ mEq/day) were not significantly lower in the CyA group. At 7 days plasma Mg concentrations were similar ($1.25 \pm .10$ vs $1.30 \pm .05$ mEq/L in control), while plasma renin activity (12.8 ± 3.1 vs 21.2 ± 1.8 ng/ml/hr, $p<.05$) and aldosterone (18.8 ± 7.2 vs 58.7 ± 4.9 ng/dl, $p<.01$) were reduced in the CyA group.

Thus short-term CyA treatment in the rat does not lead to hypomagnesemia or urinary Mg wasting.

CALCIUM CHANNEL BLOCKER INHIBITION OF CYCLOSPORIN A (CyA) UPTAKE IN A RENAL TUBULAR CELL LINE, LLC-PK₁. J.E. Scoble*, J.C.M. Senior*, P. Sweny*, Z. Varghese*, J.F. Moorhead. Renal Research Unit, Royal Free Hospital London, U.K.

CyA nephrotoxicity is a major disadvantage to its use in renal transplantation and may be manifest by disorders of tubular cell morphology and function. Recent work has suggested a beneficial *in vivo* effect of calcium channel blockers in human renal transplant CyA nephrotoxicity. We examined the action of CyA on a pig kidney cell line, LLC-PK₁, thereby excluding any haemodynamic factors. CyA levels were measured by an established HPLC assay. CyA at 1,000 ng/ml did not affect cell growth in either normal or high calcium medium. Medium containing 2,000 ng/ml CyA \pm Verapamil was added to confluent monolayers and uptake expressed as ng CyA/mg cell protein, mean \pm SEM for groups (A) CyA (B) CyA \pm 0.5 mM Verapamil (C) CyA \pm 1 mM Verapamil, * $p<.01$ vs A, ** $p<.001$ vs A.

	5 min	30 min	60 min
A)	49.7 \pm 6.7	112.0 \pm 3.0	105.0 \pm 12.6
B)	53.5 \pm 14.7	71.0 \pm 6.4*	68.0 \pm 10.5
C)	61.5 \pm 22.0	49.0 \pm 9.5*	72.7 \pm 9.0*

In vitro Verapamil inhibits the uptake of CyA by LLC-PK₁ cells and this may have therapeutic implications for the amelioration of CyA nephrotoxicity.

EFFECT OF CYCLOSPORINE (CSA) ON FRACTIONAL EXCRETION OF SODIUM (FE_{Na}) IN RENAL TRANSPLANT PATIENTS. Bharat V. Shah*, Changgi D. Hong, M. Roy First, Dept. of Internal Med., University of Cincinnati Medical Center, Cincinnati, Ohio.

Nephrotoxicity of CSA is clinically well known and of major concern for clinicians. Its mechanism of nephrotoxicity is not well understood and there is no satisfactory method to prevent or predict it. Experimental animal and human studies indicate that CSA causes an increase in renal vascular resistance, a decrease in renal blood flow and a reduction of glomerular filtration. A retrospective analysis of FE_{Na} was performed in 126 consecutive renal allograft recipients treated with CSA to determine whether or not the pattern of FE_{Na} in CSA-treated patients differed from that observed in patients treated with conventional immunosuppression (Kid. Int. 16:167, 1979), and to study the behavior of FE_{Na} in relation to the episodes of acute reversible CSA nephrotoxicity (17 cases). It is shown that CSA induced a significant early decline in FE_{Na} compared with conventional immunosuppression, which lasted for 3 days; that 76% of acute reversible CSA nephrotoxicity was associated with a significant drop in FE_{Na}, which occurred an average 2.5 days before the rise in serum creatinine; and that acute CSA nephrotoxicity developed significantly more frequently in males ($p<.02$) for an unknown reason. Close monitoring of FE_{Na} may provide a clinically useful early warning for on-coming CSA nephrotoxicity in renal allograft.

HIGH CARBOXY (CPTH) AND MARKEDLY LOWER AMINO TERMINAL (NPTH) PARATHORMONE LEVELS IN RENAL TRANSPLANT (RT) PATIENTS (RTP'S) RESPOND TO ORAL CALCIUM (Ca) THERAPY (CT) WITH REDUCTION IN PHOSPHATE WASTING (PW). R. Steiner,* M. Ziegler,* B. Catherwood,* N. Halasz,* and L. Defetos.* UCSD Med. Ctr., VA Hosp., San Diego, CA. (Intr. by D.D. Fanestil.)

Hyperparathyroidism (HPT) in RTP's often is of uncertain clinical significance. We measured CPTH (pg/dL), NPTH (pg/dL), Ca (mg/dL), and PW as FEPO₄ serially (74 times) in 10 RTP's with PW (creatinine [Cr] 1.4±0.2 mg/dL [mean±SE], 15±4.2 mo. post RT) during control and during CT with dihydrotachysterol or 1-25 (OH)₂D₃. The CPTH/NPTH ratio was high (median .09) with significant inter-RTP variation and no relation to Cr (r=.23, p=.86). All 10 RTP's were HPT (>200) by CPTH, but 5 had normal NPTH (<200). Paired data from maximum NPTH before CT and minimum NPTH after 2 mo. CT showed CPTH 4122±1409 vs 1499±400*, FEPO₄ .61±.09 vs 48±.06*, Ca 9.4±.2 vs 9.9±.2. Likewise, data from maximum CPTH off CT and minimum CPTH on CT showed NPTH 498±139 vs 221±79*, FEPO₄ .58±.09 vs .45±.06*, and Ca 9.2±.3 vs 10.0±.2*. After stopping CT no change persisted in Ca, FEPO₄, or NPTH. These together with pilot data (ASN '84) in 22 RTP's suggest that RTP's with normal NPTH (75±10 vs 759±192†) have lower CPTH (811±109 vs 4479±834†), higher Ca (9.8±.1 vs 9.4±.1*), and lower FEPO₄ (.37±.06) vs 75±.09†). Conclusions: In RTP's (1) CPTH overestimates HPT, if any, as defined by NPTH, which may explain "PTH refractoriness" seen in some RTP's; (2) with minimal azotemia factors besides GFR increase the CPTH/NPTH ratio; (3) HPT and PW are not fixed but respond to chronic CT; (4) marked PW suggests the presence of true HPT as defined by elevated NPTH. (*p<.05; †p<.01).

REDUCTION OF CYA NEPHROTOXICITY IN AN ISCHEMIC RAT KIDNEY MODEL BY 16, 16-DIMETHYL PROSTAGLANDIN E₂ (dmPGE₂). N.T. Stowe, K. Jojima*, M.D. Cressman, J. Pestana*, M.O. Magnusson*, A.C. Novick Cleveland Clinic Foundation, Cleveland, Ohio

Cyclosporine (CyA) nephrotoxicity is enhanced in the presence of renal ischemia. The purpose of this study was to determine if CyA-induced nephrotoxicity in the presence of renal ischemia would be reduced by an angiotensin converting enzyme inhibitor, captopril or by a prostaglandin analogue dmPGE₂. Male Sprague Dawley rats underwent uninephrectomy and 45 min ischemia of the remaining kidney. CyA (50 mg/kg, p.o.) alone, with captopril (10 mg/kg, p.o.) or with dmPGE₂ (15 ug/kg, s.c.) was administered for 11 days. RBF and GFR were then measured under anesthesia (Inactin) using a flow probe and inulin clearance respectively. The combination of renal ischemia and CyA (n=11) resulted in a significantly lower GFR of 0.29 ± 0.05 (S.E.M.) ml/min (p<.05) and a RBF of 5.4 ± 0.5 ml/min compared to ischemia controls. When captopril (n=7) was given, GFR remained at 0.35 ± 0.06 ml/min and RBF at 5.4 ± 0.4 ml/min. However, with dmPGE₂ (n=9), GFR was significantly elevated over the untreated and captopril groups to 0.71 ± 0.08 ml/min (p<.05) with a RBF of 6.9 ± 0.7 ml/min. These results, obtained in an ischemic kidney model support the reported protective effect of dmPGE₂ in reducing CyA nephrotoxicity as demonstrated in a nonischemic CyA rat model. Furthermore, PGE₂, at the doses tested, proved to be superior than captopril in reducing CyA nephrotoxicity.

POLYGLLOBULISM IN RENAL GRAFT RECIPIENTS DUE TO A DIRECT EFFECT OF CYCLOSPORIN A

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Polyglobulism develops in about 6% of renal graft recipients on Cyclosporin A (CyA) and prednisolone therapy. We studied if this finding is due to an increased release of erythropoietin (EPO) from renal graft or a consequence of a direct effect to the immunosuppressive therapy. 12 transplant recipients with polyglobulism were studied (mean serum creatinine 1.9±0.8 mg/dl; Hb 17.5±0.6 mg/dl; mean CyA blood trough level 430 ng/ml-RIA method, mean prednisolone dose 7.5 mg/die). The mean EPO serum level was significantly lower (35.6±16 miu/ml) than in the control group without polyglobulism (EPO=83.3±26 miu/ml, n=12, Hb 14.2±0.5 mg/dl, matched kidney graft function, immunosuppressive therapy, posttransplant period, age and sex; p 0.0005). Peripheral blood erythroid stem cells (BFU-E) were significantly increased (1205±629 cells/ml) in all polyglobulic patients investigated in comparison with the control group (450±245 cells/ml). Normal pO₂ levels excluded hypoxic cause of polyglobulism. Our investigations show that polyglobulism in renal graft recipients is not caused by an increased release of erythropoietin from the kidney graft but seems obviously to be due to an enhancing effect of the immunosuppressive therapy on the erythroid stem cell compartment associated with regulatorily diminished erythropoietin serum levels. A genetic predisposition in developing polyglobulism might be possible as 80% of these patients have the HLA-A₂ locus.

ERYTHROPOIESIS AND RADIOIMMUNOASSAYABLE RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEPO) IN RENAL TRANSPLANT (Tx) RECIPIENTS. C.H. Sun*, W. Paul*, H.J. Ward, M.A. Koyle* and D.B.N. Lee. Harbor-UCLA Med Ctr, Torrance, VA Med Ctr, Sepulveda, and SmithKline Bio-Science Lab, Van Nuys, California.

Recent studies demonstrated that large doses of exogenous rHuEPO (ExoEPO, 150-500 U/kg) corrects anemia in uremic patients. We measured serial changes in endogenous rHuEPO (EndEPO) in 31 Tx recipients and relate these changes to the post-Tx resolution of anemia. Following recovery of Tx function, hematocrit (Hct), %, increased from 20±2(SE) to normal (M=40, F=36) and serum ferritin (FER), ng/ml, decreased from 389±166 to 95±54 over 58±10 d. EPO, mU/ml, rose from 14±2 to 148±39 by 2nd post-Tx d (PTD) and resolved, as rapidly, by 6th PTD. It occurred even in the absence of Tx function and was not aborted by blood transfusion and probably reflected overall graft response to hypoxia, blood loss and exposure to uremic environment. This early peak was followed 2-24d later by a more sustained and moderate rise in EPO to 66±19. This EPO wave, which began 0-12d after the recovery of Tx function, was paralleled by rise in Hct, but dropped to normal when Hct rose to 33±1. Thereafter, Hct continued to climb in face of normal EPO levels. EPO failed to normalize in 2 patients with erythrocytosis and in 6 patients with post-Tx iron-deficiency anemia. We conclude that in post-Tx patients, modest increases in EndEPO induces rise in Hct and fall in FER similar to those observed in uremic patients given large doses of ExoEPO (calculated plasma EPO up to 100 fold higher). Once initiated, erythropoiesis is sustained by "normal" EPO levels.

SERUM IL-2 ACTIVITY IN KIDNEY GRAFT RECIPIENTS. G.Sunder-Plafmann, F.Stockenhuber, P.Balcke. 1st Medical Clinic, University of Vienna, Austria.

At present three substances are used for long-term immunosuppressive therapy in renal graft recipients. The primary effect of corticosteroids is directed at IL-1 producing monocytes, CyA blocks the release of IL-2 from T₄-lymphocytes and azathioprine inhibits the proliferation of immunological competent lymphoid cells. In rejection episodes immune response overcomes these barriers. We studied if measurement of serum IL-2 levels could be a helpful marker in detection of kidney graft rejection. Serum IL-2 activity was measured using an enzyme immuno assay in 61 renal graft recipients on prednisolone and CyA therapy and in 23 patients under prednisolone and azathioprine. 20 healthy subjects served as control group. Detectable IL-2 levels could neither be found in the control group nor in patients on prednisolone and CyA therapy with stable graft function (n=49). However, IL-2 levels were strikingly increased in all 12 patients on this therapy with acute graft rejection. Mean serum IL-2 level was 92,75±69,10 U/ml. On high dose methylprednisolone therapy IL-2 levels decreased and became undetectable. Patients on prednisolone and azathioprine with stable graft function frequently showed slightly elevated serum IL-2 activity. Increased values were found in 14 out of 23 patients without signs of graft rejection respectively (7,43±4,42 U/ml). We conclude that serum IL-2 activity is a new important marker in the immunological monitoring of renal grafting. However, in patients on conventional therapy increased IL-2 levels do not necessarily indicate graft rejection, but may reflect the different sites of drug action.

ANALYSIS OF SITE OF ACTION OF CYCLOSPORINE (CSA) ON THE T CELL ACTIVATION PATHWAY. M. Suthanthiran, A. Subramaniam,* P. Sehajpal,* and K.H. Stenzel. The Rogosin Institute, Cornell University Medical College, New York, New York.

T cell activation is dependent upon interactions among T cell surface sites and their natural ligands, accessory cell signals and phosphatidylinositol (PI) hydrolysis resulting in the Ca²⁺ signal and protein kinase C (PKC) activation. We have analyzed the site of action of CSA with respect to these events by using three distinct models of T cell activation. Highly purified human peripheral blood T cells were activated with 1) anti-CD₃ + accessory cells (accessory cell dependent T cell activation, Model I), 2) cross-linked anti-CD₃ + anti-CD₂ (accessory cell independent T cell activation, Model II), 3) ionomycin + sn-1,2-dioctanoyl glycerol (Ca²⁺ signal and PKC activation independent of PI hydrolysis, Model III). CSA mediated significant (P<0.01) inhibition of T cell proliferation in all 3 models. The half maximal inhibitory concentrations of CSA were 95, 75 and 90 ng/ml for models I, II and III, respectively. Recombinant IL-2 (100 units/ml) did not completely reverse the inhibition found with CSA. In experiments examining early T cell activation events, CSA did not interfere with the increase in intracellular free Ca²⁺ concentration but prevented the expression of IL-2 receptors. We therefore conclude that 1) CSA has a direct inhibitory effect on T cells that is independent of its effects on accessory cells, 2) events distal to PI turnover and Ca²⁺ mobilization but proximal to nuclear activation are inhibitable with CSA and 3) intracellular processes, in addition to those linked to IL-2 production, are inhibited by CSA.

PLASMA NEOPTERIN LEVELS ARE USEFUL IN IMMUNE MONITORING OF RENAL ALLOGRAFTS. M. Takasugi† C.Sun M. Koyle‡ and H. Ward. Harbor-UCLA Transplant Section and UCLA Tissue Typing Laboratory, Los Angeles

A major shortfall of the cyclosporine (Csa) era has been our inability to monitor for acute rejection and differentiate Csa-nephrotoxicity from rejection episodes. In addition to serum creatinine, we have measured plasma levels of neopterin, a macrophage secretory product which is thought to reflect activity of the humoral immune system. We measured neopterin by radioimmunoassay in a retrospective study of 65 consecutive renal allograft recipients treated with Csa. Patient groups were comprised of those with uneventful courses (control, N=10), biopsy-proven rejection (N=15), Csa nephrotoxicity (N=30), and cytomegalovirus (CMV) infection (N=10). Since neopterin levels in the plasma are higher in renal failure, all patient data were expressed as the ratio of neop/creat.

group	N	serum Cr	Csa level ^ω	Neop ^ω	Neop/creat
A. cont	10	1.4±0.4	194±61	18.3±6.6	12.8±4.5
B. AR	15	4.8±2.5 ^K	228±191 ^K	163.8±145 ^K	35.8±24 ^K
C. Csa	30	2.7±1.6	424±396 ^K	41.3±26.3 ^K	19.2±9.2 ^K
D. CMV	10	2.8±1.3	160±87	240.1±237 ^K	89.1±59 ^K

K= p<0.01; all values $\bar{x} \pm SEM$; $\omega = ng/ml$
 Plasma neopterin levels allowed differentiation between each group with elevated levels antedating rises in creatinine by 4 to 5 days in each group. We conclude that measurement of plasma neopterin is useful in immune monitoring of renal allografts and may facilitate differentiation of acute rejection episodes from Csa nephrotoxicity and CMV disease. Further study of neopterin levels in a prospective analysis is warranted.

TISSUE EOSINOPHILS (E) AND LEVELS OF EOSINOPHIL MAJOR BASIC PROTEIN (MBP) IN SERUM AND URINE DURING ACUTE ALLOGRAFT DYSFUNCTION. R. M. Ten,* V. E. Torres, G. M. Kephart,* J. A. Katzman,* K. E. Holley, G. J. Gleich.* Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

E are frequently but inconsistently increased in blood, kidney, and urine during acute allograft rejection (AR). Since participation of E in the pathogenesis of AR requires attraction and degranulation, we have correlated their presence and degranulation in renal biopsies with the serum and urine MBP levels in AR, tubular necrosis or cyclosporine toxicity (ATN-C), and kidney donors (CN). MBP, β_2 microglobulin (β_2M) were measured by RIA, ELISA. Cellular and extracellular localization of MBP was studied by IF. The extent and severity of E infiltration (E.In. 0-4) and degranulation (DGr 0-3) were semiquantitatively assessed.

	Biopsy						MPB levels	
	n	with E.In.	with DGr.				n	with >CN +2SD
		0	1	2	3	4	serum	urine
CN	7	7	0	0	0	0	10	--
ATN-C	6	5	1	0	0	0	8	0
AR	15	1	3	4	4	3	2	12

Tissue E by IF were more prevalent than suggested by H&E sections. Marked extracellular deposition of MBP was observed in some biopsies. Urine, but not serum, MPB levels were elevated in AR (28±7 ng/ml, $\bar{x} \pm SEM$), as compared to CN (4±1, p=0.006) and ATN-C (9±4, p=0.052). Urine MBP levels in ATN-C were not different from CN. In contrast, both serum and urine levels of β_2M were elevated in AR and also ATN-C. These observations support the participation of E in the pathogenesis of AR and suggest that urine levels of MBP may be helpful in the diagnosis of AR.

METHYLPREDNISOLONE PHARMACOKINETICS IN RENAL TRANSPLANT PATIENTS RECEIVING CYCLOSPORINE.

Kathleen Tornatore,* Gene Morse,* William Jusko,* and J. Joseph Walsh. SUNY at Buffalo, Depts. of Pharm., Pharmaceuticals and Med., Buffalo, New York.

Renal transplant patients generally receive standardized doses of immunosuppressive agents as per protocol. Cyclosporine has been reported to decrease the clearance of oral prednisone in renal transplant recipients. However, understanding of the effect of cyclosporine upon methylprednisolone (MePd), a glucocorticoid commonly used for intravenous (IV) therapy, is limited.

The disposition of IV MePd (Dose: 10-60 mg/d) was examined in nine renal transplant patients during the post-transplant period (10 days-1 year). Plasma samples were collected over 24 hours and analyzed for MePd via HPLC. Pharmacokinetic parameters were determined by noncompartmental analysis.

The mean total clearance of MePd was 375 ml/h/kg (range 105 - 680 ml/h/kg) and the volume of distribution averaged 1.33 ± 0.4 L/kg. The mean plasma half-life was 2.6 ± 1.2 h. When normalized for dose in mg of MePd received, the mean peak concentration at 1 h was 10.7 ± 3.6 ng/ml with an 8 h concentration ranging from 0.1 to 3.5 ng/ml.

These results indicated that an appreciable degree of variability in MePd metabolism exists in renal transplant recipients receiving cyclosporine. This observation may partially explain the unpredictable response to MePd therapy seen in acute rejection. The role of variable clearance as a predisposing factor in acute allograft rejection or chronic steroid toxicity also requires further study.

SIGNAL TRANSDUCTION AND THE ACTIVATION OF HUMAN T LYMPHOCYTES BY CROSS-LINKING CLASS I MHC MOLECULES. M.C. Wacholtz*, T. Geppert* and P.E. Lipsky* (Intr. by D. Seidin). UTHSCD, Dallas, TX.

Class I major histocompatibility complex (MHC) gene products expressed on the surface of most nucleated cells serve as recognition and restriction elements in cytotoxic T cell responses and appear to play a role in the accessory cell-T cell interactions involved in the induction of T cell proliferation. The present studies examined the possibility that MHC molecules might also serve as signal transducing structures directly stimulating functional and biochemical changes in the T cell. Purified T cells were used to examine the effects of monoclonal antibodies (Mab) directed against class I MHC determinants. Immobilizing anti-class I Mab to microtiter plates or cross-linking Mab to class I MHC molecules on the surface of T cells with secondary antibody induced IL2 responsiveness or co-stimulated proliferation with phorbol myristate acetate (PMA). Similar results were observed with Mab to β_2 microglobulin but not LFA-1. The capacity of these Mab to increase intracellular calcium ($[Ca^{2+}]_i$) was examined by flow cytometry using indo-1 loaded T cells. Reacting T cells with anti-class I Mab or an anti- β_2 microglobulin Mab and cross-linking them with secondary antibody stimulated an increase in $[Ca^{2+}]_i$. In the absence of the secondary antibody, no increase in $[Ca^{2+}]_i$ was detected. Neither control Mab nor Mab directed against surface LFA-1 increased $[Ca^{2+}]_i$. These studies demonstrate that class I MHC antigens are not only important as recognition elements, but when cross-linked can also directly transmit activation signals to the T cell.

INTERACTION OF CICLOSPORIN (CYA) AND CALCIUM ANTAGONISTS (CA). K. Wagner, M. Henkel, H.-H. Neumayer, Free University of Berlin, F.R.G.

In isolated case reports an interaction between CyA and CA has been reported. We therefore investigated the influence of the CA diltiazem (D) and nifedipine (N) on CyA therapy in two clinical trials in kidney transplant recipients. In a retrospective study CyA dose after start of D (n=23) was reduced by $43 \pm 5\%$ (p<.0001), compared to $-21 \pm 4\%$ with N (n=24). Clearance of CyA (CL_{CyA}) dropped by $50 \pm 9\%$ (D) and by $-11 \pm 3\%$ (N). After withdrawal of N CyA blood levels (RIA) were stable and 0.15 rejections per patient occurred within 100 days, whereas after withdrawal of D CyA levels dropped by $-37 \pm 6\%$ (p<.0001) and 0.3 rejections occurred per patient (p<.05). In a prospective study in 22 kidney graft recipients D (120 mg/day) raised CyA levels by $176 \pm 12\%$ (p<.001). CL_{CyA} dropped by $40 \pm 4\%$ (p<.001). GFR (inulin-single-shot) was not altered. Pharmacokinetics of orally applied CyA also showed no difference in the invasion or in the elimination rate, however, basal concentration and maximum concentration were significantly higher under D. Reduction of CyA dose by $39 \pm 4\%$ (p<.0001) led to comparable CyA levels as without D. Conclusion: N has no effect on CyA therapy, whereas D leads to drastic elevation of CyA blood levels without influencing graft function. This increment might not be due to changes in resorption, bioavailability or elimination but rather to a change in distribution volume of CyA.

FACTORS INFLUENCING EARLY KIDNEY GRAFT FUNCTION

K. Wagner, H.-H. Neumayer, Dept. of Nephrology, Free University of Berlin, F.R.G.

In 183 cadaveric kidney transplantations immunosuppressed with ciclosporin (CyA), early graft function was analyzed: 7.1% grafts never functioned; primary function (PF) occurred in 50.3% and delayed graft function (DGF) in 42.6% (p<.05). In PF donor-creatinine was significantly lower: 93 ± 3 vs 114 ± 5 $\mu\text{mol/l}$ in DGF (p<.001). Cold ischemia time (CIT) was longer in DGF: 26 ± 1 vs 22 ± 1 hrs in PF (p<.001), warm ischemia time (rewarm phase, WIT) was also longer in DGF: 40 ± 1 min compared to 35 ± 1 min in PF (p<.05). Ischemia Index (IND) was lower in PF (47 ± 2) compared to 64 ± 3 in DGF (p<.001). Patients with PF received a lower CyA dose within the first week: 52 ± 1 vs 59 ± 2 mg/kg/week in DGF (p<.01). High CyA doses resulted in a lower incidence of PF. Additional analysis in local (LD) and external donors (ED) showed the relevance of those factors: IN LD PF was 60% compared to 39% in ED (p<.01), although ED had a higher diuresis (321 ± 29 vs 216 ± 15 ml/min/LD), were younger (29 ± 1 vs 39 ± 1 yrs LD), needed less catecholamines (80% vs 93% LD) and had fewer mismatches (1.9 ± 0.1 vs 2.4 ± 0.1 LD), however, ischemia times were longer: CIT ED: 28 ± 1 vs 20 ± 1 hrs LD, p<.001, WIT ED: 41 ± 1 vs 35 ± 1 min p<.01, IND ED 71 ± 3 vs 43 ± 2 LD p<.001. Conclusion: early kidney graft function is determined mainly by ischemic damage of the graft during the preservation period as well as interactive CyA toxicity in the early posttransplant period.

INTRAVENOUS (IV) KINETICS AND ORAL BIOAVAILABILITY (F) OF CYCLOSPORINE (C) IN THE POST-OPERATIVE PERIOD OF RENAL TRANSPLANT (RT) PATIENTS. J. Joseph Walshe, Mark Holdsworth*, Rocco Venuto, Joseph Gerbasl*, and Gene Morse*. SUNY at Buffalo, Depts. of Med., Pharm. & Surgery, Buffalo, N.Y.

The bioavailability of C has been reported to vary from 5-60%. However, studies of C absorption during the first month post-renal transplantation have lacked correlation with IV C kinetics and employed RIA methodology.

In the present study, 7 cadaveric RT recipients received 3.5 mg/kg of C IV over 8h X 3 days and subsequently 8 mg/kg/24h orally 1h before breakfast. During the third IV dose, and on 3 separate days within the first month after RT, 24h pharmacokinetic studies were undertaken. Whole blood concentrations were determined by HPLC. C levels at the end of the IV infusion were 1244 ± 48 ug/ml and declined biexponentially to 275 ± 146 ng/ml at 12h and 143 ± 38 ng/ml at 24h. Total body clearance was 0.37 ± 0.13 L/min, volume of distribution was 3.2 ± 1.8 L/kg with the T 1/2 of 11.3 ± 3.4 h. Despite a similar F between the oral study periods (0.29 ± 0.1 , 0.28 ± 0.1 and 0.32 ± 0.3), intrapatient variability was great.

IV C was well tolerated, achieved adequate blood levels and was not associated with any apparent renal or pulmonary complications. However, conversion to oral therapy was complicated by a wide range of inpatient F values. Inpatient decline in F was associated with low C blood levels and prolonged hospitalization. Based on this limited study, a longer initial IV treatment period may be indicated to overcome absorption variability associated with oral C therapy during the critical post-operative period after RT.

CYCLOSPORIN REDUCES DEGRANULATION OF PMN-LEUKOCYTES IN PATIENTS FOLLOWING CADAVERIC RENAL TRANSPLANTATION. C. Wanner*, P. Schollmeyer*, H. Wilms*, W.H. Hörl*, Univ. of Freiburg, Dept. of Medicine, Freiburg, FRG (intr. by T.F. Lüscher).

It has been shown that patients after renal transplantation show reduced degranulation of PMN-leukocytes during hemodialysis.

Therefore, we investigated plasma levels of granulocyte elastase in complex with α_1 -proteinase inhibitor (E- α_1 PI), lactoferrin and myeloperoxidase in 15 patients under immunosuppression with prednisolone and ciclosporin before and during 22 days following cadaveric renal transplantation. In vitro incubation experiments were performed in order to study the specific effect of several immunosuppression drugs on PMN-leukocyte degranulation.

Compared with patients undergoing abdominal surgery (AS; n=10); transplanted patients (TP) showed significantly lower levels of E- α_1 PI and lactoferrin, whereas plasma myeloperoxidase values were unchanged. E- α_1 PI levels of AS vs TP patients were at day 1: 555 ± 99 vs 230 ± 33 ng/ml; day 5: 440 ± 42 vs 179 ± 29 ng/ml; day 9: 370 ± 57 vs 130 ± 23 ng/ml. Lactoferrin values of AS vs TP patients were at day 1: 800 ± 271 vs 245 ± 60 ng/ml; day 5: 334 ± 66 vs 129 ± 26 ng/ml; day 9: 281 ± 29 vs 120 ± 17 ng/ml. In vitro incubation experiments of whole blood and isolated PMN-granulocytes revealed a significant lower level of plasma E- α_1 PI and lactoferrin after 60, 120 and 180 min of incubation in the presence of ciclosporin compared to controls, whereas azathioprine and prednisolone showed no effects.

In summary our results document that ciclosporin reduces degranulation of PMN-leukocytes in patients following cadaveric kidney transplantation and also in in-vitro experiments. Similar results could not be demonstrated with azathioprine or steroids.

BLOOD TRANSFUSIONS ARE A CRITICAL IMMUNE MODIFICATION IN BLACK RENAL TRANSPLANT RECIPIENTS. H. Ward M. Koyle† P. Terasaki‡ M. Cecka* Harbor-UCLA Renal Transplant Section and UCLA Tissue Typing Laboratory, Los Angeles, CA.

We have previously reported on the outstanding patient and allograft outcomes that can be achieved in the cyclosporine (Csa) era. In a retrospective analysis of our single center results in black vs non-black recipients of cadaver allografts and of National and International Transplant Registry data, we identified the immune modifications which favorably affected graft outcome. Csa use, B, DR tissue match, pretransplant transfusion status & transplant center effect in 66 Harbor recipients (blk, + Csa) vs 85 non-blk Csa patients, and in over 488 blks (+Csa) vs 2591 non-blk + Csa renal transplant recipients from registry data.

group	N	graft survival ϕ (+)trans (-)trans	graft survival ϕ 0-2 mm 2-4 mm	(B, DR)
Harbor blks	66	85.6%	80.5%	86.9% 79.4%
non blk	85	83.7%	85.2%	87.3% 82.9%
Registry blk	488	75% ϵ	54.5%	65.9% 68.7%
Registry non-blk	2591	78%	75% κ	81.2% κ 80.6% κ

κ =p<0.01 blk vs non blk; ϵ = p<0.001 transfused vs non transfused; mm=mismatch; ϕ = one-year. Although our single center data does not indicate a clear benefit from transfusion, multicenter data indicates that the results in non-transfused black are no better than with azathioprine.

INHIBITORY EFFECT OF VERAPAMIL (V) ON THE ACTIVATION AND FUNCTION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBM) IS ADDITIVE WITH CYCLOSPORINE (CSA) AND MAY OCCUR THROUGH CALCIUM (Ca) INDEPENDENT MECHANISMS. M.R. Weir, R. Pepler*, D. Gomolka*, B.S. Handwerker*. Univ. of Maryland Hosp., Baltimore, MD.

Ca is essential for lymphocyte activation and function. We have studied the effects of varying concentrations (C) of V (5×10^{-7} M, 5×10^{-6} M, 5×10^{-5} M) on the immunological function of human PBM in the presence of CSA (0-600ng/ml). The proliferative response to mitogenic lectins, and the generation of cytotoxic T lymphocytes (CTL) following allogeneic stimulation were inhibited in a C-dependent fashion (CDF) with V. This inhibition was additive to the inhibition induced by CSA; a V dose of 5×10^{-6} M reduced by 50% the amount of CSA necessary to cause the same level of inhibition were no V present. Lectin induced 45 Ca uptake by PBM was inhibited in a CDF by V; in contrast CSA caused a large early increase in cytosolic Ca. Lectin-induced IL-2 receptor expression by PBM was markedly inhibited only by the highest C of V; CSA had only minimal effects. V inhibited the effector activity of both natural killer cells and previously generated CTL, while no inhibition was demonstrated with CSA. The response of the helper T cell clone HT-2 to recombinant IL-2 was significantly inhibited in a CDF by V and was synergistic with CSA. IL-2 receptor expression and response are reported to be Ca-independent. Thus, V may have Ca-independent and Ca-dependent effects on lymphocyte function. The inhibitory effects of V on the afferent limb of immunity are additive or synergistic with CSA. However V, unlike CSA, has inhibitory effects on the efferent limb of immunity and has potent ability to decrease transcellular uptake of Ca.

OMEGA-3 POLYUNSATURATED FATTY ACIDS (PUFAs)
IMPROVE LIPID PROFILE BUT NOT RENAL HAEMODYNAMICS
IN RENAL TRANSPLANT RECIPIENTS (RTR).

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Dietary fish oil supplements containing PUFAs have several biological actions which may benefit RTR. PUFAs 1) Increase renal plasma flow (RPF) and glomerular filtration rate (GFR) in normal individuals 2) Reduce cyclosporin A (CsA) nephrotoxicity in rats and 3) Improve abnormal lipid profiles. The effects of PUFAs on renal haemodynamics and lipid abnormalities in 16 RTR was studied. Patients lacked renal reserve capacity when challenged with an oral protein load. Abnormal lipid profiles were present in 11 patients [raised cholesterol (Chol) in 2, raised triglyceride (TG) in 3, raised Chol and TG in 6]. RPF (125-I hippuran clearance), GFR (51-Cr EDTA clearance) and fasting lipids were measured before and after 6 weeks supplementation with MaxEPA 0.2ml/kg/day. *p>0.001 # p=NS.

Mean±SEM	PRE-MaxEPA	POST-MaxEPA	POST-PRE
TG (mmol/l)	1.92±0.23	1.33±0.15	-0.58±0.10*
Chol "	6.60±0.45	6.24±0.34	-0.36±0.26#
HDL-Chol "	1.30±0.09	1.45±0.11	+0.15±0.08#
GFR (ml/min/1.73m ²)	55±5	52±5	-3±2#
ERPF "	360±23	346±23	-15±131#

No difference in haemodynamic response was noted when comparing CsA (n=9) and azathioprine (n=7) treated subgroups.

Conclusions: PUFAs have no effect on renal haemodynamics in either azathioprine or CsA treated RTR lacking functional reserve, but are effective in the treatment of hypertriglyceridaemia following renal transplantation.