American Journal of Pathology, Vol. 129, No. 1, October 1987

The Pathophysiology of Protein-Overload Proteinuria

JAN J. WEENING, COEN VAN GULDENER, MOHAMED R. DAHA, NGAISAH KLAR, ANNEMIEKE VAN DER WAL, and FRANS A. PRINS

From the Departments of Pathology and Nephrology, State University of Leiden, Leiden, The Netherlands

Alterations in glomerular function and structure were studied in protein-overload nephrosis in the rat induced by intraperitoneal administration of bovine serum albumin (BSA). Fractional clearance (C/GFR) studies using inulin and tracer proteins of different molecular size and charge revealed in proteinuric rats 1) unchanged glomerular filtration rate and renal plasma flow; 2) a 34-fold increase in C/GFR of rat serum albumin, reaching values similar to BSA; 3) a 2-fold increase in C/GFR for anionic horse radish peroxidase (HRP), but normal values for neutral and cationic HRP, and 4) an 11- and 3-fold increase for heterologous IgG and IgM, respectively. Glomerular epithelial cells showed degenerative changes, but the distribution of anionic sites in the glomerular basement membrane was found to be unaltered, as determined by polyethyleneimine binding studies. In summary, an elevation of serum albumin concentration resulted in an increased transcapillary albumin transport. This was found to lead to degenerative changes of glomerular epithelial cells with development of large pore defects, which were completely reversible. (Am J Pathol 1987, 129:64–73)

THE GLOMERULAR capillary wall (GCW) constitutes a semipermeable, size- and charge-selective filter between the glomerular capillary lumen and the urinary space.[1,2] Under normal conditions, the ultrafiltrate is virtually devoid of proteins. Increased permeability to plasma proteins may be caused by a number of immunopathologic, toxic, or hemodynamic events leading to damage of the endothelial and/or epithelial cells and disruption of the glomerular basement membrane (GBM).[3,4] Increased passage of proteins from the capillary lumen to the urinary space has also been observed in man and in laboratory animals after parenteral administration of high amounts of proteins.[5–26] Studies on experimental models of protein-overload proteinuria have shown that increased transcapillary movement of proteins causes degenerative changes of glomerular epithelial cells characterized by swelling, vacuolization, increased reabsorption droplets, loss of foot processes, and lifting from the underlying GBM.[11,12,20] Recent studies have reported on functional and structural glomerular alterations in rat models of protein-overload proteinuria, providing conflicting evidence with regard to changes in size- and charge-selective properties of the GCW. In BSA-induced proteinuria sieving curves of neutral dextrans were found to be normal, suggestive of an intact sieving filter.[23] In contrast, in the same model increased filtration of IgG[24] and of anionic ferritin[22] was found, and morphologic studies revealed detachment of glomerular epithelium from the underlying GBM,[20,26] a lesion which is usually associated with a large pore defect.[27–29] Immunoelectrophoresis of urinary proteins revealed that albumin constitutes the majority of filtered proteins, but a considerable proportion of the globulin fraction also reaches the urine.[13,16,19,20] In addition, the role of hemodynamic factors, such as possibly increased flows and pressures, has not been studied in detail.

This article describes pathophysiologic mechanisms in protein-overload proteinuria in female Wistar rats rendered proteinuric by daily administration of bovine serum albumin (BSA). Hematocrits, serum total protein, rat serum albumin (RSA), and BSA concentrations were monitored during and after BSA administration. Alterations in charge- and size-selective properties of the GCW after two doses of BSA were studied by measuring proteinuria and fractional

Supported by grants from the Dutch Kidney Foundation and the Prevention Fund.

Accepted for publication May 18, 1987.

Address reprint requests to Jan J. Weening, MD, Department of Pathology, P.O. Box 9603, 2300 RC Leiden, The Netherlands.

clearances of a number of protein tracers. Structural changes were studied by light and electron microscopy. Distribution of anionic sites within the GBM was studied by binding of polyethyleneimine (PEI), a strongly cationic polymer.[30] Finally, the role of hemodynamics in the pathogenesis of this model of proteinuria was evaluated by determining changes in glomerular filtration rate (GFR) and renal plasma flow (RPF) at 4 hours after one, two, or three daily intraperitoneal doses of BSA.

Materials and Methods

Animals

Female Wistar rats were used at 3 months of age with a weight range of 190–220 g. The animals were housed in metabolic cages and had free access to standard rat food (Hope Farms Inc., Woerden, The Netherlands) and tap water. Urine was collected for 24 hours and analyzed for protein content by the biuret method.

Induction of Proteinuria

Proteinuria was induced by daily intraperitoneal injections of 1.25 g BSA (Sigma Chemical Comp., Rijswijk, The Netherlands) dissolved in 0.9% NaCl in a volume of 5 ml. Control rats received saline only.

Experimental Design

The rats were divided into four groups.

Group A (n = 5) was used for quantitative analysis of serum and urinary proteins and hematocrits during 6 consecutive days after two intraperitoneal BSA doses at Day 0 and Day 1.

Group B (n = 30) was used for measuring GFR, RPF, and the fractional clearances of RSA, BSA, three differently charged horse radish peroxidases (HRP), exogenous rabbit IgG, and human monoclonal IgM at Day 2, when proteinuria was maximal. The protein tracers were characterized for molecular radius and charge by column chromatography and isoelectric focusing as described below.

Group C (n = 12) was used for light- and electron-microscopic studies 16 hours after one or two doses of BSA and 6 days after the first dose of BSA. Glomerular distribution of glycosaminoglycans in laminae rarae interna and externa was studied by staining with PEI and morphometric analysis in proteinuric animals after two doses of BSA and in controls.

Group D (n=14) was used for sequential determination of changes in GFR, RPF, and filtration fraction (FF) at 4 hours after one, two, or 3 daily intraperitoneal doses of BSA.

Analysis of Proteins

In animals of group A, blood was collected daily by tail vein punction. Total serum and urinary protein was measured by the method described by Lowry et al.[31] BSA and RSA were measured by radial immunodiffusion.[32]

Clearance Experiments

Physicochemical characteristics of the proteins used in the clearance experiments are listed in Table 1. HRP was purified, chemically modified, and characterized as described.[29,33] Rabbit IgG was purified by DEAE-column chromatography and gel filtration. Human monoclonal IgM was purified from serum of a multiple myeloma patient by precipitation in 0.01 M sodium acetate, pH 6.0, followed by DEAE-column chromatography and gel filtration. Rabbit IgG and human IgM were characterized for molecular radius by an S300 gel filtration column (Pharmacia, Woerden, the Netherlands), and isoelectric points (pI) were determined by isoelectric focusing in a 1% agarose gel (LKB Produkter, Zoetermeer, Netherlands following the manufacturer's manual. The fractional clearances of RSA, BSA, IgG, and IgM were determined in the animals also receiving nHRP.

Clearance experiments were performed as described before[29] in animals of group B at Day 2, 16 hours after the last dose of BSA. At this time proteinuria was maximal. The animals were anesthesized by Inactin (Promonta, Hamburg, FRG) at a 100 mg/kg dose and placed on a heated table. Body temperature was monitored via a rectal thermometer (Telethermometer, Yellow Springs Instruments, Denver, Co) and maintained between 37 and 38 C. Tracheostomy was performed and two polyethylene tubings (PE 10, Clay Adams, Parsipanny, NJ) were inserted into the left jugular vein for infusion of inulin and protein

Table 1—Physicochemical Characteristics of Tracer Proteins

	a_e	pI
Rat serum albumin	36	4.3
Bovine serum albumin	36	4.3
Rabbit IgG	55	6.8
Human IgM	85	7.3
Anionic HRP	29	3.4
Native HRP	30	7.2
Cationic HRP	30.5	9.0

a_e, molecular radius as determined by gel filtration (see text); pI, isoelectric point as determined by isoelectric focusing in polyacrylamide or agarose gels (see text); HRP, horse radish peroxidase.

tracers. PE50 tubing in the right femoral artery was connected to a transducer (Elema Schonander EMT 34, Siemens, Amsterdam, NL). Arterial blood pressure was recorded with a Mingograph 34 (Siemens). The left femoral artery was cannulated for withdrawal of arterial blood. Urine was collected from the left ureter. Inulin (6%) was infused at a constant rate of 0.034 ml/min after a starting bolus of 0.4 ml. After a 60-minute equilibration period, transition time was determined by lissamine green infusion. The HRP tracers were administered as a small bolus (0.2–0.6 ml) followed by continuous infusion at a rate of 0.069–0.026 ml/min. The clearance rate of inulin and protein tracers was determined over a 30-minute period, during which time arterial blood was obtained by continuous withdrawal from the left femoral artery. Urine samples were collected from the left kidney during a similar time period (corrected for transition time). At the end of the 30-minute period, 0.3-ml blood samples were collected from the renal vein and the aorta in heparinized syringes to calculate RPF from the renal extraction of inulin and the GFR. The kidney was then perfused with saline and homogenized as described[29] for the determination of reabsorbed peroxidase tracers. Inulin concentration in urine and plasma samples was determined by the anthrone colorimetric assay.[34] HRP concentration in urine, plasma, and kidney homogenate was measured by enzymatic activity.[35]

One-kidney GFR and RPF were then calculated from the inulin concentration obtained in the urine, peripheral arterial, and renal venous plasma samples by standard formulas: for GFR, the ratio was calculated of urine volume times urine concentration of inulin over the time-averaged plasma inulin concentration; for RPF, GFR was multiplied by the ratio of arterial plasma inulin concentration over the arterial minus the renal vein inulin concentration, both collected at the same time at the end of the clearance period. The clearance (C) of each peroxidase tracer was calculated as the ratio of the amount excreted in the urine plus the reabsorbed fraction determined in the kidney homogenate over the time-averaged concentration in the peripheral artery. Fractional clearance was defined as C/GFR. Plasma samples were also analyzed for total plasma protein. Separate blood samples were taken for measurement of hematocrits (Hct). Rabbit IgG and human IgM were measured by radioimmunoassay.

Light and Electron Microscopy

Structural glomerular changes in protein-overload nephrosis were studied by light, immunofluorescence (IF), and electron microscopy 16 hours after one and two injections of BSA and at 6 days after the first dose, when proteinuria had returned to background levels. Under Inactin anesthesia a small biopsy specimen of kidney cortex was taken from the left kidney and frozen for IF studies. Subsequently, both kidneys were fixed by perfusion at 120 mm Hg with 1.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Renal tissue was then dehydrated and embedded in epoxy resin. Semithin sections were stained with toluidene blue. Thin sections were stained with lead citrate and uranyl acetate and examined in a Philips 201 electron microscope.

Immunofluorescence was performed on frozen sections using monospecific fluoroisothiocyanate (FITC)-conjugated antisera to RSA and BSA and to rat IgG and C3 (Nordic, Tilburg, Netherlands).

Glycosaminoglycans in the GBM were visualized and studied by morphometric analysis in kidneys of 3 rats at 16 hours after administration of two doses of BSA and in 3 control rats after saline injections. Sixteen hours after the second BSA injection, the animals were anesthesized with Inactin, and 0.2 ml of a 0.05% solution of PEI in phosphate-buffered saline was administered intravenously under arterial blood pressure control. After 15 minutes, the kidney was perfused with a mixture of 1% phosphotungstic acid and 0.5% glutaraldehyde in phosphate buffer as described before.[29] Renal tissue was processed as described above. Of each kidney, 8 blocks of Epon-embedded cortex were cut, and at least one representative glomerulus per block was used for morphometric analysis of PEI-stained anionic sites by means of a MOP AM 0.3 graphic tablet (Kontron, Munich, FRG). In total, 440,000 ± 36,000 nm of GBM were counted per animal for anionic sites in the laminae rarae interna and externa.

Renal Function

In order to assess changes in GFR and RPF at 4 hours after one, two, or three BSA injections, we used a separate group of rats to measure inulin clearance and extraction (group D). Clearance experiments were performed as described above.

Statistics

Statistical analysis of differences between experimental and control groups was performed by means of the unpaired Student *t* test.

Results

Fluctuations in total serum protein, RSA, BSA, and hematocrits upon administration of two doses of BSA are depicted in Figure 1. Simultaneously with a

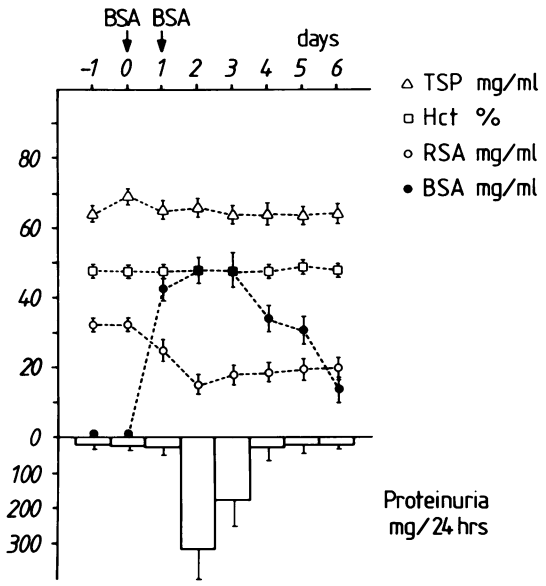

Figure 1—Changes in total serum protein (TSP), hematocrits (Hct), RSA, BSA, and urinary protein loss upon intraperitoneal administration of 0.5 mg BSA. Values are the mean of 5 rats ± standard deviation (SD) of the mean.

rise in BSA serum concentration to a maximum of 44 mg/ml, RSA concentration decreased proportionally. Total serum protein content and hematocrits remained almost unchanged during the 6 days follow up period. Urinary protein loss did not rise after the first injection of BSA (16 ± 5 mg), but increased significantly at Day 2 to a value of 320 ± 93 ($P < 0.01$) mg. Immunoelectrophoresis showed this to be largely albumin. At Day 4, urinary protein loss had returned in most animals to baseline values.

Values of the clearance studies of protein tracers are given in Tables 2–4 and Figures 2 and 3. GFR and RPF were virtually unchanged. The fractional clearance of RSA rose from 0.000055 to 0.001853, ie, approximately 34-fold ($P < 0.01$), whereas the fractional clearance of BSA was 0.00136. The fractional clearances of aHRP, IgG, and IgM showed an increase from 0.6 to 1.4 ($P < 0.05$), from 0.000072 to 0.000787 ($P < 0.01$) and from 0.000097 to 0.000306 ($P < 0.01$), respectively. Fractional clearances of neutral and cationic HRP did not change signifi-

Table 2—Functional Data Collected in Clearance Studies for Group B

	Weight (g)	UP (mg/24 hr)	SBP (mm Hg)	Hct (%)	TSP (mg/ml)	GFR (ml/min)	RPF (ml/min)
Control	219 ± 17	16 ± 5	144 ± 9	49 ± 4	73.3 ± 7.2	0.76 ± 0.15	2.90 ± 0.60
Experimental	206 ± 18	281 ± 64*	132 ± 11	49 ± 4	78.2 ± 10.7	0.76 ± 0.12	3.46 ± 0.87

Values are mean ± SD; n = 15 per group.
UP, urinary protein loss; SBP, systolic blood pressure; Hct, hematocrit; GFR, one-kidney glomerular filtration rate; RPF, one-kidney renal plasma flow; TSP, total serum protein.
*$P < 0.05$.

Table 3—Fractional Clearance Values for Anionic (a), Native (n), and Cationic (c) HRP

	GFR	C·HRP−c	C·HRP+c	C·HRP−c/GFR	C·HRP+c/GFR
a HRP Control (n = 5)	0.69 ± 0.16	0.004 ± 0.001	0.005 ± 0.001	0.006 ± 0.002	0.008 ± 0.002
a HRP Experimental (n = 5)	0.74 ± 0.09	0.010 ± 0.002*	0.013 ± 0.003*	0.011 ± 0.003*	0.015 ± 0.003*
n HRP Control (n = 5)	0.74 ± 0.12	0.060 ± 0.018	0.067 ± 0.020	0.085 ± 0.026	0.092 ± 0.029
n HRP Experimental (n = 5)	0.74 ± 0.13	0.074 ± 0.020	0.081 ± 0.024	0.107 ± 0.042	0.114 ± 0.045
c HRP Control (n = 5)	0.84 ± 0.15	0.250 ± 0.027	0.256 ± 0.027	0.250 ± 0.026	0.288 ± 0.034
c HRP Experimental (n = 5)	0.84 ± 0.15	0.280 ± 0.032	0.288 ± 0.032	0.305 ± 0.061	0.309 ± 0.061

−c, without correction for reabsorbed tracer; +c, with correction for reabsorbed tracer (see text); n = 5 per group; values are mean ± SD.
*$P < 0.05$.

Table 4—Fractional Clearance (C/GFR) Values for IgG, IgM, RSA, and BSA in Control and Proteinuric Rats

	IgG		IgM		RSA		BSA	
	−c	+c	−c	+c	−c	+c	−c	+c
Control	48 ± 22	72 ± 14	45 ± 31	97 ± 22	31 ± 14	55 ± 14	—	—
Experimental	343 ± 140*	787 ± 200*	85 ± 79*	306 ± 159*	900 ± 316	1853 ± 203	409 ± 130	1360 ± 283

Values are mean ± SD × 10^{+6}; −c, without correction for reabsorbed trace; +c, with correction; n = 5 per group.
*$P < 0.05$. For GFR values see nHRP groups in Table 3.

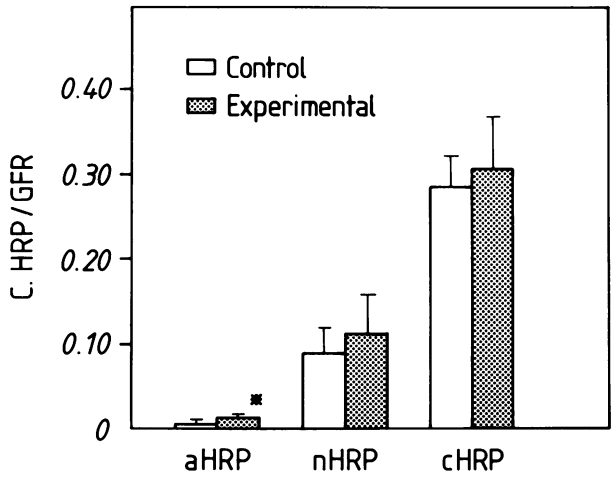

Figure 2—Fractional clearance of anionic (*a*), native (*n*), and cationic (*c*) HRP in proteinuric and control rats. Values are the mean of 5 animals in each group ± SD of the mean.

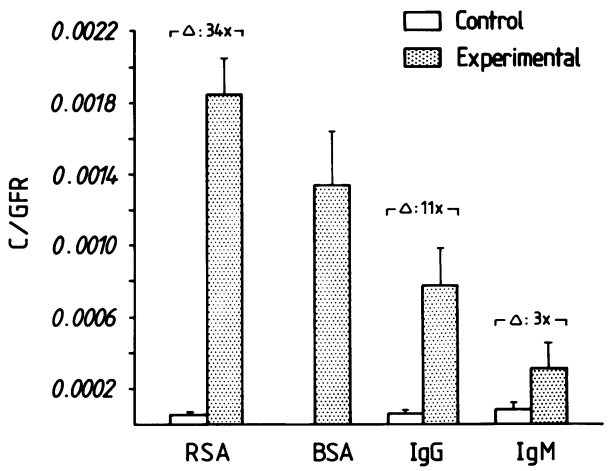

Figure 3—Fractional clearances of RSA, BSA, IgG, and IgM in proteinuric and control rats. Values are the mean of 5 animals in each group ± SD of the mean.

cantly. A considerable proportion of some of the filtered proteins was found in the kidney homogenate (Table 4). By immunofluorescence techniques it was not possible to detect the exact localization within the kidney of these tracer proteins. Because of the uncertainty, whether kidney bound proteins were filtered and reabsorbed by tubules or bound to vessel walls and interstitium, we have given the fractional clearance values with and without correction for kidney bound tracer. Kidney bound fractions were 43% of total for aHRP, 72% for IgG, and 54% for IgM, but these percentages were found to be similar for proteinuric and for control animals, thus not affecting the net increase in fractional clearance due to BSA overload.

Light microscopy revealed numerous protein reabsorption droplets in glomerular and tubular epithelial cells of proteinuric animals (Figure 4). The reabsorption droplets stained strongly for RSA and BSA and weakly for rat IgG. Rat C3 was absent. No glomerular hypercellularity was found. Ultrastructural studies showed glomerular epithelial cell swelling, partial obliteration of foot processes, an increased number of pinocytic vesicles, occasional lifting from the underlying GBM, a variable number of protein reabsorption droplets, and microtubular condensation in animals which had received two doses of BSA (Figure 5). Minor changes were found after only one dose of BSA. Morphometric analysis of PEI staining of the GBM revealed similar numbers of anionic sites in

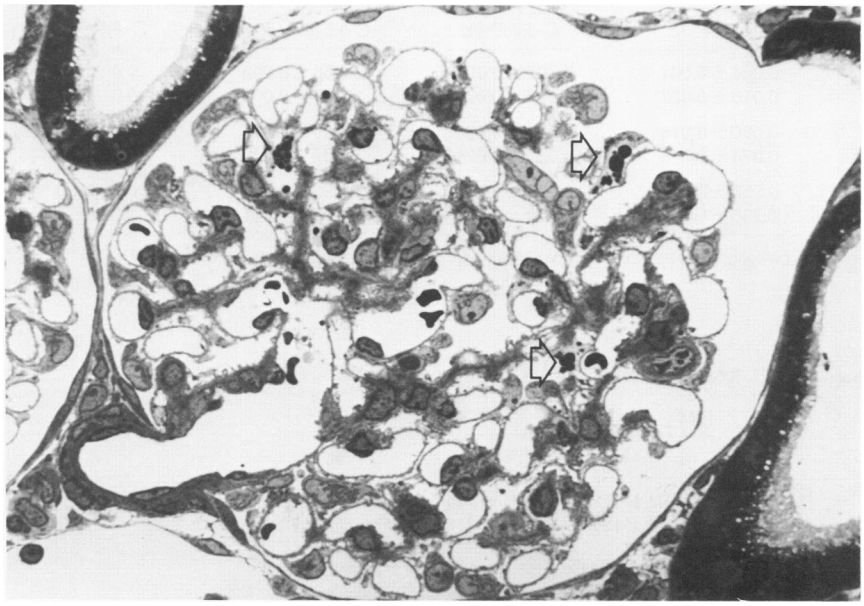

Figure 4—Light micrograph of a glomerulus from a proteinuric rat after two doses of 1.25 g BSA, showing numerous protein reabsorption droplets (*arrows*). (Toluidene blue, ×640)

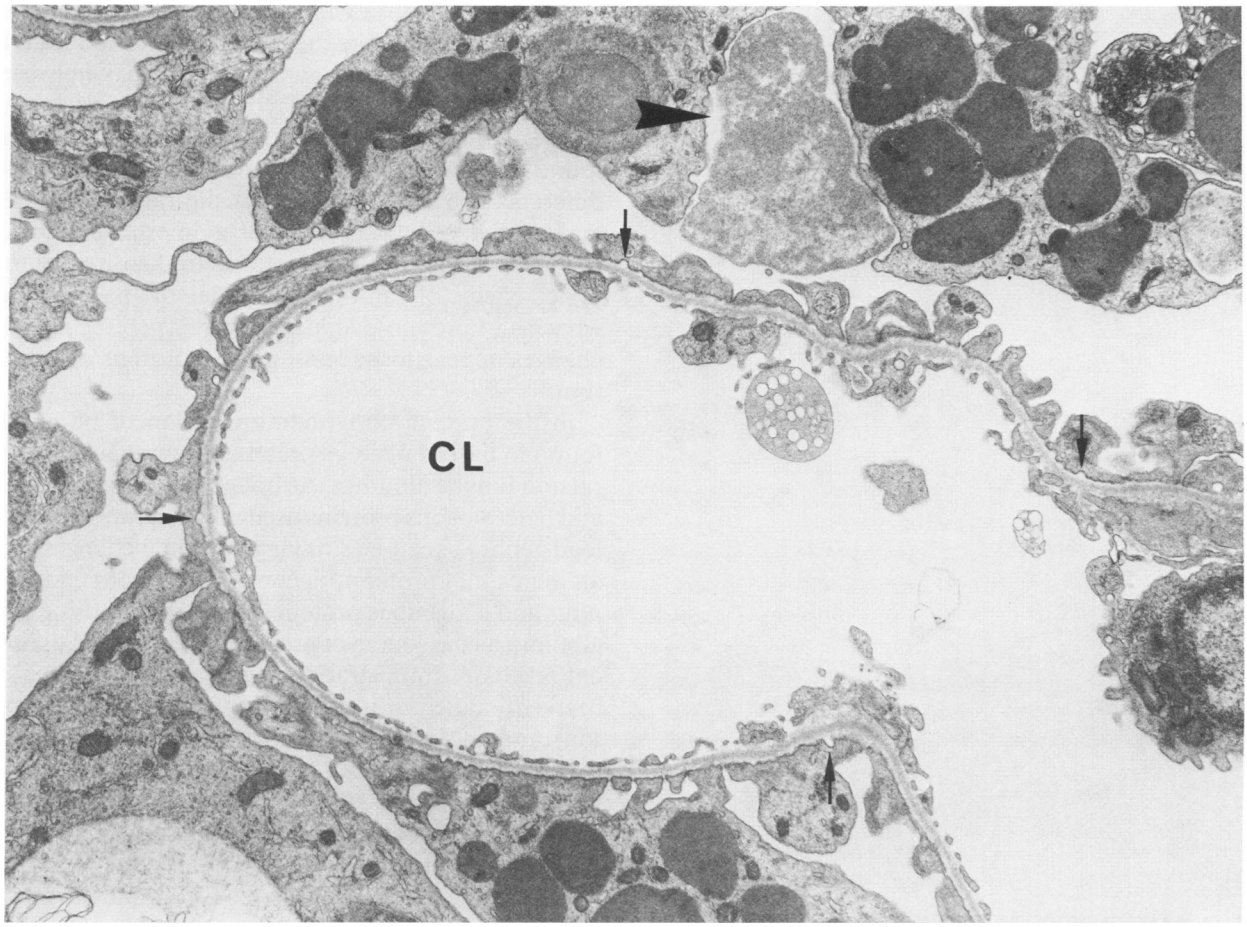

Figure 5—Electron micrograph of a glomerular capillary loop from the same rat as in Figure 1, showing areas of foot process obliteration, a high number of developing pinocytic vesicles (*arrows*), protein reabsorption droplets in the epithelial cells, and accumulation of flocculent proteinaceous material (*arrowhead*) in the urinary space. *CL*, capillary lumen. (Uranyl acetate and lead citrate, ×10,500)

laminae rarae interna and externa in proteinuric and control animals (Table 5, Figure 6). At 6 days, electronmicroscopy showed epithelial cells to be almost completely normal. Occasionally a few reabsorption droplets were found.

Renal function as determined by inulin clearance and extraction at 4 hours after one, two, or three bolus injections of BSA showed no overt changes (Table 6). At surgery, interstitial tissue was remarkably edematous in the animals that had been given three doses of BSA.

Discussion

Parenteral administration of BSA to female Wistar rats caused a transient and massive increase in urinary protein loss, which was found to contain predominantly rat and bovine albumin. These findings confirm previous studies in rats exposed to BSA[8,12,13,16–26] and studies in rabbits given injections of human serum albumin.[36] The almost immediate disappearance of proteinuria and glomerular epithelial cell lesions at approximately 48 hours after the last BSA injection was found by most other investigators in this model, but not by Marks and Drummond,[14] who found prolonged proteinuria and eventually severe glomerular lesions in the rat strain they studied. Possibly this is due to the source of protein (human serum albumin) and to strain differences. Sex and strain have been found to be important determinants of the glomerular response to high parenteral doses of protein.[18]

Proteinuria was found to be associated with structural changes of the glomerular epithelial cells, resem-

Table 5—Distribution of PEI Bound Anionic Sites per Nanometer in Laminae Rarae Interna (Iri) and Externa (Ire) of the GBM (n = 3 Animals per Group)

	Iri	Ire
Control	8.1 ± 1.4	10.5 ± 1.3
Experimental	7.1 ± 2.0 (NS)	9.8 ± 2.1 (NS)

NS, not significant.

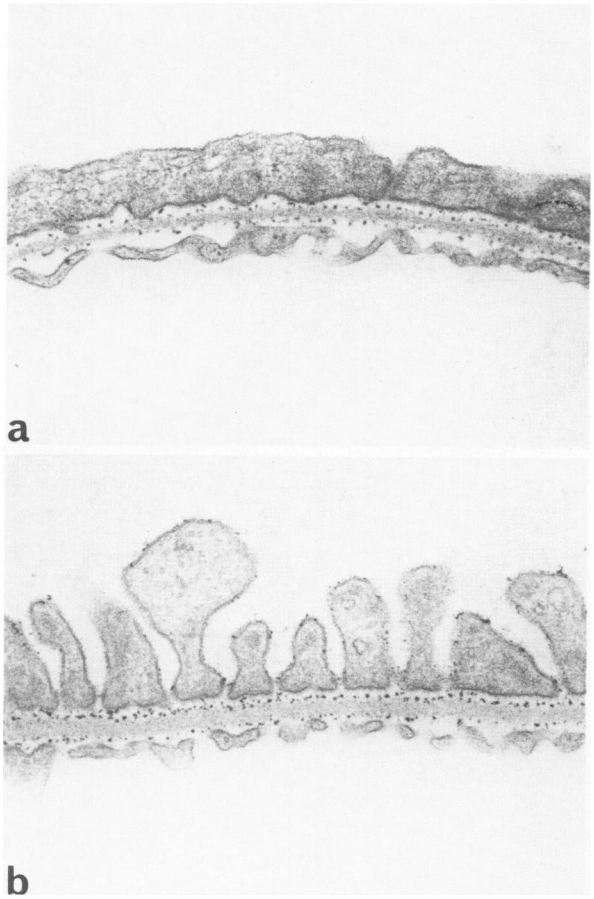

Figure 6—Electron micrographs of segments of the GCW from a proteinuric (**a**) and a control (**b**) rat after staining with PEI and phosphotungstic acid. (×28,500)

bling those seen in aminonucleoside nephrosis[27,28] and adriamycin nephrosis.[29,37] In protein overload proteinuria, these lesions seemed to be somewhat milder and especially detachment of epithelial cells from the GBM seems to be less frequent than in the toxic models of nophrosis.[27,29–37] Davies et al[26] performed morphometric analysis of glomerular ultrastructural changes in BSA-induced overload nephrosis. They found the extent of glomerular epithelial cell changes to correlate with the amount of BSA administered and with the extent of proteinuria. Andrews[38] compared glomerular lesions of protein overload proteinuria with those of aminonucleoside nephrosis in male Sprague–Dawley rats and found a striking difference in glomerular pathology between the two models. He described markedly less severe glomerular lesions in the overload model than those found by us and others, which may again be due to the differences in strain and sex of the rats studied. In aminonucleoside nephrosis all glomeruli exhibited severe epithelial cell damage, due to the direct cytotoxic effect of aminonucleoside of puromycin; whereas in protein-overload nephrosis epithelial cell changes appear to be less uniform in their distribution.

In the present study, administration of BSA was found to induce massive proteinuria containing both rat and bovine albumin. Although most homologous and heterologous proteins used in the models of overload nephrosis caused similar glomerular changes and an increase of proteinuria consisting of both endogenous and exogenous proteins, investigators using egg albumin as the source of protein have reported different results. Administration of egg albumin, which is markedly different in molecular weight (48,000 dalton) and molecular charge (pI 7.0) from albumin (69,000 and 4.3, respectively) has been found to cause massive urinary loss of the injected egg albumin, but no glomerular changes and no increased excretion of proteins other than the egg albumin.[10,26]

Recently, Mori et al[39] described a unique model of protein-overload proteinuria in rats bearing a pituitary tumor secreting prolactin, growth hormone, and adrenocorticotropic hormone. Growth hormone generated by the pituitary tumor in these rats induces liver hyperplasia associated with hyperproduction of albumin. The ensuing condition resembles homologous albumin protein-overload proteinuria. The animals develop massive proteinuria of over 500 mg per day containing predominantly albumin but also globulins. The glomerular epithelium shows degenerative changes resembling the changes observed in the present study. No studies on glomerular hemodynamics or on permeability characteristics to high molecular weight proteins or dextran sieving curves have been performed in this model.

Table 6—Renal Function at 4 Hours After One, Two, or Three Doses of BSA (Group D)

	UP (mg/24 hr)	SBP (mm Hg)	Hct (%)	TSP (mg/ml)	GFR (ml/min)	RPF (ml/min)
Control (n = 5)	15 ± 4	146 ± 8	48 ± 3	73.8 ± 6.8	0.72 ± 0.10	2.70 ± 0.41
1 × 1.25 g BSA (n = 3)	19 ± 4	143 ± 6	49 ± 4	76.2 ± 5.2	0.74 ± 0.09	3.23 ± 0.52
2 × 1.25 g BSA (n = 3)	193 ± 37*	140 ± 8	47 ± 3	78.2 ± 9.0	0.74 ± 0.12	3.42 ± 0.40
3 × 1.25 g BSA (n = 3)	345 ± 81*	139 ± 6	47 ± 4	77.6 ± 8.1	0.78 ± 0.15	3.41 ± 0.82

*$P < 0.05$ versus control values.
Values are mean + SD; GFR and RPF are one-kidney values.

Lippman [10] and Davies et al[26] have shown that administration of BSA in rats caused massive proteinuria consisting of approximately equivalent amounts of BSA and RSA. In addition, filtration of hemoglobin[10] and of the globulin fraction[26] was increased. The present study has quantified these alterations in filtration and excretion of proteins in BSA-induced overload nephrosis by measuring the fractional clearances of a number of endogenous and exogenous proteins of different molecular weight and charge. The results support the observations of others that raising serum albumin concentration by exogenous or rat albumin administration results in increased albumin excretion. As shown by others in time sequential studies,[11,12,17,20,26] morphologic changes of the glomerulus are a consequence of increased protein filtration. They demonstrated that upon protein administration increased epithelial cell pinocytosis, lysosomal activity, and vacuolization occur. Later, the epithelial cells lose their fine architecture by flattening and retraction of foot processes. Ultimately, the cells retract from the GBM, and occasionally bare segments of GBM can been seen. At the height of proteiniuria, when the structural lesions as described above have fully developed, a large pore defect has been shown by using electron-microscopic studies[22,24] and in the present study by measuring the fractional clearances of high-molecular-weight proteins. Anionic HRP, rabbit IgG, and human IgM were found to be filtered in increased amounts. Fractional clearance values of neutral and cationic HRP were not changed, probably because the filtration of these proteins is rather high under normal conditions and an additional fraction filtered through a large pore defect does not increase significantly the amount already filtered across the intact GCW. A similar explanation has been given for the normal fractional clearances found for small and medium-sized neutral dextrans in models of nephrosis in which a sieving defect can be determined only by measuring high molecular radius dextrans.[28,29] The HRP clearances indicate that charge selectivity remains relatively intact.

The occurrence of a functional sieving defect is concordant with the structural changes in the GCW and with the finding of increased filtration of IgG[24] and anionic ferritin with a molecular radius of 6 nm.[22] In contrast, Bliss and Brewer[23] found normal dextran sieving curves in BSA-induced nephrosis. An explanation for this discrepancy is not at hand.

The unchanged distribution of PEI binding anionic sites in the GBM confirms the findings of Rollason and Brewer[21] in BSA-induced overload nephrosis. They studied glomerular polyanion and anionic sites by different histochemical methods with light and electron microscopy. No changes were found for most of the cationic tracers they used, but a loss of anionic charges from the GCW was documented by using alcian blue, confirming studies of Bliss and Brewer[25] using lysozyme. Also, a decreased binding of colloidal iron was found with light microscopy, confirming previous studies of Roy et al.[15] The marked decrease in lysozyme binding[21] has also been found in aminonucleoside nephrosis.[40] In aminonucleoside nephrosis no changes in GBM glycosaminoglycans have been found when studied by biochemical or ultrastructural methods using dyes other than lysozyme.[4]

The reduction in colloidal iron staining at light microscopy has been found in many proteinuric conditions and is usually attributed to simplification of the epithelial cell surface, since at electron microscopy, a strong binding of colloidal iron can still be found, not very different from controls.[4,15,26,29] Kerjaschki et al recently described reduced sialic acid content of podocalyxin, the major sialoprotein of the GCW, in aminonucleoside nephrosis.[41] The anionic molecular charges of glycosaminoglycans and cell-surface sialoproteins are thought to constitute the charge barrier of the GCW; but, in addition, they are likely to contribute to the integrity of the molecular sieve of the GCW. The negatively charged components play an important role in attachment of cells to extracellular matrices, and disturbance by binding of strongly cationic substances to anionic sites[42] or removal of sialic acid by neuraminidase[43] causes defects in charge and size selectivity. Loss of sialic acid after treatment with neuraminidase has been shown to cause detachment of epithelial cells from the underlying GBM,[43] similar to what can be seen in puromycin aminonucleoside nephrosis[27,43] and in adriamycin nephrosis.[29] These sites of detachment are considered to be large pore defects because they allow high-molecular-weight proteins access to the urinary space.[27-29,43]

The loss of sialoproteins, as visualized by a reduction in colloidal iron staining, does not have to be associated with a loss of charge selectivity, as was shown in adriamycin nephrotic rats, which developed severe sieving defects and massive proteinuria but no loss of charge selective properties of the GCW.[29]

High protein diets are known to increase intraglomerular flows and pressures resulting in increased GFR and RPF.[44,45] These changes in glomerular permselectivity have been shown to be associated with increased transcapillary movement of proteins and may lead to structural damage of the GCW and the mesangium.[46] Such alterations in glomerular hemodynamics have been excluded by the present study as a possible pathogenetic mechanism in the induc-

tion of protein-overload proteinuria, since parenteral administration of BSA was found to leave GFR and RPF largely unchanged.

In conclusion, parenteral administration of large doses of BSA leads to an increased transcapillary movement of albumin to the urinary space. Increased glomerular epithelial cell absorption of albumin leads to degenerative changes of the glomerular epithelium, resulting in large pore defects. These defects allow macromolecules other than albumin to enter the urinary space. All these phenomena were found to be reversible. Other studies[14] have shown progression of glomerular lesions and proteinuria in rats given 2 g of BSA. This may be due to genetically determined differences in the glomerular response (hemodynamic? metabolic?) to protein overload and epithelial cell damage.

References

1. Rennke HG, Venkatachalam MA: Structural determinants of glomerular permselectivity. Fed Proc 1977, 36:2619–2926
2. Brenner BM, Hostetter TH, Humes HD: Glomerular permselectivity: barrier function based on discrimination of molecular size and charge. Am J Physiol 1978, F455–F460
3. Brenner BM, Hostetter TH, Humes HD: Molecular basis of proteinuria of glomerular origin. N Engl J Med 1978, 298:826–833
4. Kanwar YS: Biophysiology of glomerular filtration and proteinuria. Lab Invest 1984, 51:7–21
5. McLaine PN, Marks MI, Baliah T, Drummond KN: Hyperproteinemic proteinuria induced by plasma infusion. Pediatr Res 1969, 3:597
6. Lippman RW: Mechanism of proteinuria: Effect of parenteral bovine albumin injections on hemoglobin excretion in rats. Am J Physiol 1948, 154:532–536
7. Addis T: The mechanism of proteinuria. Proc Nat Acad Sci USA 1949, 35:194–199
8. Lippman RW: Mechanism of proteinuria: II. Identity of urinary protein in the rat following parenteral protein injection. Proc Soc Exp Biol Med 1949, 71:546–549
9. Baxter JH, Cotzias GC: Effects of proteinuria on the kidney. J Exp Med 1949, 89:643–668
10. Lippman RW, Ureen HJ, Oliver J: Mechanism of proteinuria. III. Comparison of the functional and structural aspects of the effects of certain intraperitoneally administered proteins on hemoglobin excretion in the rat. J Exp Med 1951, 93:325–336
11. Ashworth CT, James JA: Glomerular excretion of macromolecular substances. Am J Pathol 1961, 39:307–316
12. Anderson MS, Recant L: Fine structural alterations in the rat kidney following intraperitoneal bovine albumin. Am J Pathol 1962, 40:555–569
13. Fisher ER, Hellstrom HR: Mechanism of proteinuria: Functional and ultrastructural correlation of effects of infusion of homologous and heterologous protein (bovine serum albumin) in the rat. Lab Invest 1962, 11:617–637
14. Marks MI, Drummond KN: Nephropathy and persistent proteinuria after albumin administration in the rat. Lab Invest 1970, 23:416–420
15. Roy LP, Vernier RL, Michael AF: Effect of protein-load proteinuria on glomerular polyanion. Proc Soc Exp Biol Med 1972, 870–874
16. Davies DJ, Brewer DB, Hardwicke J: Urinary proteins and glomerular morphometry in protein overload proteinuria. Lab Invest 1978, 38:232–243
17. Rollason TP, Brewer DB: A scanning and transmission electron microscopic study of glomerular damage in the rat following heterologous serum albumin overload. J Pathol 1981, 134:39–56
18. Lawrence GM, Brewer DB: Effect of strain and sex on the induction of hyperalbuminaemic proteinuria in the rat. Clin Sci 1981, 61:751–756
19. Lawrence GM, Brewer DB: A biochemical and immunological investigation into the physiological basis of the increased albumin filtration induced in hyperalbuminaemic female Wistar rats. Clin Sci 1982, 62:495–502
20. Lawrence GM, Brewer DB: Studies on the relationship between proteinuria and glomerular ultrastructural change in hyperalbuminaemic female Wistar rats. J Pathol 1982, 138:365–383
21. Rollason TP, Brewer DB: A study of glomerular basement membrane anionic sites and glomerular visceral epithelial cell coat in protein overload proteinuria in the rat. J Pathol 1984, 142:301–316
22. Bliss DJ, Brewer DB: Ultrastructural localization of anionic and cationic ferritin in the rat glomerular basement membrane in protein-overload proteinuria. J Pathol 1984, 143:57–68
23. Bliss DJ, Brewer DB: Increased albumin and normal dextran clearances in protein-overload proteinuria in the rat. Clin Sci 1985, 69:321–326
24. Howie AJ, Bliss DJ, Brewer DB: The glomerular ultrastructural distribution of immunoglobulin G in hyperalbuminaemic (protein-overload) proteinuria. J Pathol 1985, 145:213–227
25. Bliss DJ, Brewer DB: Glomerular lysozyme binding in protein-overload proteinuria. Virchows Arch (Cell Pathol) 1985, 48:351–359
26. Davies DJ, Messina A, Thumwood CM, Ryan GB: Glomerular podocytic injury in protein overload proteinuria. Pathology 1985, 17:412–419
27. Caulfield JP, Reid J, Farquhar MG: Alterations of the glomerular epithelium in aminonucleoside nephrosis: Evidence for formation of occluding junctions and epithelial cell detachment. Lab Invest 1976, 34:43–59
28. Olson JL, Rennke HG, Venkatachalam MA: Alterations in the charge and size selectivity barrier of the glomerular filter in aminonucleoside nephrosis in rats. Lab Invest 1981, 44:271–279
29. Weening JJ, Rennke HG: Glomerular permeability and polyanion in adriamycin nephrosis in the rat. Kidney Int 1983, 24:152–159
30. Schurer JW, Kalicharan D, Hoedemaeker PhJ, Molenaar I: Demonstration of anionic sites in basement membranes and in collagen fibrils. J Histochem Cytochem 1978, 26:688–689
31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem 1951, 193:265–275
32. Mancini G, Carbonara AD, Heremans JF: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965, 2:235–239
33. Rennke HG, Patel Y, Venkatachalam MA: Glomerular filtration of proteins: Clearance of anionic, neutral and cationic horseradish peroxidase in the rat. Kidn Int 1978, 13:278–288
34. Fuehr J, Kaczmarczyk J, Kruettgen CD: Eine einfache colorimetrische Methode zur Inulin Bestimmung fuer Nierenclearance-untersuchungen bei Stoffwechselgesunden und Diabetikern. Klin Wochenschr 1955, 33:729–730

35. Herzog V, Fahimi HD: A new sensitive colorimetric assay for peroxidase using 3,3′-diaminobenzidine as hydrogen donor. Anal Biochem 1973, 55:554–562
36. Lannigan R, McQueen EG: The effect on the renal glomerular epithelial cells of proteinuria induced by infusions of human serum albumin in rabbits and rats. Br J Exp Pathol 1962, 43:549–556
37. Bertani T, Poggi A, Pozzoni R, Delaini F, Sacchi G, Thoua Y, Mecca G, Remuzzi G, Donati MB: Adriamycin-induced nephrotic syndrome in rats: Sequence of pathologic events. Lab Invest 1982, 46:16–23
38. Andrews PM: A scanning and transmission electron microscopic comparison of puromycin aminonucleoside-induced nephrosis to hyperalbuminemia-induced proteinuria with emphasis on kidney podocyte pedicle loss. Lab Invest 1977, 36:183–197
39. Mori H, Yamashita H, Nakanishi C, Koizumi K, Makino S, Kishimoto Y, Hayashi Y: Proteinuria induced by transplantable rat pituitary tumor MtT SA5: Model for homologous protein-overload proteinuria. Lab Invest 1986, 54:636–644
40. Caulfield JP, Farquhar MG: Loss of anionic sites from the glomerular basement membrane in aminonucleoside nephrosis. Lab Invest 1978, 39:505–512
41. Kerjaschki D, Vernillo AT, Farquhar MG: Reduced sialylation of podocalyxin—the major sialoprotein of the rat kidney glomerulus—in aminonucleoside nephrosis. Am J Pathol 1985, 118:343–349
42. Barnes JL, Radnik RA, Gilchrist EP, Venkatachalam MA: Size and charge selective permeability defects induced in glomerular basement membrane by a polycation. Kidney Int 1984, 25:11–19
43. Kanwar YS, Rosenzweig LJ: Altered glomerular permeability as a result of focal detachment of the visceral epithelium. Kidney Int 1982, 21:565–574
44. Medlar EM, Blatherwick NR: The pathogenesis of dietary nephritis in the rat. Am J Pathol 1937, 13:881–895
45. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. Am J Physiol 1981, 241:F85–F95
46. Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 1982, 307:651–659

Acknowledgments

The authors thank Mrs. Marian Kantebeen for secretarial assistance.