Kidney International, Vol. 40 (1991), pp. 461–469

Role for "uremic toxin" in the progressive loss of intact nephrons in chronic renal failure

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Role for "uremic toxin" in the progressive loss of intact nephrons in chronic renal failure. We studied the effect on the progression of glomerular sclerosis of two different experimental maneuvers, peritoneal dialysis and oral adsorbent, which remove circulating substances in different fashions. Munich-Wistar rats with established glomerular sclerosis, verified by renal biopsy analysis at seven weeks after subtotal nephrectomy, were treated for four weeks with either peritoneal dialysis (PD) or oral charcoal adsorbent (AST-120). Treatment was initiated at eight weeks. Rats were paired in treatment and control groups according to the similarity in the degree of sclerosis determined at biopsy with a minimum of 50 glomeruli analyzed. Systolic blood pressure and BUN and creatinine clearance, measured at seven to eight weeks, were not different among groups. In Group 2 rats, PD was performed with 1.5% dextrose for eight one-hour cycles, six days per week, while Group 1 control rats had zero indwelling time of the dialysate. Group 4 rats received AST-120, an oral adsorbent charcoal, mixed 5% by weight with standard rat chow and given ad libitum from 8 to 12 weeks after subtotal nephrectomy, while control Group 3 rats received only rat chow. Whole kidney GFR at 12 weeks was significantly higher in Group 2 PD versus Group 1 control (0.50 ± 0.08 vs. 0.30 ± 0.05 ml/min, $P < 0.05$). There was no statistical difference for BUN and whole kidney creatinine or inulin clearance in Group 4 AST-120 treated versus Group 3 control rats. Light microscopic studies in autopsy specimens revealed that both PD and AST-120 attenuated progression of glomerular sclerosis. Thus, at autopsy, the sclerosis index averaged 1.78 ± 0.21 versus 1.30 ± 0.19 in Group 1 control and Group 2 PD, respectively ($P < 0.05$), and 1.42 ± 0.16 versus 0.99 ± 0.13 in Group 3 control and Group 4 AST-120 treated rats, respectively ($P < 0.05$). Since the dialysis replaces primarily the filtration function of the nephrons removed at the onset of the study, the present observations support the possibility that the biologically-active circulating substances, or so-called "uremic toxins", are involved in the advancement of glomerular sclerosis and are ultrafiltrable in nature.

Nearly a quarter century ago Kurnick and Lindsey performed a now classic experiment using a parabiotic mouse model [1]. Kidneys of a normal mouse connected in circulation with an anephric mouse developed hypertrophy. These findings provided convincing evidence for the involvement of circulating substances in compensatory renal hypertrophy. In experimental animals in whom a large population of nephrons is removed, a typical hypertrophy develops in remnant nephrons in association with progressive destruction of the glomerular architecture [2–5]. The structural injury is characterized by accumulation of mesangial matrix and relentless advancement of glomerular sclerosis. Because this phenomenon occurs in otherwise healthy remnant glomeruli, the notion has recently been formulated that progressive glomerular sclerosis in the chronically failing kidney resulting from a variety of primary diseases represents adaptive responses to reduced functioning nephrons [6]. The possibility that, like hypertrophy, this sclerosis process may also involve circulating substances has been raised on the basis of observations in human transplanted kidneys. Thus, Pinto et al [7] noted that the incidence of recurrence of focal glomerular sclerosis, a unique primary form of glomerular sclerosis, in transplanted kidneys was unusually high, much greater than the incidence of de novo focal glomerular sclerosis in the general population or in kidney transplant recipients with renal failure secondary to other causes. The authors speculated that "some toxic circulating factor" could have lead to the development of focal glomerular sclerosis.

If circulating substances indeed promote glomerular hypertrophy and sclerosis in chronic renal disease of other causes, as well, these substances would represent a key mechanism completing the vicious cycle of progressive glomerular damage leading to the end stage kidney (Fig. 1). Impaired renal function due to glomerular sclerosis would result in the accumulation of such circulating substances which, in turn, would promote glomerular sclerosis by augmenting mesangial matrix accumulation and obliteration of the glomerular capillary tuft.

In the present study, therefore, we examined the possibility that the progressive glomerular sclerosis of remnant nephrons indeed involves, at least in part, circulating substances. Although still far from identifying the specific circulating substance(s) of interest, we sought to narrow the candidates by characterizing the nature of such substances. For these reasons, we employed two experimental maneuvers, peritoneal dialysis and oral adsorbent administration, each of which removes somewhat different classes of materials from the circulation.

Received for publication October 29, 1990
and in revised form April 17, 1991
Accepted for publication April 19, 1991

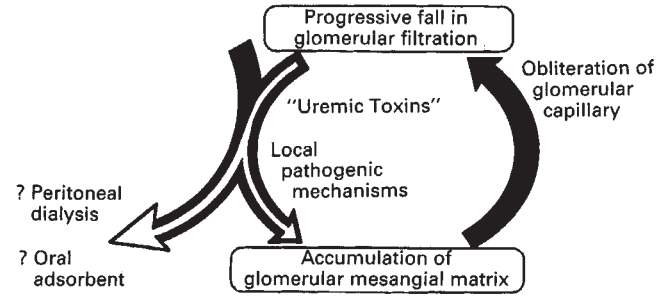

Fig. 1. *Schematic presentation of the hypothesis tested in the present study.* If circulating substances indeed promote accumulation of glomerular mesangial matrix (left side of circle) resulting in obliteration of the glomerular capillary, the progressive fall in glomerular filtration resulting from the glomerular sclerosis would, in turn, result in further accumulation of such circulating substances. Thus, the vicious and self-perpetuating cycle of progressive injury would be completed. The present study examined whether removal of such circulating "uremic toxins" (left inner arrow), via peritoneal dialysis or oral adsorbent treatment could interrupt this chain of events.

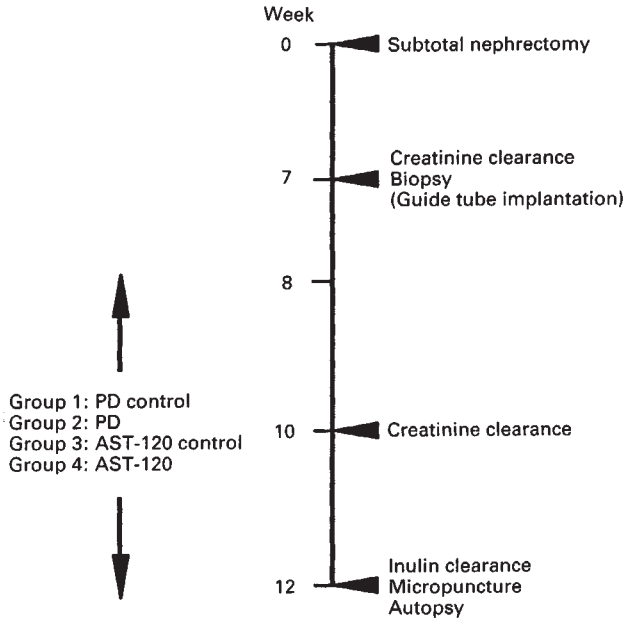

Fig. 2. *Schematic presentation of the experimental protocols employed in the four experimental groups.*

Methods

Experimental groups

Studies were performed on four groups of adult male Munich-Wistar rats, weighing 200 to 220 g, which were maintained on standard laboratory rat chow. The experimental protocols employed in the four groups are outlined in Figure 2. All surgical procedures were performed under sterile conditions. All rats were subjected to subtotal nephrectomy under pentobarbital anesthesia (Nembutal, Abbott Laboratories, North Chicago, Illinois, USA; 25 to 30 mg/kg body weight, i.p.). Subtotal nephrectomy was performed by right nephrectomy and ligation of two or three branches of the left renal artery by silk ligature to leave approximately ⅙ to ⅛ of total kidney mass. Seven weeks after the nephrectomy, each rat underwent creatinine clearance (C_{Cr}) measurement and renal biopsy.[1] Kidney biopsy was performed as described by Adler et al [8]. Briefly, a small portion of the upper pole of the kidney was surgically removed, and bleeding from the biopsy wound promptly controlled with application of gelform (The Upjohn Company, Kalamazoo, Michigan, USA). A guide tube for peritoneal dialysis (PD) was surgically implanted at this time in Group 1 and 2 rats as described below. One week later, a four week course of PD (Group 2) or sham procedure (Control:Group 1) was started. Group 1 rats were pair-fed with Group 2 rats using the regimen described by us earlier [9].

Eight weeks after subtotal nephrectomy, oral charcoal adsorbent AST-120 (Kureha Chemical Industry, Tokyo, Japan), mixed with standard rat chow in 5% wt/wt, was given ad libitum to Group 4 rats and continued for the remainder of the study. Group 3 rats had free access to standard rat chow without AST-120 and served as a control for Group 4.

At eight weeks, when each group of animals was placed on its specified protocol, systolic blood pressure was measured in all animals by the tail cuff method [10]. At ten weeks, measurements of BUN and creatinine clearance were repeated. Upon completion of study, at twelve weeks after subtotal nephrectomy, the micropuncture measurements specified below were performed on surface remnant nephrons in each rat. Animals were then sacrificed with overdose anesthesia, and the kidneys were removed for microscopic study.

Correlation between inulin and endogenous creatinine clearance

To estimate whole kidney GFR through a non-invasive procedure during the course of disease, endogenous creatinine clearance was measured in awake rats in the current study. For this purpose, the degree of correlation between endogenous creatinine and inulin clearance in rats with remnant kidneys was ascertained in a separate group of 29 subtotally nephrectomized rats. Male Sprague-Dawley rats (220 to 330 g) were subjected to subtotal nephrectomy by right nephrectomy and ligation with silk ligature of branches of the left renal artery to remove renal mass in various degrees. Within 60 days after nephrectomy, rats with various BUN levels were placed in metabolic cages for 24-hour urine collection. Serum creatinine level was measured immediately before and after the urine collection. Rats were then anesthetized and subjected to C_{In} measurement as described below. C_{Cr} and C_{In} correlated closely with each other ($r = 0.966$, $P < 0.01$; Fig. 3).

Implantation of guide tube

In Group 1 and Group 2 animals, chronic access to the peritoneal cavity for peritoneal dialysis was established by implantation of a silicone guide tube. Silicone (Medical Adhesive Silicone Type A, Dow Corning Corp., Midland, Michigan, USA) was solidified around a ~15 cm-length silicone catheter

[1] Animals with comparable whole kidney sclerosis index assessed on biopsy specimens were paired, so that one was included in Group 1 and its partner in Group 2. Likewise, one of a given pair was included in Group 3, while its partner was in Group 4. Animals which did not have a matching partner, that is, ones with sclerosis index values deviating markedly from those of any others, were excluded from the study.

(I.D. 3.27 mm, O.D. 4.54 mm, Silastic Medical-Grade Tubing, Dow Corning Corp.) to shape an oval disk (~3 × 2 cm, ~2.5 mm thickness) at a distance ~2 cm away from the edge of the catheter. This constructed catheter and all surgical instruments were sterilized by ethylene oxide gas. Rats were anesthetized with sodium pentobarbital as for subtotal nephrectomy. Following laparotomy through an approximately 6 cm-length midline incision, the skin in the left lower quadrant area was separated from the underlying fascia and muscle layer to allow subsequent placement of the catheter disk on the outer surface of the abdominal muscle wall. The constructed catheter was passed under the skin and exteriorized at the back of the neck. The disk-end of the catheter was then guided into the peritoneal cavity through a ~5 mm-diameter tunnel created by an electric cautery at a site ~2 cm left from midline. The catheter edge was then cut to allow minimum exposure of the catheter to the peritoneal cavity and firmly anchored on the inner surface of the abdominal muscle wall with a 3-0 nylon suture. Several stitches were then made with a 3-0 nylon suture around the silastic disc to anchor the disc firmly on the outer surface of the abdominal muscle wall. An occluding plastic obturator tube (Dobbhoff, Biosearch, Sommerville, New Jersey, USA) was then advanced through the implanted guide tube from its external end until ~5 mm of the obturator tube appeared in the abdomen. Omentum readily accessible for manipulation was resected to prevent subsequent clogging of the implanted guide tube. After these procedures, the abdominal incision was closed with a 3-0 silk. The exteriorized catheter was tied to the surrounding skin at the neck. The tube obturator was capped with a needle cap and immobilized within the guide tube by applying a firm 3-0 silk ligature around the guide tube. When animals were fully recovered from anesthesia, they were returned to their cages.

Procedures of peritoneal dialysis

During the subsequent one week period after surgical implantation of guide tube, animals were made accustomed to PD procedures by being held gently for approximately 15 minutes two to three times every other day. All the tools used for dialysis were sterilized by autoclave or Betadine (Purdue Frederick, Norwalk, Connecticut, USA). On the day of PD, the obturator tube was replaced by a dialysis catheter (size 8 Fr, Dobbhoff, Biosearch), and the animals were placed in a cage (~30 cm × ~60 cm) within which they were allowed to move freely. By infusing warmed Impersol (1.5% dextrose solution, Abbott Laboratories) in a volume approximating 10% of body weight into the peritoneal cavity through the dialysis catheter, PD was initiated. PD was performed daily in sessions for eight exchanges/day, with a 60-minute indwelling time in Group 2, whereas no indwelling time was allowed in Group 1 control animals. Body weight was checked at the completion of each day of PD to ensure that dialysate had been adequately removed. At the completion of the daily dialysis session, the inner dialysis tube was replaced by a new sterile obturator tube to prevent infection and occlusion of the implanted guide tube. Efficacy of this PD procedure was verified by measuring C_{Cr} of dialysis ($N = 6$). The data showed that PD indeed was efficient, achieving C_{Cr} of 0.42 ± 0.07 ml/min during the dialysis period. Furthermore, the typically high BUN levels immediately before dialysis session were uniformly reduced by ~50% upon completion of dialysis.

Characterization of oral adsorbent AST-120

Because of the multiple clefts on the AST-120 particles which were engineered to drastically increase the surface area for adsorption, attempts to quantitate various adsorbed substances by elution have thus far been unsuccessful. To calculate the maximum estimate of the amount of protein adsorbed by AST-120 and then subsequently lost in feces, the particle density of AST-120 isolated from rat feces was measured. Five male Munich-Wistar rats were fed standard chow (24% protein) containing 5% wt/wt AST-120 and maintained in metabolic cages to collect feces for four days. Feces were then dispersed in five volumes of distilled water for one hour at room temperature, and the supernatant was removed. This step was repeated three times. Further isolation was made by a fluidized bed using upflow of water, sieving trays, and a tilted trough. Finally, recovered AST-120 was dried in an oven at 105°C for four hours, and the volume and weight were measured.

The density of recovered AST-120 was compared to that of AST-120 before feeding. The value for the density of AST-120 before feeding was 0.801 g/cc, and the values of recovered AST-120 from feces averaged 1.084 ± 0.002 g/cc. Thus, AST-120 weight gain by adsorption was 35.4%.

In the present study, Group 3 control and Group 4 AST-120 rats ate 17.6 ± 0.5 versus 20.5 ± 0.8 g/day ($P < 0.05$) of standard rat chow or standard rat chow containing 5% AST-120, respectively. The standard rat chow contains 24% protein, so that the daily protein intake of Group 3 and Group 4 rats was estimated to be, on average, 4.22 and 4.68 g/day, respectively. Average daily AST-120 intake in Group 4 rats was 1.03 g/day, an amount estimated to adsorb 0.365 g/day of food content (that is, 1.03 × 0.345 g/day). This latter value is smaller than the difference in total (20.5 − 17.6 = 2.9 g/day) or protein (4.68 − 4.22 = 0.46 g/day) intake between the two groups. Overall, the daily net calorie and protein intake of Group 4 AST-120 rats is estimated, if any, to have been higher than that of Group 3 control rats.

The effects of AST-120 on serum levels of total protein, albumin, triglycerides, cholesterol, calcium, and a few other parameters in normal rats treated for six months have been reported in Japanese literature [11]. No statistically significant influence was observed on any of these parameters.

Micropuncture measurements

For the micropuncture measurements performed on the day of completion of study, each animal was anesthetized with an intraperitoneal injection of Inactin (Byk, Konstanz, Germany; 100 mg/kg) and underwent preparatory surgery as previously described [12]. Thus, following tracheotomy, indwelling polyethylene catheters (PE-50, Clay Adams, Parsippany, New Jersey, USA) were placed into the left and right jugular veins for infusion of plasma and inulin. The left femoral artery was catheterized to monitor mean systemic arterial pressure (MAP), which was measured using an electronic transducer (Model 5, Instrumentation for Physiology and Medicine, San Diego, California, USA) connected to a recorder (Model 2200S, Gould Inc., Cleveland, Ohio, USA). The left ureter was cannulated with PE-10 tubing for subsequent collection of urine. The left kidney was suspended on a Lucite holder, its surface illuminated with a fiberoptic light source, and bathed with 0.9% NaCl.

Table 1. Body weight and systolic blood pressure

	Body weight *g*			Systolic blood pressure *mm Hg*	
	0 week	8 weeks	12 weeks	8 weeks	12 weeks
Group 1 ($N = 6$) PD control	203 ± 4	254 ± 11	255 ± 11	125 ± 6	161 ± 10
Group 2 ($N = 6$) PD	206 ± 5	252 ± 6	240 ± 9	138 ± 7	162 ± 8
Group 3 ($N = 7$) AST-120 control	214 ± 2	264 ± 5	276 ± 4	144 ± 8	168 ± 7
Group 4 ($N = 8$) AST-120	219 ± 4	271 ± 7	295 ± 10[a]	141 ± 6	157 ± 8

Values are given as mean ± SE
[a] $P < 0.05$ for Group 3 vs. Group 4

Volume losses due to surgical preparation were replaced with plasma, infused in a volume equal to 1% of body weight over 30 minutes, followed by a continuous infusion at the rate of 1.2 ml/hr [13]. Following a priming dose (0.4 ml) of 5% inulin containing 0.9% NaCl solution, a maintenance infusion of this solution was started and continued throughout each micropuncture experiment at a rate of 1.2 ml/hr. Following an equilibration period (30 min), rats underwent assessment of glomerular function.

Timed (~1 min) samples of fluid were collected from the surface proximal tubules for determination of flow rate, inulin concentration, and calculation of tubule-to-plasma inulin concentration ratio, hence the single nephron glomerular filtration rate (SNGFR). The rate of fluid collection was adjusted to maintain a column of polymer oil, three to four tubule diameters in length, in a constant position just distal to the site of puncture. In conjunction with the tubule fluid collections, femoral arterial blood samples were also obtained for the determination of hematocrit and plasma inulin concentrations.

Urine was collected for determination of urine flow rate and inulin concentration, which were used for the calculation of whole kidney inulin clearance (C_{In}). Two consecutive collections with each ≈10 min duration were performed.

Hydraulic pressures were monitored in single capillaries of surface glomeruli (P_{GC}) with a continuous-recording servo-null micropipette transducer system (Model 5, Instrumentation for Physiology and Medicine). Micropipettes with outer tip diameters of 1 to 2 μm containing 2.0 M NaCl were used. Hydraulic output from the servo-null system was converted electronically to a recorder (Model 2200S, Gould) by means of a pressure transducer.

Histological studies

Tissue from renal biopsy at seven weeks after subtotal nephrectomy and the left remnant kidney obtained at the completion of the study at 12 weeks were immersion fixed in 10% neutral-buffered formalin. After routine processing, the specimens were embedded in paraffin, and 3 μ serial sections were made and stained with periodic acid-Schiff (PAS).

A semi-quantitative score (sclerosis index) was used to evaluate the degree of glomerular sclerosis using the method of Raij, Azar and Keane [14]. On a single thin section, 50 to 60 glomeruli for biopsy and 100 to 120 for autopsy specimens were examined, and the severity of sclerosis was graded from 0 to 4+ for each glomerulus. The scores from all glomeruli were averaged to obtain a whole kidney sclerosis index for all animals studied. In biopsies from 12 rats, single sections contained less than 50 glomeruli. In these animals, therefore, two separate sections > 100 μ apart (average glomerular diameter) were examined to obtain a minimum of 50 glomeruli for analysis. A 1+ lesion represented involvement of up to 25% of the glomerulus, while 4+ represented sclerosis of 75 to 100% of the glomerulus. In Groups 1 and 2, the entire glomerular corpuscular body was also evaluated for sclerosis in randomly chosen individual glomeruli, using 3-μm serially sectioned specimens. A minimum of ten sections displaying even distribution throughout each glomerulus was examined to obtain sclerosis index. A single score was then obtained for each glomerulus by averaging the scores from these multiple sections. Interstitial changes of fibrosis and tubular atrophy and dilation were assessed qualitatively.

Analytical measurements

BUN and creatinine were measured using a BUN analyzer (BUN Analyzer II, Beckman Instruments, Inc., Fullerton, California, USA) and a creatinine analyzer (Creatinine Analyzer II), respectively. Serum cholesterol was measured with an enzymatic method using a Monarch analyzer (Instrumentation Laboratories). The volume of fluid collected from proximal tubules by micropuncture was estimated from the length of the fluid column in a constant-bore capillary tube of known internal diameter. The concentration of inulin in the tubule fluid was measured by the microfluorescence method [15]. Inulin concentrations in plasma and urine were determined by the macroanthrone method [16].

Statistical analysis

Results are expressed as mean ± 1 SEM. Comparisons between two groups were made using the unpaired or paired *t*-test, whichever was appropriate. The results were deemed statistically significant when the P value was <0.05.

Results

Effect of peritoneal dialysis on glomerular function and structure (Comparison between Group 1 vs. Group 2)

Values for body weight and systolic blood pressure measured immediately before subtotal nephrectomy (0 week), at the onset of specified therapy (8 weeks), and at the time of autopsy (12 weeks) are presented in Table 1. In Group 1 PD control and

Table 2. BUN level and whole kidney clearance

	BUN *mg/dl*			Creatinine clearance *ml/min*		Inulin clearance *ml/min*
	8 weeks[a]	10 weeks[a]	10 weeks[b]	7 weeks	10 weeks	12 weeks
Group 1 (N = 6) PD control	75 ± 7	89 ± 16	—	0.59 ± 0.07	0.40 ± 0.06	0.30 ± 0.05
Group 2 (N = 6) PD	69 ± 6	59 ± 6[c]	27 ± 2	0.42 ± 0.07	0.52 ± 0.07	0.50 ± 0.08[c]
Group 3 (N = 7) AST-120 control	69 ± 3	78 ± 4	—	0.54 ± 0.02	0.46 ± 0.03	0.31 ± 0.05
Group 4 (N = 8) AST-120	68 ± 5	74 ± 4	—	0.61 ± 0.05	0.47 ± 0.02	0.39 ± 0.04

Values are given as mean ± SE.
Values were obtained immediately before ([a]) or after ([b]) daily PD session
[c] $P < 0.05$ for Group 1 vs. Group 2

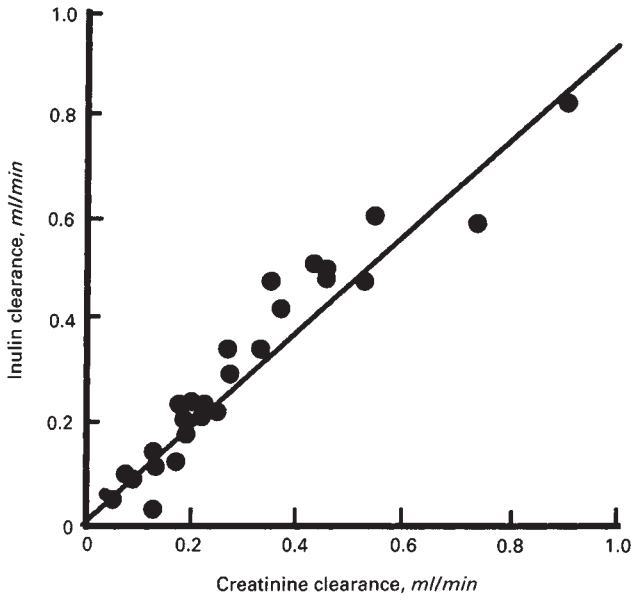

Fig. 3. *Correlation between inulin and endogenous creatinine clearance in rats with remnant kidney.* Each value represents the average of three determinations of C_{In} and C_{Cr} made in each rat in a separate group within 60 days of subtotal nephrectomy. The data show that these two parameters correlated closely with each other (r = 0.966, $P < 0.01$).

Group 2 PD animals, values for body weight were comparable at the onset of experiment (average: 203 ± 4 vs. 206 ± 5 g), increased comparably by some 25% during the eight-week post-subtotal nephrectomy period (to 254 ± 11 vs. 252 ± 6 g), and remained essentially unchanged for the remaining four week period between biopsy and autopsy (at autopsy: 255 ± 11 vs. 240 ± 9 g). Values for systolic blood pressure were numerically but not statistically significantly higher at the time of biopsy (8 weeks) in Group 2 than Group 1 (125 ± 6 vs. 138 ± 7 mm Hg, $P > 0.05$); they remained comparable at 12 weeks (161 ± 10 vs. 162 ± 8 mm Hg). Urine volume assessed at 10 weeks after nephrectomy in PD rats was significantly higher than that of control rats (28.2 ± 4.5 vs. 16.4 ± 1.8 ml/24 hr, $P < 0.05$). Values for BUN and remnant kidney creatinine or inulin clearance measured during the study are given in Table 2. Thus, both BUN, measured one week after biopsy at eight weeks, and C_{Cr}, at seven weeks at the time of biopsy, were statistically indistinguishable between the two groups [BUN: 75 ± 7 and 69 ± 6 mg/dl in Groups 1 and 2, respectively, P not significant (NS); C_{Cr} 0.59 ± 0.07 and 0.42 ± 0.07 ml/min, respectively, NS]. In Group 1 animals, in which no dialysis was performed, BUN typically increased, on average, to 89 ± 16 mg/dl, while C_{Cr} or C_{In} progressively declined. Thus, values for C_{In} averaged 0.30 ± 0.05 ml/min at 12 weeks (Table 2). In Group 2 dialyzed animals, values for BUN remained below pre-biopsy levels and were significantly lower than those in Group 1 at 10 weeks (89 ± 16 vs. 59 ± 6 mg/dl, $P < 0.05$). It is apparent that this lowered BUN in Group 2 reflects not only the function of daily peritoneal dialysis, which removes urea, but also the intrinsic renal filtration function which was greater in Group 2 than in Group 1 toward the end of study. Thus, C_{In} measured immediately before sacrifice at 12 weeks, was on average some 67% higher in Group 2 than Group 1 (0.30 ± 0.05 vs. 0.50 ± 0.08 ml/min, $P < 0.05$).

Values for SNGFR and P_{GC} measured in surface nephrons at 12 weeks in Groups 1 and 2 animals are given in Figures 4 A and B, respectively. Values for these single nephron functions varied widely in both Group 1 and Group 2 remnant kidneys. No statistical difference was, therefore, evident between these two groups for either SNGFR or P_{GC}.

Arterial blood hematocrit (N = 6 in each group) and serum cholesterol levels (N = 4 in each group) were determined at 12 weeks at the end of the study. Values for hematocrit were comparable between PD (45 ± 1 vol %) and control groups (44 ± 1 vol %, $P > 0.1$). Likewise, there was no statistically significant difference between treated and control groups: 78.3 ± 10.3 mg/dl versus 103.3 ± 10.3 in Group 1 versus Group 2 ($P > 0.05$), respectively, and 119.6 ± 9.2 mg/dl versus 127.3 ± 8.1 in Group 3 versus Group 4 ($P > 0.4$).

Wet kidney weight measured at the completion of the study was comparable between PD and control rats, averaging 1.43 ± 0.10 versus 1.31 ± 0.10 g ($P > 0.1$), respectively. Whole kidney sclerosis index was obtained from biopsy (7 weeks) and autopsy (12 weeks) specimens of the same animals and are summarized in Figure 5. Thus, whereas these two groups were comparable in this index at the time of biopsy, values for this index became substantially and significantly lower in Group 2 PD than Group 1 PD control rats (1.30 ± 0.19 vs. 1.78 ± 0.21, $P < 0.05$). As can

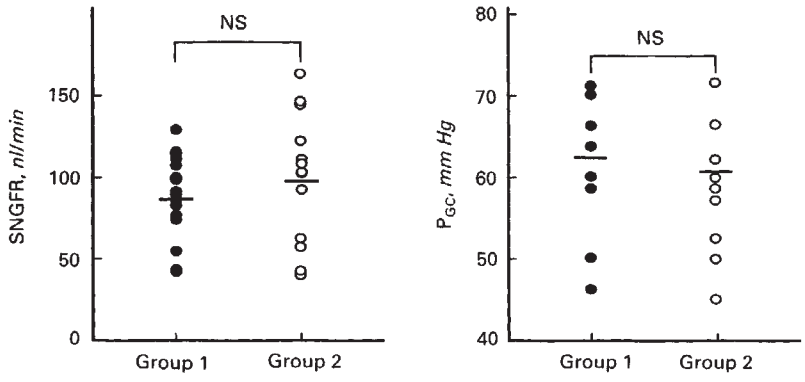

Fig. 4. *The effect of peritoneal dialysis on single nephron GFR (SNGFR) and glomerular capillary pressure (P_{GC}) of remnant superficial nephrons.* Each circle represents the data from a single nephron. There was no statistically significant difference in these parameters between Group 1 and Group 2 rats.

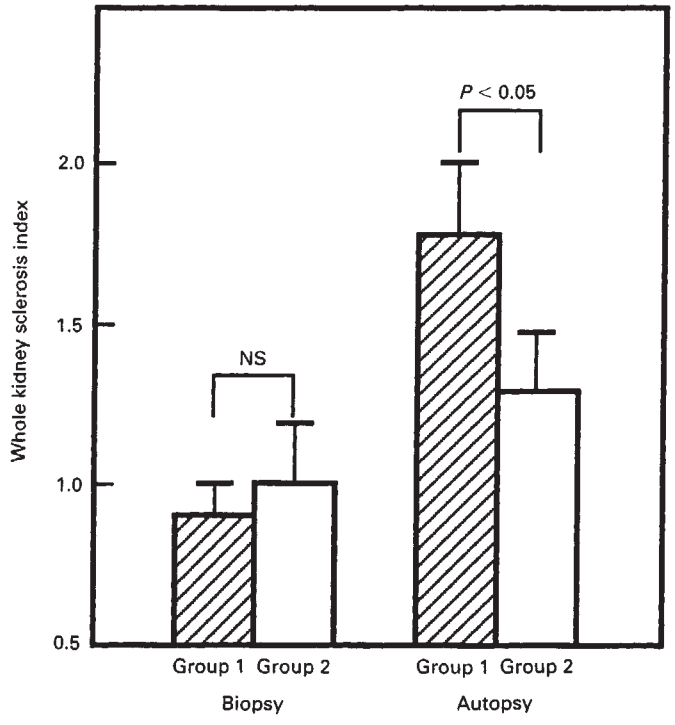

Fig. 5. *Whole kidney glomerular sclerosis index in Group 1 and Group 2 rats.* Whole kidney sclerosis index was comparable between Group 1 and 2 rats at biopsy. At autopsy five weeks later, there was significantly less sclerosis in Group 2 PD versus control Group 1 rats.

be seen in Figure 5, therefore, the increase in sclerosis index, which occurred during the five-week post-biopsy period, was roughly three times greater in Group 1 than Group 2.

We assessed the sclerosis index at the individual glomerular level in Group 1 PD control and Group 2 PD rats in accord with the method described in detail elsewhere [17]. This three dimensional approach was developed to characterize the morphology of each glomerulus, since, due to the segmental nature of the lesion, the morphological appearance of a given glomerulus on a single thin-section specimen does not necessarily represent that of its entire corpuscle. Glomeruli from biopsy and autopsy specimens were grouped separately into four different rankings of severity of glomerular sclerosis, the first rank with sclerosis index 0–1, the second 1–2, the third 2–3, and the most severe rank with 3–4. Then the percentages of glomeruli within each rank of glomeruli were calculated against the total number of glomeruli examined in the biopsy. The percent population of glomeruli with sclerosis index falling into each rank is shown in Figure 6. Thus, whereas the percent population of least sclerotic glomeruli (sclerosis index: 0–1) decreased significantly from 56 ± 14 to 20 ± 5%, that of the most advanced sclerosis increased significantly, on average, from 3 ± 2 to 36 ± 2% in control rats during the five week period. In contrast, the pattern of percent distribution of glomeruli with variably ranked sclerosis was essentially unchanged in PD rats from biopsy specimen to autopsy specimen. Interstitial changes, consisting of interstitial fibrosis and tubular atrophy and dilation, typical of chronic renal diseases, paralleled the severity of the glomerular lesions, with milder interstitial changes in both treated groups compared to control. Together with the above-mentioned markedly attenuated progression of glomerular sclerosis at the whole kidney level in these rats, PD was found to be effective in attenuating the advancement of sclerosis in glomeruli at all stages of sclerosis.

Effect of oral adsorbent on glomerular function and structure (Comparison between Group 3 vs. Group 4)

As shown in Table 1, values for body weight of Group 4 AST-120 treated and Group 3 untreated control animals were comparable until eight weeks. During the remaining four week period of AST-120 treatment, values for body weight increased in Group 4 AST-120 treated rats to a greater extent than in Group 3 untreated control rats, averaging 276 ± 4 versus 295 ± 10 g at 12 weeks ($P < 0.05$), a finding consistent with the observation that Group 4 AST-120 treated rats had a greater food intake than Group 3 rats (17.6 ± 0.5 vs. 20.5 ± 0.7 g/day, $P < 0.05$). There was no statistically significant difference between Group 3 untreated control and Group 4 AST-120 treated rats in systolic blood pressure at the time of biopsy (144 ± 8 and 141 ± 6 mm Hg) or autopsy (168 ± 7 and 157 ± 8 mm Hg), although Group 4 AST-120 treated rats at autopsy showed a tendency to have lower SBP than those in Group 3 untreated control rats. In contrast to the PD rats discussed above, AST-120 rats had significantly lower urine volume, measured at 10 weeks, than their controls (18.2 ± 1.7 vs. 22.4 ± 1.0 ml/24 hr, $P < 0.05$). Values for BUN increased slightly and comparably in Group 3 untreated control and Group 4 AST-120 treated rats, from 69 ± 3 and 68 ± 5 mm Hg to 78 ± 4 and 74 ± 4 mm Hg,

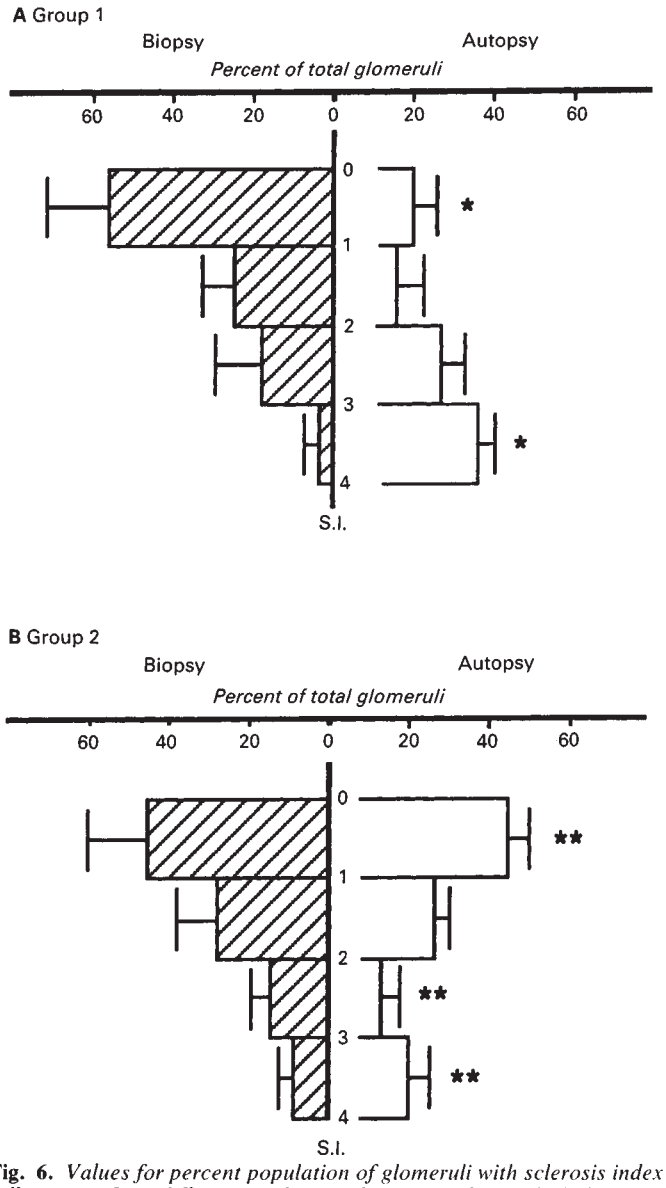

Fig. 6. *Values for percent population of glomeruli with sclerosis index falling into four different rankings of severity.* Sclerosis index was measured three-dimensionally in individual glomeruli of Group 1 PD control and Group 2 PD rats at biopsy and autopsy. The vertical scale represents the glomerular sclerosis index, 0 being no sclerosis and 4 being complete sclerosis. Values were obtained at the time of biopsy (7 weeks) and at the completion of study (autopsy, 12 weeks). Statistical comparison was made between biopsy and autopsy specimens from the same animals (*$P < 0.05$ vs. biopsy; **vs. Group 1, comparing changes).

respectively, from week 8 to week 10 (Table 2). Measurement of whole kidney filtration function, that is, C_{Cr} (measured at biopsy and 10 weeks) and C_{In} (measured at 12 weeks) showed no appreciable difference between Group 3 untreated control and Group 4 AST-120 treated rats. Values for hematocrit at completion of the study were comparable between control (48 ± 1 vol %) and AST-120-treated rats (50 ± 2 vol %, $P > 0.1$). Micropuncture measurement performed at 12 weeks showed that values for P_{GC}, as in Group 1 and 2, although varying markedly, were significantly lower in Group 4 AST-120 treated than in Group 3 untreated control rats (Fig. 7B). Values for SNGFR at 12 weeks in Group 4 AST-120 treated rats were lower than those in Group 3 untreated controls. The difference was not statistically significant due in part to wide variability within each group (Fig. 7A).

Whereas Group 3 untreated controls and Group 4 AST-120 treated rats had comparable whole kidney sclerosis index at the time of biopsy (0.55 ± 0.10 vs. 0.61 ± 0.05, P = NS; Fig. 8), this parameter became substantially and significantly lower at 12 weeks in Group 4 AST-120 treated than in Group 3 untreated control rats (1.42 ± 0.16 vs. 0.99 ± 0.13, $P < 0.05$; Fig. 7).

The sclerosis index was examined in these two groups at the individual glomerular level, as well. Virtually no glomeruli examined in Group 3 untreated control and Group 4 AST-120 treated at biopsy had sclerosis index higher than 2.0. Thus, it was not possible to assess the effect of AST-120 on glomeruli at various stages of sclerosis.

Discussion

The salutary influence of PD on glomerular structure was paralleled by a readily discernible protection of glomerular function. Thus, whole kidney inulin clearance rate, C_{In}, was significantly higher in Group 2 PD rats than Group 1 control rats at the end of the four week course of PD. Administration of oral adsorbent, AST-120, attenuated the progression of glomerular sclerosis to a degree comparable to that of PD. Thus, our observation with this oral charcoal adsorbent echoes the earlier findings by Kanai et al [18] and Yoshida et al [19]. The increases in sclerosis index from biopsy to autopsy specimens in Group 4 rats given AST-120, as well as Group 2 PD-treated rats, were less than half of those found in their corresponding untreated control groups, that is, Group 3 and Group 1, respectively. Nevertheless, unlike Group 2 PD-treated animals, no appreciable salutary effect of AST-120 was demonstrated on glomerular filtration rate in Group 4 animals (Table 2). This variation may be readily explained when one considers that both of these therapeutic measures potentially affect at least two of the opposing parameters determining glomerular filtration rate. Thus, their capability to protect glomeruli from sclerosis, hence from loss of filtration surface area, may serve to preserve filtration function. However, lower glomerular capillary pressure is expected to promote reduction in GFR. In this regard, unlike PD, AST-120 did suppress glomerular capillary pressure. This suppression, therefore, appears to be sufficiently profound to offset the salutary effect on the glomerular structure that would otherwise be expected to result in higher GFR in Group 4 AST-120 treated than Group 3 untreated animals at the completion of study. It is likely that this apparent difference in the effect of PD versus AST-120 on P_{GC} reflects, at least in part, differences in their effect on the external balance of salt and water as well as nutrients. Nevertheless, the marked nephron-to-nephron variability in glomerular pressures and flows prevailing in this model of chronic renal failure [12] precluded assessment of other single glomerular hemodynamic parameters which may also account for this difference between the two groups. The observed comparable GFR values between Groups 1 and 3, despite higher P_{GC} and an apparently lower degree of sclerosis in Group 3 than in Group 1, also remain undetermined. The observed effects of PD and AST-120 result not only from the intrinsic capacity of dialysis and adsorbent to remove

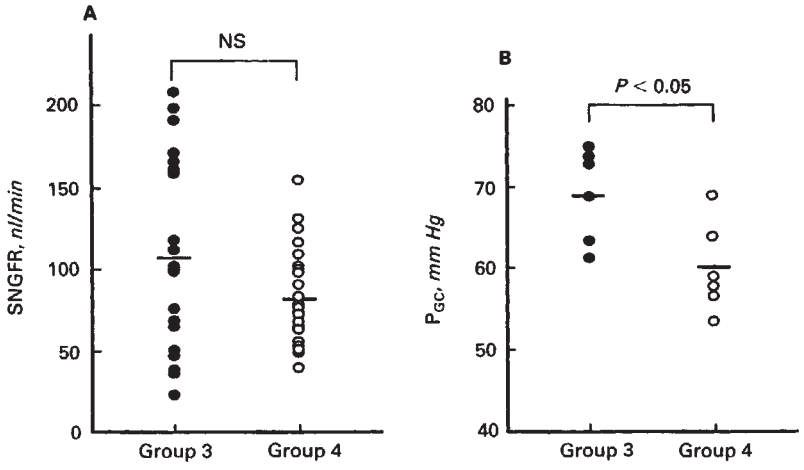

Fig. 7. *The effect of oral adsorbent AST-120 on single nephron GFR (SNGFR) and glomerular capillary pressure (P_{GC}) of remnant superficial nephrons.* Each circle represents the data from a single nephron. There was no statistically significant difference in the values of SNGFR between Group 3 control and Group 4 AST-120 rats. The values for P_{GC} in Group 4 rats were significantly lower than those in Group 3 rats ($P < 0.05$).

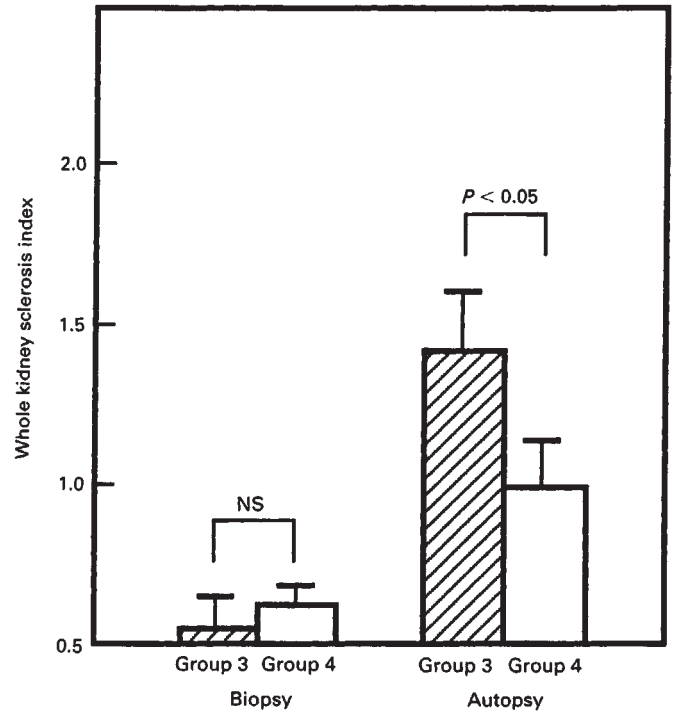

Fig. 8. *Whole kidney glomerular sclerosis index in Group 3 and Group 4 rats.* Whole kidney sclerosis index was comparable between Group 3 and Group 4 rats at biopsy. At autopsy five weeks later, there was significantly less sclerosis in Group 4 AST-120 rats versus control Group 3.

various metabolites but also from their influence on the animals' dietary habits. Unlike PD, AST-120 *per se* has virtually no capacity to remove water. Moreover, AST-120, unlike PD, promoted food intake in animals, as Group 4 animals, given AST-120, had significantly greater food intake and body weight gain than Group 3 control animals. In contrast, Group 2 PD-treated and Group 1 untreated rats had comparable body weight gains.

Given the notion, derived from experimental observations in the parabiotic system and other animal models [1, 20, 21], that the hypertrophy of remnant nephrons following nephrectomy involves circulating substances and that the phenomenon of glomerular hypertrophy and sclerosis are closely linked [11, 22–24], the series of findings in the current study are hardly surprising. Thus, the circulating substances which have the capacity to promote glomerular hypertrophy and sclerosis may accumulate in the body following substantial loss of functioning nephrons. Furthermore, both dialysis and oral adsorbent AST-120, by replacing the renal function of removing such circulating substances, attenuate the glomerular hypertrophy and sclerosis. As dialysis essentially replaces only the glomerular filtration function of the removed nephrons, the substances concerned appear ultrafiltrable in nature. The oral adsorbent AST-120 does not have capacity to adsorb water but, instead, promotes net food intake and body weight gain. Therefore, of the ultrafiltrable substances that PD could remove from the circulation, substances such as water, salt or nutrients, are unlikely candidates.

Of note, the possibility exists that the circulating substances involved in hypertrophy and/or sclerosis could be inhibitors of glomerular growth and sclerosis. Nephron loss would then lead to suppression of the production of such circulating inhibitory substances. However, our observations negate this possibility, since it appears highly unlikely that dialysis or AST-120, with their biologically inert constituents, could replace the hypothetical nephron function of releasing biologically-active inhibitory substances. Thus, our observations support the notion that nephrectomy increases circulating levels of stimulatory, rather than reducing inhibitory, substances affecting growth and sclerosis. Furthermore, it has become apparent that the circulating substance(s) are not BUN or creatinine *per se*, as these two parameters were comparable between Group 4 AST-120 treated and Group 3 untreated animals.

It should be noted that the circulating substances, in and of themselves, may or may not have the potential to promote glomerular hypertrophy or sclerosis. If they do, they are clearly not among currently known growth factors, since these growth factors lack specificity for effects on the kidney. Alternatively, the kidney may have a selective high affinity for the circulating substances, which in turn evoke local release of growth factors,

known or unknown, in an autocrine or paracrine fashion. Of note, the circulating substances under discussion should, in no way, be considered an alternative to any of the variety of local pathogenic mechanisms proposed today. Since training the rats and establishing and maintaining a close kinship between the investigator and the rats were prerequisite for the successful conduct of dialysis, a few parameters were collected, mostly at the time of sacrifice, clearly precluding any conclusion on the currently debated local mechanisms. Instead, our results point to a pathophysiologic significance of "uremic toxins" as a mechanism capable of provoking the debated local mechanisms.

Collectively, we speculate on the possible mechanism of the progression of glomerular sclerosis: The initial pathogenic insults lead to a loss of functioning nephrons through disease-specific pathophysiologic processes resulting in increased levels of circulating substances. These substances promote the production of mesangial matrix, which *per se* is self-damaging in nature, by provoking a series of local pathogenic mechanisms leading to further loss of nephrons. This loss of nephrons, in turn, causes accumulation in the circulation of the substance(s) capable of causing compensatory glomerular hypertrophy and sclerosis. The ultimate outcome of this vicious cycle is the end stage kidney. Removal of circulating substances by peritoneal dialysis or oral adsorbent disrupts this vicious cycle, thereby exerting a protective effect on intact glomeruli against progressive structural deterioration.

Acknowledgments

Portions of this study were presented at the Annual Meeting of the American Society of Nephrology, San Antonio, TX December 1988 and Washington, D.C. December 1989, and published in abstract form (*Kidney Int* 35:434, 1989 and 37:332, 1990). The authors thank Dr. J.J. Lipman and Dr. P.E. Teschan, Department of Medicine, Vanderbilt University, Nashville for advice during the establishment of the protocol for peritoneal dialysis employed in the present study, and Dr. M. Takahashi for kindly offering data for the adsorbing capacity of AST-120 and for the correlation between creatinine versus inulin clearance.

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