Glomerular Injury and Tubular Loss in Adriamycin Nephrosis

BASIT JAVAID, JEAN L. OLSON, and TIMOTHY W. MEYER

Departments of Medicine, VA Palo Alto Health Care System and Stanford University, Palo Alto; and Department of Pathology, University of California, San Francisco, California.

Abstract. Glomerular injury manifested by sustained proteinuria usually leads to tubule injury and reduction of the GFR. The current study explored the link between these processes in rats with adriamycin nephrosis. One group of nephrotic rats received a vasopressin V2 receptor blocker (V2X) from 4 to 16 wk after injection of adriamycin, whereas a second group received no treatment (NoRx). V2 receptor blockade increased urine volume without affecting protein excretion. At 16 wk, both groups of nephrotic rats exhibited a marked reduction in GFR in comparison with normal controls (V2X, 0.22 ± 0.19 ml/min; NoRx, 0.20 ± 0.11 ml/min; control, 1.23 ± 0.11 ml/min). Morphologic studies revealed that the majority of glomeruli in nephrotic rats were no longer connected to normal tubule segments (V2X, 81 ± 21%; NoRx, 85 ± 18%; control, 1 ± 2%). Glomeruli without tubules were not, however, globally sclerosed. Disruption of the glomerular tubular junction was associated with the presence of amorphous material separating damaged tubule cells from the basement membrane. Serial sections revealed that this material spread from extensive areas of adhesion between the glomerular tuft and capsule to invest the tubular neck. Reduction of the GFR was strongly correlated with the fraction of glomeruli not connected to normal tubules ($r^2 = 0.82; P < 0.0001$). V2 receptor blockade did not preserve renal function or structure. These findings suggest that local extension of glomerular injury to destroy the tubule neck is an important cause of loss of renal function in adriamycin nephrosis.

In human renal disease, sustained heavy proteinuria is usually accompanied by rapid loss of renal function. Rats that are given adriamycin provide an experimental model of the association between proteinuria and loss of function, as described by Bertani et al. (1,2) and Okuda et al. (3). These animals develop heavy proteinuria within a few weeks after adriamycin administration. Protein excretion rates remain high thereafter, and the GFR declines gradually. Previous studies showed that the appearance of proteinuria is associated with injury to the glomerular visceral epithelial cells and that the subsequent decline in GFR is associated with both glomerular sclerosis and widespread tubulointerstitial injury (1–6). The current study examined further the structural basis for the decline in GFR that accompanies sustained proteinuria in adriamycin nephrosis. Its major aim was to explore the link between proteinuric glomerular injury and subsequent tubular atrophy. The serial section technique was used to determine whether the decline in GFR was associated with the loss of glomerular connections to normal tubules. A recently developed vasopressin V2 receptor blocker was used to test whether increasing urine flow rate and reducing urine concentration could preserve tubule structure and limit loss of function.

Materials and Methods

Eighteen male Munich Wistar rats weighing 250 to 290 g received a single dose of adriamycin (3 mg/kg) by tail-vein infusion under anesthesia provided by thiopental (50 mg/kg intraperitoneally). Six additional rats received normal saline by the same route and served as normal controls. After 4 wk, animals that were given adriamycin were divided into two groups, matched for their mean body weight and urinary protein-to-creatinine ratio. One group then received the V2 receptor blocker SR121463 (V2X), whereas the other group received no treatment (NoRx). Rats in the current study received SR121463B, which is a close congener of SR121463A, a nonpeptide V2 receptor blocker that has been shown to be active in several in vitro and in vivo models (7). All rats had free access to water and powdered rat chow. SR121463 was administered by mixing it in the chow. On the basis of preliminary studies in control rats, the initial mix provided 1.5 mg of SR121463 in 20 g of chow. The mix was changed to 2.0 mg of SR121463 in 20 g of chow after 4 wk of treatment and then to 2.5 mg of SR 121463 per 20 g of chow after 8 wk of treatment. These adjustments were made to maintain the urine volume of treated nephrotic rats two to three times greater than that of untreated nephrotic rats. The duration of SR121463 treatment was 12 wk, so that the study lasted a total of 16 wk after adriamycin or saline injection. Urine was collected every 4 wk, except that no collection was made at 16 wk in two rats in each nephrotic group. Urine protein was measured by the Coomassie blue method, and urine creatinine was measured by use of a Beckman Creatinine 2 analyzer (Palo Alto, CA). Systolic BP was measured by tail cuff at 10 and 14 wk. One rat in the untreated nephrotic group died before the study was completed. At 16 wk, GFR was measured under anesthesia provided by inactin. A dose of 100 mg/kg intraperitoneally was given to normal rats, and a reduced dose of 45 mg/kg intraperitoneally was given to nephrotic rats. A PE-50 tubing catheter was inserted in the left femoral artery to record arterial pressure and to obtain arterial blood samples. After tracheotomy, catheters were inserted in the jugular veins for infusion of rat plasma, saline, and radiolabeled insulin, and a PE-10 catheter was installed into the left ureter for collection of urine. Plasma was infused in an amount equal to 1% body weight over 40 to 45 min, followed by reduction of the infusion rate to 0.4 ml/h for the duration of the study. Saline was infused at 2.4 ml/h throughout the
stained with toluidine blue so that the tissue could be examined at 6-

medulla was embedded in Epon. These blocks were sectioned serially

were fixed further in 10% formalin for embedding in paraffin and in 1.25% glutaraldehyde in 0.1 M cacodylate for embed-

during in Epon. Periodic acid-Schiff–stained and Alcian blue–stained

sections were prepared from paraffin-embedded slices. For each kid-

perfusion with 2.5% paraformaldehyde and 0.1% glutaraldehyde in

of the kidney were fixed further in 10% formalin for embedding in

long axis at approximately 2-mm intervals. Slices from the midportion

were carried out over two to three 30-min periods. No GFR value was

P

V

V2 antagonist (\( \dagger \)) and in control rats (\( \bigcirc \)). *, \( P < 0.05 \) versus control. (B) Twenty-four-h urine volume. V2 receptor blockade (\( \bullet \)) increased urine volume above values

Figure 1. Metabolic cage studies. (A) Urine protein-to-creatinine ratio. Values in rats that received an injection of adriamycin and received the V2 antagonist (\( \bullet \)) and in rats that received an injection of adriamycin and received no treatment (NoRx; \( \bigcirc \)) were increased to a similar degree above control (\( \square \)). *, \( P < 0.05 \) versus control. (B) Twenty-four-h urine volume. V2 receptor blockade (\( \bullet \)) increased urine volume above values

Studies were carried out in a separate group of three rats to examine renal injury at an earlier time point in the course of nephrosis. These rats received a single dose of adriamycin and no other treatment. Proteinuria was measured at 4 wk, and GFR measurements and morphologic studies were performed at 6 wk by use of the methods described above.

Statistical Analyses

ANOVA was used to assess the significance of differences between groups. The paired \( t \) test was used to assess the significance of differences within each group. Values are expressed as the mean ± SD throughout. \( P < 0.05 \) was considered to denote statistical significance.

Results

Values for the urine protein to creatinine ratio and for urine volume are depicted in Figure 1. As expected, rats that were
given adriamycin developed heavy proteinuria, reflected by a marked increase in the urine protein-to-creatinine ratio. Treatment with a V2 receptor antagonist beginning at 4 wk led to increased urine volume without affecting the urine protein-to-creatinine ratio. The protein-to-creatinine ratio was used to assess proteinuria because of concern that differences in urine volume might influence the completeness of urine collection in metabolic cages and thereby affect measured 24-h protein excretion. Values for protein excretion, however, proved to have only a slightly different pattern than the protein-to-creatinine ratios. Values in rats treated with the V2 receptor antagonist were slightly greater than those in untreated rats, but the difference was statistically significant only at 16 wk (V2X versus NoRx: 8 wk, 599 ± 140 versus 488 ± 213 mg/d; 12 wk, 586 ± 143 versus 574 ± 163 mg/d; 16 wk, 652 ± 124 versus 474 ± 214 mg/d). Values obtained in control rats ranged from 27 ± 6 to 45 ± 15 mg/d during the same interval.

Nephrotic rats exhibited impaired growth, but there was no difference between the treated and untreated groups at any time point. Final body weight averaged 281 ± 41 g in nephrotic rats that received the V2 receptor antagonist, 264 ± 41 g in untreated nephrotic rats, and 375 ± 15 g in normal rats. Systolic BP measured by the tail-cuff method was not different among the three groups at 10 wk (V2X, 140 ± 12 mmHg; NoRx, 147 ± 12 mmHg; control, 144 ± 11 mmHg) or at 14 wk (V2X, 146 ± 14 mmHg; NoRx, 152 ± 17 mmHg; control, 138 ± 8 mmHg).

Studies performed at 16 wk showed loss of renal function in nephrotic rats. Single-kidney GFR values in treated and untreated nephrotic rats averaged only 0.22 ± 0.19 and 0.20 ± 0.11 ml/min, respectively. These values were not different from each other but were markedly lower than the single-kidney GFR of 1.23 ± 0.11 ml/min in normal control rats. Mean arterial pressure under anesthesia in treated rats (139 ± 23 mmHg) but not in untreated rats (131 ± 22 mmHg) was significantly greater than that in normal rats (115 ± 11 mmHg); the values in treated and untreated rats were not significantly different. Renal insufficiency was associated with similar reduction of the hematocrit in both groups of nephrotic rats (V2X, 33 ± 7%; NoRx, 31 ± 4%; control, 45 ± 2%).

Loss of renal function in rats with sustained nephrosis was associated with widespread structural abnormalities. Consistent with the finding of functional studies, no difference was noted between untreated nephrotic animals and animals that received the V2 receptor antagonist. Interstitial volume was increased, and the number of tubule profiles that appeared to be normal was markedly reduced. The widened interstitium as noted by separation of the tubules from one another was due to both fibrosis and edema. In many cases, tubule profiles exhibited atrophic changes characterized by thickening of the basement membrane, loss of brush border, and attenuation of cell cytoplasm. In some cases, the atrophy was so severe as to have resulted in the loss of the lumina. In other cases, tubule lumina were filled by casts. There were occasional lymphoid aggregates. Glomeruli showed severe injury, manifested by occlusion of capillary lumina by hyalinosis or foam cells and by extensive loss of podocytes. Such loss of podocytes was associated with adhesion of the tuft to Bowman’s capsule, accompanied by the accumulation of amorphous material between the tuft and the capsular basement membrane, the structure of which could often not be delineated clearly (Figure 2). The appearance of the amorphous material on toluidine blue–stained sections varied from near lucent to approximately the density of basement membrane, and in some areas it exhibited delicate stranding. These strands stained lightly with periodic acid-Schiff. Alcian blue also stained the amorphous material. Parietal epithelial cells reflected back onto the tuft at the edges of adhesions, and the amorphous material extended circumferentially beyond the adherent segments, separating the parietal epithelium from the capsular basement membrane. In many areas, a layer of small, spindled cells rimmed the outer aspect of Bowman’s capsule.

Global glomerular sclerosis rarely was seen. Serial sections showed, however, that many glomeruli were no longer connected to normal tubules. In most cases, atubular glomeruli exhibited extensive adhesion of the tuft to the capsule with

Figure 2. Representative glomerular injury. (A) Glomerular profile showing extensive adhesion of the tuft to capsule with paraglomerular accumulation of amorphous material (*). Hypertrophied parietal epithelial cells reflect onto the tuft at the limits of the adhesion (arrowheads). (B) Glomerular profile showing Alcian blue staining of amorphous material in the areas of tuft-to-capsule adhesion and disrupted proximal tubule (asterisks). Magnification, ×240 (toluidine blue in A; Alcian blue in B).
accumulation of amorphous material as described above (Figure 3A). A smaller number of atubular glomeruli exhibited reduction of tuft volume with less extensive capsular adhesions (Figure 3B). Occasionally, the shrunken tuft occupied a small portion of Bowman’s space, giving the glomerulus the appearance of a cyst containing a tuft remnant (Figure 3C). In addition to atubular glomeruli, there were many glomeruli that were connected to atrophic tubule segments (Figure 3D). In these cases, the tubule neck was almost invariably surrounded by amorphous material, which was in continuity with the site of a tuft to capsule adhesion. Where this material extended along the tubule, tubule epithelial cells either were missing entirely or exhibited damage characterized by attenuation, the presence of intracellular vacuoles, and the loss of brush border. The site of the tubular junction sometimes was marked only by individual cells, which often had a stellate appearance, floating in amorphous material that seemed to occupy most of the space delimited by the former tubule basement membrane (Figure 4A). Injury extended for a variable distance away from the glomeruli along the tubules. In some cases, accumulation of amorphous material and tubule cell injury extended only a short length or even extended only part way around the tubule at its junction with the glomerulus (Figure 4B). In other cases, the damage extended further. Remarkably, in some of these cases, tubule structure returned nearly to normal at a considerable distance from the glomerulus (Figure 5), whereas in other cases, the tubule could be traced only as an atrophic structure without any lumen. Examination of sections from a subset of 14 individual nephrons indicated that in approximately half of the cases in which the tubule was atrophic at the glomerular junction, more normal structure was preserved at some distance from the glomerulus.

Electron microscopy was used to examine a glomerulus that showed representative injury. On light microscopy, the glomerular tuft proximate to the tubule junction was embedded in amorphous material that extended along the tubule neck. Electron microscopy revealed that this material was variably composed of delicate strands of basement membrane–like material, cell debris, and granular material resembling proteoglycan and was in some areas devoid of any substance (Figure 6). Similar material dissected down the tubule neck so that normal tubule cell attachment to the basement membrane was disrupted. Injury to tubule cells in contact with the amorphous material was manifested by loss of the brush border, intracellular edema, autophagosomes, and attenuation of the cell cytoplasm. An abrupt transition was noted with attachment of a damaged tubule cell to a cell without apparent injury. The amorphous material around the tubule neck contained lymphocytes, macrophages, and occasional spindled cells. Spindled cells that showed features suggestive of myofibroblasts also were present in the interstitium along the outer aspect of the tubule basement membrane and Bowman’s capsule.

Quantitative measures of the extent of structural abnormalities are summarized in Table 1. As expected, almost all glomeruli in control rats were connected to normal tubules. There were a few very small atubular glomeruli and no glomeruli connected to atrophic tubule segments. In contrast, only

Figure 3. Examples of atubular glomeruli. (A) Typical appearance of the midsection of an atubular glomerulus. There is extensive adhesion of the tuft to the capsule with paraglomerular accumulation of amorphous material, but the tuft is not collapsed. (B) Less-common appearance of an atubular glomerulus. The tuft is smaller and has shrunken capillary loops, whereas the area of adhesion is less extensive. (C) An unusual atubular glomerulus with a small tuft remnant within an enlarged Bowman’s space, giving a cystic appearance. Magnifications: ×290 in A and; ×240 in C (toluidine blue for all).
the minority of glomeruli in rats that remained nephrotic for >3 mo remained connected to normal proximal tubule segments. In these animals, approximately half of the glomeruli were no longer connected to tubules, and approximately one third were connected to atrophic tubule segments. Treatment with the V2 receptor antagonist, which failed to limit reduction of the GFR, also failed to limit reduction in the number of glomeruli connected to normal tubules. The average volume of glomeruli connected to normal-appearing tubule segments was not significantly greater in nephrotic rats than that in control rats, but the range of volume values encountered in nephrotic rats was larger than that in normal rats. Within the nephrotic rats, connection of the glomerulus to an atrophic tubule segment was not associated with a significant change in glomerular volume. Atubular glomeruli in nephrotic rats were reduced in size but were not as small as the few atubular glomeruli in normal rats. Of note, neither atubular glomeruli nor glomeruli connected to atrophic tubules were distinguished by marked expansion of Bowman’s space reflected by a reduction in the tuft-to-capsule volume ratio. A few glomeruli did have a cystic appearance, as described above and illustrated in Figure 3C. In these glomeruli, the tuft-to-capsule volume ratio was as low as 0.05. The number of glomeruli with cystic appearance and very low tuft-to-volume ratios, however, was small. Average values for the tuft-to-capsule volume ratio were only modestly reduced in atubular glomeruli, and this reduction was significant only in the untreated group.

Correlations between structural parameters and renal function are summarized in Table 2. Overall, the GFR was negatively correlated with various features of injury, and these features of injury were positively correlated with each other. The strongest correlation was that of the GFR with the portion of glomeruli attached to normal tubule segments, as illustrated in Figure 7. GFR also was strongly correlated with fractional tubule cell volume, and fractional tubule cell volume was, in turn, correlated with the portion of glomeruli attached to normal tubule segments. Fractional interstitial volume, as measured by the current technique, was somewhat less strongly correlated with the portion of glomeruli attached to normal tubule segments and was only weakly correlated with the GFR.

A small group of animals were killed at 6 wk after infusion of adriamycin, to confirm that the tubule loss described above develops only with sustained nephrosis. At 4 wk after infusion of adriamycin, these animals exhibited a urine protein-to-creatinine ratio of 65 ± 6, which was similar to that observed in the groups of rats studied for 16 wk. In accord with reports elsewhere, kidneys perfused at 6 wk showed only mild interstitial widening and relatively little tubule injury. Glomeruli showed epithelial cell injury with bleb formation. There were focal, segmental adhesions. The extent of these adhesions, however, and of the accumulation of amorphous material between the tuft and capsule was much less than that at 16 wk.

Examination of serial sections revealed that the adhesions developed in all sectors of the tuft and had no tendency to be localized to the area of the vascular pole. Examination of 30 glomeruli in each of three rats disclosed no glomeruli that were connected to atrophic tubule segments or were atubular.

**Discussion**

Previous studies have shown that reduction of the GFR in chronic adriamycin nephrosis is associated with development of both glomerular sclerosis and tubulointerstitial injury (1–6). The major finding of the current study is that reduction of the GFR is closely correlated with the extent to which glomeruli are no longer connected to normal tubules. Most glomeruli examined in the current study exhibited injury characterized by tuft-to-capsule adhesions, epithelial cell injury, and segmental occlusion of capillary loops. However, glomerular volume...
often was only moderately reduced, and many capillary loops remained patent even after the connection to the proximal tubule had been obliterated. These findings suggest that nephron function often ceased because of tubule loss before glomerular injury was far enough advanced to preclude filtration.

The question of how tubules are lost remains unsettled. In adriamycin nephrosis, tubule injury generally is considered to be secondary to glomerular injury and proteinuria, although a toxic effect of adriamycin on tubule cells has not been completely excluded. Obstruction by casts is one mechanism by which proteinuria could injure tubules, as was originally noted by Bertani et al. (1,2). The precise site at which casts begin to form and the conditions governing their formation are not known, but casts have been localized mostly to the distal nephron in adriamycin nephrosis (1,2). We therefore considered it possible that reducing urine concentration with a V2 receptor antagonist would limit cast formation and consequent tubule injury in nephrotic rats. Results showed, however, that V2 receptor antagonism in a dose sufficient to increase urine flow approximately threefold had no effect on the course of injury. It should be noted that studies elsewhere have suggested other mechanisms by which V2 receptor antagonism could lessen renal injury. In particular, Okada et al. (10) found that V2 receptor antagonism lessened proteinuria in rats given adriamycin, a finding that was not confirmed in the current study. Other workers have found that a forced increase in water intake reduces both glomerular and tubulointerstitial injury in rats subjected to renal ablation (11,12). An increase of water intake and urine excretion of approximately the same magnitude induced by V2 blockade in nephrotic rats had no beneficial effect in the current study.

There are a number of other mechanisms whereby proteinuric glomerular injury could cause tubule injury. The most frequently proposed is that tubule uptake of filtered proteins or protein-bound substances injures proximal tubules (13–17). Recent studies have shown that tubule injury is as extensive in analbuminemic rats as in normal rats given adriamycin, although the analbuminemic rats excrete less total protein and no albumin (18). These findings suggest that if filtered protein causes tubular injury, albumin cannot be the major protein involved. A second hypothesis is that diminished perfusion downstream from injured glomeruli leads to tubule ischemia (19,20). Morphologic studies are consistent with the hypothesis that ischemic injury leads to tubule loss in renal artery disease (21). Clear evidence of tubule cell ischemia in proteinuric renal disease, however, has not been obtained. Recently, Kriz et al. (22,23) proposed an additional mechanism whereby proteinuric glomerular injury can lead to tubule loss. They found that visceral epithelial cell injury and adhesion of the tuft to capsule is followed by accumulation of amorphous material in a paraglomerular space that sometimes extends along the capsule to invest the neck of the tubule. Tubule degeneration was observed where the material extended down the outside of the tubule, separating the tubule cells from the tubule basement membrane. These changes were demonstrated most extensively in Fawn hooded and Milan normotensive rats but were

![Figure 5. Resumption of normal tubule structure at a distance from an injured glomerulus. (A) Tubule cells are atrophic and separated from the basement membrane by amorphous, nearly lucent material (*) at the level of the glomerular tubular junction. (B) At 30 μ from A, the tubule has a lumen but is again atrophic and separated from the basement membrane by amorphous material (*). (C) At 48 μ from A, amorphous material (*) is observed along one aspect of the tubule, and tubule cells are atrophic where this material separates them from the basement membrane. As the tubule is followed in a distal direction, however, this material is no longer seen, and tubule structure returns to normal (+). Magnification, ×290 (toluidine blue).](image-url)
also observed in several other rodent models and in a number of human cases (23).

The findings of the current study seem to be consistent with the hypothesis of Kriz et al. (23). Small tuft-to-capsule adhesions were observed early in the course of adriamycin nephrosis. Subsequently, these adhesions enlarged and were associated with accumulation of amorphous material that spread circumferentially along the capsule in a paraglomerular space beneath a layer of fibroblast-like cells. Tubule injury was present where the amorphous material reached the tubule neck and dissected between the tubule cells and basement membrane. Indeed, the lesions seen in the current study looked very similar to those illustrated by Kriz et al. (23). There were some differences, which may have been accounted for in part by

Table 1. Morphometric findings at 16 wk

<table>
<thead>
<tr>
<th>Finding</th>
<th>Normal Control</th>
<th>Nephrotic, No Treatment</th>
<th>Nephrotic, V2 Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney weight (g)</td>
<td>1.51 ± 0.14</td>
<td>1.28 ± 0.21</td>
<td>1.26 ± 0.31</td>
</tr>
<tr>
<td>Prevalence of glomeruli (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>attached to normal tubule</td>
<td>99 ± 2</td>
<td>16 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19 ± 21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>attached to no tubule</td>
<td>1 ± 2</td>
<td>49 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58 ± 20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>attached to atrophic tubule</td>
<td>0 ± 0</td>
<td>34 ± 12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23 ± 18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume of glomeruli (10&lt;sup&gt;6&lt;/sup&gt; mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>attached to normal tubule</td>
<td>1.64 ± 0.15</td>
<td>1.94 ± 0.72</td>
<td>1.80 ± 0.71</td>
</tr>
<tr>
<td>attached to no tubule</td>
<td>0.40 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.06 ± 0.26&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.15 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>attached to atrophic tubule</td>
<td>—</td>
<td>1.50 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.92 ± 0.70</td>
</tr>
<tr>
<td>Vg/Vc ratio for glomeruli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>attached to normal tubule</td>
<td>0.77 ± 0.03</td>
<td>0.83 ± 0.04</td>
<td>0.80 ± 0.17</td>
</tr>
<tr>
<td>attached to no tubule</td>
<td>0.70 ± 0.12</td>
<td>0.67 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>attached to atrophic tubule</td>
<td>—</td>
<td>0.82 ± 0.06</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td>Fractional volume (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of the interstitium</td>
<td>12 ± 2</td>
<td>38 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>of tubular cells</td>
<td>56 ± 5</td>
<td>33 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Vg/Vc ratio, the ratio of the tuft volume to the volume enclosed by the capsule. Kidney weight is weight of left kidney.

<sup>b</sup> P < 0.05 versus normal control.

<sup>c</sup> P < 0.05 versus glomeruli with normal tubules in the same group.
differences in the animal models studied. In Fawn hooded rats, Kriz et al. (22) found that adhesions began most often in the area of the vascular pole. It has been speculated that this pattern may be typical of injury of hemodynamic origin. In any case, spread of injury from the vascular pole in the Fawn hooded rat usually resulted in global collapse of the tuft before the tubule junction was obliterated. Marked dilation of Bowman’s space was thought to occur in the lesser number of glomeruli in which the tubule was lost before tuft was destroyed, so that the primary tubule loss was reflected by the appearance of cystic glomeruli. In adriamycin nephrosis, we found that tuft-to-capulse adhesions were not localized primarily around the vascular pole. As the adhesions and the associated capsular lesions spread, tubule loss often preceded collapse of the entire tuft, which suggests that tubule neck injury was a major contributor to the reduction in GFR. Moreover, in the great majority of cases, glomeruli that had lost tubules did not have a cystic appearance. Thus, the high prevalence of atubular glomeruli and glomeruli attached to severely atrophic tubules could be detected only by examination of serial sections.

It should be noted that separation of degenerated epithelial cells from the tubular basement membrane by amorphous material at the tubule neck is not simply a common feature of advanced tubule injury. Atubular glomeruli were first described by Marcussen et al. in rats given lithium (24) and in rats given cisplatin (25). Toxic injury in these models led to tubule loss and glomerular shrinkage without the tubule neck lesions observed in the current study of nephrotic injury. We recently observed large numbers of atubular glomeruli and glomeruli attached to atrophic tubules in rats recovering from acute ischemic injury (26). Again, severe tubule atrophy in this setting was not associated with accumulation of amorphous material around the tubule neck, as observed in the current study of nephrosis. It also should be emphasized that the amorphous material investing the tubule neck in nephrotic rats was continuous with sites of glomerular tuft adhesion. Together, these observations suggest that most tubule loss in the current study was caused by the local extension of glomerular injury rather than by the toxic action of filtered macromolecules, by ischemia, or by cast obstruction at some point further along the nephron. We presume that one or more of these latter processes were responsible for tubule injury in the smaller portion of nephrons in which tubule loss was not associated with prominent tuft adhesion and paraglomerular accumulation of amorphous material. It is, of course, likely that some nephrons were subject to more than one form of injury, and it is possible that amorphous material accumulated around some glomeruli after the attached tubule had been damaged by another process.

Important questions concerning the paraglomerular lesion described above remain to be addressed. One is the source of the amorphous material that seems to spread outward from sites of adhesion. Kriz et al. (23) hypothesized that this material accumulates as a result of misdirected filtration from capillary loops that are denuded of epithelial cells and adherent to the capsule. It also seems possible that contact of denuded capillaries with the capsule evokes a reaction by parietal epithelial cells and other surrounding cell types, which then are stimulated to produce a variety of growth factors and matrix constituents (27–29). The Alcian blue staining of paraglomerular material suggests that it could contain acid mucous substances such as chondroitin sulfate and hyaluronic acid. Further studies clearly are needed to define better its composition. Further studies also are needed to elucidate how penetration of the amorphous material into the paratubular space injures tubule cells. Mechanical compression of the tubule seems not to be required, because injury is observed where the material comes in contact with only part of the tubule circumference. One possibility is that the material disrupts contacts between the tubule epithelial cells and underlying basement membrane, which are essential for maintenance of normal cell structure (30).

Table 2. Correlations among functional and structural measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min) against glomeruli with normal tubules (%)</td>
<td>0.82</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fractional volume of tubular cells (%)</td>
<td>0.67</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fractional volume of the interstitial (%)</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>Glomeruli with normal tubules against fractional volume of tubular cells (%)</td>
<td>0.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fractional volume of the interstitial (%)</td>
<td>0.28</td>
<td>0.03</td>
</tr>
<tr>
<td>Fractional volume of tubular cells against fractional volume of the interstitial (%)</td>
<td>0.58</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Figure 7. Correlation of the GFR with the fraction of glomeruli attached to normal-appearing proximal tubule segments. Values are from the nephrotic rats that received the V2 receptor blocker (●) or no treatment (○). Regression: $y = 0.008x + 0.06$; $r^2 = 0.82$; $P < 0.0001$. 

It should be noted that separation of degenerated epithelial cells from the tubular basement membrane by amorphous material at the tubule neck is not simply a common feature of advanced tubule injury. Atubular glomeruli were first described by Marcussen et al. in rats given lithium (24) and in rats given cisplatin (25). Toxic injury in these models led to tubule loss and glomerular shrinkage without the tubule neck lesions observed in the current study of nephrotic injury. We recently observed large numbers of atubular glomeruli and glomeruli attached to atrophic tubules in rats recovering from acute ischemic injury (26). Again, severe tubule atrophy in this setting was not associated with accumulation of amorphous material around the tubule neck, as observed in the current study of nephrosis. It also should be emphasized that the amorphous material investing the tubule neck in nephrotic rats was continuous with sites of glomerular tuft adhesion. Together, these observations suggest that most tubule loss in the current study was caused by the local extension of glomerular injury rather than by the toxic action of filtered macromolecules, by ischemia, or by cast obstruction at some point further along the nephron. We presume that one or more of these latter processes were responsible for tubule injury in the smaller portion of nephrons in which tubule loss was not associated with prominent tuft adhesion and paraglomerular accumulation of amorphous material. It is, of course, likely that some nephrons were subject to more than one form of injury, and it is possible that amorphous material accumulated around some glomeruli after the attached tubule had been damaged by another process.

Important questions concerning the paraglomerular lesion described above remain to be addressed. One is the source of the amorphous material that seems to spread outward from sites of adhesion. Kriz et al. (23) hypothesized that this material accumulates as a result of misdirected filtration from capillary loops that are denuded of epithelial cells and adherent to the capsule. It also seems possible that contact of denuded capillaries with the capsule evokes a reaction by parietal epithelial cells and other surrounding cell types, which then are stimulated to produce a variety of growth factors and matrix constituents (27–29). The Alcian blue staining of paraglomerular material suggests that it could contain acid mucous substances such as chondroitin sulfate and hyaluronic acid. Further studies clearly are needed to define better its composition. Further studies also are needed to elucidate how penetration of the amorphous material into the paratubular space injures tubule cells. Mechanical compression of the tubule seems not to be required, because injury is observed where the material comes in contact with only part of the tubule circumference. One possibility is that the material disrupts contacts between the tubule epithelial cells and underlying basement membrane, which are essential for maintenance of normal cell structure (30).
Reduction of the GFR was strongly correlated with disruption of the tubule at its junction to the glomerulus. Disruption of the tubule seemed in most cases to be caused by local extension of glomerular injury. Reduction of the GFR thus was related to the extent of glomerular injury. Correlation of GFR to glomerular injury might be expected in an experimental disease triggered by toxic injury to glomerular epithelial cells. There has been considerable discussion, however, of an apparent discordance between reduction of the GFR and the extent of glomerular injury in many disease processes. Some studies, particularly in human disease, have suggested that reduction of the GFR is regularly associated with the degree of tubule atrophy and interstitial expansion but much less reliably is associated with the severity of glomerular injury (19,31,32). We believe that these studies, although they have proved to be a valuable stimulus to the investigation of tubular and interstitial injury, may have underestimated the extent to which tubulo-interstitial injury and glomerular injury advance together as renal disease progresses. In many studies, loss of renal function has been correlated with tubular atrophy and interstitial fibrosis but not with glomerular injury, as judged by the prevalence of global sclerosis. Similar results would have been obtained in the current study if global sclerosis had been used as an index of glomerular injury in chronic adriamycin nephrosis. Examination of serial sections, however, revealed a strong correlation between loss of renal function and glomerular injury. In effect, the strength of the correlation between function and glomerular injury was improved by increasing the precision of the structural studies. The extension of paraglomerular injury to destroy the tubule neck, as originally described by Kriz (23), could account for cessation of nephron function before the glomerular tuft was globally sclerosed. The extent to which this mechanism contributes to loss of function in humans with proteinuric renal disease remains to be determined.

Acknowledgments
This work was supported by the Research Service of the VA Palo Alto Health Care System and by a grant from the National Institutes of Health (RO1 DK52841). SR121463 was kindly provided by Sanofi. B.J. was supported by a National Kidney Foundation Fellowship. Miaofen Chou and Helen Kwan provided expert technical assistance.

References


