Glomerular Epithelial Cell Changes in Early Postischemic Acute Renal Failure in Rabbits and Man

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Spreading and flattening of glomerular podocyte cell bodies and major processes and an apparent lack of foot processes were observed by scanning electron microscopy in a reversible pedicle-clamping model of acute renal failure in ADH-treated rabbits and in biopsy specimens taken 1 hour after transplantation from patients who later showed clinical signs of "acute tubular necrosis." Glomerular changes were quantified by morphometry in A) normal rabbit kidneys, B) rabbit kidneys obtained 2 hours after 1 hour of left pedicle clamping and right nephrectomy, C) kidneys similar to Group B except that the animals were treated with an agent that reliably lessens the eventual severity of renal failure (clonidine, 30 μg/kg given intravenously ½ hour before unclamping), D) 1-hour-posttransplantation biopsy specimens from human kidneys that functioned well after transplantation (recipient serum creatinine <2.5 mg/dl on Day 3), and E) 1-hour-posttransplant biopsy specimens from kidneys that later manifested posttransplantation ischemic acute renal failure (recipient serum creatinine ⩾2.5 mg/dl on Day 3). The fraction of glomerular capillary surface covered only by podocyte processes smaller than 1 μ (and not by cell bodies and wider processes) was .65 ± .02 (SEM) in A; .48 ± .03 in B; .64 ± .03 in C; .57 ± .01 in D; and .38 ± .04 in E (A vs B, $P < .01$; B vs C, $P < .02$; D vs E, $P < .01$). In Groups D and E there was a significant negative correlation between the fraction of glomerular capillary surface covered only by podocyte processes less than 1 μ in width and serum creatinine on the third posttransplantation day ($r = -.86$, $P < .01$ by the Spearman rank test). It is concluded that podocyte changes are seen by scanning electron microscopy early in clinical and experimental postischemic acute renal failure and are more pronounced in those groups that eventually develop more severe renal failure. It is unclear whether these changes reflect a decrease in glomerular hydraulic permeability or an increase in glomerular permeability to protein. (Am J Pathol 1981, 103:163–173)

ISCHEMIC ACUTE RENAL FAILURE ("acute tubular necrosis") occurs commonly following renal transplantation. Although it is reversible and does not appear to interfere with long-term function of the graft,[1] the unpredictability of this nonimmunologic type of acute renal failure increases the difficulty of diagnosing and treating acute rejection and surgical complications in the early posttransplantation period. In renal biopsy specimens obtained 1 hour after transplantation, standard light microscopy, immunofluorescence, and transmission electron microscopy, although helpful in diagnosing hyperacute rejection or pre-existing disease, are of little value in predicting the occurrence or severity of posttransplantation ischemic acute renal failure. We report here the finding of an alteration in glomerular podocyte structure, seen in biopsy specimens taken 1 hour after transplantation, which can be reproducibly quantified by scanning electron microscopy and which correlates with posttransplantation renal function. We first observed this lesion in a model of postischemic acute renal failure in the rabbit which resembles ischemic acute renal failure in man.[2,3]

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In normal glomeruli more than 50% of the total epithelial surface of the capillary loops is covered only by a fine meshwork of epithelial cell (podocyte) processes less than 1 μ in width. We have found that very early in the course of ischemic acute renal failure there is a significant reduction in the glomerular capillary surface area covered only by the fine podocyte processes less than 1 μ in width. It appears that in both rabbits and man podocyte abnormalities are more severe 1–2 hours after the ischemic insult in those groups that will later develop more severe renal impairment. The podocyte abnormalities observed by scanning electron microscopy in experimental and clinical ischemic acute renal failure resemble those described in proteinuric states[4] but are less severe. Transmission electron microscopy shows that there is little foot process "fusion" and that much of the apparent foot process loss seen by scanning electron microscopy in early ischemic acute renal failure is due to the covering over of intact foot processes by flattened podocyte cell bodies and major processes. It is possible that the podocyte cell bodies and major processes closely apposed to the foot processes may act as a sheetlike additional barrier to glomerular filtration after filtrate has passed through the slit pores between foot processes. Alternatively, the podocyte alterations may reflect increased glomerular permeability to protein, which would lead to an increased propensity for obstructive tubular casts to form. Abnormalities in the slit diaphragm may also contribute to altered capillary permeability.

Materials and Methods

The groups studied (A-E) are listed in Table 1.

Animal Studies

Twenty-five female New Zealand White rabbits weighing 2.5–3.0 kg were perfusion-fixed with 1.5% glutaraldehyde through a catheter inserted in the aorta under pentobarbital anesthesia as previously described.[5] Prior to study the rabbits had free access to Purina chow and tap water. In 4 animals (Group A1) no drug treatments were given and no surgical procedures were carried out. The remaining 21 rabbits were treated on the day before study with a long-acting vasopressin preparation, pitressin tannate in oil (1.0 U/kg subcutaneously). In 7 of these 21 vasopressin-pretreated rabbits (Group A2), no surgical procedures were carried out before perfusion fixation. In another 8 (Group B) the right kidney was removed and the left renal artery and vein were clamped for 1 hour. Perfusion fixation was carried out 2 hours after unclamping. The remaining 6 animals (Group C) were like those in Group B, except that clonidine (30 μg/kg intravenously in sterile 0.15 M saline, 0.3 ml/kg) was given ½ hour before unclamping. Previous studies from our laboratories have shown that this drug significantly lessens the severity of postischemic acute renal failure in vasopressin-pretreated rabbits, apparently by reducing microvascular damage and cast formation.[5,6] Light and transmission electron microscopic studies were performed as previously described.[7] Scanning electron microscopic studies were performed as described below.

Human Studies

Superficial wedge kidney biopsies are routinely performed in this medical center approximately 1 hour after the renal transplant vascular anastomoses are completed. Biopsy specimens from 21 patients who underwent transplant operations between May 31, 1979, and May 26, 1980, were divided and fixed by one of us (K.S.) immediately after they were obtained. The tissue wedge was divided with a razor blade and processed by standard means for light-microscopic, immunofluorescence, and transmission electron microscopic examination.[3,7] Approximately a quarter of the biopsy specimen was immersion-fixed in phosphate-buffered 3% glutaraldehyde for scanning electron microscopic examination. We found razor-cut, immersion-fixed tissue quite satisfactory for the study of the glomerular podocytes. The razor blade tends to shear off Bowman's capsule, leaving the underlying glomerular tuft intact.

Paraffin sections of formalin-fixed tissue from the 21 cases were ranked independently and without knowledge of clinical history by two of the authors (K.S. and L.R.) for the following 10 histologic changes[3,10]: interstitial edema, loss of proximal tubular brush border, tubular necrosis, tubular dilatation, tubularization of Bowman's capsule, dilatation of Bowman's space, glomerular sclerosis, juxtaglomerular apparatus hyperplasia, and the presence of polymorphs or starch particles in glomerular capillary loops. There was a significant correlation between observers for all lesions ($P < .05$), and thus the rankings of the two observers were combined for the final assessment.

Two patients (Numbers 16 and 19) had their transplanted kidneys removed in the first 24 hours after transplantation for the reasons cited in Table 2. Four additional patients (Numbers 17, 18, 20, and 21) had signs of acute rejection (fever, rising serum creatinine often after an earlier decline, poor perfusion on renal

scan) by the third posttransplantation day and were treated for rejection. (The diagnosis of rejection and the decision to treat these four patients for rejection were made by the surgeons caring for the patients without knowledge of the scanning electron microscopic findings.) These 6 patients were excluded from our analysis of postischemic acute renal failure. All of the 15 remaining transplants eventually functioned. However, most patients exhibited some degree of postischmic acute renal failure after transplantation, and Table 2 indicates the number of posttransplantation dialyses required to manage these patients. No patient was dialyzed before the third day after transplantation. Therefore, the serum creatinine on the morning of the third day was used as the best available clinical index of the severity of posttransplantation acute renal failure. The 15 patients were divided into two groups on this basis. Six patients had serum creatinine levels below 2.5 on the third posttransplantation day (Group D). Nine had serum creatinine levels above 2.5 on the third day (Group E). Before transplantation, all recipients had end-stage renal disease with pre-dialysis serum creatinine levels greater than 10 mg/dl. None of the donors had proteinuria or received nephrotoxic drugs before harvesting.

Scanning Electron Microscopy

Glutaraldehyde-fixed tissues were rinsed in buffer, postfixed in osmium tetroxide, and then dehydrated in a graded series of acetone solutions. After three changes of 100% acetone, the specimens were critical-point-dried, mounted on stubs with conductive tape, sputter-coated with 125–150 Å of gold palladium, and examined in a JEOL JSM-35C scanning electron microscope operating at an accelerating voltage of 25 kv with a 0° specimen tilt angle. The photography and morphometric analysis were done without knowledge of group assignments or clinical history.

Duplicates of photomicrographs of glomeruli taken at magnifications of $\times 1800$ to $\times 4000$ and printed on paper of standard weight were used for morphometry. For each field, regions of capillary surface covered by cell bodies and major podocyte processes greater than or equal to 1 μ wide, and the remaining regions covered only by individual pedicels and processes thinner than 1 μ were cut out and weighed separately so that we could determine the fraction of total capillary area covered by pedicels and small processes. Photographs of at least 6 superficial glomeruli from each rabbit and 2–10 glomeruli from each transplant biopsy were assessed in this way.

One representative specimen from Groups A1, A2, and D (Case 6), and 2 from Groups B and E (Cases 11 and 14) were frozen in 100% ethanol and then cryofractured to provide a better view of the endothelial surface of the glomerular capillaries.[8] Photomicrographs were taken at $\times 30{,}000$ and assessed as described by Avasthi, Evan, and Hay.[9]

Statistical Analysis

Since there are no data that indicate that quantitative podocyte changes are normally distributed, the standard t test and linear correlation test could not be used. Instead, the nonparametric Wilcoxon two-sample test was used to test for differences between groups, and the Spearman rank test was used for correlations.[3]

Results

Light Microscopy

By light microscopy there were no discernible differences between Groups B and C or Groups D and E.* There was no correlation in the human biopsy series between light or fluorescence microscopic findings in the 1-hour post-renal-transplantation biopsy specimens and the functional status of the kidneys three days later. These findings are consistent with our previous studies showing that no differences could be detected by routine histologic techniques between postischemic kidneys from clonidine-treated and untreated rabbits until significant differences in creatinine clearance appeared 6 hours after the ischemic insult,[5,6] and with studies by others showing that posttransplantation function cannot be predicted from light and fluorescence microscopic changes in 1-hour posttransplantation biopsy specimens.[10] No light-microscopic feature correlated with the podocyte changes described below in any of the groups.

Scanning Electron Microscopy

By scanning electron microscopy, Groups A1 and A2 appeared identical and were combined for purposes of subsequent data analysis. In these normal rabbit kidneys the podocyte cell bodies were nearly spherical and hung off the capillary loops in a berry-like fashion (Figure 1). The fraction of glomerular capillary surface covered by foot processes and podocyte processes narrower than 1 μ was $.65 \pm .02$ (SEM) ($n = 11$).

In the postischemic kidneys of Group B, this fraction was reduced to $.45 \pm .03$ ($n = 7$) ($P < .01$). Cell bodies were flattened, and in many areas the glomerular capillaries were covered by a continuous sheet of

* Additional microscopic and clinical data may be obtained by writing to the first author.

Table 1—Groups Studied and Fraction of Glomerular Capillary Surface Covered Only by Podocyte Processes Narrower than 1μ in Each, Determined by Scanning Electron Microscopy and Morphometry

A. Normal rabbit kidneys (n = 11)	.65 ± .02 (SE)	
		$P < .01$
B. Rabbit kidneys (left) obtained 2 hours after 1 hour of left pedicle clamping and right nephrectomy (n = 8)	.48 ± .03	
		$P < .02$
C. Rabbit kidneys like those in B except that clonidine 0.030 mg/kg i.v. was given 30 minutes before unclamping (n = 6)	.64 ± .03	
D. One-hour posttransplantation biopsy specimens from human renal transplants that functioned promptly (serum creatinine below 2.5 mg/dl on the third day posttransplantation) (n = 6)	.57 ± .01	
		$P < .01$
E. One-hour posttransplantation biopsy specimens from human renal transplants that had posttransplantation acute renal failure (serum creatinine above 2.5 mg/dl on third day posttransplantation) without signs of rejection (n = 9)	.38 ± .04	

podocyte cytoplasm, and the usual branching structure and foot processes were not seen (Figure 2). In the clonidine-treated postischemic kidneys (Group C), some slight flattening of cell bodies was seen, but the fraction of glomerular capillary surface area covered by foot processes and podocyte processes thinner than 1 μ was not different from that in the control Group A (.64 ± .03, n = 6).

In 1-hour biopsy specimens from all of the human renal transplants that functioned promptly (Group D) the podocytes appeared similar to those in Groups A and C (although cell bodies were hemispherical rather than spherical) and the fraction of capillary surface covered by processes <1 μ in width was greater than .50. In 1-hour biopsy specimens from 7 out of 9 of the transplants that exhibited posttransplantation renal failure (Group E), podocyte abnormalities similar to those in the rabbit kidneys of Group B were observed

Table 2—Data From Biopsy Series

Case No.	Group	Type of donor	Time of biopsy after vascular anastomosis (min)	% Glomerular capillary surface covered only by processes <1 μ wide†	Creatinine 3rd Posttransplantation Day (mg/dl)†	No. Posttransplantation dialyses	Reason for exclusion from statistical analysis
1	D	Living, related	60	52	2.0	0	
2	D	Living, related	60	59	1.2	0	
3	D	Living, related	60	61	0.4	0	
4	D	Living, related	60	55	1.0	0	
5	D	Cadaver	55	57	2.3	0	
6	D	Living, related	60	55	1.3	0	
7	E	Cadaver	40	41	9.0	1	
8	E	Cadaver	60	31	8.4	3	
9	E	Cadaver	90	25	11.3	10	
10	E	Cadaver	60	42	6.4	0	
11	E	Living, related	30	52	3.1	0	
12	E	Cadaver	45	56	5.1	0	
13	E	Cadaver	30	40	7.4	0	
14	E	Cadaver	60	18	8.2	2	
15	E	Cadaver	60	35	6.6	0	
16*		Cadaver	60	35	—	—	Perfusion injury, problems with venous anastomosis, and recipient hypotension led to immediate nephrectomy
17*		Cadaver	60	56	15.2	5	Early episode of rejection
18*		Cadaver	60	60	9.5	8	Early episode of rejection
19*		Cadaver	60	44	—	—	Kidney removed because of recipient bleeding
20*		Cadaver	50	44	9.7	2	Early episode of rejection
21*		Cadaver	70	51	4.1	1	Early episode of rejection

* Excluded from statistical analysis for reasons stated in right-hand column.

† These two variables are significantly correlated in groups D and E ($r = -.86$, n = 15, $P < .01$ by Spearman rank correlation test).

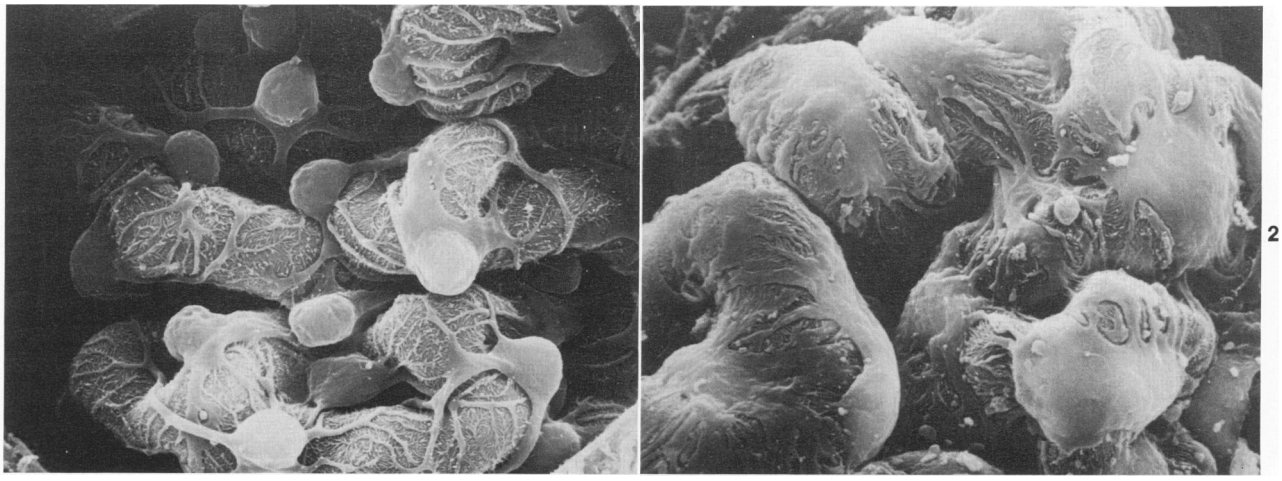

Figure 1—Scanning electron micrograph of a glomerulus from a normal rabbit (Group A). The podocyte cell bodies hang off the capillary loops in berrylike fashion. Most of the capillary surface is covered by foot processes and podocyte processes narrower than 1 μ. (× 1500) **Figure 2**—Scanning electron micrograph of a glomerulus from a rabbit in Group B. Cell bodies are flattened and are indistinguishable from the broad irregular processes that emanate from them and cover most of the capillary surface. Few foot processes and podocyte processes narrower than 1 μ are seen.

(Figure 3), and the fraction of capillary surface covered by processes <1 μ in width was less than .50. In some areas there were defects in the sheetlike covering of podocyte cytoplasm that allowed underlying, relatively normal foot processes to be seen, suggesting that the principal alteration was flattening of cell bodies and major processes rather than foot process fusion or obliteration. There was a significant rank correlation in Groups D and E between the fraction of capillary surface covered by podocyte processes thinner than 1 μ and serum creatinine on the third day after transplantation ($r = -.86$, $P < .01$; Table 2). Overall, the mean fraction of capillary area covered by processes <1 μ in width was .57 ± .01 in Group D and .38 ± .04 in Group E ($P < .01$) (Tables 1 and 2).

No significant endothelial cell abnormalities were observed in the rabbit groups by transmission or scanning electron microscopy. Cryofracture to display the glomerular capillary endothelium by scanning electron microscopy was carried out in only the cases in the human biopsy series (Cases D6, E11, and E14). In these cases an apparent decrease in size and density of the endothelial fenestrae was observed (Figure 4). Fenestral density (number per 5 sq cm in a × 30,000 photomicrograph) and average fenestral diameter (in Angstroms) were 38.8 and 463 in Case D6, 17.4 and 694 in Case E11, and 17.1 and 662 in Case E14. Normal values for these measurements in man are unknown, and measurements in animals are based on perfusion-fixed rather than immersion-fixed tissues.[9]

Transmission Electron Microscopy

Transmission electron microscopic studies showed that the podocyte cell bodies in Groups A, C, and D were roughly spherical or hemispherical and were connected to the foot processes enveloping the capillary loops by long, thin cytoplasmic processes. In Groups B and E the podocyte cell bodies were flattened and closely applied to the foot processes enveloping the loops. Thick cytoplasmic processes were seen immediately above the foot processes in most areas. The lack of foot processes seen by scanning electron microscopy (Figures 2 and 3) apparently was largely due to concealment of foot processes by these overlying thick processes and flattened cell bodies, rather than to true "fusion" of foot processes, since fewer than half of the foot processes covering capillary loops were "fused" as seen by transmission electron microscopy, even in cases with severe podocyte changes seen by scanning electron microscopy (Figures 5 and 6B). Wrinkling of the basement membrane along the mesangial reflections was frequently observed in Group B, suggesting contraction of the mesangium (Figure 5). Higher magnification transmission electron microscopic examination showed normal slit diaphragms between foot processes in Group A (Figure 6A) and retraction, reduplication, or absence of the slit diaphragms in Group B (Figure 6B). Similar changes in postischemic acute renal failure in the rat have been reported by Barnes and colleagues in a recent abstract.[11] Only minor abnormalities in the slit diaphragm were observed in Group C (Figure 6C). Abnormalities in the slit diaphragm were also observed in Group E, but these were less easy to interpret, since this diaphragm is difficult to demonstrate in immersion-fixed renal biopsy material.

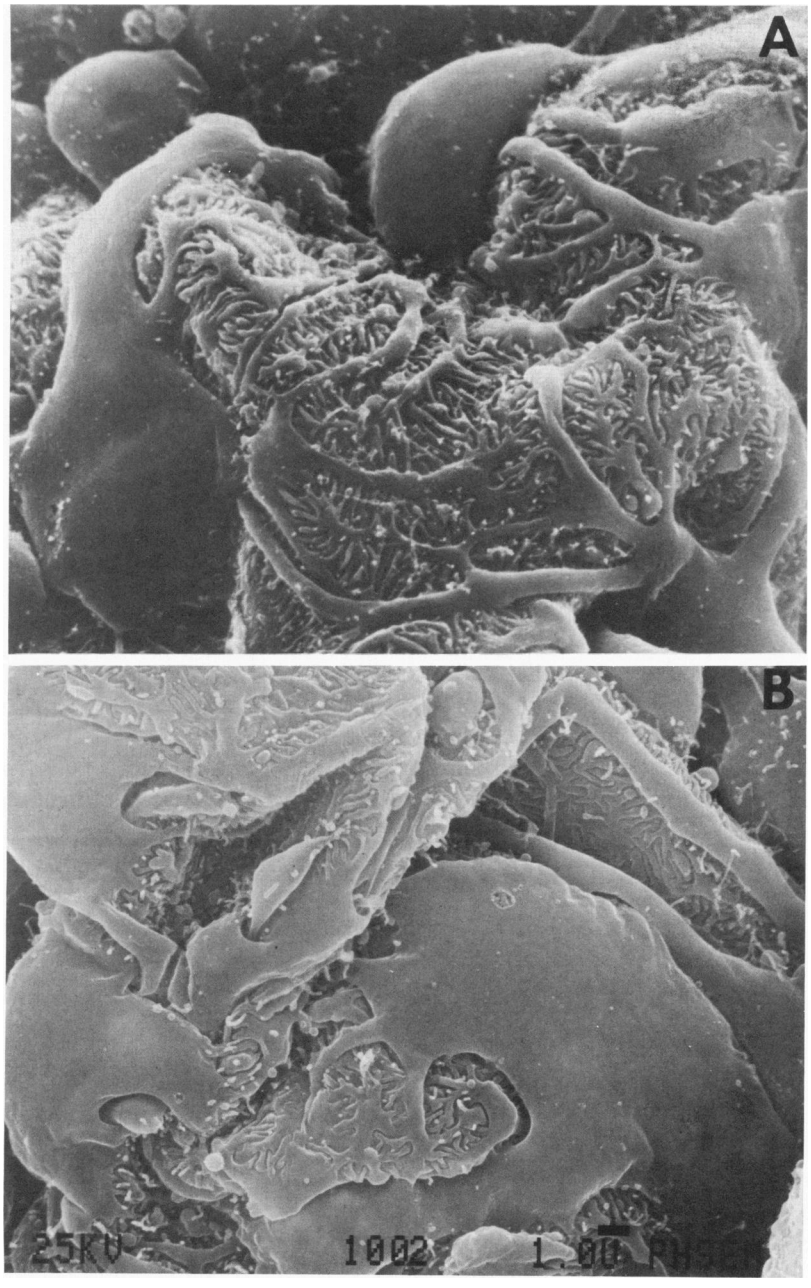

Figure 3A—Normal human glomerular capillary loops from a pretransplantation biopsy specimen from a cadaveric donor. Podocyte cell bodies are nearly hemispherical or octopuslike in appearance; more than half of the capillary surface is covered by processes less than 1 μ in width. (×4000) **B**—Scanning electron micrograph of a glomerulus from a human transplant kidney in Group E. Podocyte changes similar to those in Figure 2 are seen. Defects in some of the podocyte major processes allow underlying relatively normal foot processes to be observed. Elsewhere they are hidden by the overlying broad podocyte major processes greater than 1 μ in width. (×4000, bar = 1 μ)

Discussion

In this report we describe an alteration in glomerular epithelial cells that is found very early following an ischemic insult in both man and experimental animals and appears to provide prognostic information relating to subsequent renal function. These changes cannot be properly appreciated with the use of transmission electron microscopy, because of the complex three-dimensional shape of the podocytes, and there is no change observable by light microscopy that has similar predictive value. The investigations reported in this paper support the concept that the eventual decrease in glomerular filtration that follows an ischemic insult is related to morphologic changes in the glomerular podocytes that occur soon after the insult.

In 1974 Cox and associates[12] reported finding abnormalities in glomerular podocyte structure by scanning electron microscopy in unilateral acute renal failure produced by infusing norepinephrine into one renal artery of dogs for 2 hours. The authors suggested that renal failure in this model was largely the result of decreased glomerular capillary permeability, and that the absence of the renal podocyte branching and foot

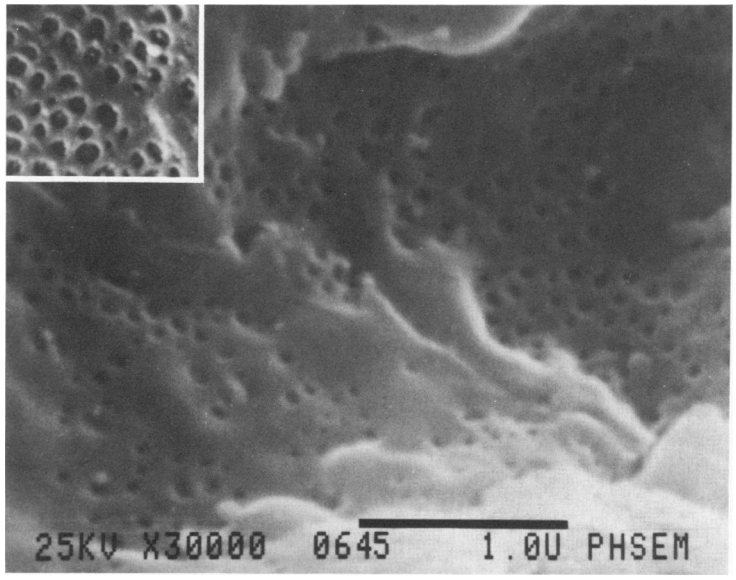

Figure 4—Reduction in number and irregularity in size of glomerular capillary endothelial fenestrae from Case 14, Group E. **Inset** shows larger and more numerous fenestrae from a rabbit in Group B. (Both reduced equally from ×30,000)

process formation which they observed represented the morphologic counterpart of this decrease in permeability. This original 2-hour norepinephrine infusion model was irreversible and characterized by cortical infarcts. Subsequently, Cronin et al[13] used a shorter 40-minute infusion of norepinephrine to produce a reversible model and did not find podocyte abnormalities. Other investigators found evidence for decreased ultrafiltration coefficient (K_f) in uranyl nitrate and gentamicin-induced acute renal failure.[14,15] The decrease in the ultrafiltration coefficient brought about by these two compounds is allegedly associated with predominantly endothelial, rather than epithelial, (podocyte) changes,[9,16] although in one recent study neither endothelial nor epithelial changes were found.[17] Glomerular changes in the pedicle-clamping model of acute renal failure observed by scanning electron microscopy have not been reported, although recent studies by Savin et al suggest that the ultrafiltration coefficient is also reduced in this type of postischemic acute renal failure.[18]

It is possible that the podocyte changes observed in this study represent the morphologic counterpart of decreased glomerular permeability. However, temporal considerations suggest another possibility involving proteinuria and tubular obstruction. In previous studies we have shown that statistically significant differences in creatinine clearance and number of tubular casts between clonidine-treated and untreated rabbits similar to those in Groups C and B do not appear until 4–6 hours after the ischemic episode.[5,6] (From this time onward the clonidine-treated group has significantly better renal function.) Thus, the significant difference in severity of podocyte alterations at 2 hours after the ischemic episode in the present study suggests that podocyte alterations precede, rather than coincide with, the eventual significant differences in renal function and number of cast-filled tubules in the two groups. Thus, the link between podocyte changes and decreased glomerular filtration rate is likely to be indirect. The podocyte changes may reflect increased glomerular permeability to large molecules, which could account in part for the proteinuria that is present in early postischemic acute renal failure.[19,20] Ryan and Karnovsky[21] have shown that renal ischemia leads to at least a transient increase in glomerular permeability to proteins, and proteinuria up to 200 mg/dl was observed in some of the rabbits in Group B. If the concentration of the serum proteins in the tubular fluid were elevated, this would increase the propensity for Tamm-Horsfall protein in the tubular fluid to aggregate and form hyaline casts.[22] Hoyer and Seiler have pointed out that under most circumstances the conditions required for hyaline cast formation are probably present only transiently in a small number of tubules, and once casts form they are passed into the urine without difficulty.[22] However, in postischemic acute renal failure there is sluggish flow of tubular fluid during and after the ischemic insult. This by itself would predispose to cast formation and in combination with elevated concentrations of serum proteins, sodium, and chloride in the tubular fluid, could lead to the formation of very long obstructive hyaline casts, which have been shown to be an important feature of postischemic acute renal failure.[5]

Support for this theory would have to come from differential protein clearance determinations to estab-

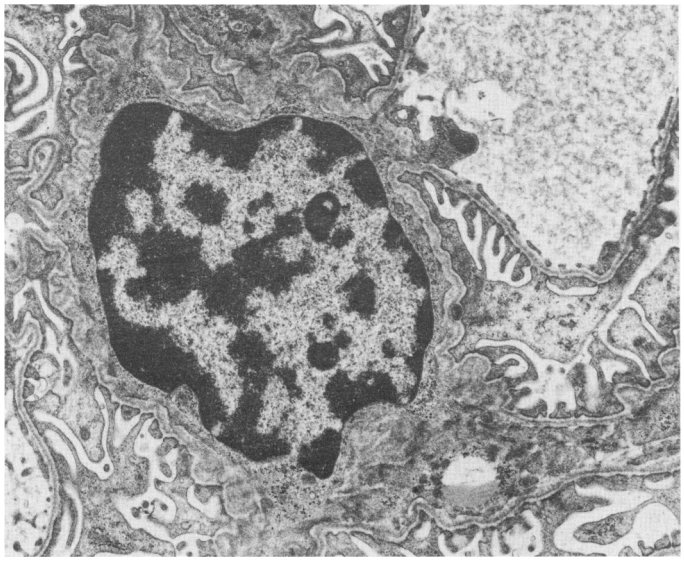

Figure 5—Transmission electron micrograph of mesangial area from a rabbit in Group B showing wrinkling of the basement membrane along the mesangial reflections, an indicator of mesangial cell contraction. There is only a minor degree of foot process fusion in adjacent capillary loops. (×9600)

lish that the proteinuria was in part of glomerular origin and not due entirely to decreased tubular reabsorption of protein or to protein discharged into the tubule at a postglomerular site.[23,24] Unfortunately, such investigations were not carried out in the present study. The interpretation of protein clearance data might be difficult, since many of the most "proteinuric" nephrons would become blocked by casts and thus not contribute fluid to the urine samples used to calculate clearances. However, it is also possible that the remaining unobstructed nephrons would also develop defects in glomerular permeability to protein akin to those observed in models of reduced renal mass.[25,26]

In most proteinuric states 50–98% of the epithelial cell foot processes are fused.[27] Fusion appears to relate to loss of the normal negative charge of the capillary loop basement membrane and in the experimental animal can occur within 10 minutes after infusion of polycations such as protamine.[28] The epithelial cell alterations in postischemic acute renal failure appear to be less severe than those in most proteinuric states. There is less foot process fusion,[29] and the major alteration in podocyte structure appears to be a flattening of cell bodies and expansion of major processes, so that they almost completely envelope the capillary loops. It is possible that this enveloping of the glomerular capillaries produces a mechanical interference with filtration.

The changes in slit diaphragm between foot processes that we observed in postischemic acute renal failure appear identical to those described in aminonucleoside nephrosis in the rat,[30] a condition characterized by proteinuria, oliguria, and azotemia.[31,32] It remains to be proven that these slit diaphragm abnormalities occur in proteinuric states unattended by a decrease in glomerular filtration rate. The fact that hypoproteinemia reduces K_f[33] and that experimental models of proteinuria are associated with reduced K_f[34,35] increases the difficulty of separating the glomerular ultrastructural changes associated with proteinuria from those associated with reduced K_f.

Regardless of whether the podocyte changes observed are a manifestation of decreased hydraulic permeability or increased permeability to proteins, the predictive value of these changes is likely to be clinically useful. In this and previous studies neither urine flow in the immediate posttransplantation period nor recorded warm or cold ischemia times allowed prediction of renal function three days later. Currently, it is often difficult to decide whether or not to subject patients with early posttransplant renal failure to the hazards of antirejection therapy.[36] If, as the present study suggests, the severity of podocyte changes is a predictor of the severity of postischemic acute renal failure, treatment for rejection could be considered in those patients with posttransplantation renal failure who have few podocyte changes in their 1-hour biopsy.

Some kidneys sustain such severe ischemic changes that they never function in the recipient. In England, where "beating heart" donors are not used, 15–30% of cadaver kidneys fall into this category.[37] The evaluation of podocyte changes in pretransplantation biopsy specimens of donor kidneys may help to identify such kidneys so that one may avoid transplanting them. However, an undetected proteinuric state in the donor or the administration of protamine[28] (used to reverse

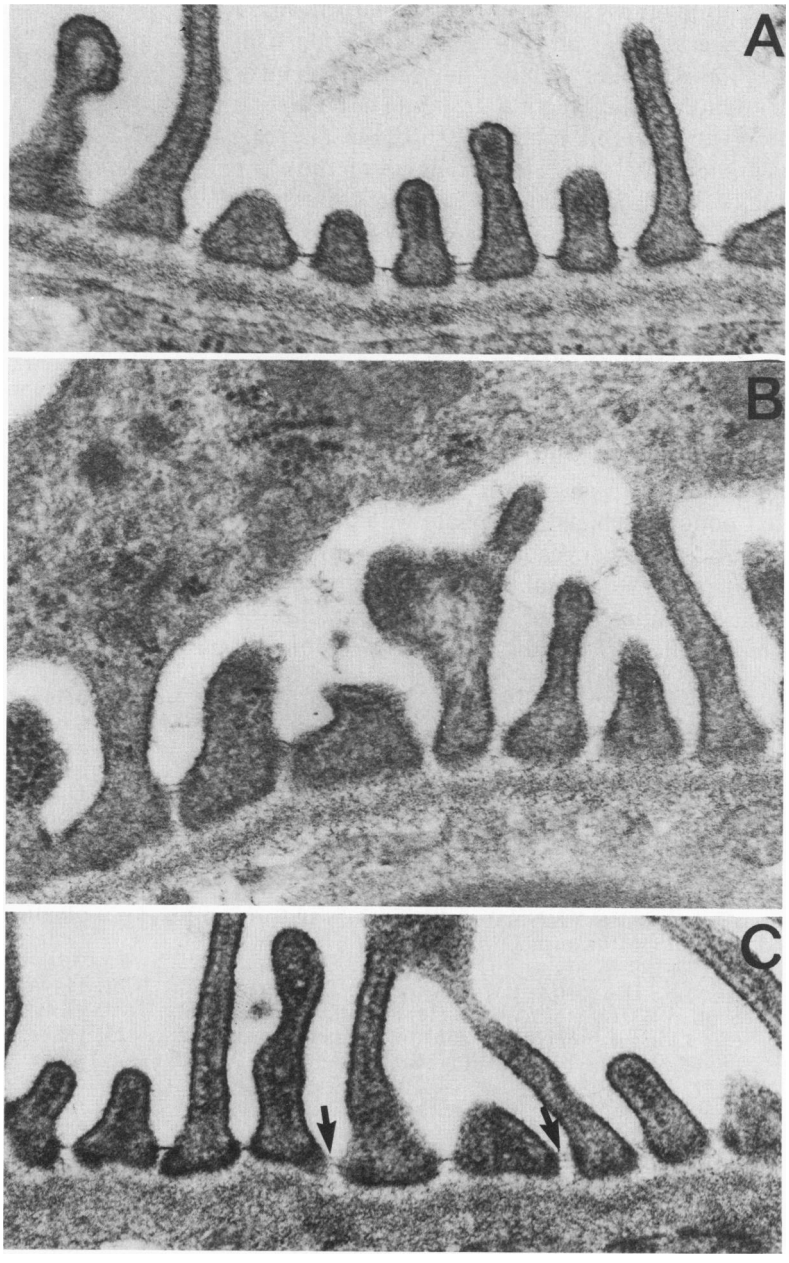

Figure 6A—Normal slit diaphragms between foot processes in a rabbit from Group A2. (×60,000) **B**—Retraction, reduplication, and loss of slit diaphragms in a rabbit from Group B. The foot processes are covered by an overlying major podocyte process. (×60,000) **C**—Reduplication of slit diaphragms (*arrows*) in a rabbit from Group C. This was the only abnormality in slit diaphragms observed in this group. (×60,000)

heparinization) may also produce podocyte changes similar to those produced by ischemia and would not preclude successful transplantation. Delayed fixation also brings about a number of confusing morphologic changes in the glomeruli. This is the reason why postmortem material is unsatisfactory for assessment of glomeruli by scanning electron microscopy.[38]

The endothelial cell changes in Cases 6, 11, and 14 (Figure 6) suggest the possibility that in man both epithelial and endothelial alterations may play a role in posttransplantation ischemic acute renal failure. This idea is not entirely new. Ten years ago, Weymouth et al[39] noted severe epithelial and endothelial changes by transmission electron microscopy in the 1-hour posttransplantation biopsy specimen from a patient who developed reversible ischemic posttransplantation renal failure. Unlike the podocyte changes, which we have found persist for at least 6 hours (Solez, unpublished), glomerular endothelial changes are short-lived in experimental postischemic acute renal failure,[40] and this probably accounts for our inability to demonstrate endothelial alterations in rabbit kidneys examined 2 hours after the ischemic insult. If the endothelial changes we observed in 1-hour posttrans-

plantation biopsy specimens are similarly transient, or if they are largely an artifact of immersion-fixation, then these changes probably have little or no influence on eventual renal function. It should be noted that our observations on endothelial changes must be regarded as preliminary, since they are based on the examination of only 1–2 specimens per group, rather than 2–6 specimens per group, as in the study of Avasthi et al.[9]

Two important questions arise from our current study: 1) What is the cause of the podocyte alterations observed in postischemic acute renal failure? Angiotensin II produces similar morphologic changes[41,42] and is known to reduce the ultrafiltration coefficient[43] and cause proteinuria.[44] However, other possible mediators such as vasopressin,[6] thromboxane,[45] or calcium[46] may also play a role. 2) Can one demonstrate that podocyte changes correlate with and precede or coincide with changes in *a*) ultrafiltration coefficient or *b*) protein permeability in the same glomeruli? Such a demonstration, using micropuncture or isolated perfused glomeruli, is necessary before one can conclude that podocyte changes are directly related to altered glomerular capillary permeability. These important issues merit further study.

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