Glomerular Epithelial Abnormalities Associated With the Onset of Proteinuria in Aminonucleoside Nephrosis

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A sequential ultrastructural study has been made of glomerular podocytic epithelium before and after the onset of proteinuria induced by daily subcutaneous injections of low doses of puromycin aminonucleoside (PAN). At 4 days, before the onset of proteinuria, the principal change was extensive replacement of podocytic foot processes by broad expanses of epithelial cytoplasm. At 5 days, when proteinuria had developed, the epithelial cells showed in addition multiple cytoplasmic droplets, many large balloonlike vacuoles, some of which were ruptured, and many foci of epithelial detachment from the glomerular basement membrane (GBM). Of particular significance, the onset of proteinuria coincided precisely with the development of areas of epithelial detachment that led to direct continuity between externally denuded GBM and the urinary space. It seems likely that these areas are the primary sites of protein leakage across the GBM in this experimental model. (Am J Pathol 1987, 126:220–229)

THERE IS continuing controversy over the pathogenesis of proteinuria in human glomerular disease, particularly in minimal change disease (lipoid nephrosis). The nephrosis induced in rats by the injection of puromycin aminonucleoside (PAN) has many features in common with minimal change disease, and has been used extensively in the study of the fundamental processes causing proteinuria in this condition. In PAN nephrosis, Farquhar and Palade[1] originally proposed that the primary lesion was a generalized increase in leakiness of the glomerular basement membrane (GBM) because intravenously administered ferritin particles were observed within the GBM in PAN nephrosis but not in control animals.[1] However, the major morphologic change in PAN nephrosis (as in minimal change disease) is the replacement of discrete glomerular podocytic foot processes by continuous expanses of flattened epithelial cytoplasm.[1–5] Venkatachalam and his colleagues[2,3] noted the penetration of intravenously injected horseradish peroxidase or catalase into basal pockets of epithelial cytoplasm overlying the GBM and into cytoplasmic vacuoles within the abnormal glomerular epithelial cells and therefore suggested that plasma proteins which penetrated the abnormally leaky GBM were then transferred across the epithelial layer to the urinary space through a system of cytoplasmic vacuoles.

Ryan and Karnovsky[4] subsequently described areas of focal loss of the epithelial covering on the outside of the GBM, the development of which coincided with the onset of proteinuria in PAN nephrosis. At such sites the GBM was directly exposed to the urinary space; and because intravenously injected ferritin was found to penetrate deeply into the GBM only in segments of the GBM that lacked an epithelial covering, it was proposed that proteinuria in PAN nephrosis is primarily due to a glomerular epithelial lesion that results in plasma protein leakage across the GBM at these sites.[4] Ryan et al[6] later confirmed that endogenous albumin and immunoglobulin G tra-

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versed the GBM in PAN nephrosis only in areas showing epithelial detachment. Because the epithelial layer appears likely to be the principal structure in controlling the overall hydraulic conductivity across the glomerular capillary wall,[7] it was proposed that the leakage of plasma proteins across the GBM resulted from focally increased bulk flow across the capillary wall at these sites where the epithelial covering had been lost.[4] Kanwar and Rosenzweig[8] later confirmed that in PAN nephrosis, ferritin leaked across the glomerular capillary wall to the urinary space only through segments of GBM in areas where the epithelium was detached. In the meantime, there had been increasing interest in the role of intrinsic negative charge in the glomerular capillary wall, first, in controlling normal glomerular permselectivity, and second, in the extent to which loss of such negative charge could be responsible for proteinuria (see reviews[9,10]). But from their analysis of the available data, Kanwar and Rosenzweig[8] concluded that the increased permeability of the glomerular capillary wall (GCW) in PAN nephrosis was "due to the epithelial detachment process itself and not to changes in either the composition of the GBM or other components of the GCW." Further, they agreed that "epithelial detachment could lead to major glomerular hemodynamic changes that effectively override the barrier properties of the GBM, leading to enhanced bulk flow of plasma macromolecules from capillary lumens through the GCW into the urinary spaces."

Caulfield et al[5] had earlier reported these focal areas of epithelial detachment in PAN nephrosis but, in contrast to the studies described above, said that such lesions occurred only late in the disease, several days after the onset of proteinuria. These investigators questioned the proposal by Venkatachalam et al[2,3] of a vacuolar pathway across the epithelial layer, stating that "pockets (epithelial detachment) are not seen early in the disease at the time of onset of proteinuria."

It is important to note that different groups have used different experimental protocols to induce PAN nephrosis in rats. Daily subcutaneous injections of 1.67 mg/100 g body weight PAN were administered in the studies of Farquhar and Palade,[1] Venkatachalam et al,[2,3] and Caulfield et al[5]; with this dosage, proteinuria usually occurred at 5–8 days.[5] In contrast, a single larger dose (15 mg/100 g body weight) of PAN was administered intravenously in the studies of Ryan et al[4,6] and Kanwar and Rosenzweig[8]; after this, proteinuria usually occurred at 4–5 days.[4] It could be argued that, because it may be associated with a greater delay in the onset of proteinuria, the daily low-dose protocol is the more suitable experimental model because there may be less telescoping of events into a shorter time frame when compared with the single higher dose model. Thus, if the morphologic changes leading to proteinuria develop more gradually, the low-dose model could allow for easier recognition of any separation of the onset of epithelial detachment from the time of onset of proteinuria. Accordingly, the detection of epithelial detachment coinciding with the time of onset of proteinuria following the daily low-dose protocol would be of considerable value in determining its significance in the pathogenesis of proteinuria. This issue has been examined critically in the present detailed sequential study of glomerular ultrastructure in the daily low-dose model of PAN nephrosis. The results demonstrate how the outer surface of the GBM comes to be denuded of epithelium and exposed to the urinary space and document the temporal relationship of these structural changes to the development of proteinuria.

Materials and Methods

Experimental Protocol

The first experimental group comprised 17 female Sprague–Dawley rats, weighing 120–170 g; these were placed in metabolic cages for acclimatization for 2 days before commencing experiments with unrestricted access to standard food and water. Twenty-four-hour urine samples were collected throughout, and urinary protein excretion was measured daily by the sulfosalicylic acid method.[11] Baseline urinary protein excretion values were determined, and then 15 rats were given subcutaneous injections daily of puromycin aminonucleoside (PAN) (6-dimethylamino-9[3′-amino-3′-deoxyribosyl]purine) (Sigma Chemical Company, St. Louis, Mo), 1.67 mg/100 g body weight, as a 0.5% solution in sterile saline, as described by Farquhar and her colleagues.[1,5] The remaining 2 animals were given saline only. In preliminary studies using this protocol, it was found that in most animals proteinuria developed on Day 5. Therefore, the PAN-injected rats were killed as follows: 4 on Day 4; 6 on Day 5; 2 on Day 7; 2 on Day 10; 1 on Day 12, this last animal having received no injections after Day 10. The 2 control animals were killed on Day 6. At the time of killing, each animal was anesthetized with intraperitoneal sodium pentobarbital (Nembutal, Abbott Laboratories, Sydney). The abdomen was then opened by a ventral midline incision, and renal perfusion fixation was performed by the protocol (Method 1) and apparatus of Griffith Bulger and Trump.[12] The kidneys were flushed briefly with

Hanks' balanced salt solution, pH 7.3, at room temperature, followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer, usually at a pressure of 180 mm Hg for 10 minutes, but in 2 animals (fixed on Day 5) a pressure of only 120 mm Hg was used. Cortical tissue was cut into 1 × 1 × 3 mm strips and fixed for an additional 3 hours in the same glutaraldehyde fixative at room temperature. After washing overnight at 4 C in phosphate buffer containing 0.1 M sucrose, tissue was postfixed in 2% aqueous osmium tetroxide for 1 hour at 4 C and dehydrated before processing for transmission and scanning electron microscopy.

A second experimental group comprised 4 female Munich-Wistar rats, weighing 150-270 g. These were chosen because they have a number of glomeruli at or close to the renal surface; such glomeruli are amenable to rapid fixation *in situ* during good blood flow by dripping fixative onto the renal surface in anesthetized animals.[4,13] This technique obviates the need for perfusion fixation while avoiding the glomerular capillary collapse that occurs during routine immersion fixation. After acclimitization in metabolic cages, 2 rats were given daily injections of PAN, as above, and 2 were given saline only. At the time of onset of proteinuria (Day 5) in the experimental rats, all animals in the group were anesthetized; abdomen was opened, and the capsule was gently stripped from the central surface of the left kidney. Glutaraldehyde fixative (2.5%) at 37 C was dripped continuously on to the surface of the kidney for 30 minutes; the animals showed no ill effects and maintained normal respiration throughout the fixation period. The superficial rim of fixed cortical tissue, approximately 1 mm deep, was then cut into elongated strips to facilitate orientation of the blocks and identification of the renal surface. The tissue was then processed for electron microscopy as described above.

Morphologic Techniques

For transmission electron microscopy, fixed tissue strips were dehydrated through graded acetone and embedded in Araldite-Epon. Thick sections (0.5-1.0 μ) were cut with glass knives and stained with 1.0% methylene blue for light microscopy. Thin sections were cut with a diamond knife, stained with uranyl acetate and alkaline lead citrate, and examined in a Siemens Elmiskop 102. Transmission electron microscopy was performed on multiple glomeruli in each animal in each group. Particular attention was directed toward the following groups of animals: 1) the four PAN-treated Sprague-Dawley rats killed on Day 4, in which approximately 500 sections through a total of 26 glomeruli were examined, and a total of 2 glomeruli were serially sectioned (50 serial sections in each glomerulus were examined); 2) the 6 PAN-treated Sprague-Dawley rats killed on Day 5, in which approximately 500 sections through a total of 68 glomeruli were examined, and a total of 8 glomeruli were serially sectioned (30-100 serial sections in each glomerulus were examined); and 3) the 2 PAN-treated Munich-Wistar rats killed on Day 5, in which a total of 7 superficial glomeruli were serially sectioned (50-200 serial sections in each glomerulus were examined).

For scanning electron microscopy, fixed tissue strips were dehydrated through graded ethanediol, followed by Cellosolve, and critical-point-dried with liquid carbon dioxide as the transitional medium. The strips were mounted on stubs, sputter-coated with gold, and examined in a Siemens Autoscan. Scanning electron microscopy was performed on multiple glomeruli in each animal in each group. Particular attention was again directed toward the above three major groups of PAN-treated animals, with a total of 30 glomeruli being examined in the Sprague-Dawley rats killed on Day 4, 30 glomeruli in the Sprague-Dawley rats killed on Day 5, and 6 superficial glomeruli in the Munich-Wistar rats killed on Day 5.

Results

Proteinuria

Baseline urinary protein excretion in Sprague-Dawley rats before the administration of PAN never exceeded 1 mg/24 hr and remained at this low rate in the 2 control rats until they were killed at 6 days. After commencing the daily injections of PAN in the 15 experimental rats, increased urinary protein excretion was first detected on Day 4 in 2 rats: the values in these animals were 3.3 and 2.7 mg/24 hr, each having increased more than fourfold from values on Day 3 of 0.8 and 0.6 mg/24 hr, respectively. The 4 experimental rats killed on Day 4 had urinary protein excretion rates ranging from 0.3 to 0.9 mg/24 hr, all within the normal range. All other experimental animals had increased urinary protein excretion rates on Day 5, with values ranging from 2.7 to 18.5 mg/24 hr. The 6 experimental rats killed on Day 5 had urinary protein excretion rates ranging from 2.7 to 7.2 mg/24 hr, including values of 5.2 and 4.0 mg/24 hr, respectively, for the 2 animals in which increased protein excretion had developed on Day 4. The 5 experimental rats remaining on Day 7 had urinary protein excretion rates ranging from 17 to 112 mg/24 hr; the 2 rats killed on Day 7 had values of 30 and 54 mg/24 hours. The 3

remaining experimental rats on Day 10 had urinary protein excretion rates ranging from 95 to 125 mg/24 hours; the 2 rats killed on Day 10 had values of 105 and 125 mg/24 hours. The last remaining experimental rat killed on Day 12 had a urinary protein excretion of 200 mg/24 hr.

Baseline urinary protein excretion in Munich-Wistar rats before the administration of PAN was slightly higher than in Sprague-Dawley rats but did not exceed 2 mg/24 hr. The values in the 2 control animals remained at these levels, being 0.5 and 0.7 mg/24 hr when they were killed on Day 5. The rats injected with PAN showed increased urinary protein excretion on Day 5, when they were killed; the values in these animals were 16 and 17 mg/24 hr, each having increased eightfold from 2 mg/24 hr on Day 4.

Light Microscopy

The glomeruli of the saline-injected Sprague-Dawley and Munich-Wistar control rats appeared normal.

In the glomeruli of PAN-treated Sprague-Dawley rats at 4 days, before the onset of proteinuria, the only detectable changes were a few veils of cytoplasm ballooning out from visceral epithelial cells and the presence of an occasional protein droplet in the epithelial cells of fewer than 10% of glomeruli.

At the time of onset of proteinuria at 5 days, in both the Sprague-Dawley and the Munich-Wistar groups of rats, balloonlike cytoplasmic veils were much more prominent, but a particularly obvious change by light microscopy was the presence of a varying number of protein droplets in more than 80% of glomeruli. By Day 7, all glomeruli showed multiple protein droplets, but this number decreased to 50% by Day 10 and to 20% by Day 12.

Transmission Electron Microscopy

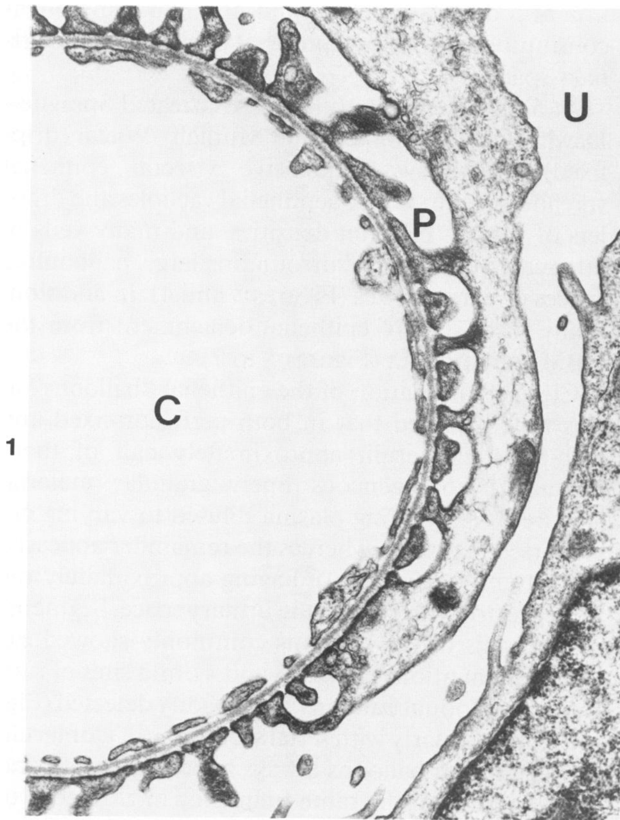
The glomeruli of the saline-treated Sprague-Dawley and Munich-Wistar control rats appeared normal (Figure 1).

The glomeruli of PAN-treated Sprague-Dawley rats at 4 days showed extensive replacement of podocytic foot processes by broad expanses of epithelial cytoplasm (Figure 2). The epithelium also showed a small number of cytoplasmic vacuoles (Figure 2) and, rarely, larger balloonlike spaces. These vacuoles and balloonlike spaces contained homogeneous finely granular material; no sites of rupture to the urinary space were detected. Rarely, small foci of epithelial detachment from the GBM were detected, but at this time not to the extent seen after the onset of proteinuria at 5 days (see below) and never showing direct continuity between subepithelial pockets and the urinary space.

At 5 days the glomeruli of PAN-treated Sprague-Dawley (perfusion-fixed) and Munich-Wistar (drip-fixed) rats showed extensive visceral epithelial spreading, multiple intraepithelial vacuoles and droplets of various electron densities, and many veils of attenuated epithelium surrounding large, balloonlike spaces of various sizes (Figures 3 and 4). In addition, many focal sites of epithelial detachment from the GBM were present (Figures 5 to 7).

Closer examination of the epithelial "balloons" at this time revealed that in both perfusion-fixed and drip-fixed glomeruli, approximately half of them contained homogeneous finely granular material (Figure 3) resembling plasma diluted to varying degrees (see Figure 3), whereas the remainder appeared almost empty (Figure 4), having approximately the same electron-lucency as the urinary space. Segments of the walls of the balloons commonly showed extreme attenuation (Figures 3 and 4), and sites of rupture of individual balloons were readily detected (Figure 8), particularly with serial sectioning of glomeruli. Such ruptured balloons always appeared empty and were found with the same frequency in glomeruli of Sprague-Dawley kidneys, perfusion-fixed at either 120 or 180 mm Hg, and in Munich-Wistar drip-fixed kidneys. Similarly, multiple sites of epithelial detachment from the GBM were present in all PAN-treated rats at 5 days, regardless of the method of fixation. These sites of detachment, which produced segments of externally denuded GBM, resulted from epithelial retraction from areas between adjacent capillary loops or paramesangial regions (Figure 5), from the formation of subepithelial pockets due to epithelial lifting from the GBM (Figure 6), or from epithelial retraction leading to complete gaps in the epithelial covering of the capillary, which exposed the outer surface of the GBM directly to the urinary space (Figure 7). Serial sectioning consistently demonstrated that epithelial balloons were situated over discrete gaps in the epithelial covering of the GBM (Figure 8), and, in some sections, the gap at the base of an epithelial balloon was present in the same plane as its site of rupture at its apex (Figure 8), so that continuity between the denuded GBM and the urinary space via the lumen of the balloon was clearly evident.

A comparison was made between the total number of sites of epithelial detachment detected in the random sections of glomeruli in the PAN-injected Sprague-Dawley rats killed at Day 4, before proteinuria, and at Day 5, on the day of onset of proteinuria. In the 4 rats killed at 4 days, only 20 separate sites of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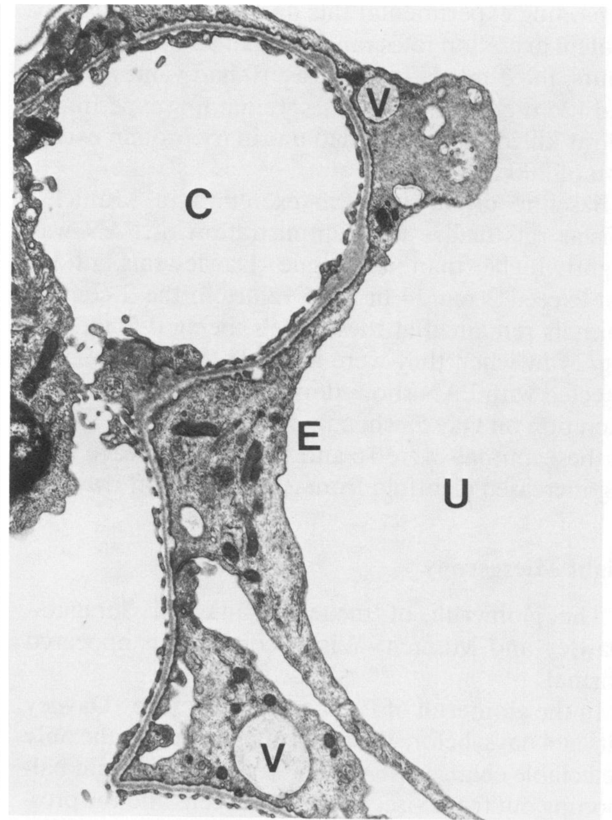
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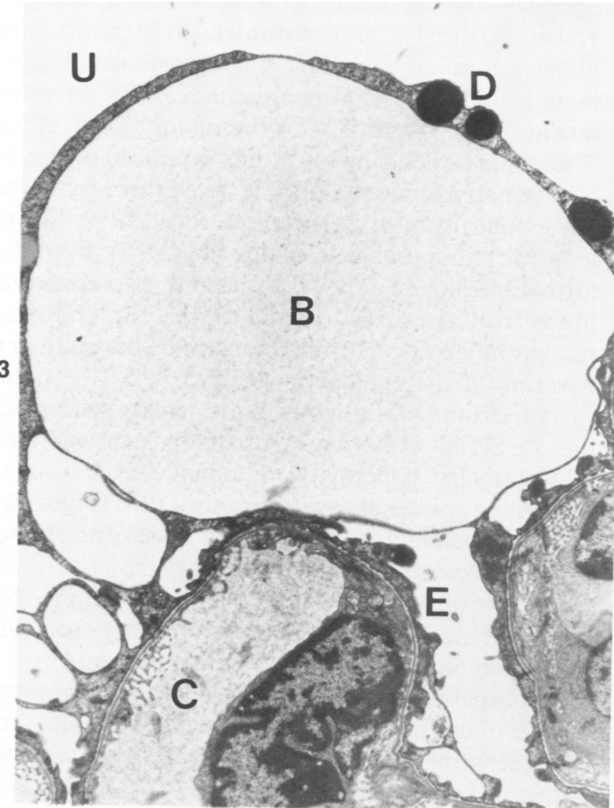
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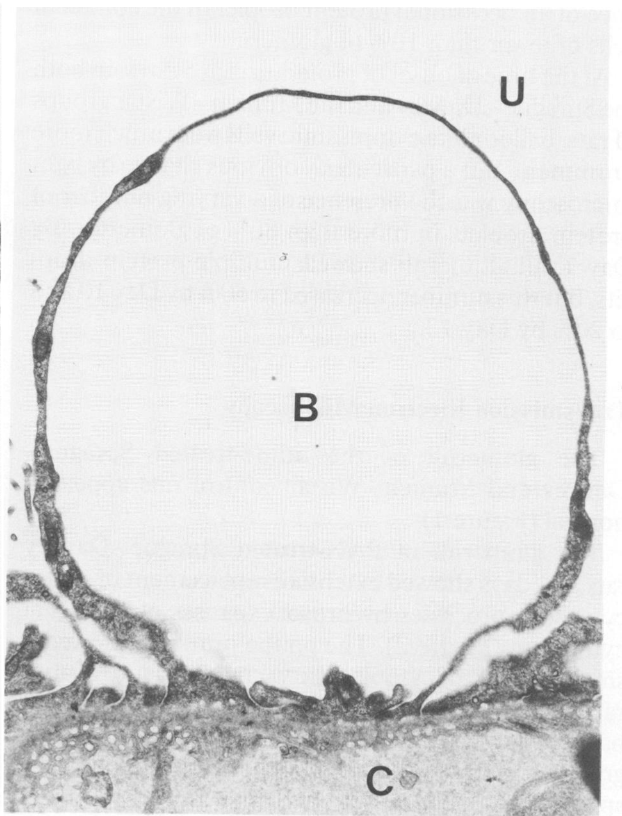
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epithelial detachment were detected in the total of approximately 500 sections of glomeruli that were examined at this stage. In the 6 rats killed on Day 5, 145 separate sites of epithelial detachment were detected in the total of approximately 500 sections of glomeruli that were examined at this stage. Statistical analysis of these data confirmed that the difference between the number of sites of externally bare GBM at 4 and at 5 days is highly significant (chi-square test, $P < 0.001$). Furthermore, as well as being considerably more difficult to detect, such sites of epithelial detachment as were found at 4 days were smaller versions of the types shown in Figures 5 and 6, ie, without any evident communication between externally denuded areas of GBM and the urinary space, in contrast to those illustrated in Figures 7 and 8.

Glomeruli of PAN-treated Sprague-Dawley rats killed at 7, 10, and 12 days were similar to those at 5 days, but visceral epithelial spreading was more extensive, and areas of epithelial retraction and GBM denudation were larger and more obvious. At 10 and 12 days, the balloonlike veils of epithelium were less apparent. Electron microscopy confirmed that vacuoles and droplets of various electron densities were prominent in glomerular epithelial cells at 7 days but were less conspicuous later, particularly at 12 days.

Scanning Electron Microscopy

The glomeruli of the saline-treated Sprague-Dawley and Munich-Wistar control rats appeared normal (Figure 9).

At 4 days, there was extensive epithelial spreading and effacement of foot processes in the glomeruli of PAN-treated Sprague-Dawley rats (Figure 10). Occasionally, there were also areas of bulging of the surface of the epithelium, presumably representing the epithelial balloons observed rarely by transmission electron microscopy at this stage. These bulges showed a relatively smooth outer surface and seldom showed any signs of perforation.

At 5 days, the glomeruli of PAN-treated Sprague-Dawley (perfusion-fixed) and Munich-Wistar (drip-fixed) rats showed almost complete effacement of epithelial foot processes by broad expanses of epithelial cytoplasm and the presence of multiple smooth-surfaced balloonlike bulges, many of which showed perforations of various sizes (Figure 11). Such perforations were irregular in shape and often very large (Figure 11). The appearance of glomeruli at 7 days was similar; but, at 10 and 12 days, balloonlike bulges were less apparent.

Discussion

The results of this investigation confirm and extend observations in previous studies that in PAN nephrosis in rats the onset of proteinuria coincides with the development of focal defects in podocytic epithelium which expose the outer surface of the GBM to the urinary space.[4,6] The probable sequence of events is that PAN causes injury to glomerular podocytic epithelial cells, which leads to a decrease in cell surface area and results in replacement of complex interdigitating foot processes by flattened continuous expanses of epithelial cytoplasm. For the most part, this architectural change follows a well-coordinated sequence, with adjacent podocytic cells forming junctional complexes with each other[14] before the slit diaphragm becomes detached and coiled up[15] after progressive reduction in the length of the intercellular slit pore as the foot processes retract. At some sites, however, foot process retraction occurs before the formation of firm junctional complexes with neighboring epithelial cells, leading to cellular separation and the formation of externally bare areas of GBM. Because the epithelial layer may be the major hydraulic conductivity barrier in the glomerular capillary wall,[7] focally increased water flux across these sites of denuded GBM could then produce proteinuria by increased bulk flow of plasma proteins to the urinary space through the defects.[4,6,8] In the present study, although epithelial spreading was well advanced by 4 days, there was no proteinuria at this time, sites of epithelial detachment were small and infrequent, and direct continuity between the bare GBM and the urinary space could not be detected. In contrast, with the onset of proteinuria at 5 days, sites of epithelial detachment were large, considerably more frequent, and, by the use of multiple random sections or serial sectioning techniques, could be shown to be in continuity with the urinary space,

Figure 1—Transmission electron micrograph (TEM) of a portion of glomerular capillary loop in a saline-treated control Sprague-Dawley rat. The glomerular capillary wall, consisting of fenestrated endothelium, basement membrane, and epithelial foot processes (*P*) appears normal. *C*, capillary lumen; *U*, urinary space. (×20,000) **Figure 2**—TEM of a glomerular capillary loop in a Sprague-Dawley rat 4 days after commencing PAN injections and prior to the onset of proteinuria. Note extensive replacement of foot processes by broad expanses of epithelium (*E*). The epithelium contains cytoplasmic vacuoles (*V*). *C*, capillary lumen; *U*, urinary space. (×10,000) **Figure 3**—TEM of a glomerular capillary loop in a Munich-Wistar rat on the day of onset of proteinuria (5 days after commencing PAN injections). There is extensive spreading of visceral epithelium (*E*). Note the intraepithelial droplets (*D*) and veil of attenuated epithelium surrounding the balloonlike space (*B*). The "balloon" contains homogeneous finely granular material resembling plasma present in capillary lumen (*C*). *U*, urinary space. (×4500) **Figure 4**—TEM of the glomerular capillary wall in a Munich-Wistar rat on the day of onset of proteinuria. The large balloonlike space (*B*) surrounded by a veil of attenuated epithelium appears empty. *C*, capillary lumen; *U*, urinary space. (×8000)

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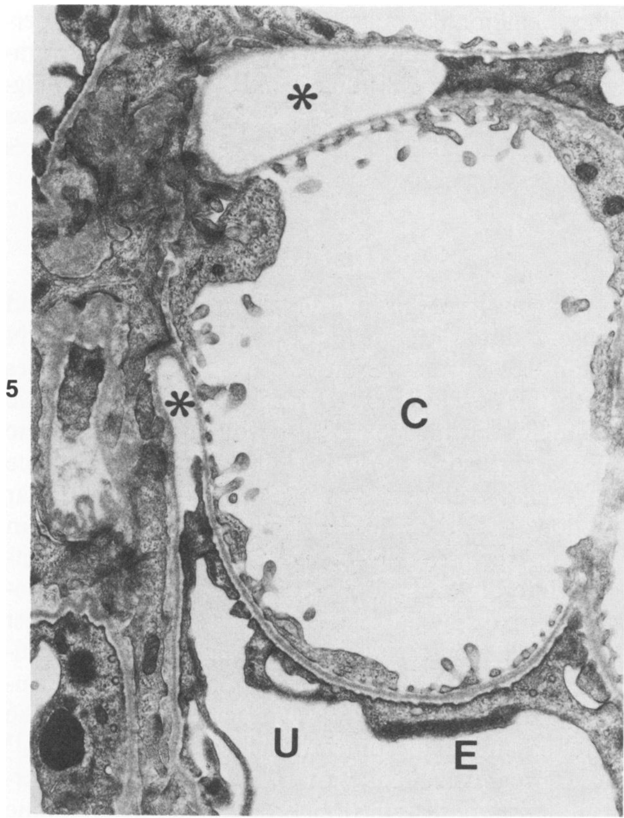

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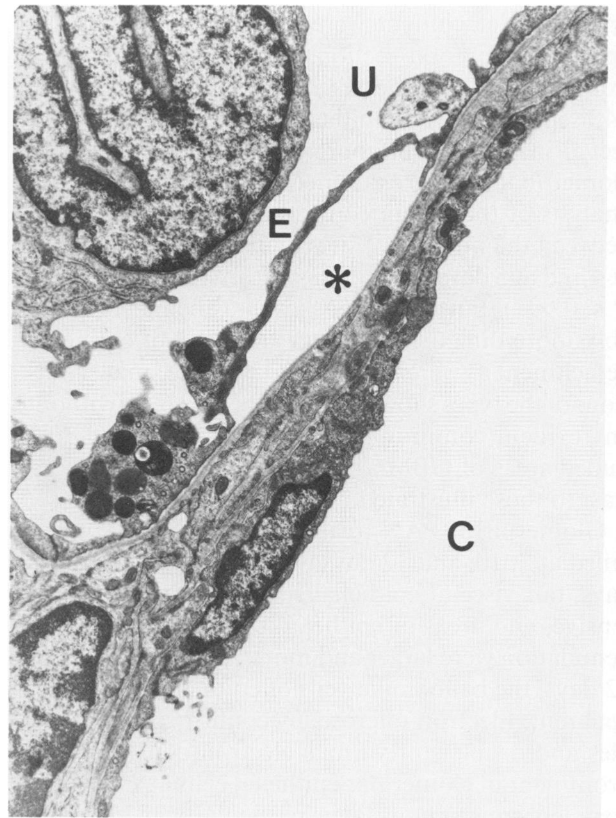

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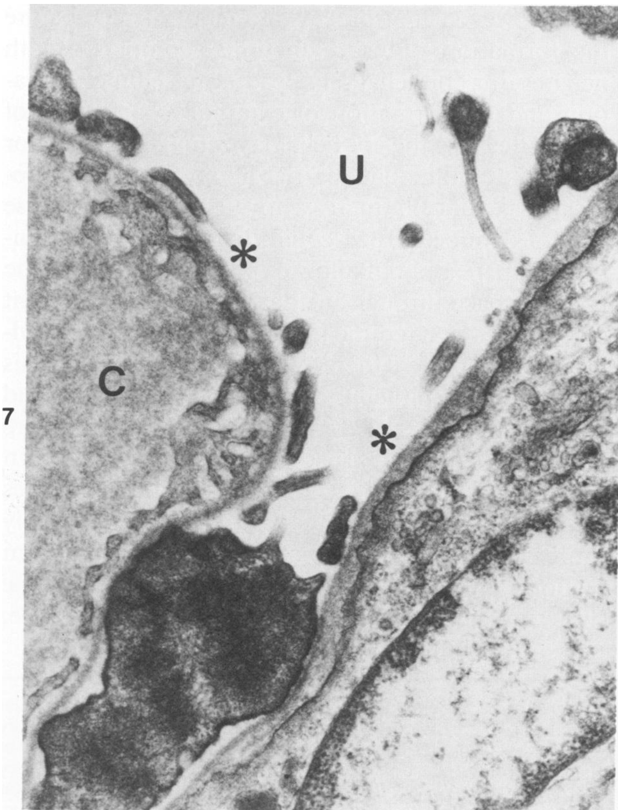

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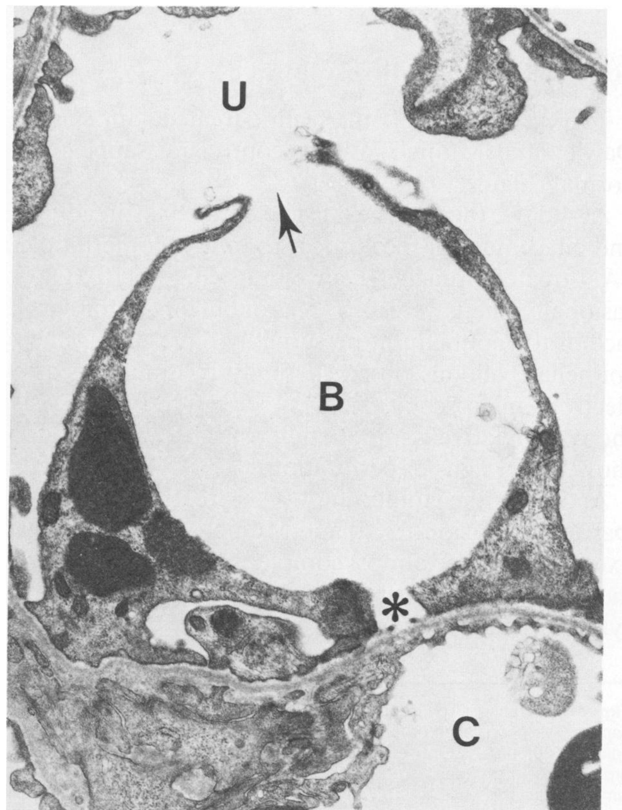


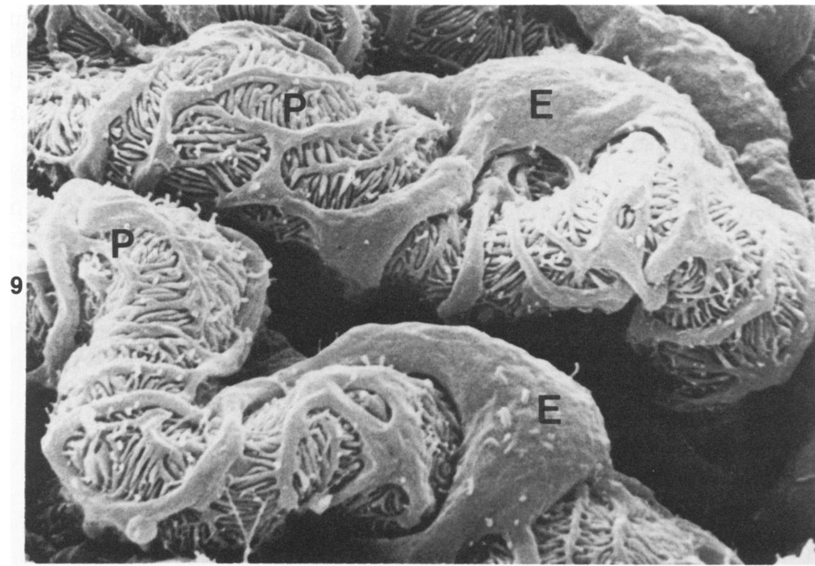
either directly or through the rupture of an overlying balloonlike vacuole.

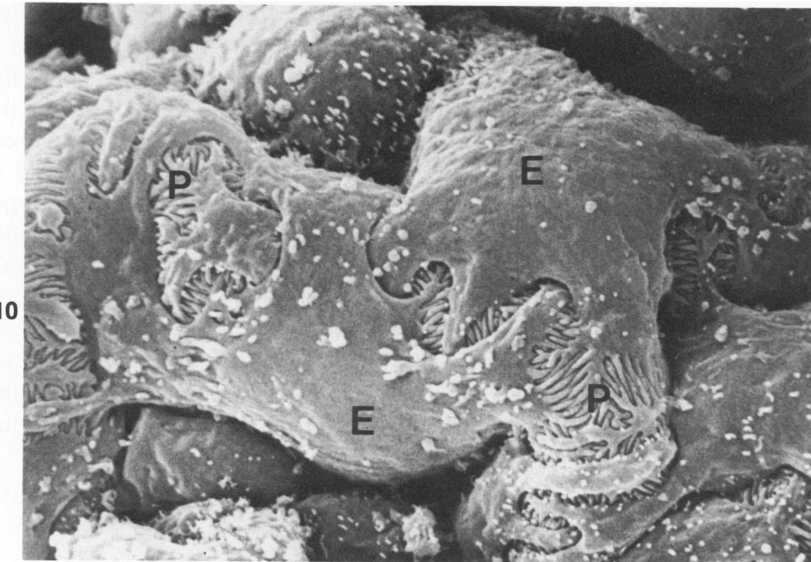
These present findings support the view first put by Venkatachalam et al[2,3] that cytoplasmic vacuoles represent a significant pathway for the passage of plasma proteins across the epithelial layer of the glomerular capillary wall in PAN nephrosis. The findings do not accord with the view of Caulfield et al,[5] raised when discussing the observations of Venkatachalam et al,[2,3] that epithelial detachment is not seen at the time of onset of proteinuria in PAN nephrosis. It must be emphasized that the animals in the present experiments were treated with the same daily low-dose PAN protocol as that used by Farquhar and Palade,[1] Caulfield et al,[5] and Venkatachalam et al.[2,5] Furthermore, identical results with respect to areas of GBM denudation and rupturing of vacuoles were obtained in the drip-fixed superficial glomeruli of Munich-Wistar rats when compared with perfusion-fixed glomeruli of Sprague-Dawley rats, thus obviating the possibility that the morphologic findings were artifacts of the perfusion fixation.

The mechanism of formation of the epithelial "balloons" in PAN nephrosis is unclear. Andrews[16] described the scanning electron microscopic appearance of similar large epithelial vacuoles in rats in the later stages (14 days) of PAN nephrosis and noted evidence of rupture of such vacuoles to the urinary space, but he did not examine earlier stages. It seems likely that these vacuoles develop over focal sites of epithelial detachment, leading first to a small basal pocket covered by flattened epithelium. There may then be slow insudation of plasma across the GBM into the pocket which progressively expands, causing "ballooning" and attenuation of the overlying epithelial cytoplasm until it eventually ruptures and the contents of the vacuole are flushed into the urinary space. In support of this, the vacuoles observed in drip-fixed Munich-Wistar kidneys before the onset of proteinuria contained homogeneous granular material resembling dilutions of the plasma present in the capillary lumen; but a day later, after the onset of proteinuria, half of the vacuoles contained plasmalike material, and the rest appeared almost empty. Presumably, this change in the appearance of the contents of the vacuoles occurs after their rupture, with consequent rapid dilution of the contents by the now relatively unrestricted increased water flux across the denuded GBM.

The present findings thus support the concept that, in PAN nephrosis, proteinuria results fundamentally from glomerular epithelial injury, which leads to the formation of focal gaps in the epithelial covering of the GBM, where increased water flux causes plasma proteins to be dragged across the GBM filter to the urinary space. Furthermore, because such focal areas of externally denuded GBM have been observed in the glomeruli of nephrotic patients with various types of glomerulonephritis, including focal glomerulosclerosis and hyalinosis,[17] amyloidosis,[18] minimal change disease, membranous glomerulonephritis, diabetic glomerulopathy, and lupus nephritis (Ryan and Davies, unpublished observations), it seems likely that formation of these bare areas of GBM also plays a significant role in the pathogenesis of proteinuria in these glomerular diseases.

Figure 5—TEM of a portion of glomerulus in a Sprague-Dawley rat on the day of onset of proteinuria. There is extensive spreading of visceral epithelium (*E*). Note focal sites (*asterisks*) of epithelial detachment from the basement membrane in paramesangial regions, *C*, capillary lumen; *U*, urinary space. (×12,000) **Figure 6**—TEM of a glomerular capillary wall in Sprague-Dawley rat on the day of onset of proteinuria. The epithelium (*E*) is detached and lifted from the basement membrane forming a subepithelial pocket (*asterisk*). *C*, capillary lumen; *U*, urinary space. (× 4500) **Figure 7**—TEM of a portion of glomerulus in a Munich-Wistar rat on the day of onset of proteinuria. At various sites (*asterisks*), the epithelium is completely absent from the basement membrane, leaving its outer surface directly exposed to the urinary space (*U*). *C*, capillary lumen. (×26,000) **Figure 8**—TEM of the glomerular capillary wall in a Sprague-Dawley rat on the day of onset of proteinuria. The large balloonlike space (*B*) overlying a focal segment of bare basement membrane (*asterisk*) is showing perforation (*arrow*) to the urinary space (*U*). *C*, capillary lumen. (×13,600)





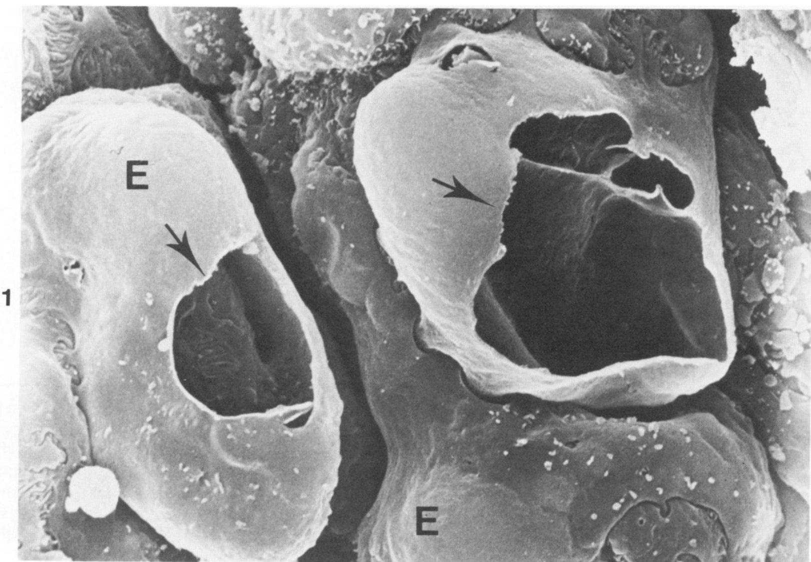


Figure 9—Scanning electron micrograph (SEM) of a glomerulus in a saline-treated control Sprague-Dawley rat. Epithelial cell bodies (*E*) give rise to primary and secondary major processes. Foot processes (*P*) arise from these, interdigitate with those from other cells, and wrap around the glomerular capillary loops. (×2250) **Figure 10**—SEM of a glomerulus in a Sprague-Dawley rat 4 days after commencing PAN injections and prior to the onset of proteinuria. There is extensive spreading of epithelial cells (*E*) and effacement of foot processes (*P*). (×2250) **Figure 11**—SEM of a glomerulus in a Sprague-Dawley rat on the day of onset of proteinuria (5 days after commencement of PAN injections). Foot processes are almost completely effaced by broad expanses of epithelial cytoplasm (*E*). Note the balloonlike bulges of epithelial cytoplasm, showing large perforations to the urinary space; the perforations have irregular edges (*arrows*). (×2250)

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