Tubulointerstitial Inflammation, Cast Formation, and Renal Parenchymal Damage in Experimental Pyelonephritis

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Some basic changes in experimental pyelonephritis were studied by transmission and scanning electron microscope. Initially, bacteria settled and multiplied in capillaries and venules. Leukocytes first marginated and then escaped from the capillaries, particularly to the wide peritubular interstitium. After opening the tubular basement membrane, the infiltrating leukocytes were immediately localized in the tubular wall between epithelial cells but were never seen between the epithelial cells and the underlying basement membrane. The inflammatory cells seemed not to be able to pass through the tight junctions of the nonnecrotic tubular epithelium. As a consequence of severe inflammatory injury, the tight junctions exhibited alterations of intermediate junction type. Where circumscribed necrosis of the tubular walls occurred, leukocytes appeared in the lumen. Thus, pus casts originated from these sites, apparently as drainage of interstitial abscesses. The secondary/regressive and regenerative/tubular changes were similar to those occurring after various tubular lesions. (Am J Pathol 1983, 113:300–308)

IN KIDNEY previously injured by ligation of the ureter, suppurative inflammation occurs if bacteria are introduced into the bloodstream. The disease known as pyelonephritis is typically a tubulointerstitial lesion showing patchy infiltration of the renal interstitium and tubules by inflammatory cells, with tubular necrosis and pus cast formation. The focal accumulation of leukocytes may result in abscess formation in the site of destroyed renal tissue. In the course of healing, the polymorphonuclear and macrophage infiltration changes to macrophages, plasmacytes, and lymphocytes; tubular atrophy and interstitial fibrosis occur. At the end of the process a corticomedullary scar and deformation of the underlying calyx develop.[1] According to recent publications, renal scarring results from early tissue destruction due to massive infiltration of the interstitium by polymorphonuclear leukocytes at the very beginning of acute pyelonephritis.[2–4] The direct damage of bacterial infection, and humoral and cellular immune mechanisms probably do not play a significant role in the evolution of pyelonephritis. The microorganisms only incite the acute inflammatory response, and early antibiotic treatment prevents renal scarring.[5]

Ultrastructural investigations on suppurative inflammation in the tubulointerstitium are scarce.[2,6] In the present work we have studied some basic phenomena of experimental pyelonephritis in rat kidney cortex with the transmission and scanning electron microscopes: the localization of bacteria in renal vessels, the migration of leukocytes from the intertubular capillaries to the interstitium, the inflammatory infiltration and injury of the renal tubules, and cast formation. Our working hypothesis was that pus casts originate partly from abscesses and partly from those nonnecrotic tubules that are infiltrated with leukocytes. One of our aims was to demonstrate the migration of inflammatory cells through the tubular basement membrane (TBM) and epithelium to the lumen. For a description of the alterations of the cortical peritubular interstitium, we refer to the work

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of Pedersen et al,[7] who divided it into a wide space loosely filled with interstitial cells and a narrow one having only interstitial cell processes. In our scanning electron microscopic observations the membrane-bound extensions of the white blood cells were named after relevant publications.[8-10]

Materials and Methods

Male Wistar rats weighing from 250 to 350 g and *Escherichia coli* (12797 CDC group American Type Collection) were used. The culture of bacteria growing in the logarithmic phase was centrifuged, and the sediment was diluted with physiologic saline to a density of 0.3. On the calibration curve relating the number of bacteria and the density of the suspension, a density of 0.3 was equal to 7.5×10^7 bacteria. We operated on the animals as in our previous work.[11] The left ureters were ligated. The ligation was removed 48 hours later. Four animals were sacrificed 1, 4, 6, 14, 24, 40, and 48 hours and 2, 4, 7, 10, 15, and 21 days after bacterial infection. *In situ* perfusion fixation for electron microscopy was performed with a solution containing dextran and glutaraldehyde.[12] Subsequently, small blocks of renal cortex were excised, fixed in 2% osmic acid, contrasted *en bloc* with uranyl acetate, and embedded in Durcupan ACM (Fluka). Semithin sections were stained with methylene blue–basic fuchsin and by Jones' silver impregnation. Ultrathin sections were stained with uranyl acetate and lead citrate. For scanning electron-microscopic examination, other blocks were postfixed in 1% osmic acid, dehydrated linearly in acetone, and critical-point–dried with liquid carbon dioxide. Finally, the specimens were gently fractured, and the fractured surfaces were coated with gold. Examinations were made with a TESLA BS 540 transmission electron microscope operating at an accelerating voltage of 80 kv and with a TESLA BS 300 scanning electron microscope at 25 kv. The remaining part of the kidney was further fixed in 4% neutral formalin and embedded in paraffin. The staining techniques were as follows: hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), Crossmon and Endes trichrome, van Gieson, elastica, Prussian blue, silver staining according to von Kossa and Jones, and the fibrin staining of Weigert.

Results

Light-Microscopic Findings

At 24 hours a small number of polymorphonuclear leukocytes (PMNs) and monocytes marginated focally to the endothelium of dilated venules and accumulated in the interstitium of the cortex and corticomedullary boundary. Between the 40th hour and fourth day abscesses destroying renal parenchyma and in the areas between the abscesses massive acute inflammatory infiltration of the interstitium and tubular walls were observed, with necrosis of some tubules, pus casts, and stasis in the peritubular capil-

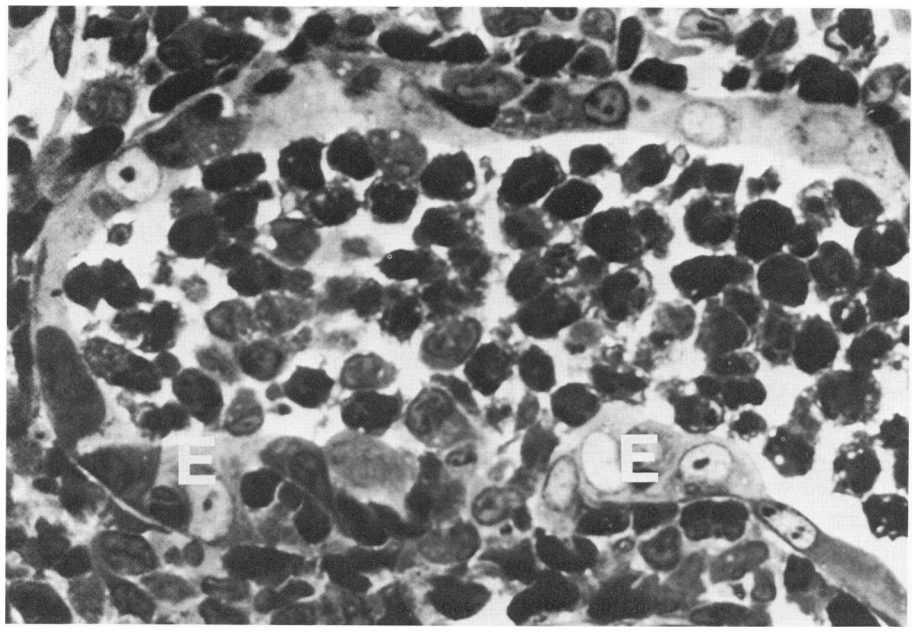

Figure 1—The interstitium is full of inflammatory cells. The dilated collecting tubule contains a cellular cast. A circumscribed tubular wall rupture has occurred between the two epithelial cells denoted by *E*. (Semithin section, methylene blue–basic fuchsin, ×880)

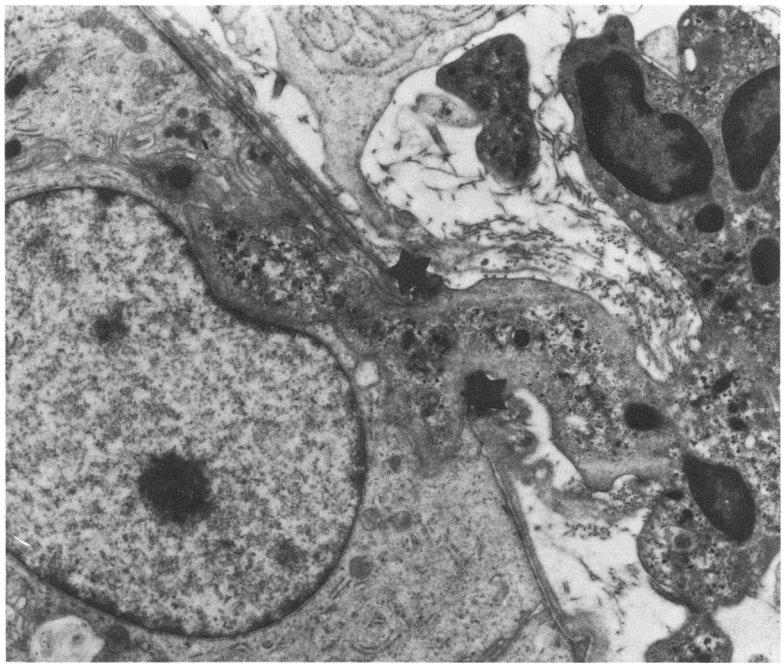

Figure 2—Changes after 7 days. The infiltrating pseudopod of a granulocyte is localized between two epithelial cells. The basement membrane is opened (*stars*), with its tortuous continuation lying irregularly around the portion of the just intruding granulocyte. (×11,000)

laries. (In this interval it was difficult to obtain an adequate perfusion because of inflammatory congestion.) After the seventh day, macrophages, lymphocytes, and plasmacytes predominated in the interstitial infiltrate, with disappearing abscesses. The casts in some dilated and dedifferentiated tubules still contained many PMNs, debris, and fewer macrophages. The same cells could be seen in the walls of these tubules and outside their TBM. At certain places the epithelium of these tubules had become necrotic, and nothing separated the cast from the interstitium full of inflammatory cells (Figure 1). Mainly in the cortex, atrophic tubules with a large mass of basement membrane material were formed, surrounded by interstitial cells.

TEM Findings

Migration of Inflammatory Cells and Cast Formation

At some distance from the abscesses, the intratubular capillaries were dilated and contained erythrocytes, PMNs, monocytes, and thrombocytes. No bacteria were found, and the marginated leukocytes adhered to the endothelium. In some places they penetrated with their pseudopods between the endothelium and through the capillary basement membrane. Finally, not only the cytoplasm but also the nucleus escaped into the extravascular space. When leukocytes were found completely in the interstitium, no gaps were seen in the capillary basement membrane or endothelium. In the early changes the leukocytes primarily infiltrated the wide interstitium. Because of the later massive infiltration, it became difficult to differentiate the wide and narrow spaces, and the tubules moved away from one another. Closer to the abscesses, rupture of the capillary walls, fibrin, and hemorrhage into the interstitium could also be seen. The PMNs and monocytes in the interstitium, either containing engulfed bacteria or not, lay close to the TBM. The inflammatory cells opened the TBM and moved first with their cytoplasm and then with their nuclei in between the epithelial cells (Figure 2). After they had crossed, the TBM seemed to become a continuous layer again. The leukocyte was usually localized in the basal region of the tubular wall, but was always separated from the TBM by a narrow layer of epithelial cytoplasm. Occasionally desmosomes disappeared, and the epithelial cells were connected only through intermediate and tight junctions. If the tubulointerstitial inflammation was severe, and there was a cast in the lumen, the structure of the tight junction changed: the fusion of the neighboring cell membranes disappeared, a fine intercellular gap developed, and the density of these cytoplasm regions decreased (Figure 3). In some places, as a consequence of the inflammation the cells of the interstitium and the tubular lumen were connected by a "bridge" of leukocytes through rupture of the tubular wall (Figure 4).

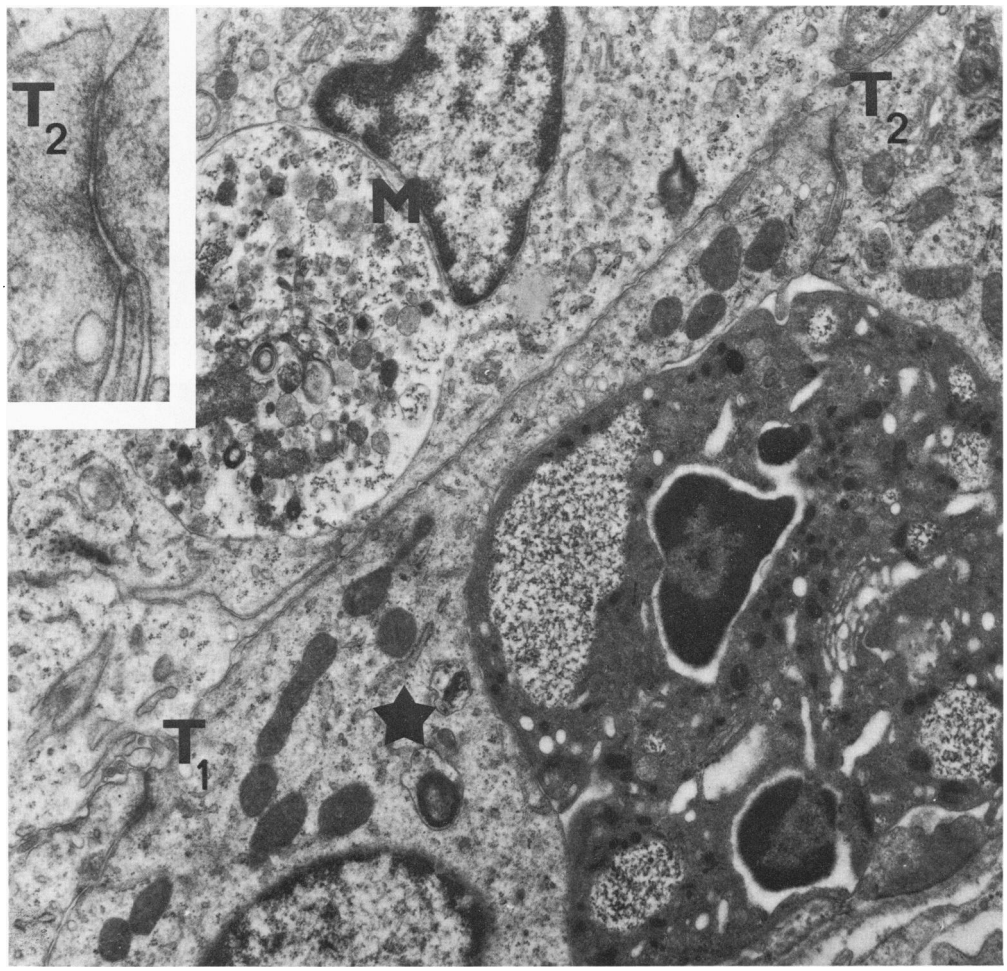

Figure 3—Changes after 4 days: The epithelium is dedifferentiated; it contains a granulocyte (*lower right*) and possibly two remnants of bacteria (*star*). The luminal surface is flattened by a cellular cast (*upper left*). The macrophage (*M*) containing a phagosome is part of the cast. Tight junction T_1 is not affected. At tight junction T_2 (also in **Inset**) cell membranes are distinct, and an intercellular gap has arisen. The density of this tight junction region is decreased. (×13,000; **Inset**, ×52,000)

Other Changes in the Parenchyma Due to Inflammation

After 40 hours, where inflammatory broadening of the interstitium was marked, the proximal tubules had lost their microvilli partially or totally. The number and size of the endocytic vesicles and the basal infoldings had decreased. In general, in those epithelial cells that had lost their brush border, the desmosomes were larger, thicker, and denser. The distribution and organization of the mitochondria became irregular. Numerous lipid droplets appeared at the base of the cells. Mostly by the side of the imigrated leukocytes, epithelial cells were poor in cell organelles but rich in free ribosomes, and thus reminiscent of embryonal cells (Figure 4). When the inflammation was intensive, phagolysosomes of various sizes and membrane-bound vacuoles could be observed. The latter contained well-recognizable bacteria. At places of tightly filled lumens with a cast, the luminal surface of the parenchymal cells was smooth, but remnants of microcilli could sometimes be identified. We observed atrophic tubules, especially from the seventh day; the lumens disappeared. Their dedifferentiated cells contained lipid droplets, and at the basal pole the quantity of free ribosomes and rough endoplasmic reticulum (RER) was increased. The TBM had become thicker and occasionally multiplied or was detached from the epithelium. Above the detachment a new thin basement membrane was always formed. The original old basement membrane was bordered outside by long processes of interstitial cells which were rich in RER. Between these collagen fibers, macrophages and chronic inflammatory cells could be seen. The outfoldings of the TBM some-

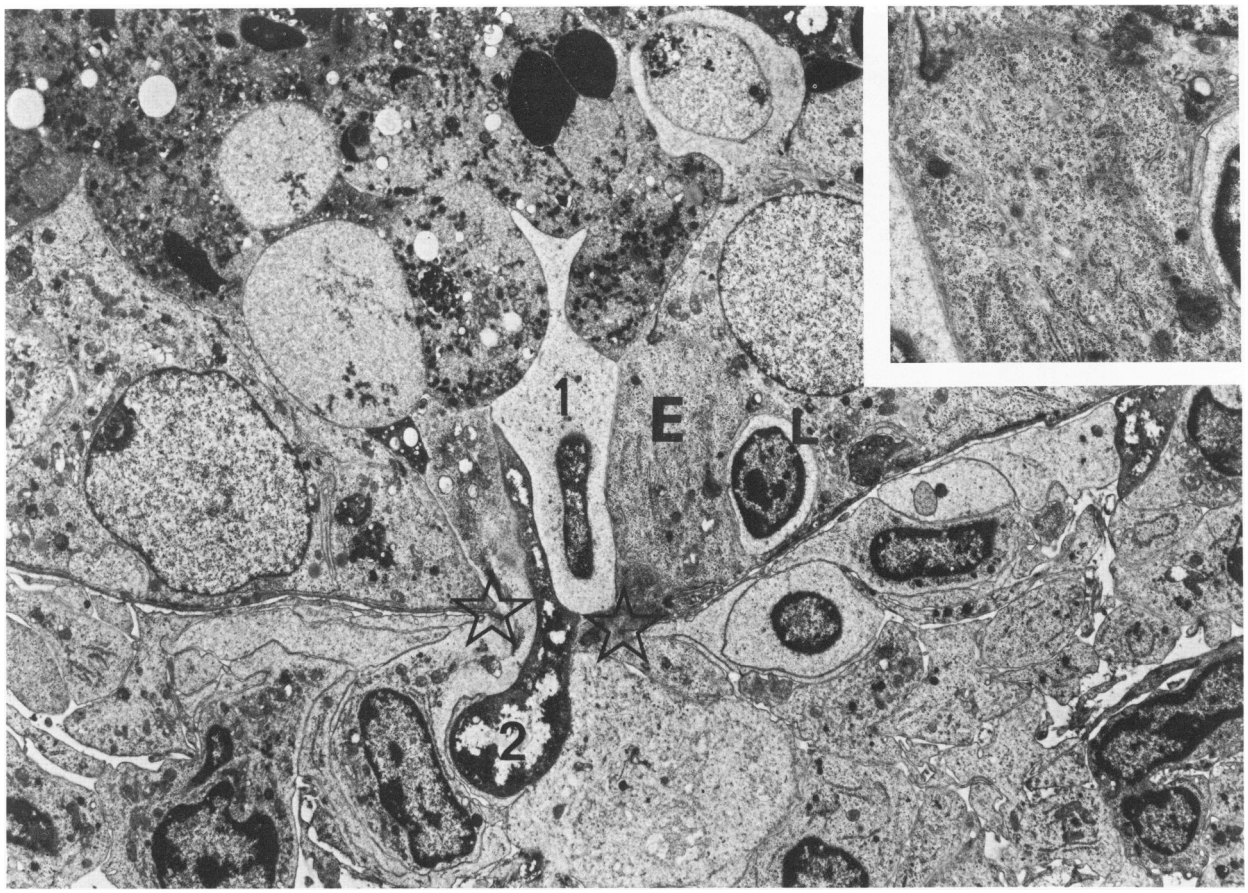

Figure 4—Changes after 15 days: Circumscribed tubular wall and basement membrane rupture can be seen between the two *stars*. A lymphocyte (*L*) is in the wall. The infiltrated interstitium (*bottom*) and the cellular cast (*top*) are connected by inflammatory cells (*1, 2*). The neighboring epithelial cell (*E*) (also in **Inset**) is of embryonal or regenerating type and is extremely rich in ribosomes. (×4400; **Inset**, ×9900)

times became separated, and they lay free in the interstitium.

SEM Findings

Bacteria were found sticking to the endothelium of intertubular capillaries and venules in continuously increasing number from the 14th till the 48th hour (Figure 5). No microorganisms could be seen on the surface of the podocytes and parietal cells of Bowman's capsule. Beginning from the 24th hour, hyperemia occurred with margination of granulocytes and, to a lesser extent, monocytes. These adhering cells were sperical or amoeboid in shape. Occasionally, one or more bacteria were attached to the microvilli of the leukocyte (Figures 6B and C). The leukocyte often extended a smooth-surfaced thick pseudopod into the interstitium, while the bulk of the cell with microvilli was still in the vessel (Figure 6A). We also saw the diapedesis of comma-shaped red blood cells, the narrower part being in the interstitium and the thicker in the venule. The inflammatory process broadened primarily the wide peritubular interstitium. At the 40th hour we detected leukocytes in the narrow spaces. The basal region of the tubular walls was at times also infiltrated by inflammatory cells (Figure 7). In some of these places tubular epithelium had pushed down its microvilli. The tubules draining the abscesses contained casts consisting of hundreds of bacteria, numerous granulocytes, and necrotic debris. Many tubules were filled only with hundreds of microorganisms. We were not able to find a fractured surface where granulocytes were migrating from the tubular wall into the lumen. From the seventh day, atrophic tubules appeared in the damaged places showing characteristic basement membrane wrinklings (Figures 8A and B). The great number of collagen fibers limited the interpretation of the scarring.

Discussion

During ultrastructural studies of tubulointerstitial immune nephritis and pyelonephritis, the migration

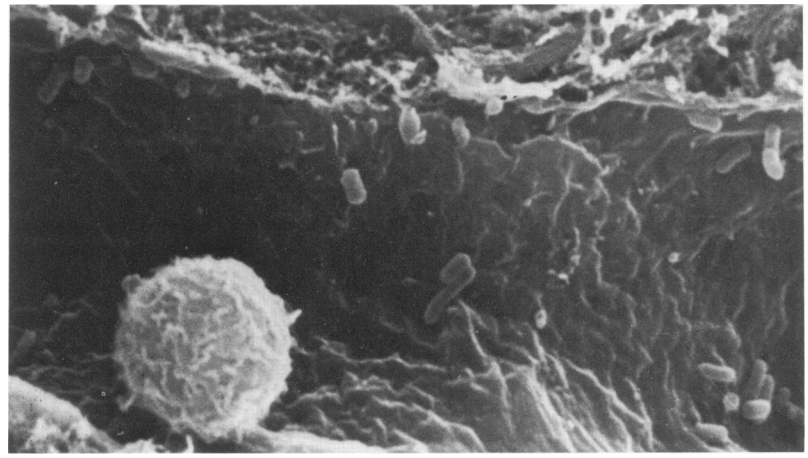

Figure 5—Changes after 2 days. Several bacteria and a granulocyte of spherical shape adhere to the endothelium of a venule. (×8000)

of leukocytes through intact basement membranes was not investigated.[2,6,13-15] After the administration of bacteria into the circulation, they adhere to the endothelium of the glomerular and intertubular capillaries and are phagocytosed within a short time.[6] Fluorescent studies also localized microorganisms in renal vessels in early phases of pyelonephritis, while later they settled in the interstitium and began to duplicate.[16,17] Although we did not observe the multiplication of bacteria themselves, their appearance in an increasing number as time passed suggests that they replicate even in the blood vessels. Under the influence of bacterial chemotaxins,[18] PMNs appear and, owing to the inflammatory mediators originating

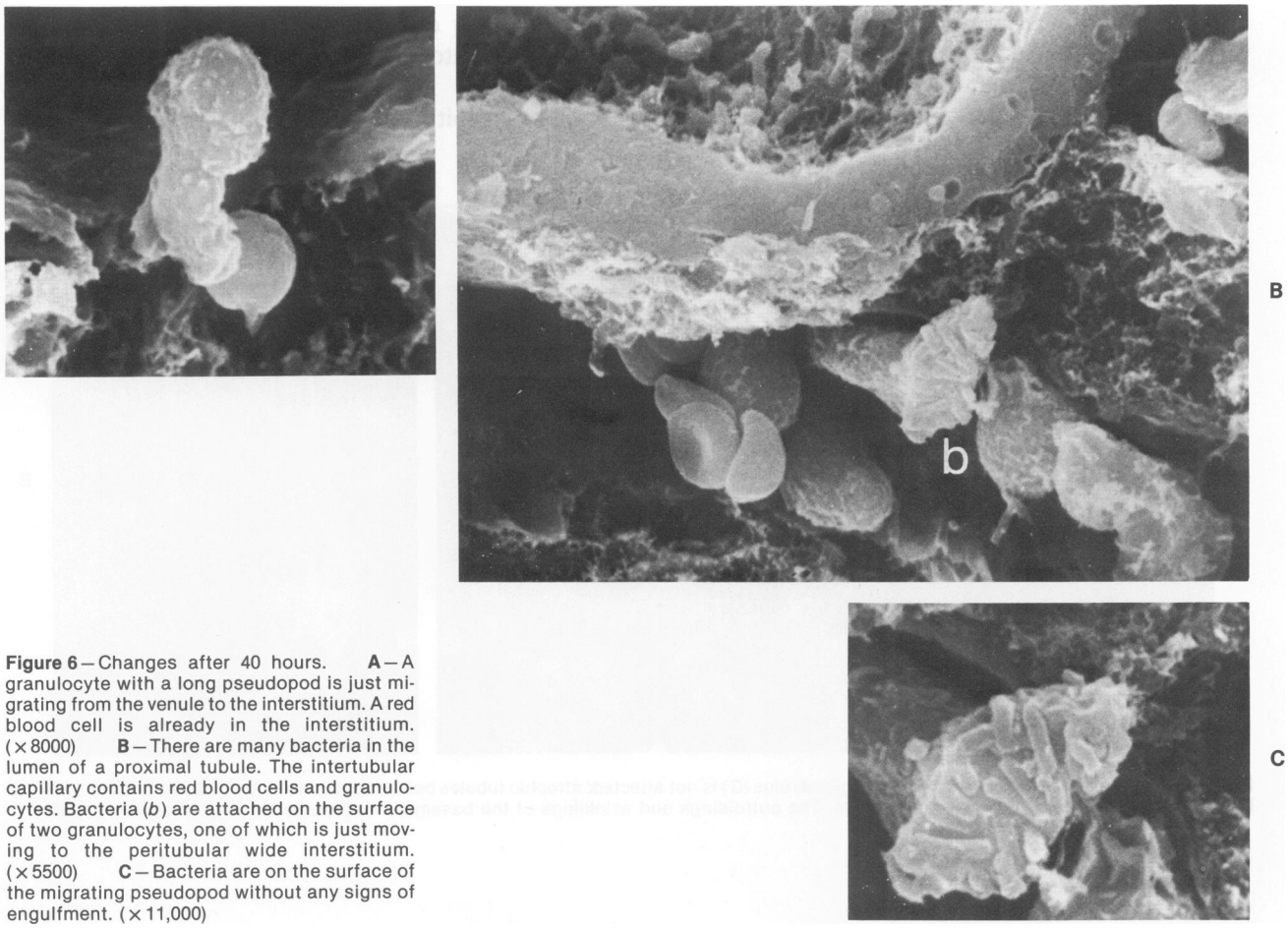

Figure 6—Changes after 40 hours. **A**—A granulocyte with a long pseudopod is just migrating from the venule to the interstitium. A red blood cell is already in the interstitium. (×8000) **B**—There are many bacteria in the lumen of a proximal tubule. The intertubular capillary contains red blood cells and granulocytes. Bacteria (*b*) are attached on the surface of two granulocytes, one of which is just moving to the peritubular wide interstitium. (×5500) **C**—Bacteria are on the surface of the migrating pseudopod without any signs of engulfment. (×11,000)

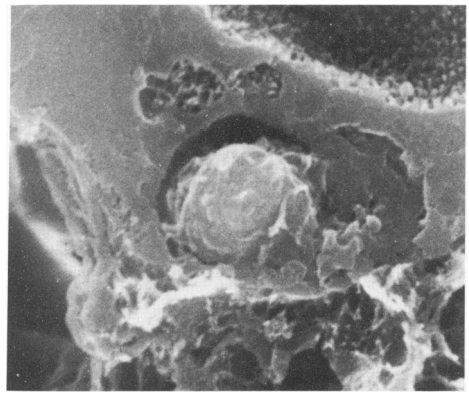

Figure 7—Changes after 2 days. A leukocyte is in the basal region of a proximal tubule. (×8000)

from the activation of complement cascade and to the lysosomal enzymes released from the stimulated neutrophils,[19] there is an increase in the permeability and injury of the capillary walls. Prostaglandins enhance the effect of permeability mediators.[20] The leukocytes migrate from the capillaries to the interstitium. The phases of this emigration that we observed in the kidney are identical to those of cell escape during inflammation described by Marchesi.[21] Under the scanning electron microscope we sometimes saw migration when several bacteria were attached to the surface of the granulocyte without any signs of engulfment. During inflammatory response, besides the increase in capillary wall permeability, local severe vascular injury might also occur with necrosis of endothelial cells.[22] Through such gaps bacteria can easily be transported on the surface of leukocytes to the interstitium, where they can multiply further. Miller and Derek[23] report that at the beginning of pyelonephritis there is a marked suppression of the cell-mediated immune response, and this corresponds with the period of rapid bacterial replication of the kidney. The massive but ineffective polymorphonuclear tubulointerstitial infiltration destroys the fine structure of the renal parenchyma irreversibly. When pyelonephritis begins, predominantly the wide peritubular interstitium is infiltrated. Later, as the inflammation becomes more intensive, infiltration of the narrow spaces also takes place, and they become widened. This widening surely decreases the blood supply of the tubules. The degeneration and dedifferentiation of the epithelial cells might be an effect of long-standing mild hypoxic injury. We do not know the reason why the inflammatory cells display a tendency to move into the lumens of the tubules. Cast formation could be considered to be a physiologic means of drainage of the interstitial suppurative inflammation. The steps involved in the migration through the tubular wall differ from those of the migration from the vessels. After opening the TBM with a pseudopod, the inflammatory cells (neutrophils, monocytes, and lymphocytes) immediately localize themselves between two epithelial cells. Our observations indicate

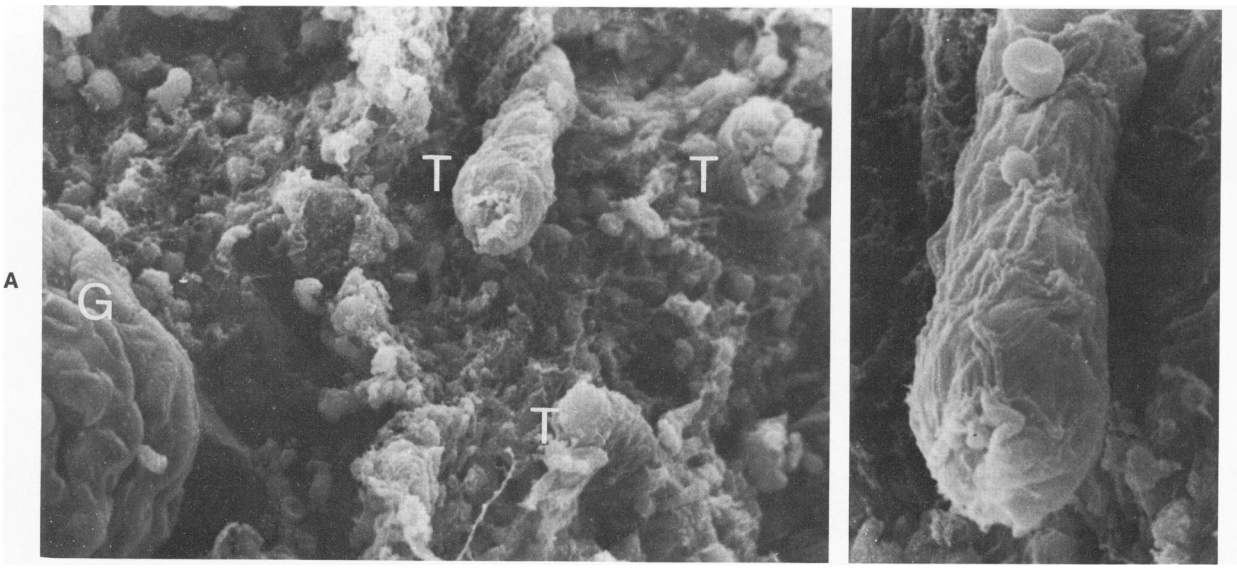

Figure 8—Changes after 10 days. **A**—The glomerulus (*G*) is not affected; atrophic tubules can be seen in the inflamed interstitium. (×1400) **B**—An atrophic tubule at higher magnification. The outfoldings and wrinklings of the basement membrane are clearly visible. (×3400)

that the connection between the tubular epithelium and its basement membrane must be very tight, in contrast to that of the capillary endothelium and endothelial basement membrane, because the infiltrating cells were not found situated between the tubular epithelium and the underlying basement membrane. When inflammatory cells were seen in the tubular wall, they were separated from the basement membrane by an epithelial layer. This feature is probably important from the point of view of tubular integrity.

We have made extensive investigations of serial thin sections of blocks in which, on the evidence of semithin sections, there was a moderate tubulointerstitial infiltration without epithelial necrosis, but with casts in the lumen. With the transmission electron microscope, we often saw infiltration of the epithelium, but opening of tight junctions and emigration into the lumen was never observed. On the basis of this and our additional scanning electron microscopic studies, we believe that inflammatory cells are not able to cross through nonnecrotic cortical tubular walls. In contrast to our previous hypothesis, we believe that casts usually originate from those regions where tubulointerstitial infiltration caused tubular wall necrosis or rupture.

For the study of morphologic alterations of junctional complexes, Bulger et al[24] recommend osmic acid fixation. Using glutaraldehyde–osmic acid fixation, we could still observe the intermediate junctionlike change of tight junctions during severe tubular injury. This might happen in the tubular epithelium when the pressure effect of the cast is added to the inflammation. In experimental malakoplakia the macrophagelike transformation of the proximal tubular epithelium has been reported.[25] In the present work we also observed large phagolysosomes or autophagic vacuoles and bacteria in the cytoplasm of the dedifferentiated tubular cells. They were visible only where the tubulointerstitial inflammation was severe and the tubular lumens were filled with pus casts. This change of the tubules can contribute to the turning of the inflammatory injury into an irreversible outcome. Others have also reported engulfed bacteria in the tubular epithelium in the course of pyelonephritis.[2,26] Cells of embryonal type seen in the tubular walls were similar to those observed in cell regeneration following tubular necrosis.[27,28] We think that these cells represent an increased regeneration process and not a serious dedifferentiation of the tubules.

The thickening and multiplication of the TBM, together with a new layer formation, is a common late result of various lesions of the tubules.[29,30]

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