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Proximal tubule morphology after single nephron obstruction in the rat kidney

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Proximal tubule morphology after single nephron obstruction in the rat kidney. This study examined the effects on proximal tubule morphology of blocking single nephrons with paraffin wax for one day, one week, or one month in the rat. Proximal tubule lumens were blocked with a short column of wax using micropuncture. Chronically blocked and control (normal) tubules were fixed by either intravascular or intraluminal perfusion of glutaraldehyde solution. Proximal tubule segments downstream to the wax block were examined by light and transmission electron microscopy. Intraluminal Alcian blue dye, serial sectioning, and nephron microdissection techniques were used to identify nephrons. One day after obstruction, all proximal tubule cells downstream to the block were injured. Some recovery was seen. S_1 and S_2 segments showed more severe damage than S_3 segments. Alcian blue, which normally is excluded from cells, entered the cytoplasm of some damaged S_1-S_2 cells. After one week of obstruction, the tubule appeared to have reconstituted itself, but cells were less differentiated than normal. One month after obstruction, blocked tubules were atrophied. Tubule cells were simplified and were surrounded by a thickened basement membrane. The results suggest that prolonged proximal tubule blockade produces injury and atrophy of the proximal tubule probably due to ischemia and interruption of normal reabsorptive activity.

Obstruction of the urinary tract results in impaired kidney structure and function [1]. With prolonged obstruction, nephron atrophy is seen. Atrophic nephrons have reduced function, and they are characterized morphologically by 1) small glomeruli and 2) tubules of reduced size with thickened basement membranes. Proximal tubule atrophy is characterized by a reduced cell height, loss of brush border, decreased complexity of basolateral membranes, and a reduced number of cell organelles, particularly mitochondria [2]. Proximal tubules in the rabbit kidney show signs of mild injury as early as one day after ureteral obstruction [3], and atrophy is clearly established after one month [4].

The reasons why nephron atrophy results from prolonged obstruction are not clear. Several factors have been postulated to play a role in causing nephron atrophy with prolonged ureteral obstruction: decreased renal blood flow, pressure-induced changes, infection, and disuse [5]. It has been especially difficult to implicate disuse, that is, lack of functional stimulation, as a mechanism for nephron atrophy, since other mechanisms (such as decreased blood flow) are well-established and certainly contribute to renal damage.

Chronic obstruction of the lumen of a single proximal tubule is accompanied by functional changes which closely resemble the changes produced by unilateral ureteral obstruction. For example, renal (or glomerular) blood flow and glomerular capillary pressure are decreased to the same extent in both cases after one day of obstruction [6–9]. It therefore was of interest to look at the morphology of single blocked nephrons.

The main purpose of the present study was to examine the morphology of kidney tubules whose lumens had been blocked with paraffin wax for one day to one month. We were primarily interested in investigating the idea that interruption of normal tubule fluid flow results in disuse atrophy. We examined proximal tubule segments downstream to a block, since these tubule segments suffer a more drastic interruption of tubule activity than upstream segments. Also, late portions of the proximal tubule would not be subjected to ischemic injury. We found that after one day of obstruction, proximal tubule cells were injured to varying degrees. After one week, there was considerable evidence of repair, but the tubule cells were more simplified than normal. After one month of obstruction, blocked tubules had atrophied. These results indicate that prolonged tubule lumen obstruction leads to tubule injury and atrophy. Since atrophy of blocked nephrons was seen in late proximal segments which presumably have a normal blood flow, disuse probably contributes to the observed atrophy.

Methods

Experiments were done on 20 male Wistar rats, weighing 212 to 356 g. The following procedure was used to obstruct the lumen of single proximal tubules of superficial cortical nephrons. The rat was anesthetized with sodium pentobarbital (40 mg/kg body wt) and was placed on a heated animal board. Rectal temperature was kept at 37 to 38°C. The left kidney was exposed by a flank incision, and was supported in a micropuncture cup. Saline (0.9% NaCl) was periodically dripped onto the kidney surface to prevent drying. To map specific nephrons,

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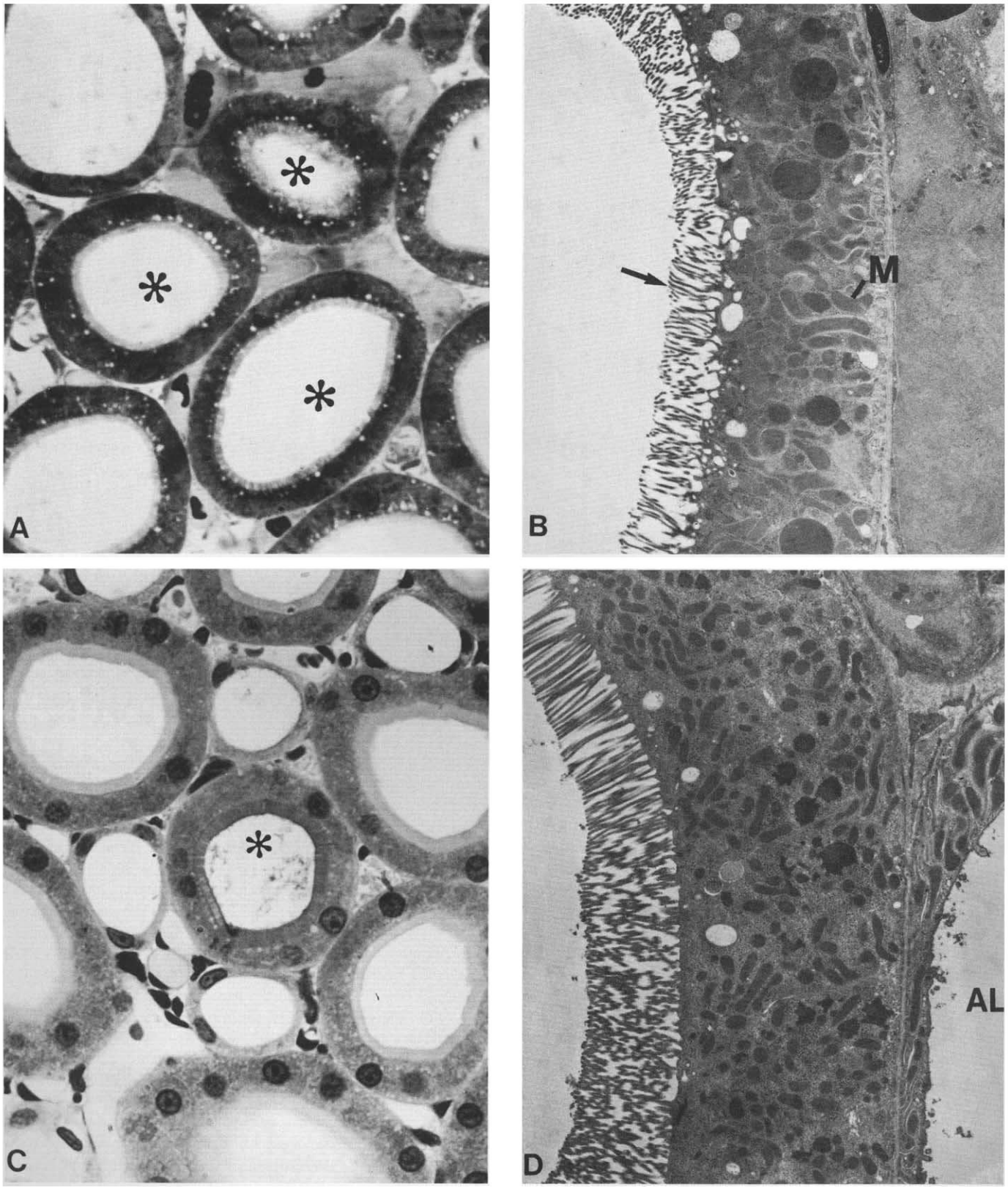


Fig. 1. *Control tubules.* **A** is a light micrograph showing several cross sections (asterisks) of S_1-S_2 segments of a tubule that had been acutely blocked and microperfused with fixative solution. **B** is a transmission electron micrograph of a well–fixed S_2 segment showing short microvilli (arrow), numerous mitochondria (M) positioned between basolateral interdigitations, and a well–defined endocytic apparatus. **C** is a light micrograph and **D** is a transmission electron micrograph of an S_3 segment. The acutely blocked and fixed tubule is marked by an asterisk in the light micrograph. Cells of this segment of the proximal tubule have long microvilli, a paucity of basolateral interdigitations, and absence of a well–developed endocytic apparatus. Ascending limb (AL). (A, ×800; B, ×6,000; C, ×800; D, ×6,000).

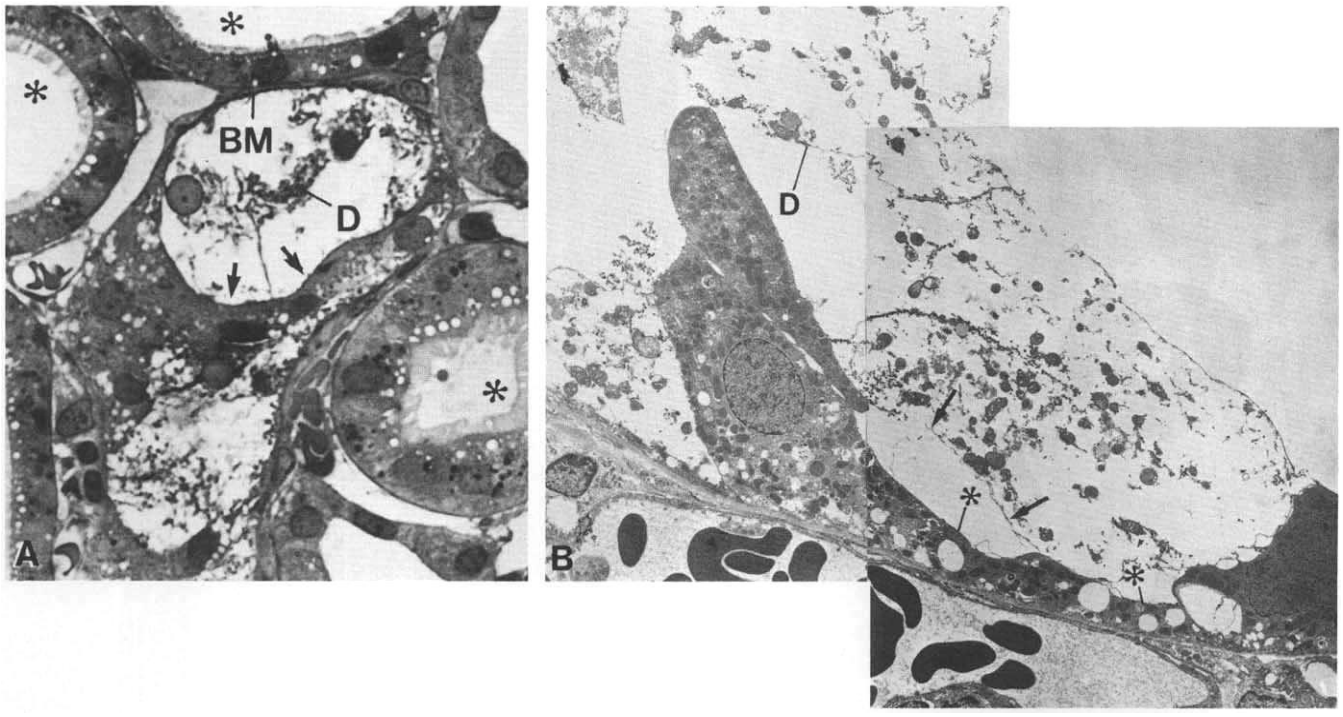


Fig. 2. *Light and electron micrographs of S_1 or S_2 segments of a nephron that had been blocked upstream with wax for one day.* By light microscopy (**A**), all cells in the blocked tubule appear to be injured. There was a loss of brush border (arrows), and cell debris (D) is present in the tubular lumen. Some regions of the tubular wall show a denuded basement membrane (BM) as a result of cell loss. Neighboring control proximal tubules (asterisks) appear normal. **B** shows an electron micrograph of an adjacent section. The cells show different degrees of damage. The attenuated cell located in the center (asterisks) covers the tubular basement membrane and may be in the process of recovery. Above this cell is another cell that is detached (arrows) from the basement membrane. This cell has a hydropic appearance, Alcian blue staining of its cytoplasm, and mitochondria with intracristal swelling, and has obviously been severely damaged. Note cellular debris (D) in the tubular lumen. (A, ×630; B, ×2700).

about half of the time we injected into the tubule lumen a small amount of Ringer's solution dyed with FD & C Green No. 3 dye (1 g/liter) with a 7 to 8 μm tip–diameter, sharpened micropipette. The tubule lumen was blocked at the same puncture site with a column of solid paraffin wax [10] stained with oil red O dye, using a 9 to 15 μm tip–diameter micropipette. The wax column was about 300 μm in length. The other half of the time, downstream segments belonging to a blocked nephron were identified simply by noting their collapse as the wax was injected. We made a careful drawing of the wax injected nephrons, so that we could identify the blocks and downstream segments at a later time. Most of the wax blocks were probably located at an early or mid-proximal convoluted tubule site, because we did not study nephrons in which downstream (late proximal) surface segments were absent. Typically, 5 to 8 nephrons were blocked per kidney. The kidney was then replaced in its original position, and secured to the body wall by a ligature passing through some of the perirenal connective tissue. The incision was closed by suturing the muscle layers with silk thread, and by apposing the cut skin edges with metal clips. The rats were allowed to recover from anesthesia and were housed in individual cages.

In all experiments we used clean techniques (sterilization of surgical instruments). In three rats, we also sterilized the micropuncture pipettes and saline solutions, and heated the wax to 100°C just before use.

Fixation of kidney tubules for morphological study was accomplished using either of two approaches: in the first, we fixed the whole kidney by intravascular perfusion; in the second, we fixed individual tubules by intraluminal perfusion. Prior to these maneuvers, the rats were deprived of food overnight, but had free access to water. They were anesthetized with Inactin (100 mg/kg body wt). The rats were placed on a heated animal board, and rectal temperature was kept at 37 to 38°C.

Intravascular fixation of kidneys [11] was done in six rats in which we had obstructed tubules one week ($N = 4$) or one month ($N = 2$) earlier. The aorta was exposed by a midline abdominal incision, and was cannulated caudal to the left renal artery. The cannula and attached tubing contained about 20 ml heparinized saline and were connected at the other end to a bottle containing 1% glutaraldehyde in Tyrode's solution [11]. The contents of the bottle were kept at a pressure of 150 to 170 mm Hg by means of a rubber bulb and mercury manometer. In rapid succession, the stopcock connecting the bottle to the aorta was opened, the aorta above the kidneys was clamped with a hemostat, and the left renal vein was cut to allow outflow of blood and fixative solution. The kidneys were perfused for 10 minutes with about 200 ml of fixative solution, and were subsequently stored in the same solution. Small blocks of kidney tissue containing obstructed or control nephrons were saved for serial sectioning or microdissection. Individual neph-

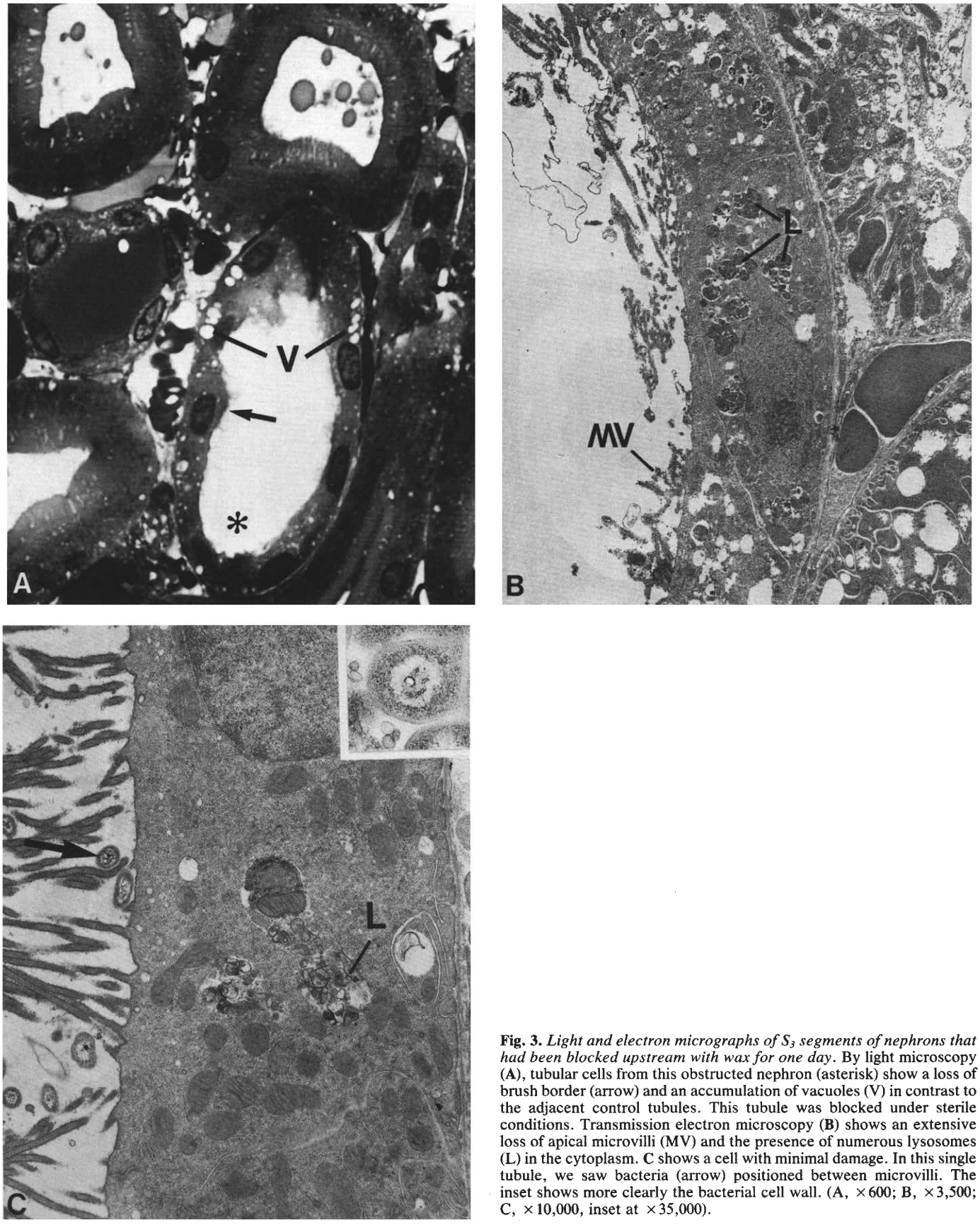
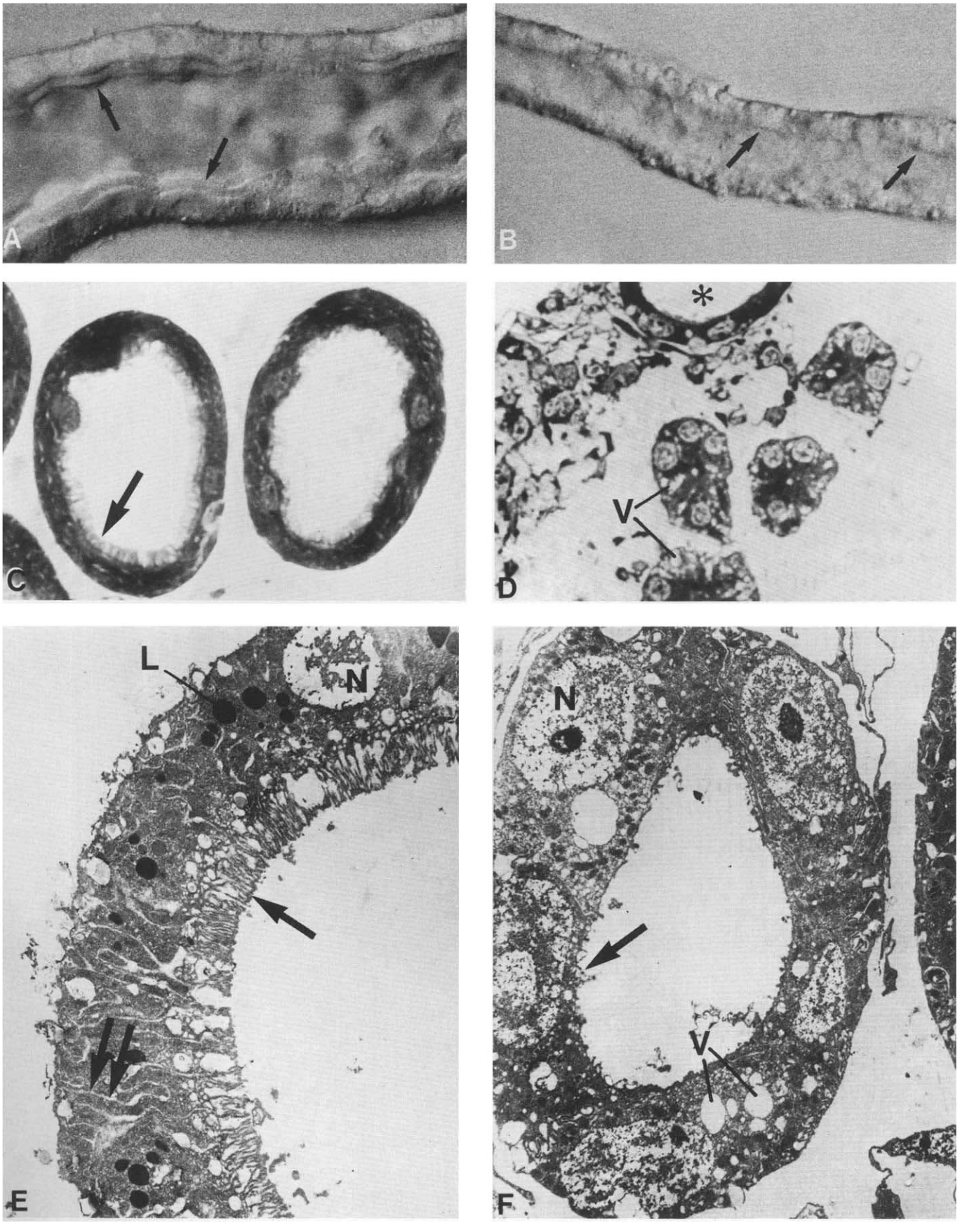


Fig. 3. *Light and electron micrographs of S_3 segments of nephrons that had been blocked upstream with wax for one day.* By light microscopy (**A**), tubular cells from this obstructed nephron (asterisk) show a loss of brush border (arrow) and an accumulation of vacuoles (V) in contrast to the adjacent control tubules. This tubule was blocked under sterile conditions. Transmission electron microscopy (**B**) shows an extensive loss of apical microvilli (MV) and the presence of numerous lysosomes (L) in the cytoplasm. **C** shows a cell with minimal damage. In this single tubule, we saw bacteria (arrow) positioned between microvilli. The inset shows more clearly the bacterial cell wall. (A, ×600; B, ×3,500; C, ×10,000, inset at ×35,000).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rons were microdissected after the tissue had been macerated with 8 N HCl for 55 minutes at 60°C [12]. Individual tubules were microdissected under a stereomicroscope. Some of these tubules were examined and photographed using a Nikon Optiphot microscope equipped for Nomarski differential interference contrast (Nikon Inc., Tokyo, Japan). Serial sections were made of four microdissected nephrons blocked for one week and three normal microdissected nephrons; the sections were evaluated by light and transmission electron microscopy.

For fixation of individual nephrons by intraluminal perfusion of fixative, rats were prepared as for micropuncture experiments [8] and the fixation method recommended by Olivetti, Dal Canton and Andreucci [13] was used. Briefly, individual nephrons were fixed by perfusing the tubule lumen with a solution containing 2% glutaraldehyde and 1% Alcian blue dye in 0.1 M cacodylate buffer (pH 7.3 to 7.4). Downstream segments of previously blocked nephrons were identified from their collapsed appearance and maps made earlier. The fixation was accomplished by injecting the fixative solution distal to the wax block, using a 7 to 8 μm tip–diameter micropipette attached to a Hampel microperfusion pump. The fixative was delivered at a rate of 20 to 30 nl $\cdot$ min^{-1} for five to 12 minutes. Fifteen nephrons blocked for one day were successfully perfused; after one week of obstruction, we were unable to fix single nephrons by intraluminal perfusion. The main criteria used for satisfactory perfusion were minimal gross leakage, filling of previously identified tubule segments (often including the distal tubule), and presence of dye in the loop of Henle (noted when the kidney was subsequently cut into small blocks of tissue). In the same animals, we also fixed normal nephrons in the same manner. In eleven control nephrons, the tubules were blocked with wax a few minutes before tubule fixation; in eight control nephrons the tubules were fixed without any injection of wax. The kidney was rapidly removed and immersed in fixative solution (1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3 to 7.4) without dye.

Small pieces of kidney tissue, each containing a single dyed tubule, were cut with a scalpel. The kidney pieces were kept in fixative for at least three hours at room temperature. They were then rinsed in 0.1 M cacodylate buffer, postfixed in 1% OsO_4, rinsed, dehydrated with graded ethanols, placed in propylene oxide, and embedded in Epon 812 epoxy resin. Thin (1 μm) Epon–embedded sections for light microscopy were stained with basic fuchsin. The fixed nephrons were marked by the Alcian blue dye. Nephrons were usually traced from the deep cortex to the kidney surface by serial sectioning. Identification of proximal tubule segments (S_1, S_2, S_3) was based on the ultrastructural criteria established by Maunsbach [14]. For transmission electron microscopy, ultrathin sections (0.04 to 0.06 μm) were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Philips 400 electron microscope.

Results

Both control (normal) and experimental (chronically blocked) tubules were usually studied in each kidney, thus providing an internal control for the various preparative procedures. In the present study, we evaluated only proximal tubule segments downstream to a wax block. We used two types of controls: nephrons acutely blocked with wax, and unblocked nephrons. We found no structural differences between these controls, and so in subsequent descriptions we will not distinguish between them. We also observed no alterations in the appearance of control nephrons studied in animals that had had nephrons blocked for one day, one week, or one month.

One day

Both light and transmission electron microscopic observations (Fig. 1) showed that control proximal tubules were well preserved by intraluminal perfusion [13]. These tubules could be easily and positively identified by the Alcian blue staining of the apical cell surface. Under the light microscope, this dye stained the brush border blue. Under the electron microscope, the dye appeared as electron dense particles lining the apical microvilli.

After one day of tubule obstruction, obvious cell damage was seen along the entire length of the proximal tubule distal to the wax block. The same degree of damage of obstructed nephrons was seen in all of the obstructed nephrons regardless of whether they were blocked using non-sterile or sterile procedures. By light microscopy, all cells from the S_1 and S_2 segments showed extensive loss of brush border (Fig. 2A). Some cells showed frank necrosis and there was cell debris in the tubule lumen. Loss of cells occasionally completely denuded the basement membrane. The cytoplasm of some cells was stained with Alcian blue; this suggests a loss of plasma membrane integrity, since this dye does not normally enter cells. Transmission electron microscopic observations (Fig. 2B) confirmed the changes seen by light microscopy. In addition, the damaged cells showed intracristal swelling of mitochondria, ruptured plasma membranes, and detachment of the cells at points from the basement membrane (Fig. 2B). Less severely damaged cells appeared to be spreading along and relining the tubular basement membrane (Fig. 2B). Commonly, areas of microvilli were seen well within the cytoplasm of proximal tubule cells, suggesting interiorization of microvilli. Thus, after one day of tubule blockade, signs of severe cell injury and recovery were seen. These changes were observed throughout the length of the downstream proximal convoluted tubule (S_1-S_2 segments), including sites remote from the wax block.

Fig. 4. B, D, *and* **F** *show downstream proximal tubular segments from nephrons that had been blocked upstream with wax for one week, and* **A, C,** *and* **E** *show control segments from the same kidneys.* **A** and **B** are Nomarski images of S_3 (pars recta) segments of proximal tubules that were microdissected after maceration in acid. The blocked segment (**B**) has a reduced tubular diameter and possesses cells of reduced size with incomplete brush border (arrows) compared to control (**A**). Routine light microscopy (**C** and **D**) and transmission electron microscopy (**E** and **F**) support the observations made with Nomarski optics. The downstream S_1-S_2 segments seen in **D** are reduced in diameter as compared to segments upstream to the wax block of the same nephron (asterisk) or to a control nephron (**C**). In addition, the cells in **D** lack a brush border and possess numerous vacuoles (V). By transmission electron microscopy (**F**), the downstream segments appear to be lined by atrophic cells that possess numerous vacuoles (V) and few microvilli (arrow), as compared to the control tubule from the same kidney (**E**). Lysosomes (L); basilar interdigitations (double arrow); Nucleus (N). (A, ×600; **B**, ×600; **C**, ×600; **D**, ×600; **E**, ×3,800; **F**, ×3,800).

In the same blocked tubules, the S_3 segments generally showed less damage than S_1-S_2 segments. All of the cells were mildly injured. At the light microscope level (Fig. 3A), we saw a loss of brush border, accumulation of cytoplasmic granules and/or vacuoles, and a reduction in cell height. Alcian blue dye did not enter the cytoplasm of these cells. With transmission electron microscopy (Fig. 3B), it was clear that the microvilli were reduced in number. The cytoplasm contained numerous autophagic lysosomes and small clear vacuoles. The cytoplasmic architecture was disorganized. Figure 3C, taken at a higher magnification, showed the least amount of damage we observed for the S_3 segment. Several profiles of bacteria were seen between the microvilli, but never within the cell. This was the only tubule in which we saw bacteria.

One week

We attempted to fix, by intraluminal perfusion, single tubules which had been blocked for one week, but were unsuccessful because the fixative solution leaked from the puncture site and would not flow down the nephron. Therefore, we used another approach to identify tubule segments belonging to a blocked nephron, namely, microdissection after maceration in acid. In this case, the whole kidney was fixed by intravascular perfusion with a glutaraldehyde solution. Both unblocked (control) and blocked nephrons were microdissected from the same kidneys and subjected to the same procedures. The blocked nephrons were more difficult to microdissect than normal nephrons; they were smaller and more fragile and were surrounded by fibrous tissue.

Cells in control proximal tubules (Figs. 4A, 4C, and 4E) had a normal appearance for well-fixed, acid-treated tissue. After one week of blockade we detected no differences in cell morphology between S_1, S_2, and S_3 segments. Light microscopic investigation of blocked tubules revealed a loss of brush border, a reduction in outer tubular diameter and cell height, a narrowed or collapsed lumen, and an excessive amount of interstitial tissue adjacent to these nephrons (Figs. 4B and 4D). Transmission electron microscope observations of the blocked proximal tubule revealed cells with a simplified morphology. The cells were characterized by a few short microvilli, reduced cell height, few basilar interdigitations, a loss of normal orientation of mitochondria, and many large vacuoles scattered throughout the cytoplasm (Fig. 4F). Overall, the tubule was clearly reduced in size (compare Figs. 4E and 4F) and possessed a population of dedifferentiated cells.

One month

Figures 5A and 5C show examples of control proximal tubules, and Figures 5A, 5B, and 5D show tubules which had been blocked with wax for one month. The whole kidney had been fixed by intravascular perfusion with glutaraldehyde solution. At this time, blocked tubules were greatly reduced in both outer and inner diameters when compared to control tubules. The epithelial cells lining the blocked tubule were simplified; for example, they lacked a brush border. There was extensive interstitial fibrosis adjacent to the blocked segments. By transmission electron microscopy (Fig. 5B), the cells appeared to lack characteristics of a normal proximal tubule cell. They lacked basilar interdigitations and had few organelles. The tubule was encased in a thickened basement membrane.

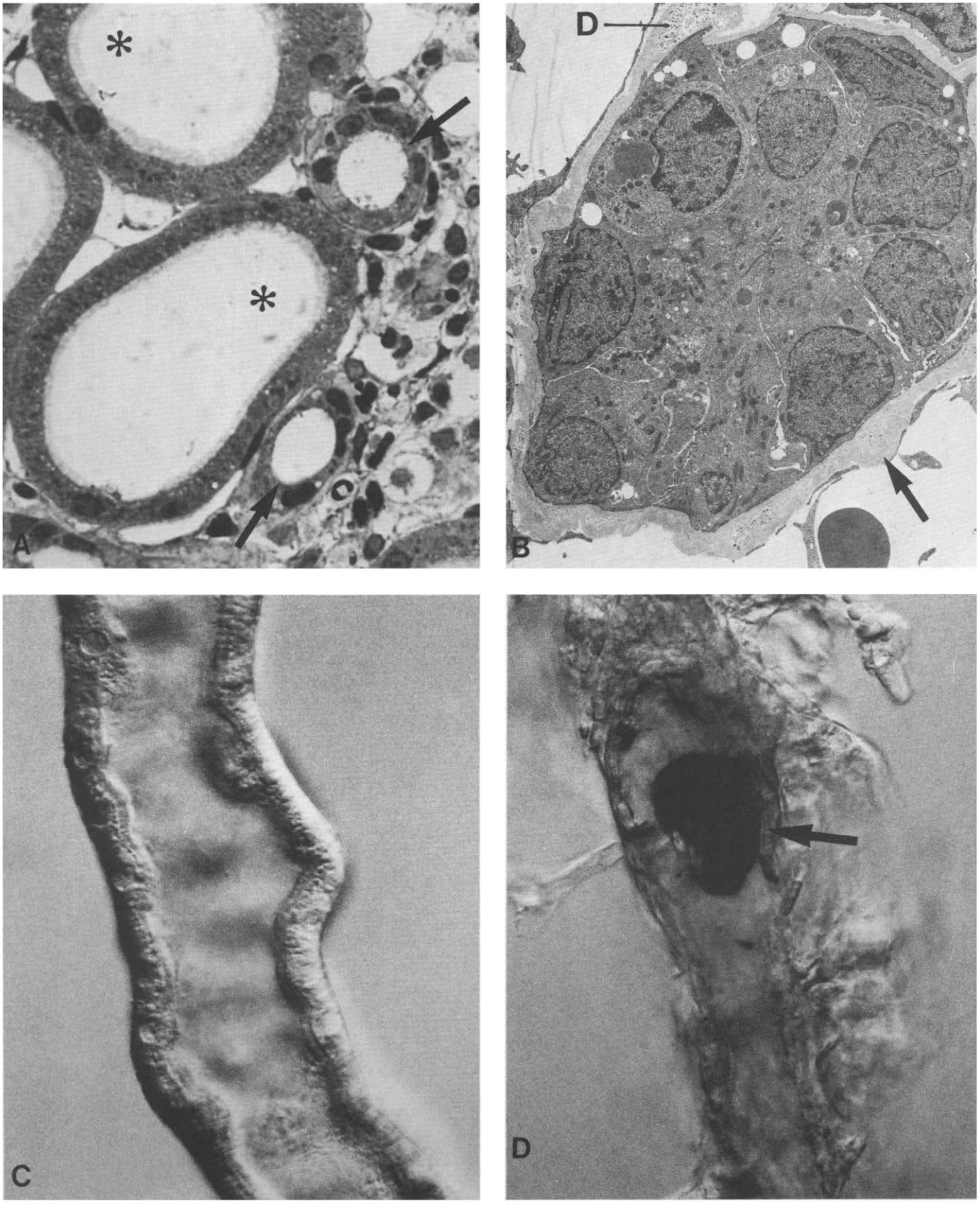
Discussion

The present study demonstrates that proximal tubule lumen blockade with paraffin wax eventually leads to tubule atrophy. Figure 6 summarizes the morphological changes which we observed in this study. One day after tubule blockade, the entire proximal tubule downstream to the block showed signs of cell injury. The damage was more severe in the convoluted portion of the proximal tubule (S_1-S_2 segments) than in the late straight portion (S_3 segment). Injury was indicated by changes such as loss of brush border, mitochondrial disorganization, changes in cytoplasmic density, changes in cell shape and basolateral interdigitations, and the appearance of autophagic vacuoles. We also found that Alcian blue stained the cytoplasm of some cells in the pars convoluta. This dye is normally excluded from cells, and so penetration of the dye suggests an abnormally leaky plasma membrane, and confirms the presence of severe damage. After one week of blockade, the obstructed tubule appeared to have reconstituted itself, since the damage appeared less severe than at one day. The cells, however, were less differentiated than normal. It was no longer possible at this time to distinguish S_1, S_2, and S_3 segments on the basis of cell structure. One month after obstruction, the blocked tubules were clearly atrophic. The simplified tubule cells were surrounded by a thickened basement membrane.

We consider it unlikely that these changes were a direct result of contact of the tubular epithelium with the wax block. The changes in tubule structure occurred downstream to the block, quite far from the wax injection site. Although we did not study upstream segments carefully, in the few instances where such segments were examined, they appeared normal at one day. We detected no evidence that blockade with wax of one nephron produced damage to neighboring nephrons. Acute injection of wax (control tubules) a few minutes before fixation did not produce any signs of tubule injury. The changes induced are more than likely related to prolonged interruption of the normal flow of tubule fluid rather than a toxic effect of the wax block.

A key question is the nature of the agent or agents that produced the tubule injury and atrophy observed in this study. For the reasons just given, we consider it unlikely that the changes were due to a direct toxic effect of the wax. We discuss several other possibilities below. Possible candidates include 1) ischemia, 2) pressure-induced changes, 3) infection, and 4) interruption of normal tubule cell reabsorptive activity.

Fig. 5. *Control proximal tubules and tubules that had been blocked upstream with wax for one month.* **A** shows the atrophic S_1-S_2 downstream segments (arrows) of a blocked tubule (identified by serial sectioning) and the adjacent control tubules (asterisks) in the same kidney section. In the blocked tubule, note the reduced size of the cells and their dedifferentiated appearance. The same atrophic segment seen by transmission electron microscopy (**B**) is characterized by a thickened basement membrane (arrow) which has trapped some cell debris (**D**). **C** and **D** show acid-treated, microdissected pars recta segments. In contrast to the normal tubule (**C**), the one month-blocked tubule is narrower, has an indistinct tubule epithelium, and is surrounded by fibrotic material. Note a small piece of wax (arrow) in the tubule lumen. (A, ×800; B, ×4,200; C, ×600; D, ×600).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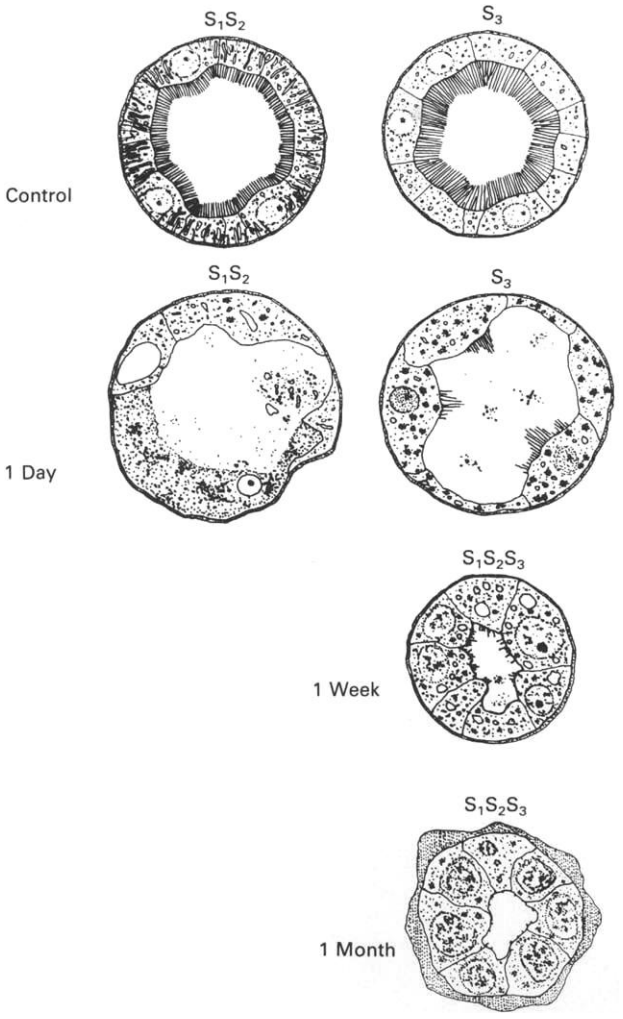

Fig. 6. *Summary of general morphological changes seen in proximal tubules blocked upstream with wax for one day to one month.*

Single nephron obstruction with paraffin wax or castor oil results in a decrease in glomerular blood flow to the blocked nephron [8, 9, 15]. The mechanisms involved are not completely understood. We have postulated that the initiating stimulus is related to a reduction in tubule fluid flow through the loop of Henle caused by the obstruction [16]. Afferent arteriolar constriction appears to be mainly responsible for the decreased glomerular pressure and blood flow in obstructed nephrons [8]. Increased local formation of angiotensin II may partly cause the vasoconstriction [9].

Many of the changes in cell morphology seen after tubule obstruction resemble changes seen after an insult, such as temporary renal artery occlusion [17–19]. An important difference between the present study and the renal ischemia models is that the inciting agent (the wax block) persists in this study, whereas in the renal ischemia models the applied insult is temporary. In the ischemia models, provided that the initial insult has not been too long in duration, damaged cells often regain a normal structure [17, 18, 20]. Because of the persistence of the wax block, recovery is apparently not possible in our study. A striking feature in both models is that neighboring cells may be injured to very different extents. The reasons for these different individual cell responses are not known. A highly localized ischemia may be one explanation. It is well known that one of the characteristic signs of ischemic injury is an irregular distribution of lesions [21]. We have recently demonstrated that glomerular blood flow is normal one to two hours after tubule obstruction with wax, is 2/3 of normal after one day, and is 1/3 of normal after one week [15]. A reduction in glomerular blood flow to 2/3 of normal at one day seems too mild to explain the striking tubule injury we observed at this time. This is especially so since the obstructed nephron will have a reduced filtration rate and rate of tubular reabsorption, and hence a lowered demand for oxygen. It may be that even though total glomerular blood flow is decreased only modestly, the distribution of blood flow to the blocked tubule may be altered sufficiently to cause local hypoxia. Our results, however, also suggest that factors in addition to ischemia may contribute to the renal injury.

Increased intrarenal pressure and stresses may contribute to the deterioration of renal structure and function after ureteral obstruction [22]. We consider it unlikely, however, that this causes the changes induced by single nephron obstruction. Although injection of the wax does dilate a single segment markedly [16], the bulk of the tubule segments are physically remote from the wax block. It is also unlikely that interstitial pressure is significantly increased by single nephron obstruction, although this has not been measured. Finally, the signs of tubule damage occurred in tubule segments downstream to the wax block; pressure must be reduced in these segments, and so a pressure–induced atrophy cannot explain the tubule damage.

We consider it unlikely that bacterial infection was responsible for the changes observed. The same changes were observed whether we used sterile or non-sterile techniques. We did see bacteria in one tubule, but despite an exhaustive search did not find bacteria in 33 other tubules prepared using non-sterile techniques. In the contaminated tubule, the bacteria were confined to the proximal tubule lumen, and were not phagocytosed by the cells. We do not know when the bacteria entered the tubule. Shimamura and Maesaka [23] injected large quantities of *E. coli* into proximal tubules of the rat kidney, and after 48 hours found that the tubules were morphologically normal, except for the presence of bacteria in the tubule lumen and cells.

Another intriguing possibility is that tubule injury and atrophy result from interference with the normal filtering–reabsorbing activity of the nephron. Perhaps some essential material or stimulus derived from the glomerular filtrate is necessary to maintain tubular integrity. Wright [24] has stated that the "kidneys thrive on work," and this suggests that a tubule whose reabsorptive work load has been compromised may atrophy. The concept of renal disuse atrophy was introduced by Hinman [5, 25], as a result of his studies on ureteral obstruction. Evidence for this idea is derived largely from the observation that a damaged kidney will maintain a low level of function in the face of its normal mate. Schleifer [26] demonstrated in the rat kidney that if proximal pars recta segments are separated from pars convoluta segments by means of a microelectrocautery, then the pars recta will show after one day an impaired ability to secrete organic anions and histological signs of atrophy. Based on these functional and structural observations,

he suggested that interruption of glomerular filtrate delivery to the late proximal tubule causes tubule damage, an idea supported by our study. At present we have no clear understanding of how disuse leads to tubule atrophy.

Our results support the view that disuse atrophy may contribute to the renal changes induced by tubule obstruction. It should be noted that signs of cell injury in blocked nephrons were observed in proximal tubule pars recta segments deep within the cortex. It is commonly accepted that superficial convoluted tubule segments are supplied primarily by the efferent arteriole of their own glomerulus [27, 28], and so a reduced glomerular blood flow might contribute importantly to injury of these segments. Proximal tubule pars recta segments, however, may be supplied largely by efferents from other glomeruli. This has been clearly demonstrated for the dog kidney [29], and is probably also true for other mammalian kidneys [30]. Since it is unlikely that obstruction of one nephron causes a decrease in blood flow to remote glomeruli, we consider it unlikely that ischemia is responsible for the morphological changes induced in the late proximal pars recta. The more severe morphological changes in the proximal convoluted tubule may be due to a combination of ischemia and disuse atrophy. Our results, therefore, support the idea that the normal structural integrity of the tubule depends on a normal filtering and reabsorbing activity.

In conclusion, obstruction of single tubule lumens with wax leads to injury and eventual atrophy of the blocked tubules. The morphological changes produced may be due to ischemia and lack of functional stimulation caused by interruption of tubule fluid flow. We believe that the same factors may contribute to the renal destruction caused by prolonged urinary tract obstruction.

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