Nonhepatic Thioacetamide Injury

II. The Morphologic Features of Proximal Renal Tubular Injury

Edward A. Barker, MD, PhD and Edward A. Smuckler, MD, PhD

Thioacetamide given orally to rats produces centrolobular hepatic necrosis and also causes death of the cells in the terminal portion of the proximal renal tubule. The morphologic changes observed during the course of the renal toxicity include the early and transient appearance of apical dense bodies, which appear to fuse to form large lysosomes, and the appearance of nucleolar hypertrophy, reminiscent of the same change seen in the hepatocytes. In addition a variety of changes described in lethally injured tubular cells in other toxicities appear. A diuresis, which lasts for 5 days, coincides with the appearance of tubular cell destruction. The mechanism of cell injury due to thioacetamide is not identified, but the temporal sequence of morphologic and physiologic change is consistent with both a relative concentration of the thioacetamide in the proximal tubule and its potential conversion to a putative proximate toxin (Am J Pathol 74:575–590, 1974).

ANALYSIS OF THE MECHANISMS involved in cell injury and neoplastic transformation has been studied by chemically induced liver modulation. Thioacetamide is one of the several agents that produce centrolobular necrosis and has been so employed. The effects of thioacetamide are not limited to the liver, however, and profound structural and functional changes occur in other organs including the kidney and the thymus. These modifications may alter the response seen in the liver and influence the host response in general. A detailed temporal analysis of these changes may permit separation of the several organ responses and provide an opportunity for an analysis of their respective significances both to host survival and to specific cell injury. A description of the thymic response of thioacetamide has been published.[1] The following report concerns the response of the kidney to this agent.

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Materials and Methods

Animals

Pathogen-free, male Sprague-Dawley rats, weighing 175 to 250 g, were maintained in our animal quarters on standard laboratory chow and water, *ad libitum*, for at least 1 week prior to experimentation. The animals were kept in plastic metabolic cages for 3 days, during which time baseline urine volumes were obtained. Following a 16-hour fast, a 2% aqueous solution of thioacetamide was administered in a dose of 2.5, 10 or 20 mg/100 g body weight, by stomach tube without anesthesia. Control animals received an equivalent volume of water. Subsequently, the animals were housed in the metabolic cages and were allowed access to standard laboratory chow and water *ad libitum*. Total daily urine volumes were measured.

For *in situ* fixation of the kidney, the rats were anesthetized with sodium pentabarbital (1.7 mg/100 g body weight, intraperitoneally), and the abdominal aorta was canulated and perfused in a retrograde manner with 50% Karnovsky's fixative.[2] The inferior vena cava was incised to allow release of the fixative, enhancing kidney perfusion. The kidneys were removed and transected with razor blades, then placed in neutral buffered formalin.

Microscopy

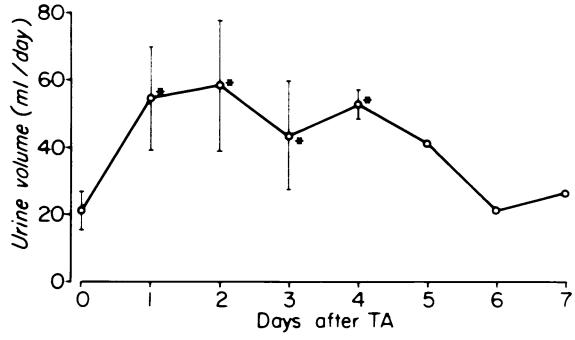
For light microscopy, coronal sections, 2 to 3 mm thick, were dehydrated and embedded in paraffin blocks; 5-μ sections were cut and stained with hematoxylin and eosin, methyl green thionin, PAS and with the Mallory trichrome procedure. Other slices were placed in 50% Karnovsky's solution, and 1 cu mm cubes from inner and outer cortex were prepared. These cubes were immersed in Karnovsky's fixative at 4 C for 3 hours, washed, postfixed for 1 hour in 1% osmium tetroxide, then processed into Epon 812 blocks. One-micron sections were prepared and stained with toluidine blue. These stained sections were used for orientation of thin sections subsequently prepared from the same blocks. The thin sections were stained with uranyl acetate and lead hydroxide, and examined with an RCA 3G electron microscope.

Results

Renal Function and Gross Pathology

A single feeding of thioacetamide at the dose range used was not associated with animal mortality during the course of these studies. Some morbidity was noted, with decreased animal activity and bristling of the fur, but otherwise the animals ate and drank in a manner similar to controls.

The external appearance of the kidneys from animals treated with thioacetamide was not remarkable. The capsules were smooth, glistening and stripped with ease. No petechial hemorrhages were noted. There was no difference in kidney weights. On cut surface, a distinct white band was noted in the inner half of the renal cortex. This region became apparent by 24 hours following intoxication and persisted for at least 4 days. Contemporaneously, diuresis occurred, beginning during



TEXT-FIG 1—Graph demonstrating mean daily urine volume (± SD) following a single dose of TA, 20 mg/100 g body weight. Asterisks indicate statistical significance ($P < 0.01$).

the first 24 hours and persisting for 5 days (Text-figure 1). Associated with this fluid loss from the body was a transient hemoconcentration.[1]

Light Microscopic Examination

Lower power inspection showed marked alterations in the inner half of the cortex; there was marked loss of normal eosinophilia as the epithelial cells died and sloughed, leaving empty tubules behind (Figures 1 and 3). This is contrasted with normal perfused kidney, in which the tubules remained open and were tinctorially uniform through the entire cortex (Figures 2 and 4). The earliest recognizable change at the light microscope level occurred 16 to 24 hours after administration of thioacetamide (TA). There was loss of tubule patency and marked swelling and rarefaction of single tubule cells and of entire tubules of the inner cortex (Figure 3). One and 2 days after poisoning, most cells of the innter cortex were affected; they were alternately swollen, vacuolated and pale, or shrunken, granular and bright pink. The brush border was uniformly disrupted; many cells were markedly distended, and some were free in the lumen. Cellular debris was prominent, and some pyknotic nuclei appeared. Three to 4 days after TA, tubule patency was again evident (*ie*, perfusion was again effective), and many damaged cells were seen free in the lumen (Figures 5 and 6). Many of the peripheral cells contained an enlarged, usually single nucleolus. Flat, attenuated, thionin-positive cells populated the periphery of the tubules; mitoses in these loci were numerous, most evident in enlarged, pleomorphic cells partially attached to the tubule edge. Occasional granular casts were present in medullary collecting ducts and tubules at this time. Seven days after TA, the tubules of the inner cortex were widely dilated, uniformly patent, and the epithelial cells were of low cuboidal type, without a brush border, or with only a thin brush border (Figure 7). Cytoplasmic thioninophilia persisted, and a few remnants of debris could be found in the lumina. By that time,

mitoses and enlarged nucleoli were scarce. Three weeks after TA, the kidney was indistinguishable from normal. At no time were any alterations in glomeruli, distal tubules, medulla, vessels or interstitium noted. The changes were qualitatively similar after all doses of TA; they differed only in degree, though they were barely evident after the low (2.5 mg/100 g body weight) dose.

Electron Microscopic Examination

Well-perfused control kidneys did not differ morphologically from previous descriptions.[2] Five hours after TA, there were several cells in the proximal convoluted tubule in the inner half of the cortex with intact plasmalemma, but which showed many small, dense bodies present in the apices of the cells. These dense bodies appeared to have a single limiting membrane but no distinguishable ultrastructure. Focally they were in contact with the plasmalemma at the luminal pole of the cells, where they appeared as electron-dense thickenings at the base of the microvilli, in some cases opening into the lumen (Figure 8). Eight hours after TA, one could find randomly scattered, isolated proximal tubule epithelial cells in the inner cortex which had disrupted luminal cell membranes, rarefied cytoplasm with dispersed organelles and distortion of cristate mitochondriales (Figure 9). The dark apical bodies were more distant from the luminal pole and were larger. Sixteen hours after TA, cytoplasmic rarefaction affected more cells and the dense bodies also appeared farther away from the luminal pole of the cell; they were invariably encased with a single membrane-lined structure, along with material of less electron density (Figures 10 and 11). The cytoplasm of these cells was light in staining; the basal infoldings were disordered and seemed reduced in number. Lateral interdigitations of the cell membrane were decreased. By 24 hours, the frequency of apical dense bodies had decreased. Numerous cytolysosomes were present; some were evident in cells of the outer cortex. In some cells, the lysosomes were enlarged, round to angular, pleomorphic, and had regions of varying electron density suggesting the incorporation of the dense bodies (Figure 11). Many of these lysosomes approximated one another, as if they were in the process of fusing. Mitochondria showed markedly variable forms with increased cristae. Aggregates of smooth endoplasmic reticulum were found in rarefied portions of cytoplasm, not infrequently bulging into the lumen (Figure 12). It was possible to find increased smooth ER in less damaged cells. Pale cells alternated with contracted, electron-dense "dark" cells containing crowded organelles and pyknotic nuclei. Nucleoli were sharply

demarcated from the remainder of the nucleoplasm, were prominent, enlarged and frequently contained small, dense granules. These nucleoli could be found in proximal tubule cells of both inner and outer cortex (Figure 13). Dilation of basilar infoldings was noted in some cells. By 48 hours occasional flat, attenuated cells bordered on the tubule basal lamina, seemingly dissecting beneath dead cells and debris, which were, in turn, lying free in the lumen. The mitochondria in this debris contained dark granules consistent with calcium salt deposition. The flat cells possessed rudimentary microvilli and many non-membrane-associated polysomal clusters (Figure 14). Myelin figures were present in contracted, dead cells. Cells showing less overt damage were found with increased smooth endoplasmic reticulum (Figure 15), particularly prominent nucleoli with granules, and a "busy" cytoplasm. Extension and coalescence of cytolysosomes was dramatic (Figure 16) and particularly prominent in cells with extensive cell swelling. No abnormalities were noted in glomeruli, distal tubules, Henle's loop, interstitium, blood vessels or collecting ducts.

Discussion

Thioacetamide produces selective renal tubular necrosis, restricted to that portion of the proximal convoluted tubule located in the inner half of the renal cortex. Morphologically, there are many similarities in this renal injury to other forms of toxin-induced renal tubular damage. Changes in fine structure, which may be nonspecific in regards to etiologic agent but are associated with lethally injured cells, include disruption of plasma membranes, vesiculation, disruption and degranulation of endoplasmic reticulum, increase in number and size of cytosomes with many partially fused forms, and mitochondrial alterations (loss of dense matrical granules, matrical rarefactions, dilatation of cristae membranes and deposition of calcium containing crystalline material). These alterations are seen in $HgCl_2$,[3-5] lead,[6] uranium,[7,8] CCl_4[9] and ethylene glycol [10] intoxications, as well as after thioacetamide administration. Alterations not seen after thioacetamide administration, but which occur in other situations, include widened microvilli and the presence of cytoplasmic lipid droplets (CCl_4), intramitochondrial calcification (vitamin D,[11] $HgCl_2$) and peritubular basal lamina thickening and ingrowth (ethylene glycol). Alterations found after TA and not usually seen in other examples of renal tubular necrosis include: a) nucleolar hypertrophy and demarcation from nucleoplasm, b) increased smooth endoplasmic reticulum and "tubular aggregates" (though these may also occur after CCl_4 or $HgCl_2$ administration), and c) the pres-

ence of electron-dense bodies in the apical portions of the cells early in the evolution of the lesion.

The apical dense bodies are single membrane-limited cytoplasmic inclusions without obvious substructure. Temporally they seem to move away from the lumen and apparently fuse to form larger lysosomes.[12] The temporal appearance and disappearance of the dense bodies coincides with the onset of diuresis and loss of proximal tubular function, but they disappear prior to the return of function, presumably in some manner related to transport through the cell in the larger single membrane-bound bodies. It is possible that these bodies represent some type of absorptive vacuole; however, neither the material that they contain nor their eventual resolution provides a clue concerning their role in this injury.

The identification of nucleolar enlargement in the livers of an animal intoxicated with thioacetamide is well known.[13,14] This increase in size is associated with an increase in the content of nucleolar RNA and of protein and has been suggested to be related to altered transport of RNA in animals so intoxicated.[15] A similar series of events could occur in the proximal tubular cells, but the role of these structural changes in the injury is not evident. It does suggest that the effect of the toxin on the liver and the proximal tubular cells has common features. The exposure of the liver to a high concentration of toxin is consistent with the absorption of the toxin from the gut and the passage through the liver prior to its distribution by blood flow. The high proportion of cardiac output passing through the kidney necessarily also exposes renal cells to a high concentration of thioacetamide on a temporal basis, thus providing for similar organ concentration.

TA is un-ionized at pHs found in urine and has a $CHCl_3/H_2O$ partition coefficient of 0.05. Under these circumstances, it is quite soluble in water. It may be filtered at the glomerulus (not bound to albumin) and passed down the nephron in the filtrate. Physiologically, TA should be progressively more concentrated, and greater back-diffusion into the tubular cells should occur as it progresses further down the proximal tubule. It is the terminal proximal tubule which demonstrates the toxin-related injury and death, suggesting that larger concentrations of TA may be achieved at that site. That some reabsorption occurs in more proximal portions of the tubule as well is suggested by the nucleolar change which occurs there. Alternatively, it is possible that the terminal proximal tubule cells are uniquely sensitive to the toxin; this possibility is given support by the fact that distal tubule and Henle's loop cells, which may be exposed to even higher concentrations of the toxin,

show no damage. The association of the apical dense bodies, the temporal sequence of thioacetamide distribution, and the potential of sulfur-dependent reducing capacity of this agent in trapping the osmium fixative suggest that resorption of thioacetamide may occur in the injured zone. We have no direct evidence for the presence of thioacetamide or one of its metabolites in these vesicles. The osmiophilia could be related to the absorption of protein or lipid. However, their absence in controls and in other forms of renal injury suggests a thioacetamide injury relationship. The bowel epithelium shows no evidence of cell injury in spite of exposure to the original dosage. It seems possible that both exposure to TA and the capacity to modify this agent or exposure in high concentration to modified thioacetamide are prerequisites for injury, and higher concentrations are necessary for lethal injury. Thioacetamide is extensively metabolized during its passage through the body.[16,17]

It seems clear that two or three not necessarily mutually exclusive possibilities may pertain to the renal lesion resulting from TA ingestion: a) TA is itself toxic to a selected population or populations of cells (centrolobular hepatocytes, terminal proximal tubule cells of the kidney and cortical thymocytes), b) TA is metabolized by these cells to an intermediate or intermediates which are toxic (thiolacetic acid would seem to be a likely candidate), or c) distribution and/or concentration phenomena of TA and/or metabolites may account for the selective toxicity. The latter possibility is not likely, at least as far as TA itself is concerned, since no damage is found in bowel epithelium after oral administration of TA. One extrapolation from the morphologic data seems warranted: that in the process of cell injury, the ion transport mechanism is damaged, leading to marked cell swelling (seen as cytoplasmic rarefaction), disruption of the cell membrane and then collapse of the cells to dense clusters of organelles (seen as dark cells). Whether this membrane damage is an early primary event or a late secondary event remains obscure.[18]

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Legends for Figures

Fig 1—A photomicrograph of the renal cortex, 3 days after TA administration, 10 mg/100 g body weight. There is striking loss of proximal tubule epithelial cells from the inner portion of the cortex only. The outer portion of the cortex is not altered and has patent tubule lumina, consistent with adequate perfusion. Compare this illustration with Figure 2 (H&E, × 32).

Fig 2—A photomicrograph of the renal cortex, normal rat. The cortex is uniformly stained and shows patent tubule lumina (H&E, × 32).

Fig 3—A photomicrograph of proximal tubules, inner zone of cortex of kidney one day after TA administration, 20 mg/100 g body weight. Most tubules are closed, in spite of evidence of adequate perfusion with fixative (most capillaries are free of blood). The brush border is not seen in damaged tubules or in tubules which are swollen shut. Numerous cells are markedly swollen and pale. Compare this to Figure 5 (H&E, × 1280).

Fig 4—A photomicrograph of the renal cortex from a normal rat. The brush border is easily visualized. Nuclei are uniform, without prominent nucleoli (H&E, × 1280).

Fig 5—A photomicrograph of the inner zone of cortex 3 days after TA, 10 mg/100 g body weight shows markedly damaged proximal tubules. Some cells have sloughed into the lumen, containing pyknotic nuclei and shrunken eosinophilic cytoplasm; other remaining peripheral cells show enlarged, prominent nucleoli (*arrows*) (H&E, × 1280).

Fig 6—A photomicrograph of inner zone of cortex of rat 4 days after TA, 10 mg/100 g body weight. Even more cells are sloughed into the lumen. Mitotic figures are present in peripheral cells (*arrows*), and flat attenuated cells are numerous along the basal lamina. Large cells with swollen vaculated cytoplasm and enlarged nuclei are present. Granular casts are present (H&E, × 1280).

Fig 7—A photomicrograph of the inner zone of the renal cortex 7 days after TA, 20 mg/100 g body weight. A few dead cells and debris remain in markedly dilated tubule lumina. Tubules are composed of low cuboidal deeply basophilic epithelium with thin or poorly developed brush border (H&E, × 1280).

Fig 8—An electron micrograph of renal inner cortex proximal tubule cells 16 hours after TA, 20 mg/100 g body weight. There is marked extensive swelling and rarefraction of many cells. A common feature at this time in the injury is the presence of dark bodies at the apices of damaged cells, which are in some places continuous with the plasmalemma between microvilli (*arrows*) (× 14,500).

Fig 9—An electron micrograph of the inner cortical zone of a rat kidney, 8 hours after TA administration, 20 mg/100 g body weight. Note the selective dissolution of proximal tubule cells with sparing of cells immediately adjacent. Note also the plasmalemma discontinuity (*arrows*) and mitochondrial distortion (× 8560).

Fig 10—An electron micrograph of inner renal cortex proximal tubule cells, 16 hours after TA, 20 mg/100 g body weight. At this stage, the dense bodies crowd the luminal portion of the cytoplasm, and are present within membrane-bound vesicles deeper in the cytoplasm (*arrows*) (× 7000).

Fig 11—An electron micrograph of inner cortex proximal tubule cells, 24 hours after TA, 25 mg/100 g body weight. Many cytosomes are present: some appear to be fusing and are enlarged (× 10,700).

Fig 12—An electron micrograph of outer renal cortex proximal tubule cells, 24 hours after TA, 20 mg/100 g body weight. Extensive networks of smooth endoplasmic reticulum are present, generally concentrated in the more rarefied cells. Frequently they lie in portions of the cell which bulge into the lumen. In other areas they form networks around single membrane-limited bodies (*arrows*). The mitochondria show intracristal dilatation (× 7000).

Fig 13—An electron micrograph of an inner cortex proximal tubule cell 24 hours after TA, 2.5 mg/100 g body weight. There is less evidence of cytoplasmic damage but note the enlarged "ropey" nucleolus, well delimited from the remainder of the nucleoplasm. Within the nucleolus are several small granules (*arrows*). This appearance is similar to nucleoli seen in hepatocytes at the same time in the evolution of toxic injury (× 9125).

Fig 14—An electron micrograph of the inner cortex, 48 hours after TA, 10 mg/100 g body weight. Debris containing recognizable mitochondria with dense granules (calcium salts) and pyknotic nuclei is present within the lumen. Cells along the basement membrane of this tubule are attenuated and posses only rudimentary microvilli. An adjacent tubule contains necrotic cells. Myelin figures are easily seen (× 3150).

Fig 15—An electron micrograph of inner renal cortex proximal tubule cells, two days after TA, 2.5 mg/100 g body weight. Note the nucleolar prominence, with intranucleolar granules. An extensive rete of smooth endoplastic reticulum ramifies throughout the cytoplasm, and Golgi complexes are prominent (× 5670).

Fig 16—An electron micrograph of the inner renal cortex proximal tubule cells 48 hours after TA, 2.5 mg/100 g body weight. Extensive enlargement of cytosegresomes, presumably due to coalescence, is evident (× 7000).

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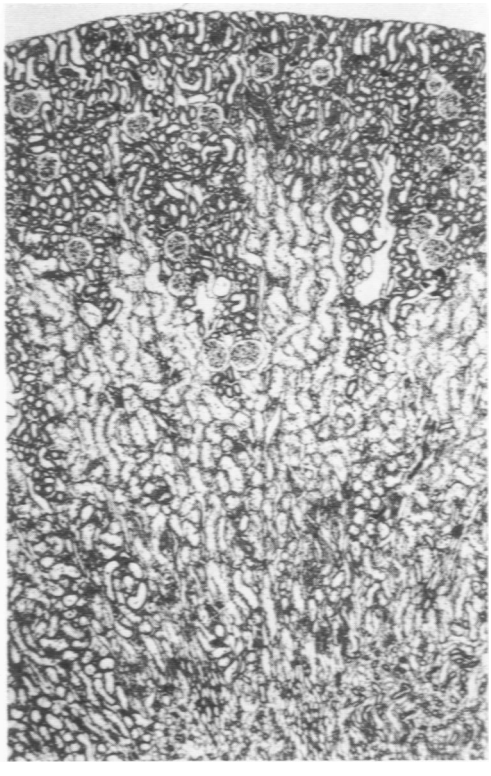

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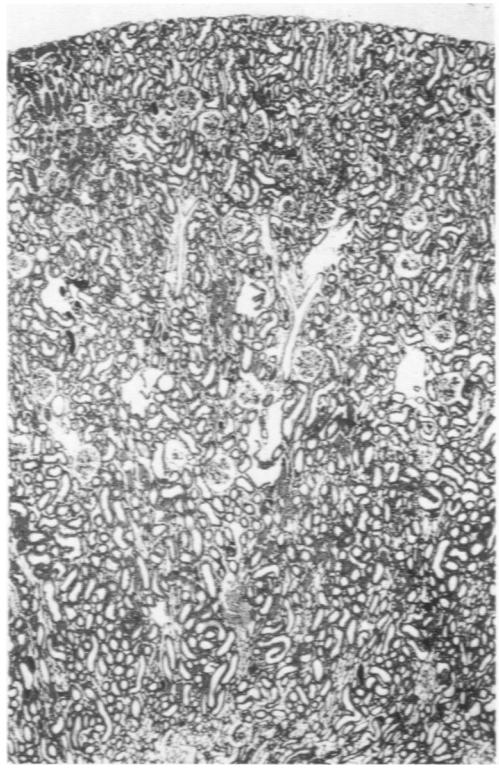

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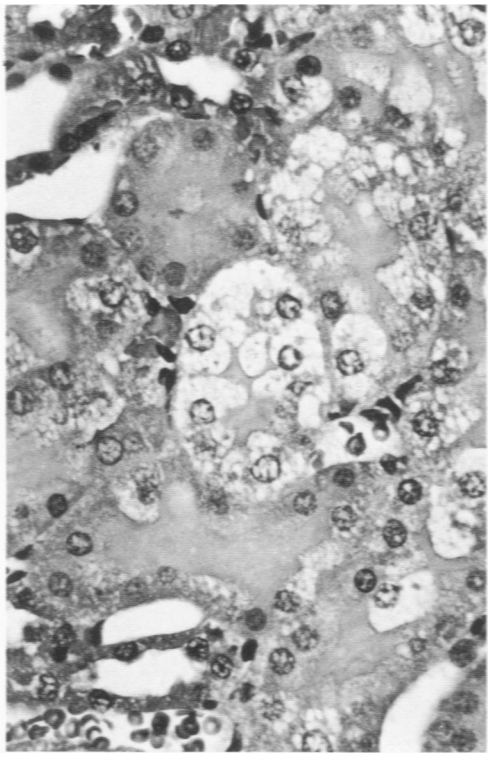

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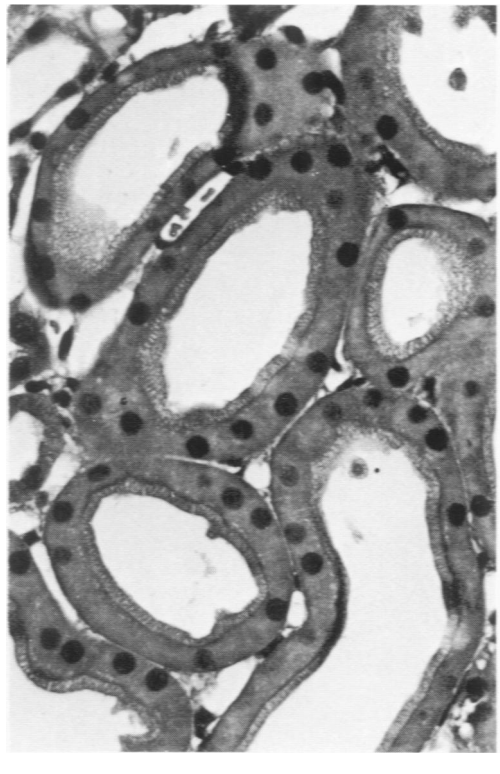

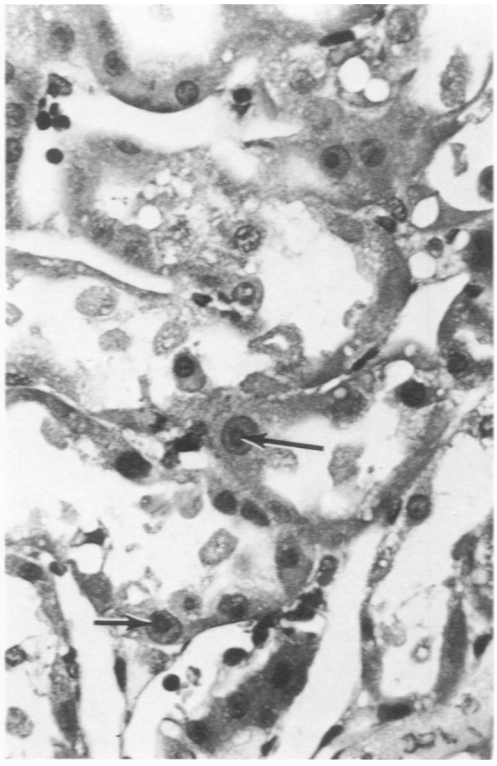

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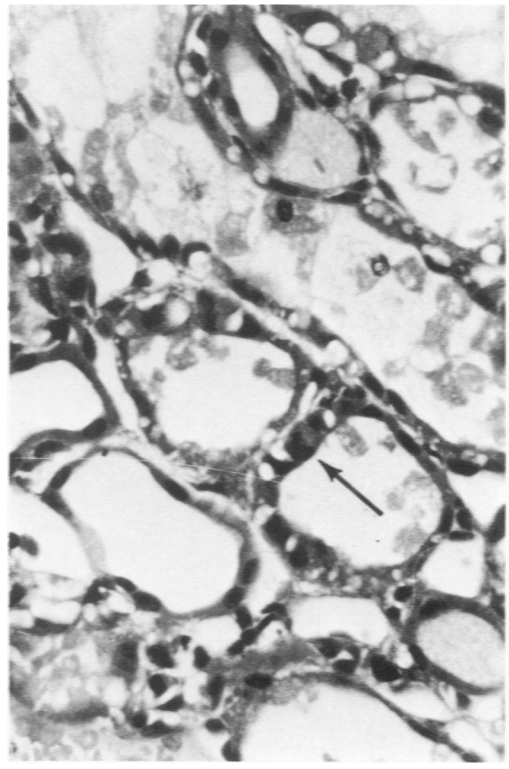

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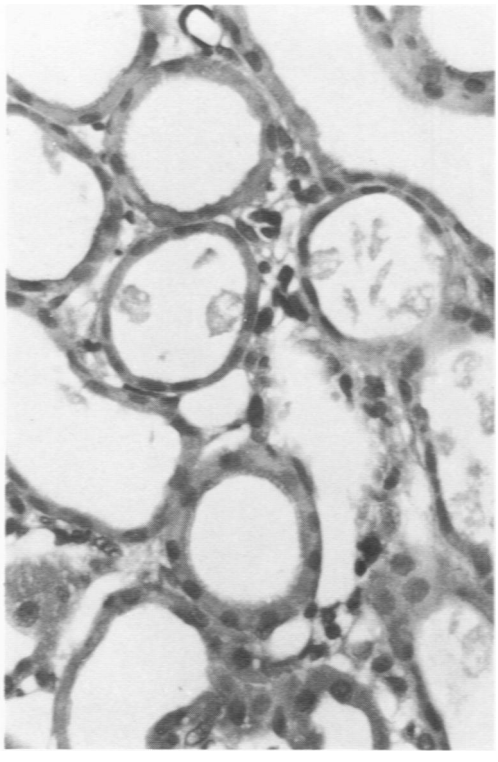

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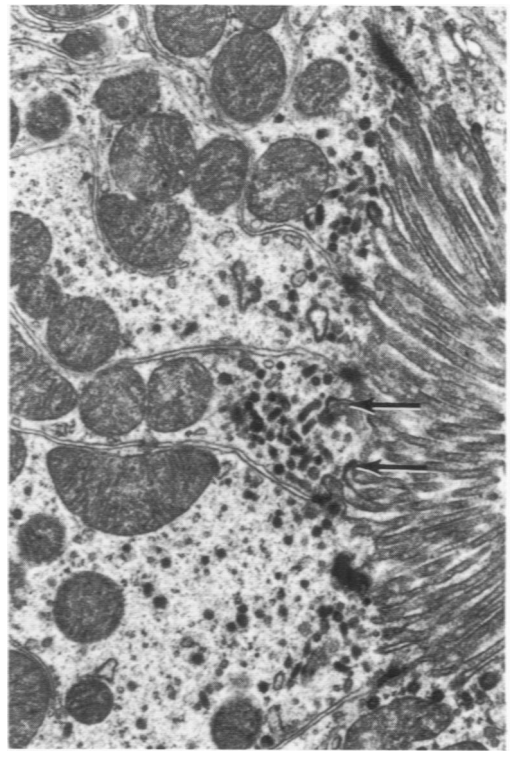

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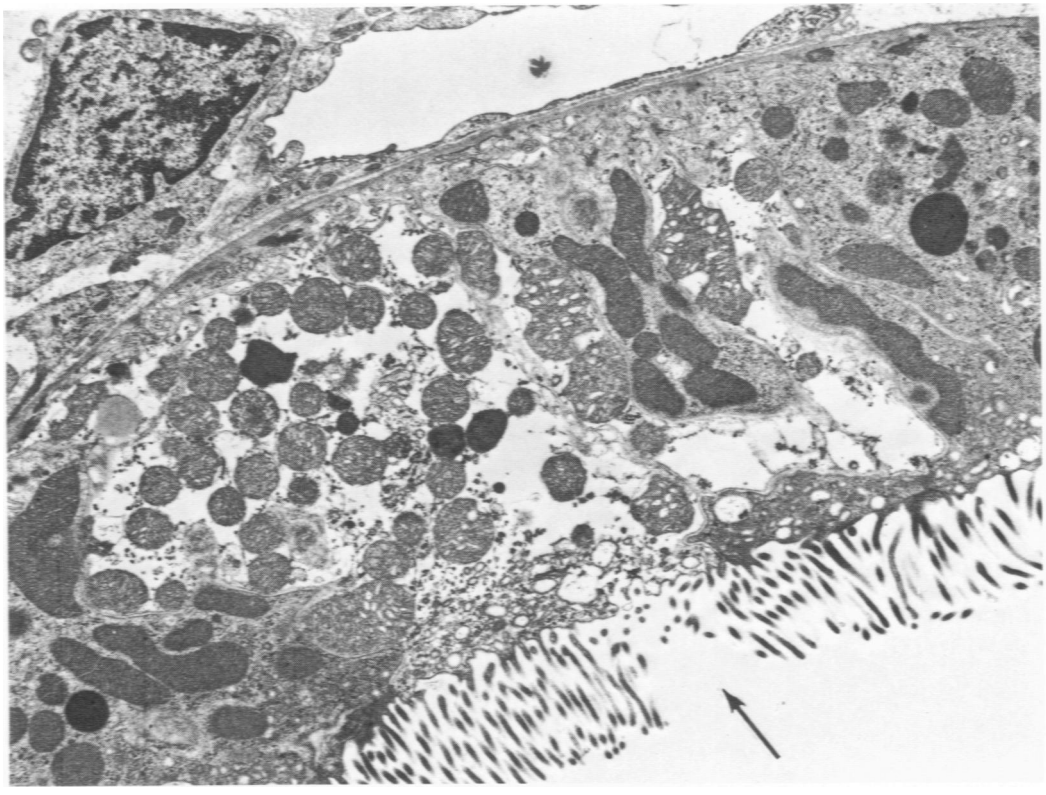

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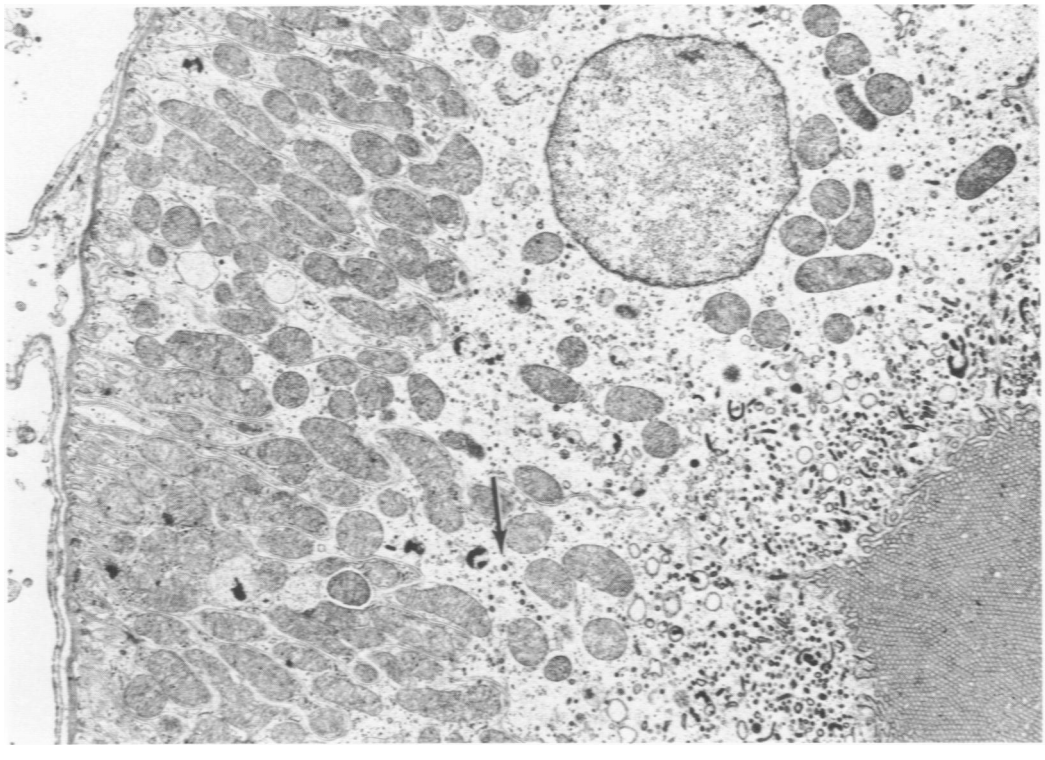

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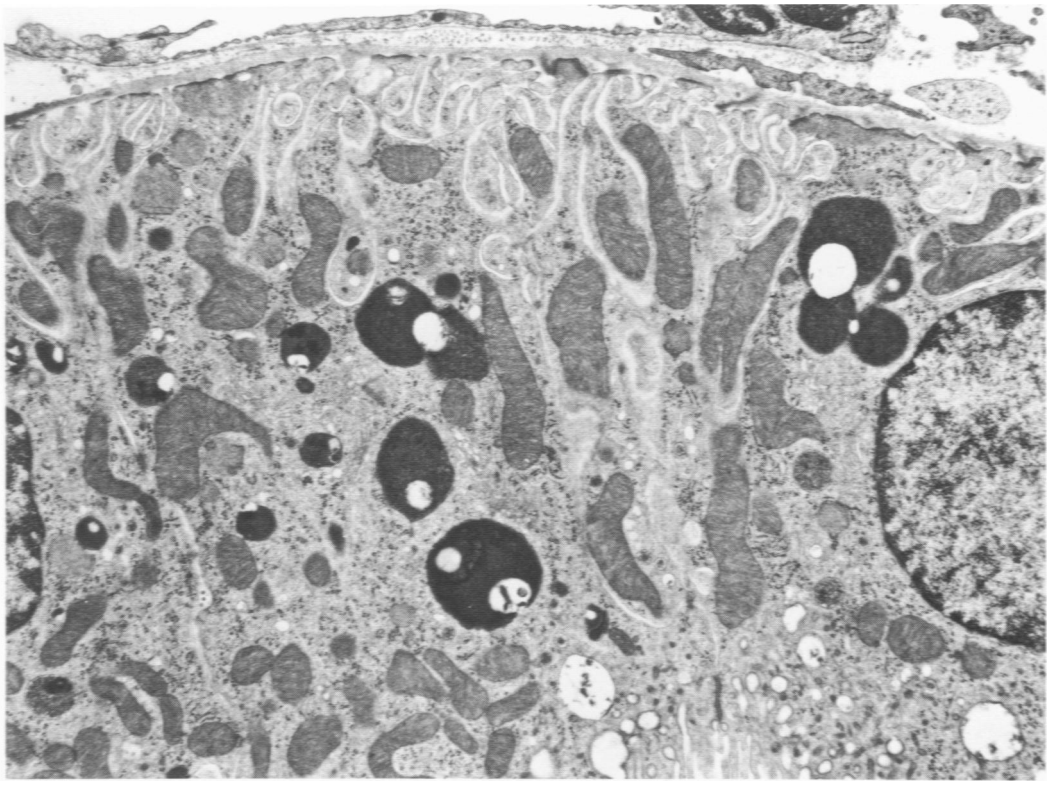

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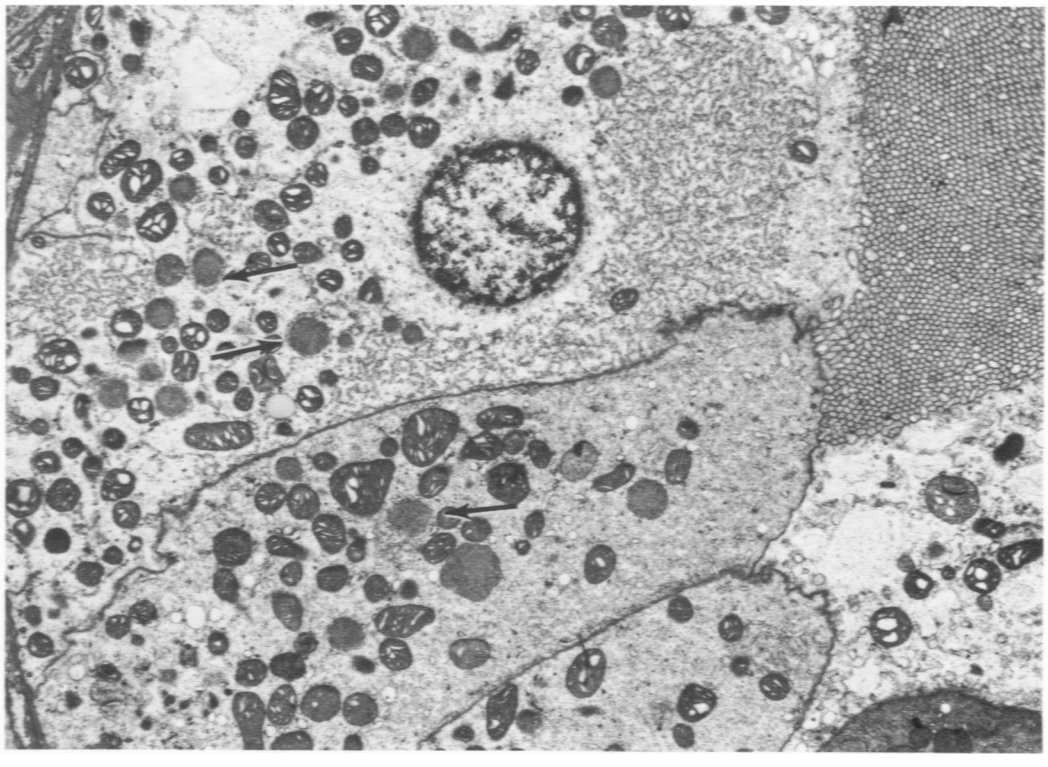

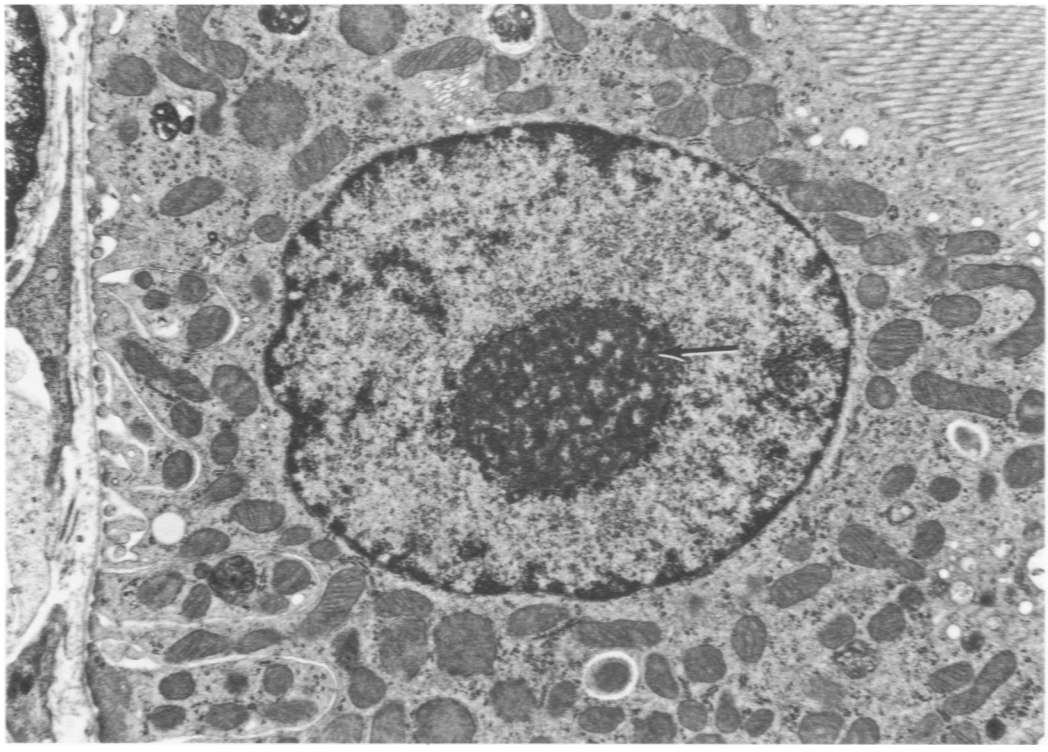

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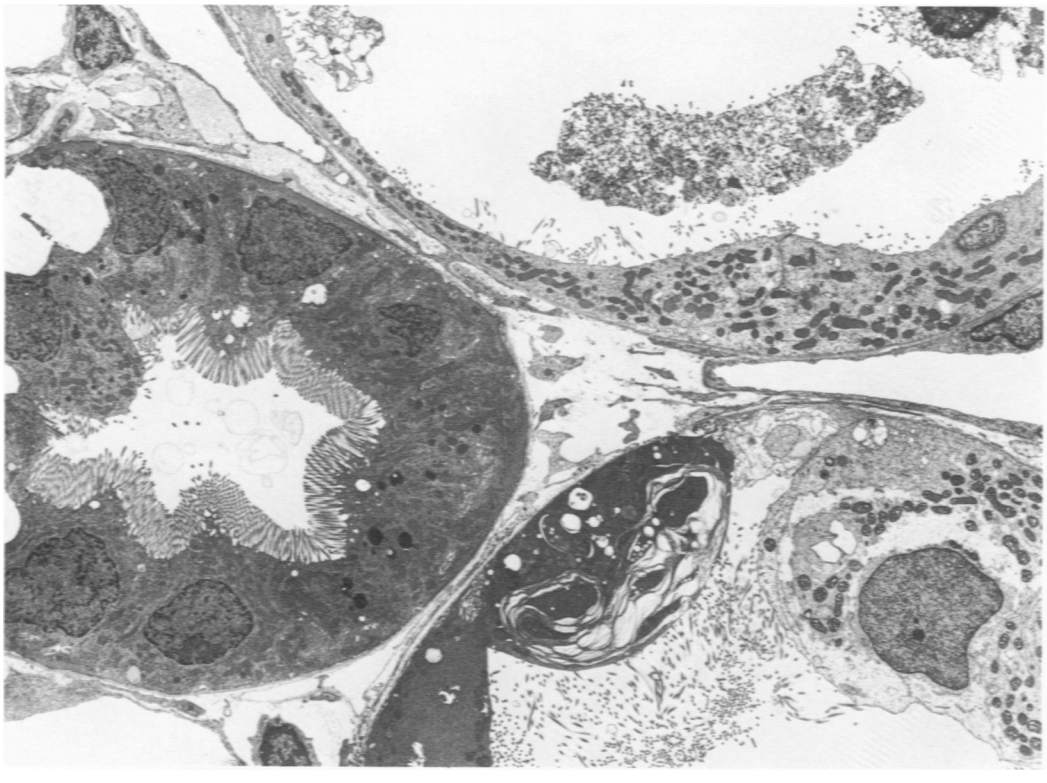

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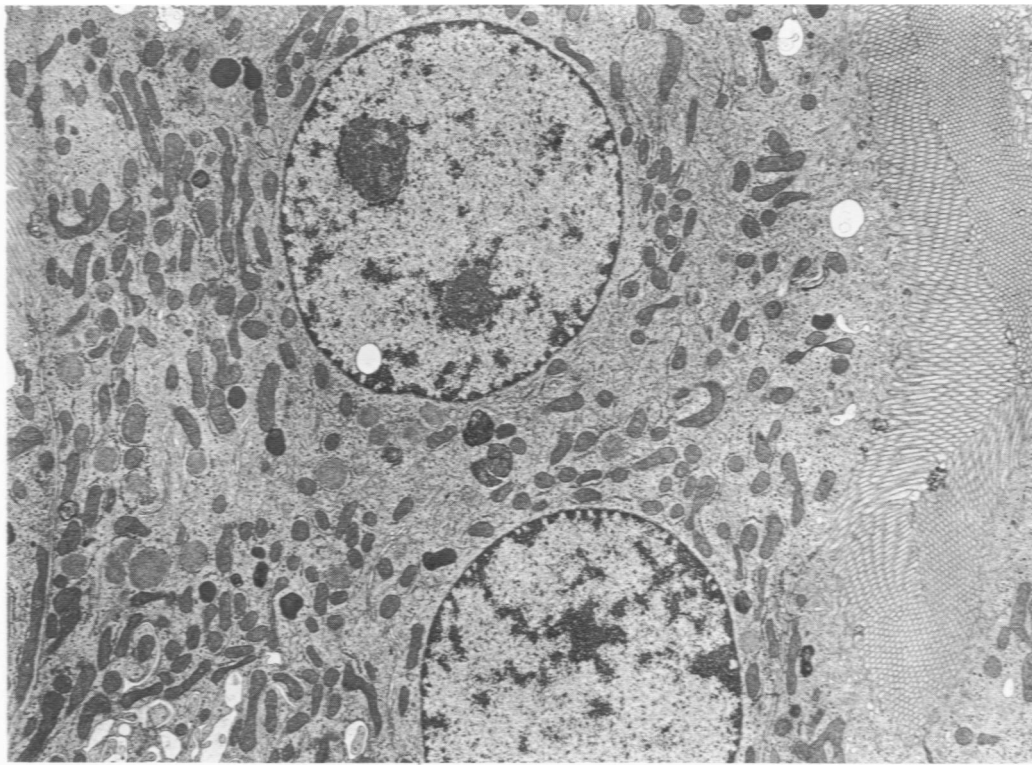

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