The Proximal Tubule Cell in Experimental Hydronephrosis. BY ALEX B. NOVIKOFF. (*From the Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, New York.*)*‡

The purpose of this report is to record several observations made in a study of hydronephrosis produced in rats by unilateral ligation of the ureter.

It has been known from the work of Wilmer (1) and Wachstein (2, 3) that the staining of the proximal tubule brush border for alkaline phosphatase activity is diminished in experimental hydronephrosis. However, the speed with which this decrease occurs has not been appreciated, possibly because earlier studies have utilized paraffin sections of tissue fixed in acetone or alcohol. With frozen sections of cold formol-calcium-fixed tissues (4), diminution of staining begins at the earliest time studied, 3 hours after ligating the ureter (Fig. 1), and is pronounced by 6 hours postligation.

Often it is tacitly assumed or explicitly stated that when staining for enzyme activity is decreased, *enzyme inhibition* has occurred. Our observations put a different aspect on such "inhibition." Alkaline phosphatase decreases in those areas of dilated proximal tubules where cytoplasmic spheres, with or without nuclei, are being extruded into the lumina (Figs. 1 and 2). Electron microscopy shows that the brush border has

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partially or completely disappeared from these areas. In some regions the microvilli are thinned out considerably (Fig. 4); in others they are completely absent (Fig. 5).

Generally, enzyme "inactivation" in a structure implies loss of biochemical function but maintenance of morphological integrity. In the situation we are describing it appears that the loss of morphological structure (brush border) includes the loss of enzymes localized in it (alkaline phosphatase and probably others).

Similar observations have been made by us in aminonucleoside-nephrotic rats. There are always some dilated proximal tubules with diminished alkaline phosphatase activity. These seem to be parts of nephrons in which internal hydronephrosis has been created by casts (*cf.* reference 5). These casts, visible in the lower portions of the nephron, possess alkaline phosphatase activity. Electron microscopy shows that in the cells of the dilated proximal tubules the brush border is thinned or absent. Extruded cytoplasmic masses, sometimes with nuclei, are present in the lumina. It seems reasonable that the microvilli are the source of the alkaline phosphatase in the casts lower in these nephrons.

It may be suggested, on the basis of Fig. 13 of Farquhar *et al.* (6), that similar cytoplasmic extrusions occur in some of the nephrons of nephrotic human kidneys.

The second observation which we have made in the hydronephrotic rat kidney may be of considerable interest in relation of the "lysosome" concept. This was first formulated in 1955 by de Duve and colleagues (7) and more fully developed by him in 1959 (8). De Duve has proposed that the granules which contain acid phosphatase and other hydrolytic enzymes are "suicide bags" whose enzymes become active during cell autolysis.

Up to a fairly advanced stage of dilatation, the proximal tubules of hydronephrotic kidneys show essentially normal acid-phosphatase "droplets" (9, 10). In the normal rat, they are roughly spherical, and in preparations stained for acid phosphatase activity their diameters range from 0.3 to 1.3 μ (average 1.0 μ). They have a roughly linear arrangement and appear more numerous in the region of the Golgi apparatus. The latter, clearly evident in Aoyama silver preparations, often has a light brown color in acid phosphatase preparations.

Electron micrographs of normal proximal tubule cells show vacuoles bounded by thin single membranes, frequently torn and usually containing some granular material. These vacuoles have the same location, size, and frequency as the acid phosphatase droplets seen in light micrographs. The product of acid phosphatase reaction (lead phosphate) can be observed in these vacuoles in electron micrographs of tissue fixed in formol-calcium and then incubated for enzyme activity (Fig. 6).

In the 6 hour hydronephrotic kidney many of these vacuoles contain within them small amounts of cytoplasm, including mitochondria which appear to be in varying states of disintegration (Figs. 7 and 8). From our observations we cannot suggest the origin of the vacuoles, or their relation to the more apical vacuoles apparently continuous with the cell membrane, or the manner in which cytoplasmic fragments come to lie within them. Clark (11) has observed similar vacuoles in the proximal tubules of newborn mice; they "consist of an amorphous material and contain concentrically lamellar structures and mitochondria."

We noted another interesting finding in those proximal tubule cells which are irreversibly damaged by hydronephrosis. About 4 to 7 weeks following removal of the ligature from a ureter obstructed for 6 hours, there are isolated areas of degenerating cells. Blood capillaries (rich in apparent ATPase activity) have extensively infiltrated into the area, and the tubule cells for the first time have lost most of their mitochondrial DPNH-tetrazolium reductase activity. In these degenerating tubule cells the acid phosphatase activity is present not in small droplets but in large irregular masses (Fig. 3). In unpublished experiments, we have observed similar enlarged acid phosphatase structures in dying liver cells following bile duct ligation and in necrotic cells of adenomata produced in the livers of rats fed the carcinogen, 3′-methyl-dimethylaminoazobenzene.

The presence of degenerating cytoplasm in vacuoles corresponding to the acid phosphatase droplets in the early stages of cell damage, and the marked development of acid phosphatase structures in cells in terminal stages of degeneration, give support to the suggestion of de Duve that lysosomes function in cell autolysis.

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Explanation of Plates

Plate 86

Fig. 1. Frozen section of cold formol-calcium–fixed kidney of the rat 3 hours following ligation of ureter. Stained for alkaline phosphatase activity by the method of Gomori (12); incubation 3 minutes at 37°C. Note cytoplasmic masses (*c*), sometimes with nuclei (*n*), extruded into lumina. At one place (arrow) a portion of the stained brush border appears to be separated. Magnification, × 350.

Fig. 2. Frozen section of cold formol-calcium–fixed kidney of the rat 6 hours following ligation of ureter. Stained for acid phosphatase activity, by the method of Gomori (12); incubation 20 minutes at 37°C. Note cytoplasmic masses (*c*), sometimes with nuclei (*n*), extruded into lumina. Most of the acid phosphatase "droplets" are of normal size. Magnification, × 730.

Fig. 3. Frozen section of cold formol-calcium–fixed kidney 7 weeks following removal of ligature which had tied the ureter for 6 hours. Stained for acid phosphatase activity, as in Fig. 2. The two tubules to the right show essentially normal acid phosphatase–rich droplets. In the center, a degenerating tubule shows large, irregular acid phosphatase–rich masses. Magnification, × 780.

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PLATE 86
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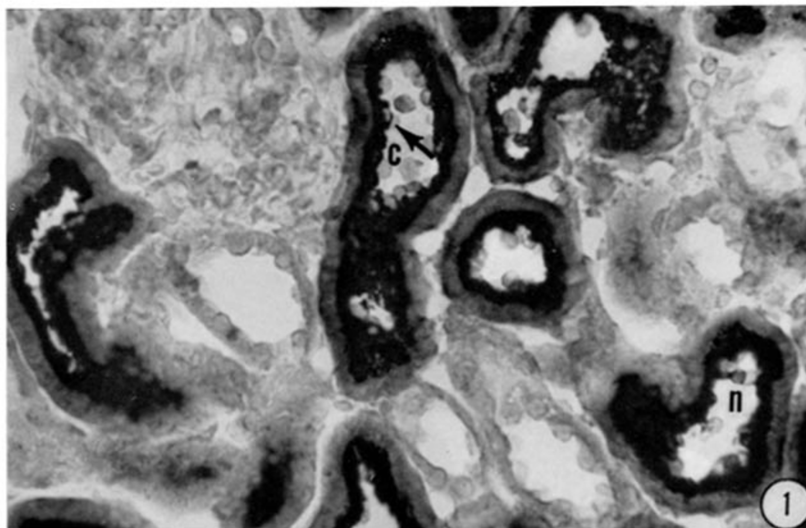

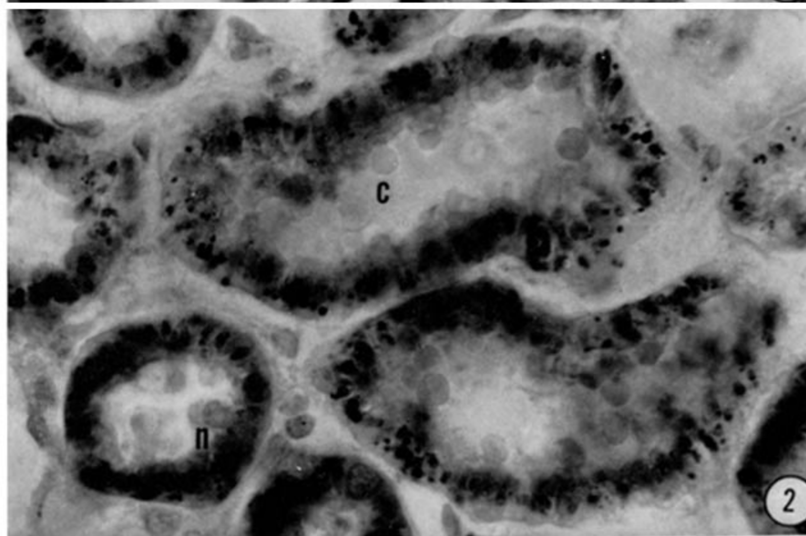

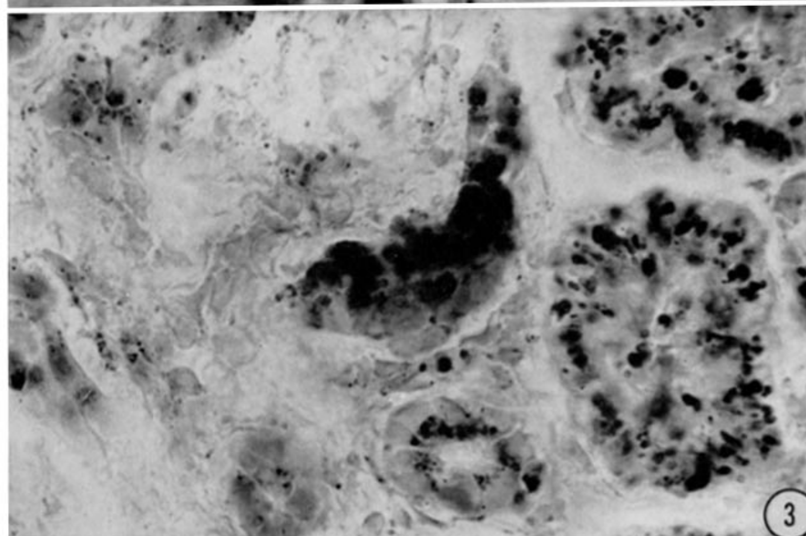

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PLATE 87

FIG. 4. Highly magnified portion of the luminal surface of a cell in a dilated proximal tubule of the rat kidney 6 hours after ureter ligation. The tissues used for this and the electron micrographs in Figs. 5, 7, and 8 were fixed in buffer osmium-tetroxide (13), 1 hour; kept in buffered formalin (14) overnight; dehydrated in alcohols and embedded in uranium-containing methacrylate (15). Sections were cut on a Servall Porter-Blum microtome and examined with an RCA EMU 3B microscope.

The cell surface appears to have lifted away from the cytoplasm, and most of the microvilli (brush border) have disappeared. In the more intact cytoplasm may be seen the mitochondria, dense particles, and vacuoles. Magnification, × 10,000.

FIG. 5. Dilated proximal and distal tubules in the rat kidney 6 hours after ligation of ureter. In the proximal tubule, the brush border and cytoplasm are completely missing above the nucleus. The mitochondria are essentially normal. Clear vacuoles are seen near the base of brush border. In the distal tubule, considerably dilated, note essentially normal mitochondria and cell membranes where adjacent cells interdigitate (16). Magnification, × 9,100.

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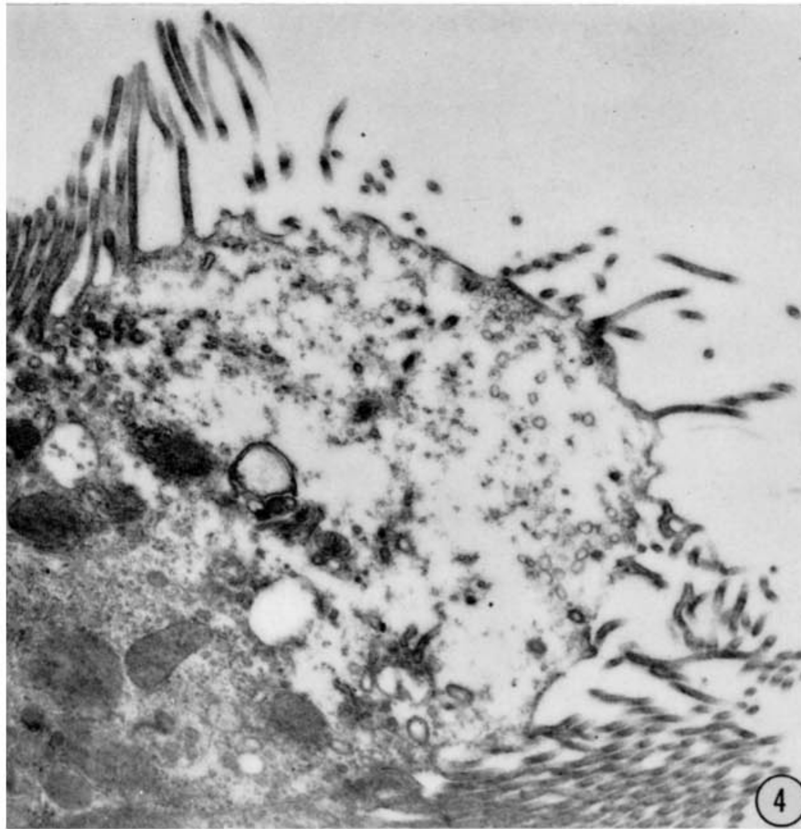

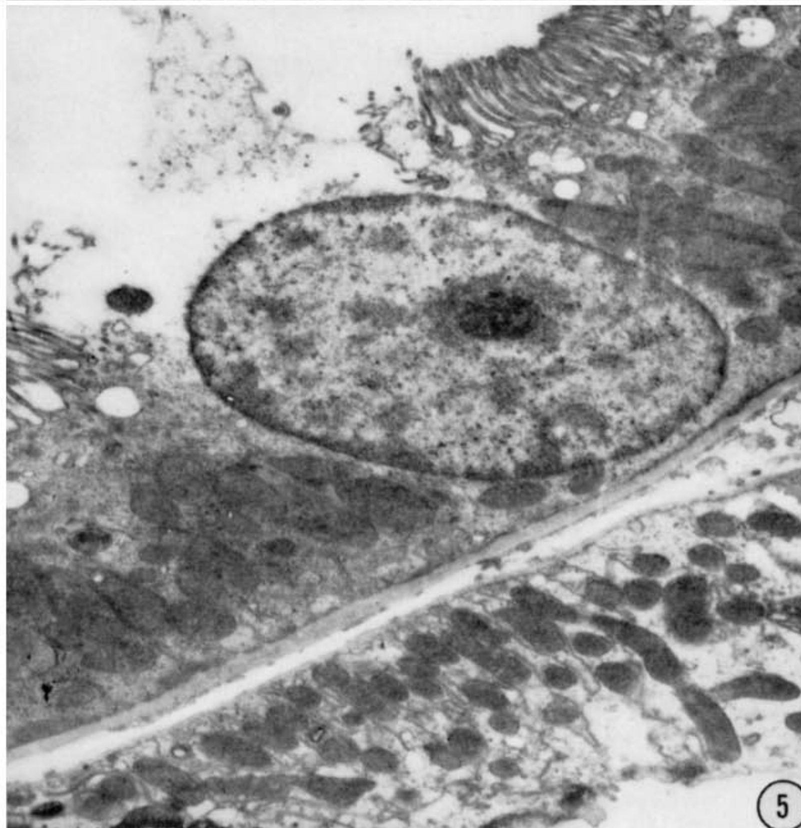

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PLATE 88

FIG. 6. Proximal tubule cell of normal rat kidney incubated for acid phosphatase activity. Tissue processed in fashion of Kaplan and Novikoff (17). Following overnight fixation in cold formol-calcium, 25 μ sections were cut on a Bausch and Lomb freezing microtome and incubated in the Gomori acid phosphatase medium for 2 hours at 2°C. They were then treated for 10 minutes with cold buffered osmium tetroxide, dehydrated in alcohols, and embedded in uranium-containing methacrylate, in the usual fashion.

Note: (1) fairly extensive damage to fine structure, probably resulting from freezing, thawing, and incubation; (2) small dense deposits of lead phosphate throughout the tissue, probably resulting largely from enzyme diffusion; and (3) the extensive lead phosphate deposit within the droplet (arrow). Magnification, × 20,000.

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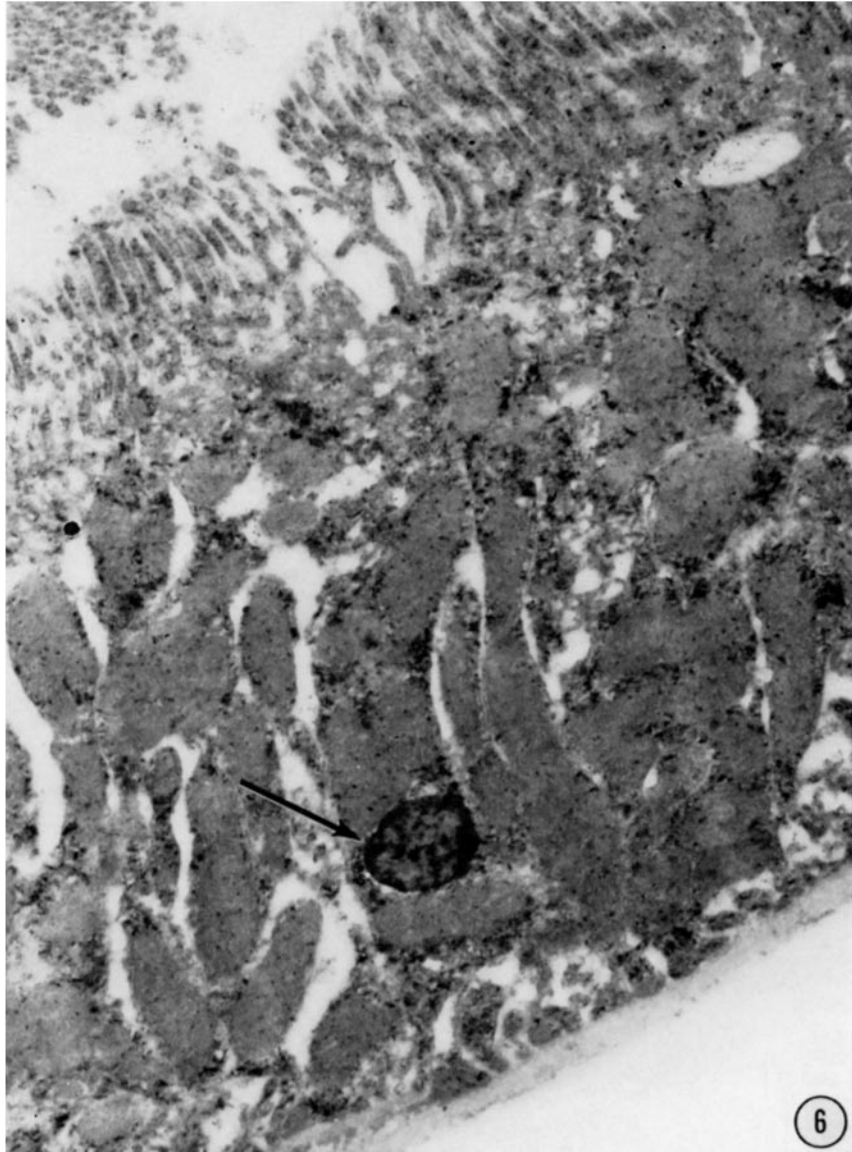

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PLATE 89

FIG. 7. Proximal tubule in the rat kidney 6 hours after ligation of ureter. Note portion of extruding cytoplasm, with nucleus, from which the brush border microvilli have largely disappeared. Elsewhere, clear vacuoles are seen near the base of the brush border. Several acid phosphatase droplets are seen. Inside of one a mitochondrion (*mi*) is seen. Note the outer membrane (*m*) of the droplet. Magnification, × 10,000.

FIG. 8. Proximal tubule in the rat kidney 6 hours after ligation of ureter. Note fairly normal mitochondria, cell membranes in area of interdigitated cells, clear vacuoles below the brush border, and nucleus (to the right). The single membranes (*m*) of the acid phosphatase droplets near the center of the photograph are not clearly visible, except in some areas. Within these droplets may be seen: (1) fairly normal mitochondria, with cristae, and (2) myelin-like layers apparently arising from degenerating mitochondria (*d*). At the left, large acid phosphatase droplets, with damaged membranes, may be seen. Magnification, × 16,000.

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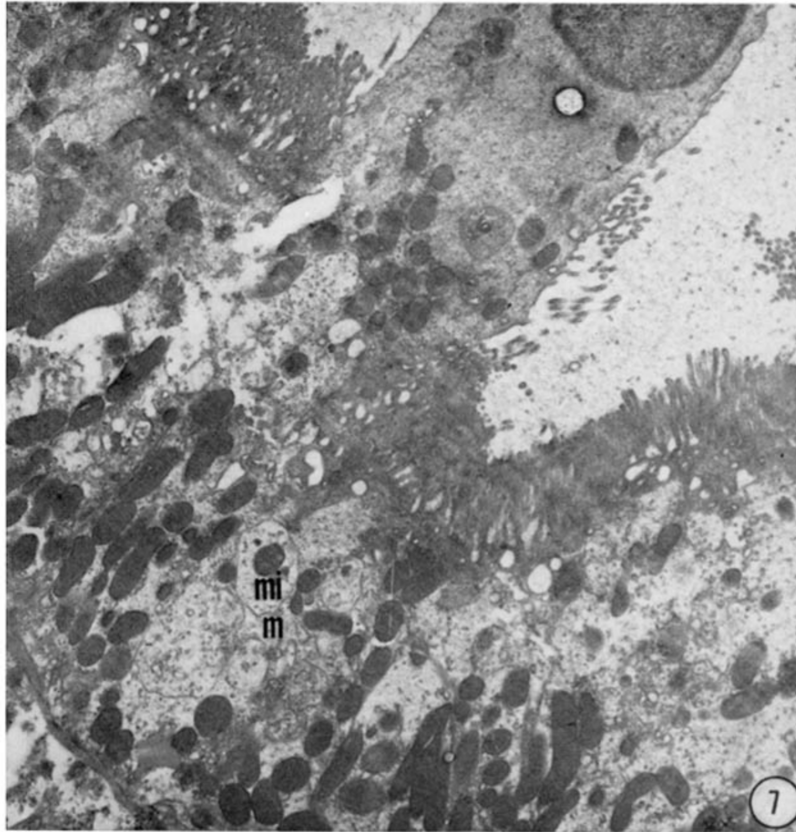

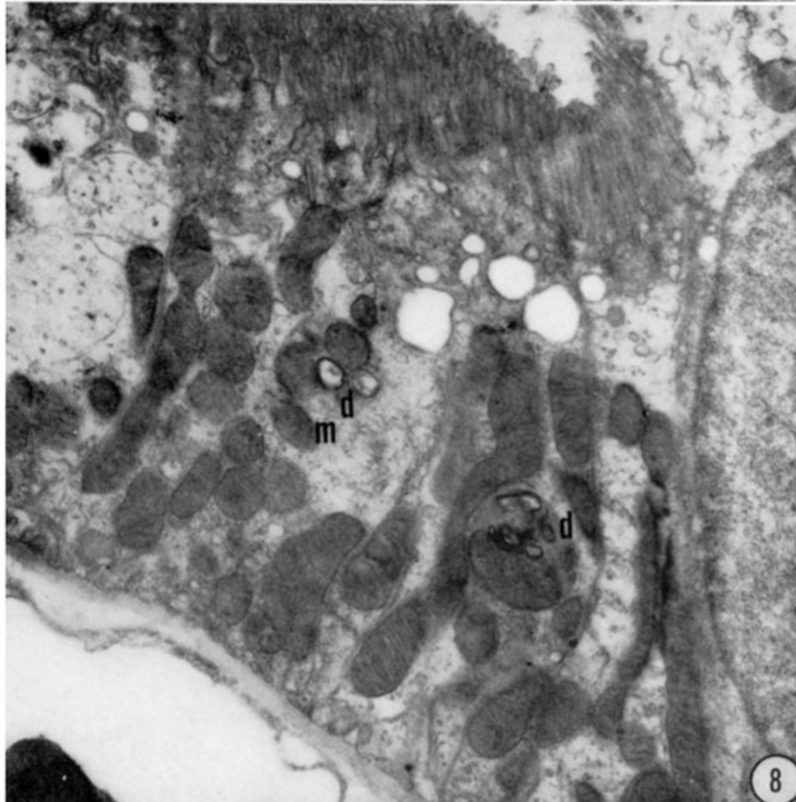

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