Effects of a Single Dose of Cyclophosphamide on Various Organs in the Rat

IV. Electron Microscopic Study of the Renal Tubules

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THE URINIFEROUS TUBULES constitute the main pathway for the elimination of many cytotoxic metabolites of cyclophosphamide (Cytoxan, Endoxan). Three hours after this alkylating agent is injected, the urine contains about 30% of the total amount of cyclophosphamide injected and its metabolites.[1] This is probably the cause of the extensive necrosis of epithelial cells of the urinary bladder of the rat injected with a single dose of cyclophosphamide.[2,3]

The observations, as well as reports of the nephrotoxicity of cyclophosphamide as evidenced by the necrosis of tubular epithelium in man [4] or in experimental animals,[5] form the background of our investigation on the effects of a single dose of this alkylating agent on the epithelial cells of the uriniferous tubule.

Materials and Methods

Female Fischer 344 rats weighing approximately 170 g were given a single intraperitoneal dose of 200 mg/kg of cyclophosphamide in distilled water, as in prior experiments.[2,3]

Three animals were sacrificed at each of the following time intervals after injection: 10, 20 and 30 minutes, and 1, 2, 5, 8, 12, 24 and 48 hours.

Control animals were injected intraperitoneally with 1.7 ml of isotonic saline solution; two animals were sacrificed at each of the following time intervals after injection: 20 minutes and 1, 5, 8 and 24 hours. Kidneys were removed under ether anesthesia. Coronal sections of the kidney cortex were placed in a drop of OsO_4, divided into small cubes and rapidly fixed in chilled phosphate-buffered 1% OsO_4 for 2 hours.[6] After dehydration in graded alcohols, tissues were embedded in a mixture of epoxy resins.[7] Thick sections were stained with toluidine blue. Thin sections were cut on a Porter-Blum ultramicrotome and stained with uranyl acetate and lead citrate,[8,9] coated with carbon and examined with a Siemens Elmiskop 1A electron microscope.

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Results

Light Microscopy

Controls. This group of animals showed no obvious architectural alterations at any time.

Cyclophosphamide-Treated Animals. A few vacuoles in the apical portions of cells of proximal and distal tubules were found in animals sacrificed after 1 hour. There were no other changes of note after this time.

Electron Microscopy

Controls. Ultrastructure of the kidneys of control animals was not altered and similar to that described under normal conditions.[10,11]

Cyclophosphamide-Treated Animals. There were some differences in the degree of ultrastructural change among the 3 batches of animals, perhaps because of some variation in the commercially purchased drug. The findings, as described, represent the maximal changes observed.

PROXIMAL TUBULES. The most striking changes were found in the proximal convoluted tubules. Ten minutes after the drug was administered, the cisternae of both smooth and rough endoplasmic reticula (SER and RER) and of the Golgi complex were moderately dilated. Free ribosomes were seen in the cytoplasm of some cells. Dilatation of endoplasmic reticulum became prominent after 20 minutes (Fig 1) and was also observed in the perinuclear spaces. Moderate enlargement of peroxisomes (microbodies) was first observed at this time. Some of the peroxisomes were seen in close contact with dilated profiles of SER and contained circular or tubular profiles at the internal surface of their limiting membrane (Fig 1 inset). In some segments of tubules, cellular alterations were more advanced: these changes ranged from more extensive dilatation of the ER to necrosis of individual cells. However, it must be pointed out that, in every area investigated, some cells had minimal or no evident damage.

One hour after cyclophosphamide was administered, the swelling of peroxisomes and dilatation of the ER cisternae increased. Cell disruption and necrosis were present in many cells, some of which were desquamated in the lumen of the tubules (*cf* Fig 5). Some epithelial cells of the proximal tubules appeared devoid of microvilli and were markedly swollen; vacuolar and hydropic changes with the formation of membrane-bound cytoplasmic vacuoles were observed. Small focal aggregates of SER were also observed in many of the cells at this time (Fig 2).

Two hours after cyclophosphamide, the changes were essentially the

same, although cell necrosis and desquamation were at their peak. Many cells were markedly swollen and had breaks in their basement lamina or luminal plasmalemma with extrusions of cell organelles. In such cells, the mitochondria were swollen and there was dilatation of the cristae. Aggregates of the SER were still seen at this time.

Between 5 and 12 hours after cyclophosphamide, some of the epithelial cells exhibited collections of peroxisomes in the apical portion of the cell (Fig 3). In numerous cells the enlargement of peroxisomes was now very marked. Peroxisomes 4 μ in diameter were frequent; many of them had a peculiar, nipple-like projection (Fig 4). The peroxisomes were usually surrounded by cisternae of SER. Nucleoids were large and distinctive. Matrix density appeared decreased in some peroxisomes. Rupture of the limiting membranes of peroxisomes was occasionally observed, particularly at 12 hours (Fig 5). Other organelles in the same cells appeared morphologically normal.

Occasionally, two peroxisomes of approximately normal size were observed within the same envelope of endoplasmic reticulum (Fig 6).

Some dilatation of ER was found in only a few of the cells from animals killed 12 hours after the injection of cyclophosphamide. Aggregates of SER were seen only occasionally after this time. Cell necrosis was markedly diminished or absent and continued to decrease. Forty-eight hours after the injection of cyclophosphamide, only minor changes were found in a few of the epithelial cells. The peroxisomes appeared normal. Throughout the experiment, the number of lysosomes in experimental cells did not differ from that in controls. There were no significant changes in nuclear or nucleolar structures.

DISTAL TUBULES. Changes in the distal tubules were not as prominent as those in the proximal tubules. During the 2 hours after injection, dilatation of ER and cell necrosis were observed. Necrotic desquamated cells were found in the lumen (Fig 7). Five hours after injection, evidence of cell necrosis was minimal. At 8 hours, the morphology returned to near normal.

THE GLOMERULI. Throughout the experiment, no significant evidence of damage was found in the glomeruli.

Discussion

There is pharmacologic evidence that the breakdown of cyclophosphamide into biologically active alkylating compounds takes place principally in the liver.[1] Therefore, the changes observed in epithelial cells of the proximal segment of uriniferous tubules were presumably the result of the cytotoxic effect of metabolites of cyclophosphamide.

This cytotoxicity resulted in necrosis of tubular epithelial cells within the first few hours after the alkylating agent was administered, in a manner similar to that observed in the urinary bladder.[2] Cell necrosis diminished markedly after 8 hours. The lysosomes did not appear to be affected; hence, this effect was probably direct and not the result of storage as, for instance, in hemoglobinuric nephropathy.[12]

The renal changes were observed at the same time or earlier than those produced in hepatocytes under identical experimental conditions.[13] Hence, renal tubular changes were not the result of hepatic alterations.

The disorganization and dilation of RER and SER were more prominent in tubular renal cells than in liver cells. The appearance of focal aggregates of smooth-surfaced endoplasmic reticulum, 1 hour after cyclophosphamide, does not seem to represent diffuse hypertrophy of the SER as observed in hepatocytes.[13] Similar changes may be observed in renal tubular epithelium [14,15] or hepatocytes [16–19] after the administration of other compounds and are usually interpreted as a response to injury. There is evidence, at least in CCl_4-treated animals, that this is the result of direct membrane damage with a decrease in microsomal enzymes and increased rates of breakdown of smooth-surfaced membranes.[17–19]

The striking enlargement of peroxisomes occurring in some animals 5–12 hours after cyclophosphamide has not been previously observed, to the best of our knowledge. No such changes were observed at any time in the peroxisomes of the hepatocytes under identical experimental conditions,[13] although an increase in the number of peroxisomes or alterations in their morphology have been observed in the liver under the influence of other drugs.[20,21] We can only speculate on the nature of the enlargement of renal peroxisomes. Although matrix density appeared to be diminished in some of the largest peroxisomes, in other large peroxisomes such a change was not observed. Therefore, it does not appear likely that the enlargement was due to altered membrane permeability. Furthermore, other adjacent organelles appeared quite normal. Therefore, the possibility that cyclophosphamide stimulates the excessive production of a peroxisome-related enzyme or enzymes must be considered.

Peroxisomes of liver and kidney cells are known to contain a number of enzymes believed to be involved in hydrogen peroxide metabolism.[22–24] Histochemical and biochemical studies to date on peroxisomes of both types of cells have apparently not disclosed any structural or enzymatic differences.[23,25–28] The conspicuous increase in the number of liver peroxisomes after the administration of clofibrate (CPIB; ethyl

chlorophenoxyisobutyrate), a hypolipidemic agent, has led to the supposition that hepatic peroxisomes are related to lipid metabolism[20] and that structural changes in these organelles are related in some way to altered lipid metabolism.[29]

The present observations suggest that the peroxisomes of the liver and kidney do not react in the same fashion to cyclophosphamide and its metabolites. We are unable to state whether the differences observed are due to a different population of microbodies, a different enzyme content or whether the liver peroxisomes may be somehow protected from the effects of the drug. These differences deserve further investigation.

The fate of the large peroxisomes is not clear. Some of them show membrane breaks (Fig 5) and, thus, probably disintegrate. Others have been observed in the apical portion of the cell and are perhaps extruded (Fig 3). The nipple-like protrusions observed in some large peroxisomes (Fig 4) are perhaps linked to the presence of two smaller peroxisomes found within the same envelope of endoplasmic reticulum (Fig 6). It appears far-fetched at this time to suggest that peroxisomes may divide, but this possibility cannot be excluded. In any event, 24 hours after injection the large peroxisomes were no longer observed.

Summary

A single dose of 200 mg/kg of cyclophosphamide, administered intraperitoneally to female Fischer 344 rats, produced changes within the cells of the proximal convoluted tubules. Dilatation of the cisternae of smooth endoplasmic reticulum, rough endoplasmic reticulum and Golgi complex was observed within 10 minutes after the drug was administered and was assumed to represent a toxic reaction to the alkylating products of the drug. This was followed by necrosis of some of the cells, which reached a peak 2 hours after injection. In the surviving cells, the most interesting changes concerned the peroxisomes, which became somewhat enlarged 20 minutes after injection and, 5–12 hours after injection, increased strikingly in size, reaching 4 μ in diameter. This increase was not observed in liver cells under identical experimental conditions and suggests possible differences between hepatic and renal peroxisomes. Possible reasons for the selective enlargement of renal peroxisomes and their possible fate are discussed.

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[Illustrations follow]

Legends for All Figures

Fig 1.—Proximal tubular cells 20 minutes after injection of cyclophosphamide: the endoplasmic reticulum appears markedly dilated. Mitochondria are ovoid and their matrix density is increased. Two peroxisomes (*P*) and several lysosomes (*Ly*) are also identified (× 14,400). **Inset.**—Portion of proximal tubular cell 20 minutes after injection of cyclophosphamide, showing a group of peroxisomes (*P*) with varying degrees of enlargement. Circular and tubular profiles appear attached to their limiting membrane (*arrows*) (× 16,000).

Fig 2.—Epithelial cell of proximal convoluted tubule 1 hour after injection of cyclophosphamide. Focal aggregates of smooth endoplasmic reticulum (*ser*) may be noted.

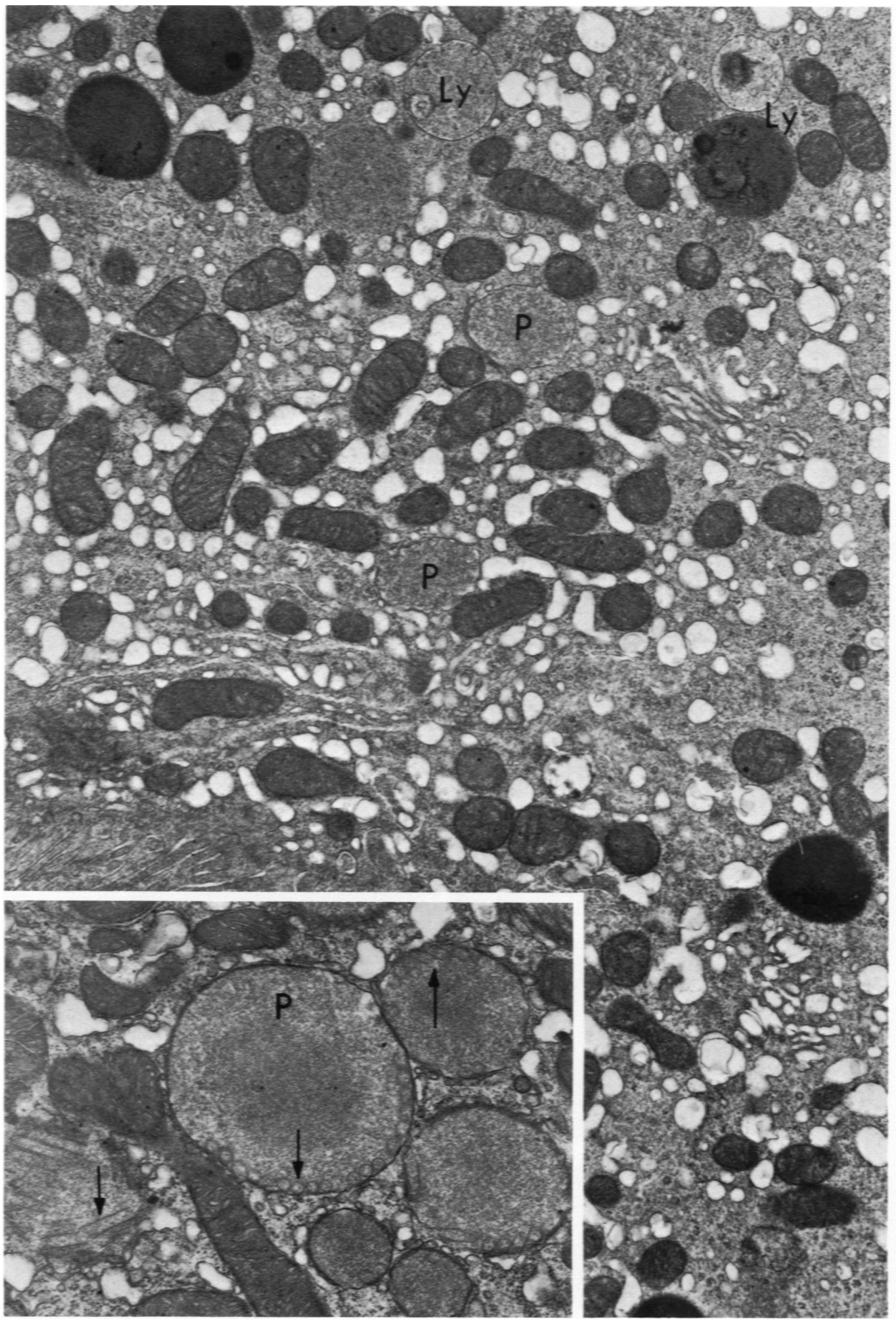
Fig 3.—Proximal tubule 5 hours after injection of cyclophosphamide. Apical portion of epithelial cell containing group of peroxisomes (*P*). Some of them surrounded by cisternae of SER appear trapped among microvilli. Lysosomes indicated by *Ly*, microvilli by *Mv* (×19,800).

Fig 4.—Portion of proximal tubular cell 12 hours after injection of cyclophosphamide, showing markedly enlarged peroxisomes surrounded by SER. Largest peroxisome shows nipple-like projection (*arrow*). Nucleus indicated by *N*, basement lamina by *BM* (× 25,500).

Fig 5.—Same kidney as Fig 4. Enlarged peroxisome with breaks (*arrows*) in its limiting membrane and markedly enlarged nucleoid (× 26,100).

Fig 6.—Same kidney as in Fig 4 and 5. Two peroxisomes of normal size, one with and one without visible nucleoid, enclosed within same envelope of SER (*arrows*) (×20,000).

Fig 7.—Distal renal tubule 1 hour after injection of cyclophosphamide, showing epithelial cells with marked hydropic changes, probably undergoing necrosis. Cast (*C*) of necrotic cells is present in lumen (× 7200).



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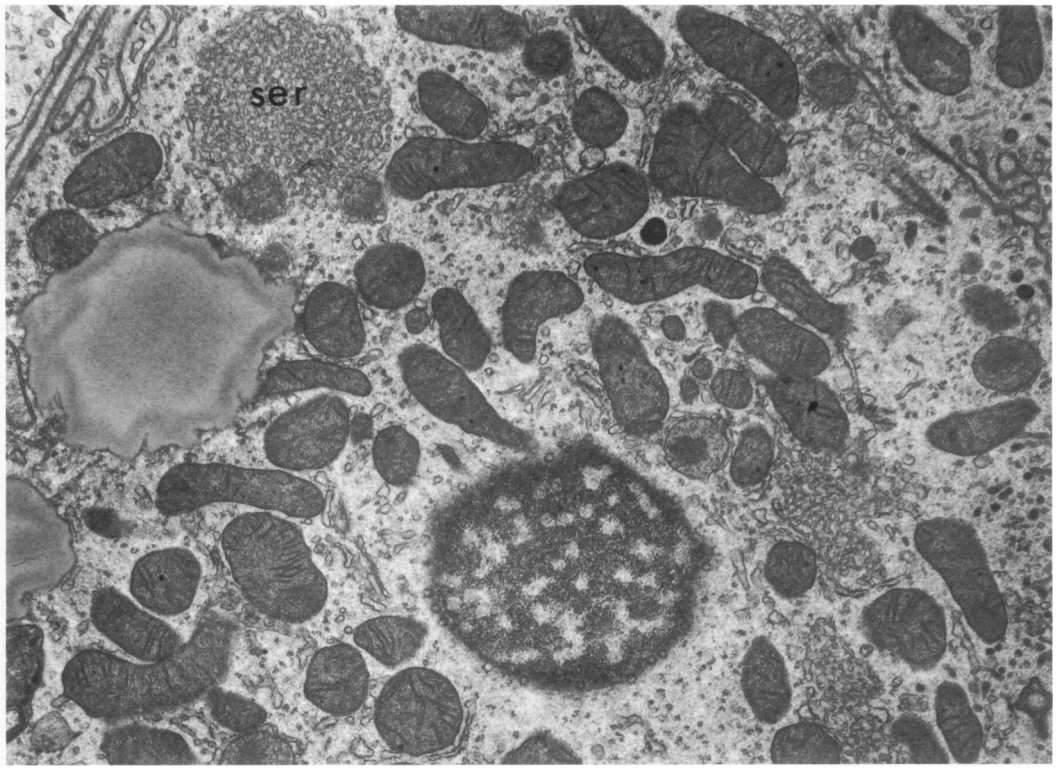

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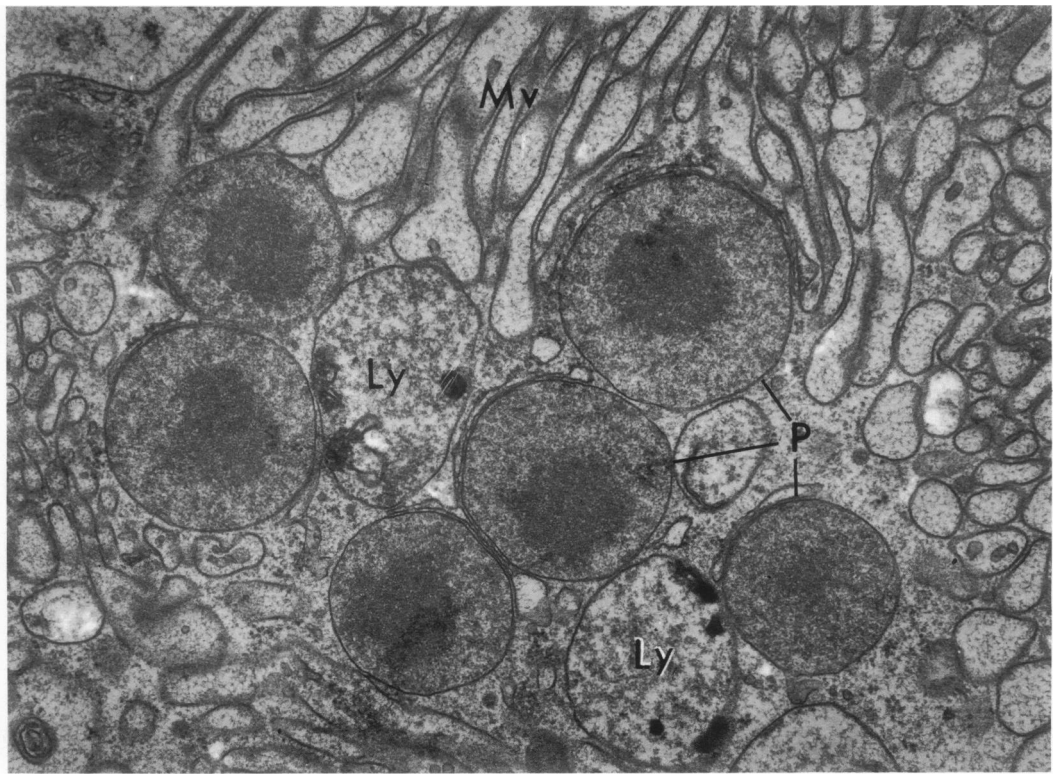

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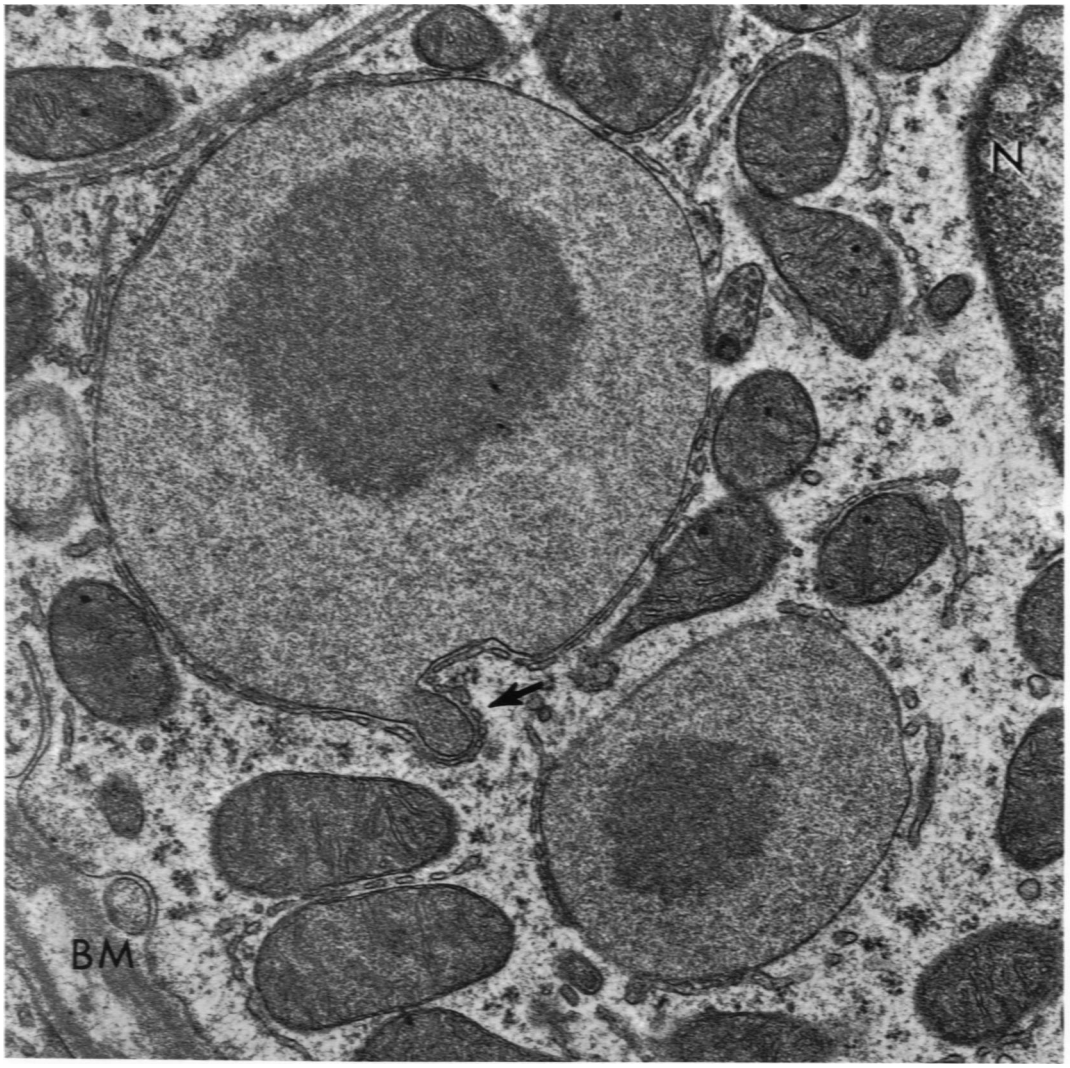

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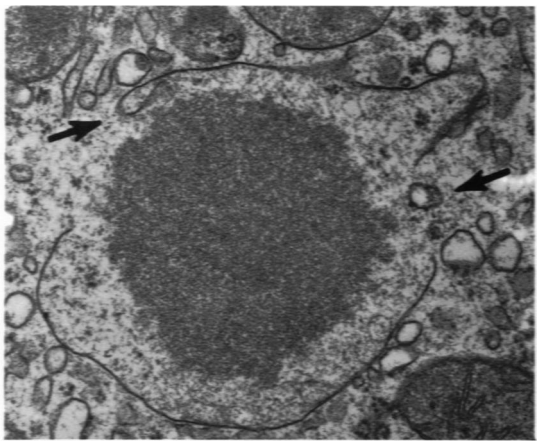

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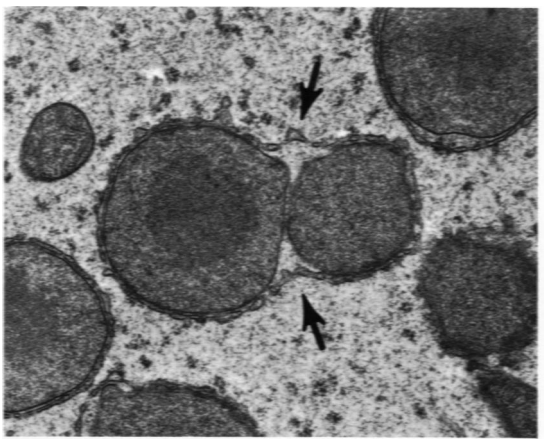

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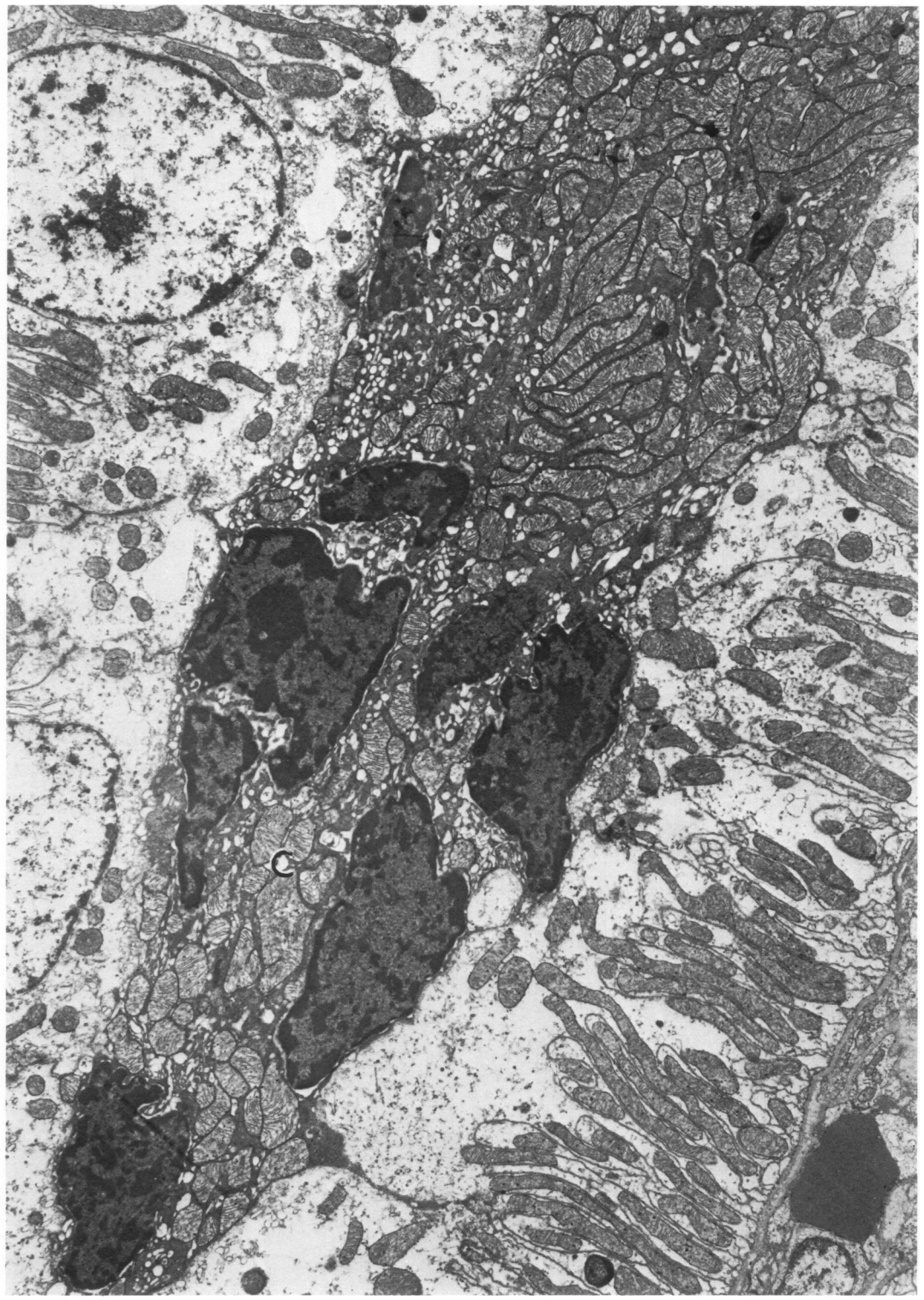

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