CYTOLYSOMES AND MITOCHONDRIAL DEGENERATION

ALEX B. NOVIKOFF and EDWARD ESSNER. From the Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, New York City

In 1957 Clark described vacuoles, in the cells of the proximal convolutions of the kidneys of the newborn mouse, which contained mitochondria within them (7). Similar bodies have been found in cells of the proximal convolutions of rat kidney 6 hours following ligation of the ureter (14). These were identified with large acid phosphatase-rich bodies seen in frozen sections of cold formaldehyde-fixed tissue incubated in the glycerophosphate-lead medium of Gomori for acid phosphatase activity (10) and examined by light microscopy (14).

Enlarged lysosomes have been encountered in acid phosphatase preparations of a variety of cells undergoing physiological and pathological lysis (14, 16, 3–5) and the term "cytolysome" has been suggested for them (16). Only in the kidney were these cytolysomes studied by electron microscopy: "Within these cytolysomes remarkable events are in progress, which can be revealed with the electron microscope. Cytoplasm has somehow found its way inside the droplets and is apparently in the process of digestion. This is suggested by what looks like a progressive degeneration of the mitochondria within the droplets" (16).

In 1962 Ashford and Porter (1) described bodies containing mitochondria in isolated rat livers perfused with glucagon. They described these bodies as lysosomes on the basis of appearance, and wrote,"This is in keeping with what is now the usual practice among cytologists. Admittedly the identification would be more definite if parallel cytochemical studies had been done to

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All figures are from the livers of 200 to 300 gm rats given 400 mg Triton WR-1339 in 2 ml saline, intravenously, 90 minutes before removal of tissue, under pentobarbital anesthesia.

FIGURE 1

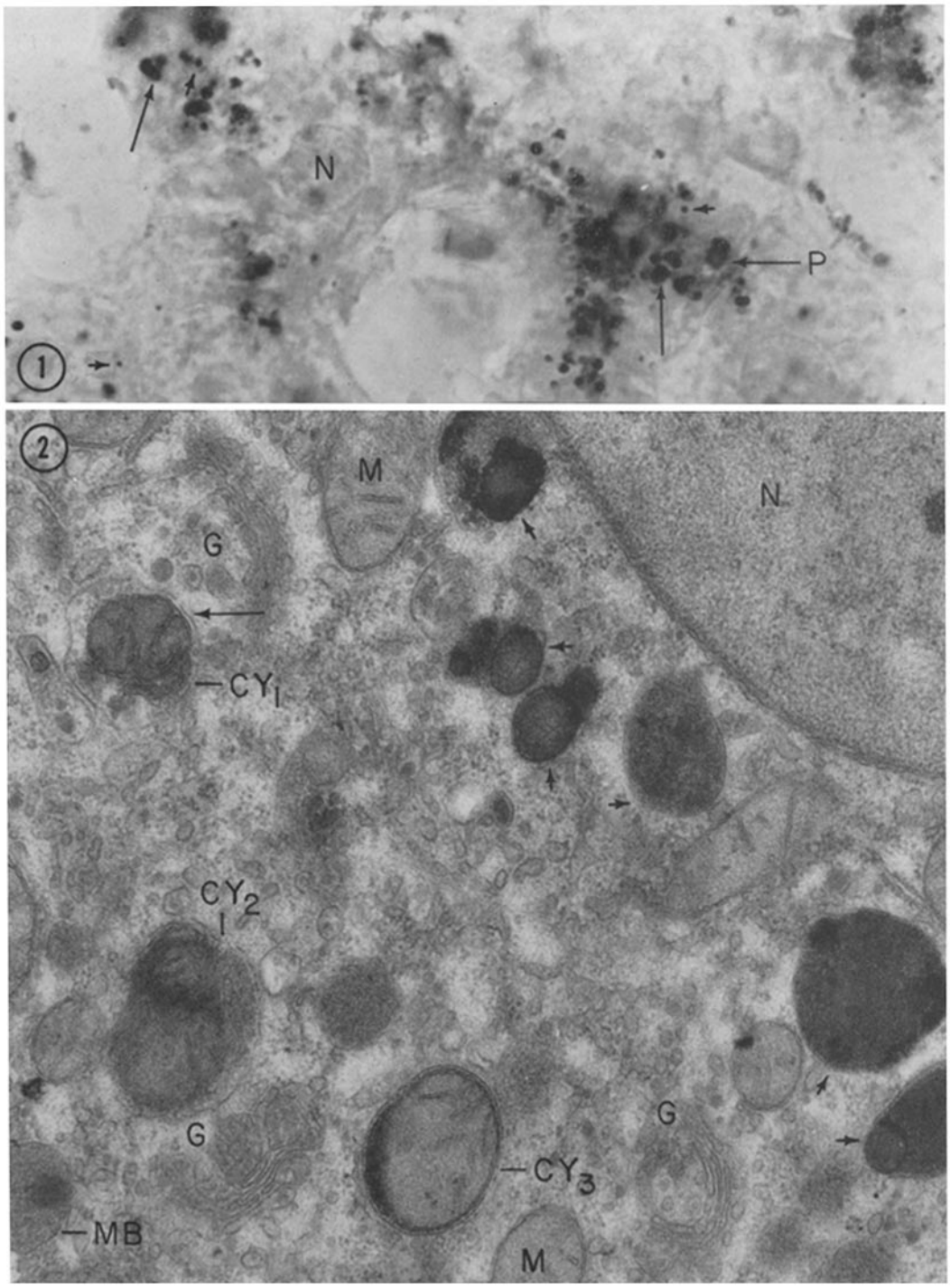
Frozen section (10 μ) of tissue fixed overnight in cold formol-calcium (2), and incubated, 20 minutes at 37°C, for acid phosphatase activity in the medium of Gomori (10). The outline of a parenchymal cell nucleus (*N*) is barely visible.

Short arrows indicate some of the lysosomes in the size range of those in normal liver, and long arrows some of the enlarged lysosomes or cytolysomes. Arrow at (*P*) indicates a cytolysome in proper focus to show restriction of reaction product to its periphery. × 1600.

FIGURE 2

Portion of hepatic cell in tissue fixed in cold osmium tetroxide-sucrose (6) for 1 hour, embedded in methacrylate, and examined following staining of the thin section with saturated $KMnO_4$. The photograph shows a portion of the nucleus (*N*), some apparently unaltered mitochondria (*M*), and a microbody (*MB*). Three regions of Golgi membranes (*G*) are seen. Note the large Golgi vacuoles, *i.e.*, dilatations from the Golgi cisternae, in the lower left region, and the similarity of their contents and material to the area at the right of the mitochondrion in vacuole CY_2. Near CY_1 the Golgi vacuoles are smaller.

In vacuoles CY_1, CY_2, and CY_3, the mitochondrial nature of the enclosed body is evident from the typical cristae; in CY_1 and CY_3 the outer mitochondrial membrane is clearly seen; and at the unmarked arrow in CY_1 the typical relations of outer and inner mitochondrial membranes are seen. In CY_3 some electron-opaque material is seen at the left of the mitochondrion. Not included in this communication, but encountered in many cells, are apparent transitions from such mitochondria to electron-opaque bodies such as seen in the other vacuoles (unmarked arrows). These are interpreted as stages in the degeneration of the mitochondria under the action of acid hydrolases in the vacuole. × 28,000.



determine the presence or absence of acid phosphatase and other acid hydrolases."

The parenchymal cells of livers in all rats injected intravenously with high doses of the detergent, Triton WR-1339 (400 mg/200 to 300 gm rat), show numerous cytolysomes (Fig. 1) in many cells of all areas of the liver examined. The time course of these and other cytological changes, as well as lipid analyses of liver and plasma, will be described elsewhere, by the authors in collaboration with Dr. Paul Roheim and Dr. Luis Biempica.

These cytolysomes differ from the lysosomes of normal liver parenchymal cells, with respect to size and distribution. They are larger (about 0.8 to 1.6 μ in diameter in the acid phosphatase preparations, as compared to 0.4 to 0.8 μ in normal liver) and they are spread more widely in the cytoplasm than the lysosomes of normal liver, which are concentrated in a narrow pericanalicular zone (11, 15, 19). The liver cells of Triton-treated animals also show lysosomes of normal size.

The livers of 6 Triton-treated animals were examined by electron microscopy. Sections from many blocks always show, in addition to normal polymorphic "dense bodies" (18), vacuoles containing mitochondria. As Ashford and Porter found in isolated livers (1), transitions are easily observed, from obvious mitochondria, many with typical cristae and some with intramitochondrial granules, to bodies containing electron-opaque material without signs of cristae or granules (Fig. 2). Almost invariably, the mitochondria-containing vacuoles, particularly those in which the mitochondria have not yet undergone extensive transformation, lie close to the Golgi apparatus (Figs. 2, 6, 3) (see also Fig. 1 in Ashford and Porter (1)). Sometimes the Golgi cisternae are relatively unswollen, and the Golgi vacuoles are unusually dilated (Fig. 2, lower left). More commonly, the Golgi cisternae are very much dilated (Fig. 3). In both instances, the impression is created that mitochondria have somehow been enclosed within a much dilated Golgi cisterna. However, the situation is complicated and the precise mechanisms involved are not clear, even from a great many electron micrographs. Sometimes, it appears that the rough surfaced reticulum forms very many smooth surfaced vesicles, some contributing to the Golgi apparatus (20) and others perhaps contributing more directly to the mitochondria-containing vacuoles.

Unequivocal demonstration of the lysosomal nature (17) of these mitochondria-containing vacuoles is derived from thin sections of liver, from 2 animals, fixed overnight in cold 4 per cent formaldehyde containing 1 per cent $CaCl_2$ (2) to which has been added 5 per cent sucrose (12), then cut on a freezing microtome at 40 μ, incubated for acid phosphatase activity (10) at pH 5.0, treated with osmium tetroxide-sucrose for 30 minutes (6), dehydrated with ethanols and embedded in methacrylate (1:7, methyl:butyl) containing uranyl nitrate (22) or in Epon 812 (13). Thin sections were prepared on a Porter-Blum microtome equipped with a diamond knife. Electron micrographs were taken with an RCA-EMU-3B electron microscope at magnifications of 5,700 to 14,500 and enlarged photographically. Electron-opaque accumulations of enzyme reaction product are present in the vacuoles (Figs. 5 to 7).

FIGURE 3

Portions of hepatic cells in tissue fixed in cold osmium tetroxide-sucrose (6) for 1 hour, embedded in Epon 812 (13), and examined following staining of thin sections with saturated uranyl acetate and with lead hydroxide (9).

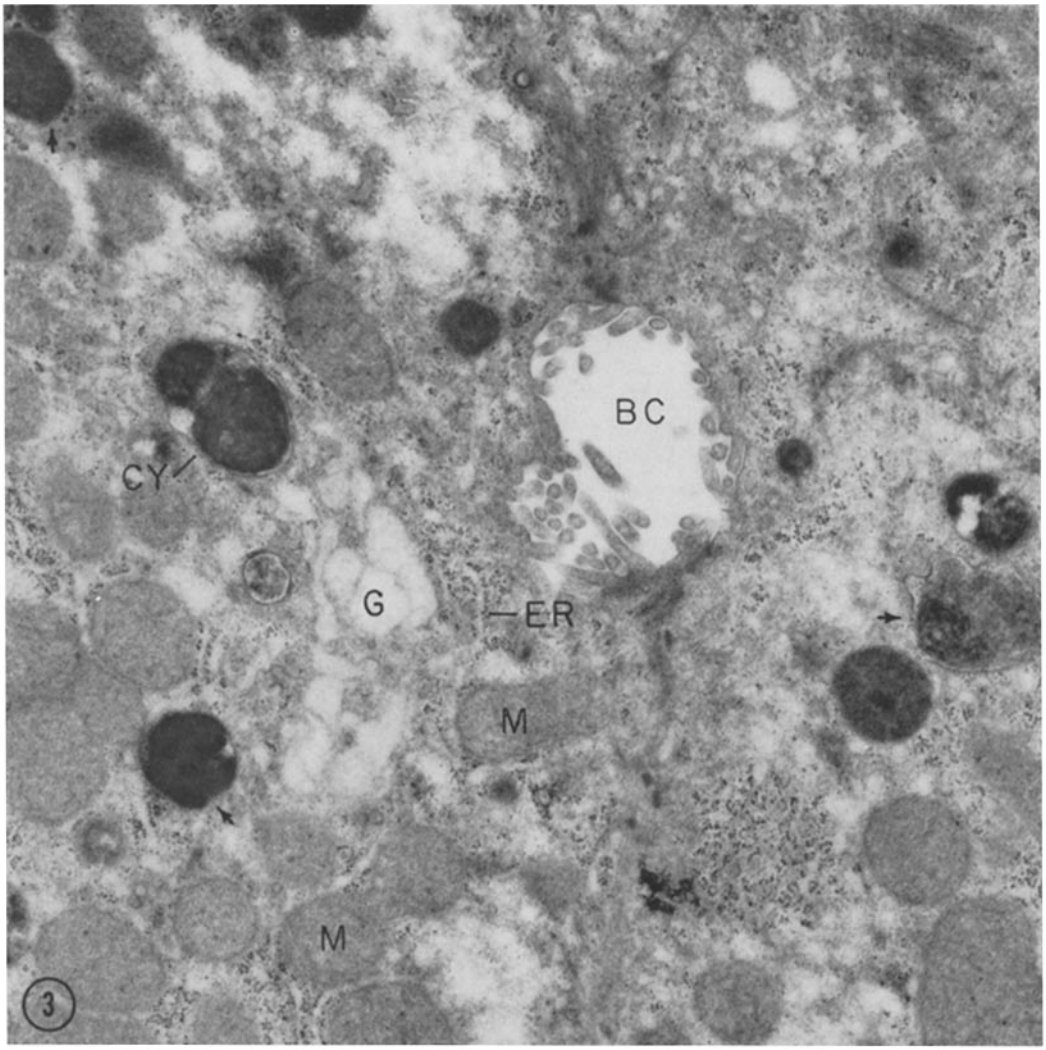
The photograph shows a somewhat dilated bile canaliculus (*BC*), ribonucleoprotein particles of the rough surfaced endoplasmic reticulum (*ER*), and mitochondria (*M*).

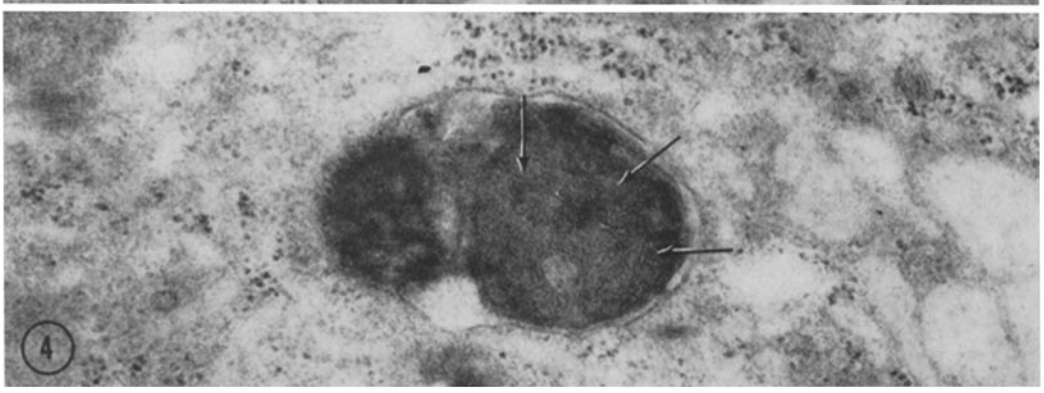
The Golgi cisternae (*G*) are swollen. Near them are two vacuoles containing bodies of high electron opacity. These and other similar bodies are indicated by arrows. The one marked *CY* is enlarged in Fig. 4. × 17,000.

FIGURE 4

Enlargement of vacuole, *CY*, of Fig. 3.

Within the electron-opaque material are membranes (arrows) that may reasonably be interpreted as cristae. × 41,000.





Ashford and Porter (1) suggest that their findings "place in question the concept of the lysosome as a well defined organelle of the liver cell." They conceive of the origin of the mitochondria-containing vacuoles as "foci of physiologic autolysis" that are "automatically surrounded by a membrane." They consider the mitochondria-containing vacuoles to resemble the pericanalicular dense bodies of normal liver (18). By implication, they suggest that the latter have developed from such vacuoles in which cytoplasm, perhaps of different character, has been degraded. In considering this suggestion, it might be noted that if the normal polymorphic dense bodies develop in this fashion, cytoplasm-containing vacuoles should be encountered in normal liver. To our knowledge, mitochondria or other recognizable cytoplasmic structures have not been described in the dense bodies of normal rat liver. Mitochondria-containing vacuoles are encountered only in pathological liver (similarly in pathological kidney (14)). Secondly, these vacuoles do not show the ferritin-like grains characteristic of many of the normal lysosomes (18). Thirdly, the mitochondria-containing lysosomes, as already indicated, are larger and show a more widespread distribution in the cytoplasm than is characteristic of normal liver lysosomes.

We think it likely that in liver cells, as in other secretory cells and neurons (20, 21), lysosomes are produced by dilatation and separation of portions of the Golgi cisternae. It remains to be determined whether these pour their contents around mitochondria and other cytoplasmic structures to produce a new vacuole membrane in the fashion suggested by Ashford and Porter, or whether the cytoplasmic structures are incorporated into the lysosome which enlarges while retaining its membrane. Cytoplasmic bodies of varying origin, appearance, and duration of existence, among different tissues or in the same tissue under different circumstances, may be considered to be lysosomes (8) if they are shown to possess acid hydrolase activities (17).

Examination by electron microscopy of the enlarged lysosomes of cells undergoing physiologic lysis (16, 17) should be of interest. If they, too, prove to be vacuoles containing degenerating cytoplasmic structures, such a finding may suggest that cytolysomes generally have a common structural and physiological significance.

Further study is also required to determine the ultimate fate of the electron-opaque residues of mitochondrial degeneration and the extent of cell injury, reversible or irreversible, that the appearance of cytolysomes signifies.

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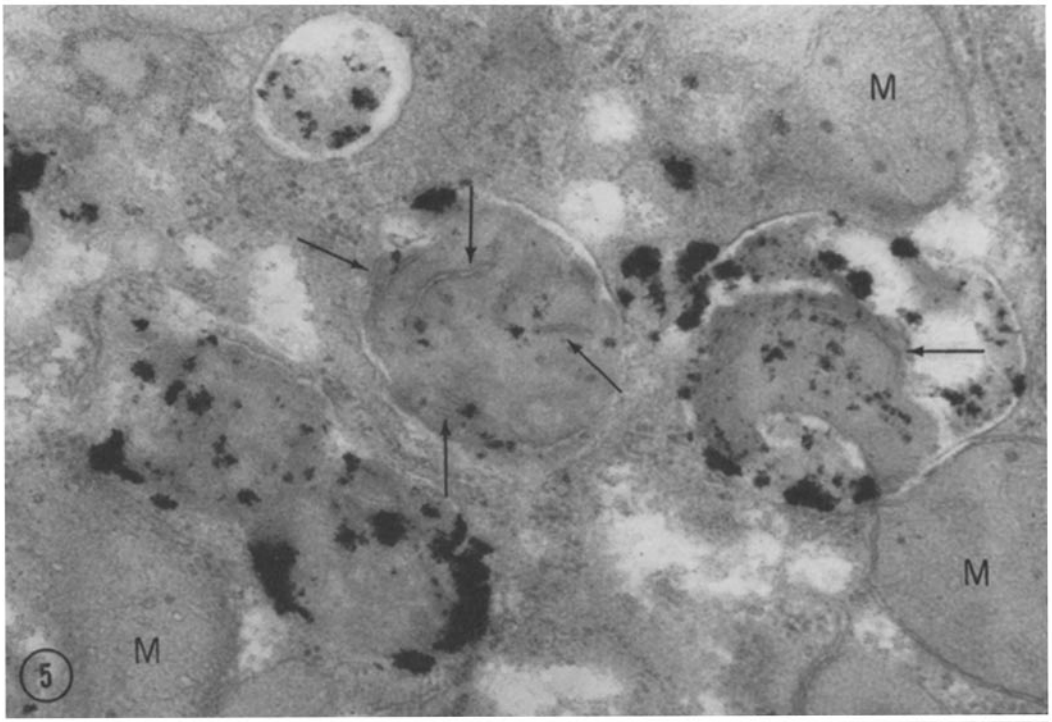
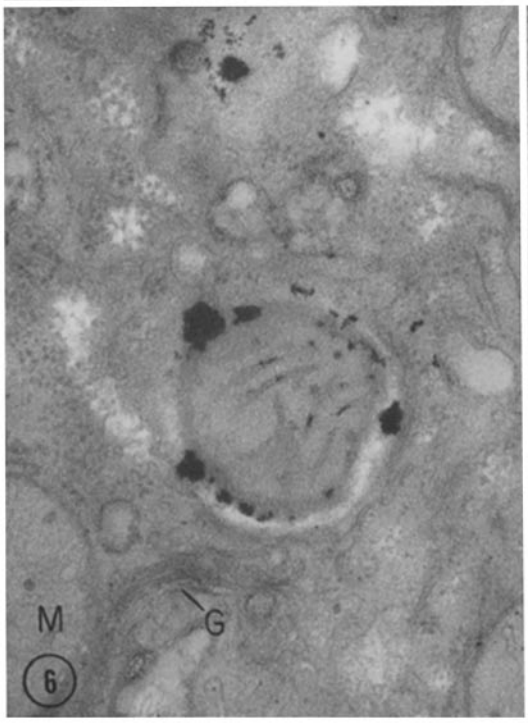
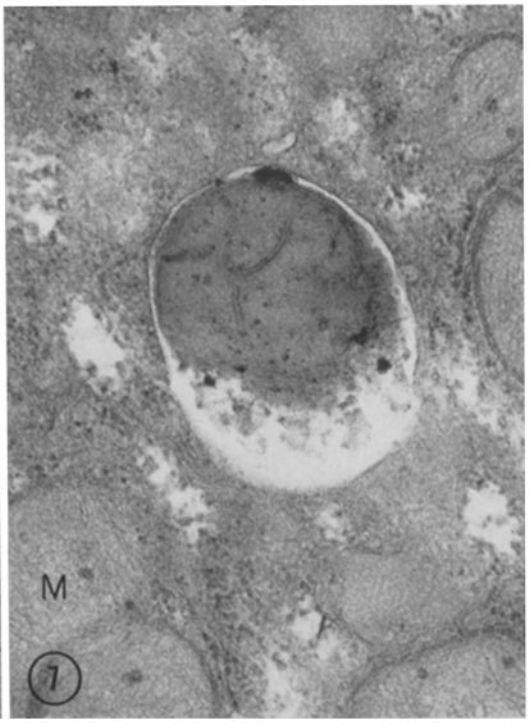
Figures 5 to 7

Areas of parenchymal cells from frozen sections incubated for acid phosphatase activity (5 minutes at 37°C), as described in the text, and then embedded in Epon 812 (13), and examined in thin sections stained with uranyl acetate and lead hydroxide (9).

These photographs show apparently unaltered mitochondria (*M*), Golgi membranes (*G*), and vacuoles in which mitochondria are enclosed. Cristae of these mitochondria are indicated by arrows in Fig. 5, and are clearly visible in Figs. 6 and 7. The electron-opaque material in these vacuoles is enzyme reaction product (lead phosphate). It appears that in Fig. 5, right, and in Fig. 7, the mitochondria are partially dissolved. Magnifications, Fig. 5, × 43,000; Fig. 6, × 39,000; Fig. 7, × 39,000.







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