

CYTOPLASMIC COMPONENTS IN HEPATIC CELL LYSOSOMES

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De Duve and his colleagues, a few years ago, originated the concept of lysosomes based on the biochemical analysis of isolated cytoplasmic particles (1). He reported that a number of hydrolytic enzymes are concentrated in a fraction consisting of small subcellular units which sediments between the usual mitochondrial and microsomal fractions. Subsequent to this discovery, some of these properties have been demonstrated in hepatic pericanalicular dense bodies, and Essner and Novikoff (2), using the electron microscope, have shown that the enzyme product of acid phosphatase is found exclusively in these bodies. They have also found that a variety of

pigment granules in human liver cells possess both the fine structure characteristic of dense bodies and the acid phosphatase activity of lysosomes (3). In this report we shall refer to polymorphic dense bodies which appear normally in peribiliary (or pericanalicular) positions in liver cells as lysosomes. 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 determine the presence or absence of acid phosphatase and other acid hydrolases.

The microbody, as distinct from the lysosome, is a cytoplasmic particle 0.4 to 0.6 micron in

diameter, which is delimited by a single membrane, contains a moderately dense matrix, and usually shows a dense central nucleoid with evidence of a lattice structure. This uniform structure renders it distinct from the smaller dense body or larger multilobulated dense body identified as the lysosome. The relationship of lysosomes and microbodies is under investigation (4) and will not be considered in this report. The morphologic differences can be readily seen by comparing lysosome *C* in Fig. 2 with microbody *mb* in Fig. 4.

De Duve (5) postulated that changes in relations of substrates and enzymes, and of the various lytic processes involving lysosomes, might occur in various physiological and pathological circumstances. It has, for example, been shown that their distribution in the hepatic cell is altered after bile duct ligation, carbon tetrachloride poisoning, or 3'-Me-DAB ingestion, in that they become more generally distributed throughout the cell (6). In some recent studies we have been able to observe the response of liver cell fine structure to "controlled" environmental conditions, and we find the alterations in the lysosome population and the fine structure of the lysosomes of sufficient interest to report separately.

Our observations come from an investigation of changes in the submicroscopic structure of rat hepatic parenchymal cells under conditions of isolation perfusion (7) and the response of the isolated organ to single hormones. In three experiments of this study, glucagon was added continuously to the perfusate for 4 hours, while in a fourth it was given in a single dose, rapidly administered. The same changes in liver cell fine structure were observed in each experiment.

PROCEDURES

The livers from Sprague-Dawley rats were placed in a perfusion machine similar to that described by Miller *et al.* (8). They were provided with a perfusate consisting of 60 per cent whole rat blood, 40 per cent Ringer's solution, penicillin, streptomycin, and heparin, hereinafter referred to as the standard perfusate. This perfusate was cycled through a chamber containing a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide which provided a continuous oxygen saturation of at least 92 per cent. The temperature of the preparation was maintained between 36° and 38°C. Glucagon (Lilly 258-234B-167-1) was added continuously to the perfusate over a 4-hour period. In two preparations, it was included at a rate of 100 micrograms per hour, while in a

third perfusion a smaller amount was used, and in a fourth short-term preparation (15 min.) it was given as a single 100 μ g dose. Biopsies were taken from each liver before the experiment started and from the right, left, and middle lobes after 4 hours of perfusion. These were trimmed to 1 \times 1 \times 2 mm blocks and fixed in 1 per cent osmium tetroxide in a phosphate buffer according to Millonig (9) for 2 hours, dehydrated in alcohols and propylene oxide, and embedded in Epon 812 (10). After sectioning on a Porter-Blum microtome the sections were stained with lead (11) and examined in a Siemens Elmiskop I electron microscope. A total of about 250 micrographs were taken of glucagon-treated material, many as survey pictures at low magnifications. These were compared with approximately 500 micrographs of liver perfused without glucagon (see below) and 400 micrographs of liver cells before perfusion, representing the starting material.

Eight perfusions, with the standard perfusate, carried out in a similar manner provide controls for judging the effect of glucagon infusion. The liver and blood donors were prepared by a 48-hour fast followed by refeeding 5 and 4 hours, respectively, before perfusion. A comparison of pre-perfusion biopsies in twenty preparations demonstrated that this produced a very uniform starting (control) material.

OBSERVATIONS AND DISCUSSION

After perfusion with glucagon, the number of bodies recognized as probable lysosomes increased greatly, and their distribution changed from that normally encountered in the liver cell (Fig. 1). In standard perfused preparations, for example, it is customary to find lysosomes gathered in groups of up to 10, in close proximity to the bile canaliculi. In cells perfused with glucagon, on the other hand, the lysosomes often numbered as many as 30 in a single section of a cell and were distributed, generally, in a central part of the cytoplasm near the nucleus. The mitochondria, with the exception of those described below, were not apparently changed.

As part of almost every lysosome in these glucagon-treated cells it is possible to recognize a mitochondrion or a remnant of one (see arrows, Fig. 1). In some instances (Figs. 2 and 4) the contained mitochondrion is essentially normal in appearance; in others it is smaller, is abnormally dense after osmium tetroxide, and shows varying degrees of structural decay. One may reasonably conclude that the mitochondria are in various stages of breakdown or hydrolysis. Other material included within the lysosomes can be identified as

representing other cytoplasmic components, small vesicles, RNP particles, and even cisternae of the ER (*A* in Fig. 2). The identification of the contents is, however, in many cases impossible.

It seems, therefore, as though each lysosome, when first formed, is a packet of cytoplasm segregated from the surrounding viable cytoplasm by a membrane or membranes. An early stage in this process is probably shown by body *B* in Fig. 3, and a recently formed body may be represented by *A* in Fig. 2. The peculiar feature of this material (isolated liver treated with glucagon) is that the incidence of lysosomes is higher than normal and that mitochondria are the favorite object for encapsulation and lysis.

The sequences in and reasons for the formation of this particular type of lysosome in such large numbers are difficult to imagine. Glucagon has recently been shown to produce a protein catabolic

effect (12). Possibly some condition it establishes in the cell leads to the development of foci of physiologic autolysis (in the sense defined by de Duve, 1) and such areas are automatically surrounded by a membrane, possibly to shield the rest of the cell from the general spread of the degradative process.

These observations and speculations place in question the concept of the lysosome as a well defined organelle of the liver cell. We would suggest instead that lysosomes represent portions of the cytoplasm (mitochondria included) set aside for hydrolysis with the general purpose of providing the protoplast with breakdown products for use in a reoriented physiology. Whether or not the microbodies, through fusion with these foci of hydrolysis, are the source of hydrolytic enzymes is a problem for future investigation.

This work was done during Dr. Ashford's tenure of a

FIGURE 1

Electron micrograph of a thin section from perfused rat liver treated with 400 μg of glucagon over a 4-hour period. This field represents the generalized distribution of lysosomes under these experimental conditions, in contrast to the usual peribiliary arrangement in the normal cell. It also demonstrates the increase in the number of lysosomes seen in thin sections under these conditions. Arrows point to lysosomes (*lys*) clearly containing remnants of mitochondria. Other structures include bile canaliculus, *bc*; Golgi apparatus, *G*; mitochondrion, *m*; nucleus, *N*. $\times 16,000$.

FIGURE 2

Electron micrograph of a thin section of perfused rat liver treated as described in Fig. 1. Several dense bodies or lysosomes show stages in the degeneration of mitochondria. Body *A* is interpreted as a rather early form and includes ergastoplasm (rough ER) within its boundaries. Body *B* is probably more advanced, but the mitochondrion still shows cristae and granules, and in some areas the outer double membrane. Body *C* represents an almost complete metamorphosis and contains small extra dense areas characteristic of lipofuscin granules. However, even in this advanced stage, cristae and granules may still be found on close examination. $\times 26,000$.

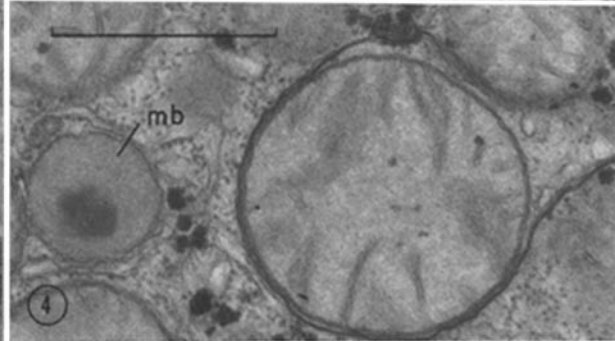
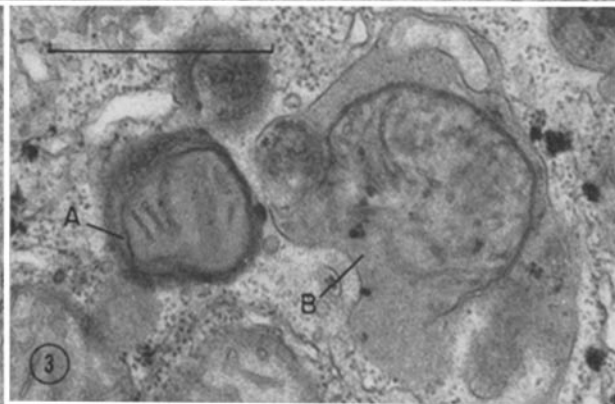
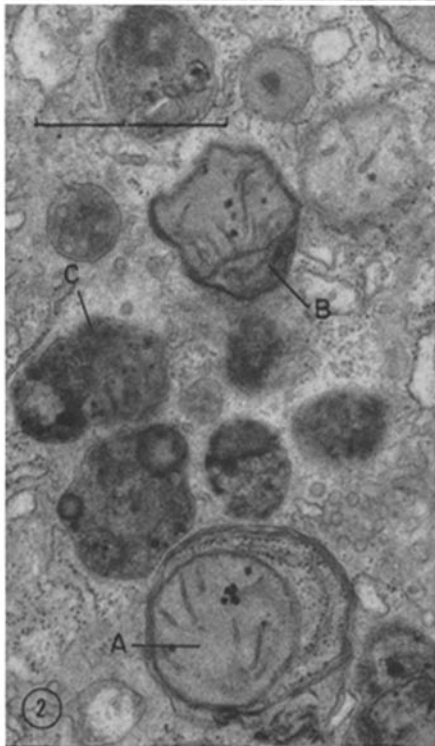
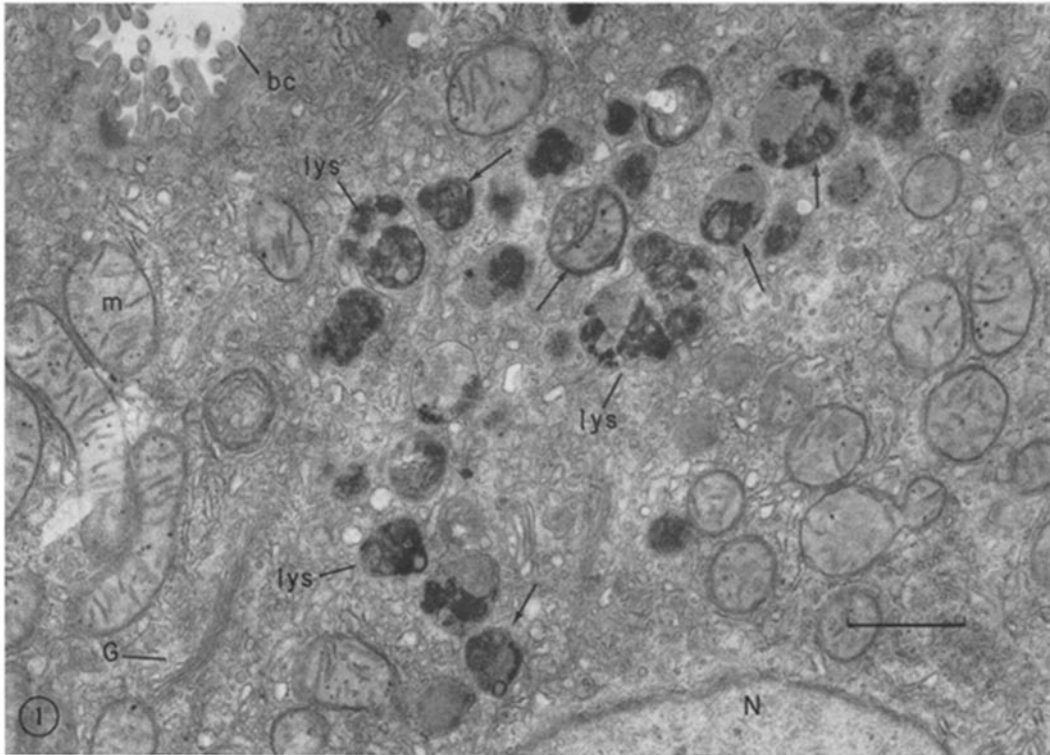
FIGURE 3

Micrograph of thin section of perfused rat liver treated with 100 μg of glucagon for 15 minutes. Lysosome *A* contains a mitochondrion and marginal condensations. Body *B* contains a mitochondrion whose limiting membrane is no longer intact, and in which surrounding material is enclosed in a limiting line interpreted to be a membrane. This is taken to represent a very early stage in lysosome formation. $\times 30,000$.

FIGURE 4

Micrograph from same cell as Fig. 3. This demonstrates an apparently normal mitochondrion which is enclosed with a small amount of cytoplasm by a dense membrane. The destruction or alteration of this mitochondrion has apparently not started. A microbody (*mb*) is at the lower left. $\times 30,000$.

Scale line = 1 micron in all figures.



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REFERENCES

1. DE DUVE, C., Lysosomes, a new group of cytoplasmic particles, in *Subcellular Particles*, (T. Hayashi, editor), New York, Ronald Press, 1959, 128.
2. ESSNER, E., and NOVIKOFF, A. B., Acid phosphatase activity in hepatic lysosomes: Electron microscopic demonstration of its reaction product, *J. Histochem. and Cytochem.*, in press.
3. ESSNER, E., and NOVIKOFF, A. B., Human hepatocellular pigments and lysosomes, *J. Ultrastruct. Research*, 1960, 3, 374.
4. NOVIKOFF, A. B., and ESSNER, E., The liver cell, Some approaches to its study, *Am. J. Med.*, 1960, 29, 102.
5. DE DUVE, C., The function of intracellular hydrolases, *Exp. Cell Research*, 1959, 17, Suppl., 169.
6. NOVIKOFF, A. B., The biochemical cytology of the liver, *Bull. New York Acad. Med.*, 1959, 35, 67.
7. ASHFORD, T. P., PORTER, K. R., and BADENHAUSEN, S., Modulations in the fine structure of the rat liver under conditions of organ isolation perfusion, in press.
8. MILLER, L. L., BLY, C. G., WATSON, M. L., and BALE, W. F., Dominant role of the liver in plasma protein synthesis; direct study of isolated perfused rat liver with aid of lysine- e - C^{14} , *J. Exp. Med.*, 1951, 94, 431.
9. MILLONIG, G., The advantages of a phosphate buffer for OsO_4 solutions in fixation, *J. Appl. Physics*, 1961, 32, 1637.
10. FINCK, H., Epoxy resins in electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1960, 7, 27.
11. LEVER, J. D., A method of staining sectioned tissues with lead for electron microscopy, *Nature*, 1960, 186, 810.
12. MILLER, L. L., Glucagon: a protein catabolic hormone in the isolated perfused rat liver, *Nature*, 1960, 185, 248.