CYTOLYSOMES IN METABOLICALLY ACTIVE CELLS

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Ashford and Porter (1) have described membrane-limited vacuoles in liver cells that contain mitochondria in a variety of degenerative states, fragments of endoplasmic reticulum, and granules. More recently, Novikoff and Essner (6) have demonstrated the presence of similar bodies in liver cells, and have shown them to be the site of acid phosphatase activity. In an earlier report, Novikoff (4) had observed them in the proximal tubule cells of the kidney. In the above reports, the tissues had been subjected to rigorous experimental procedures, *i.e.* the perfusion of isolated liver with glucagon (Ashford and Porter, 1) the intravenous injection of a detergent, Triton WR-1339 (Novikoff and Essner, 6), or the ligation of the ureter (Novikoff, 4). Novikoff and Essner have stated that "mitochondria-containing vacuoles are encountered only in pathological liver (similarly in pathological kidney)," and, since these vacuoles occur in cells undergoing cytolysis, Novikoff has suggested the name "cytolysome" for them (5). Though both lysosomes and cytolysomes display acid phosphatase activity, Novikoff has pointed out that the typical lysosome of normal liver is smaller in size, more restricted in position, contains ferritin-like granules, and does not possess cytoplasmic inclusions.

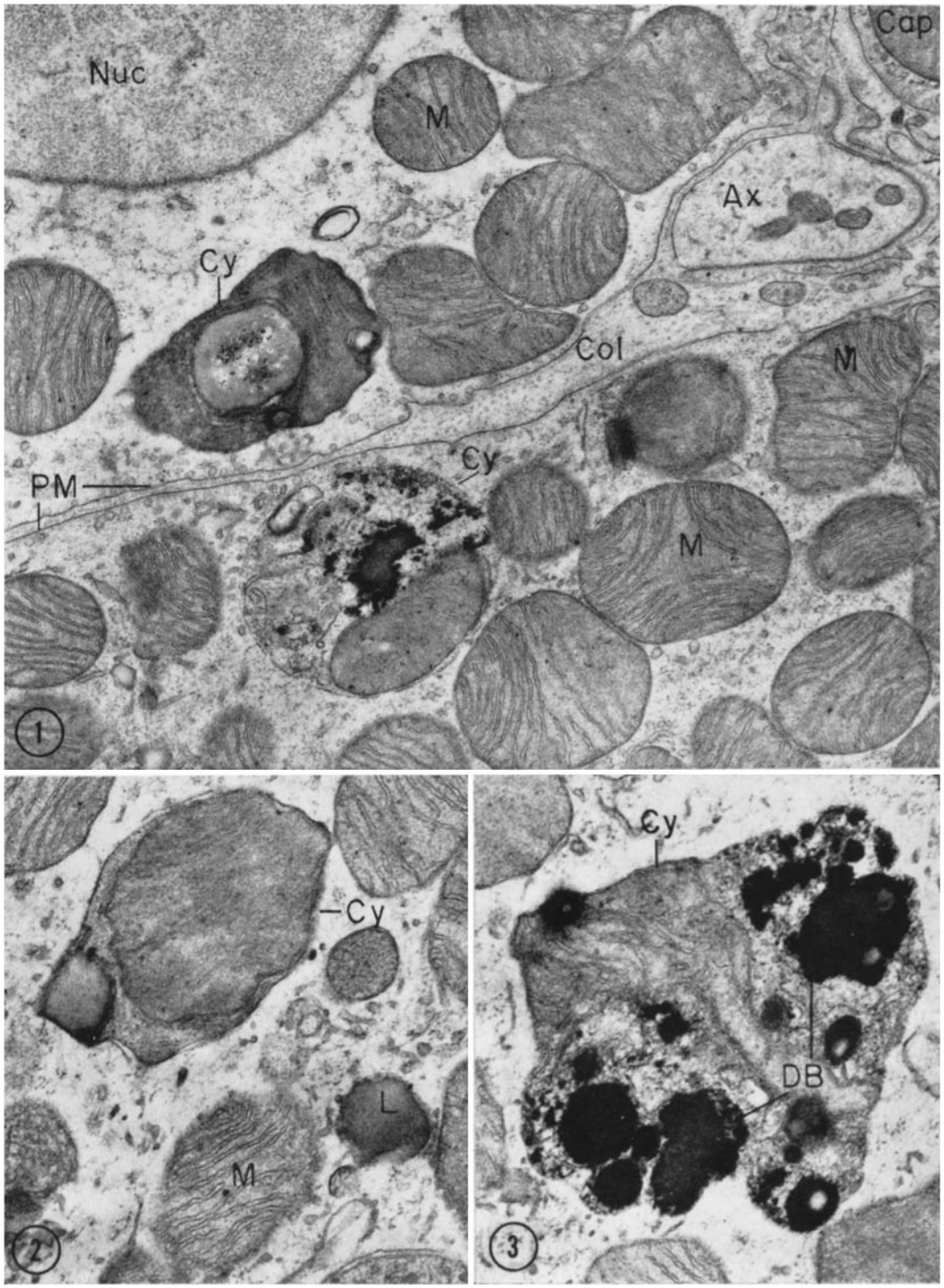
In a study of the changes in the fine structure of brown adipose cells rapidly mobilizing lipid, structures similar to cytolysomes have been encountered, and it seems useful to report their occurrence in a metabolically active, but not degenerate, tissue. Sidman and Fawcett (7), in light microscope studies, have shown that fasting mice, subjected to a cold-room temperature of 5°C, mobilize significant amounts of lipid from the interscapular fat pad. The mobilization of lipid was indicated by the depletion of cellular lipid inclusions, and was shown to be dependent upon the innervation of the fat pad.

In the study in which the present observations were made, rats were fasted for 12 hours and then placed in individual cages in a cold room (5–7°C) with water *ad libitum*, but no food. After 6 hours of exposure, the animals were sacrificed by decapitation and the interscapular fat pads excised. Animals fasted for 18 hours and maintained at 21°C were used as controls. Blocks of tissue (1 × 1 × 2 mm) were fixed for 1½ to 2 hours in cold (4°C) phosphate-buffered 1 per cent osmium tetroxide, dehydrated in increasing concentrations of ethanol, placed in 3 changes of dry acetone (½ hour each), and embedded in Vestopal W. Tissues were sectioned with a Porter-Blum or Huxley microtome, stained with lead hydroxide (2), and examined with either a Philips 100B

FIGURE 1 Portions of two brown adipose cells showing part of the nucleus of one (*Nuc*), mitochondria (*M*), and two cytolysomes (*Cy*). Between the plasma membranes of the two cells (*PM*) are collagen (*Col*), an axon (*Ax*), and a portion of a capillary (*Cap*). The cytolysomes contain mitochondria in varying stages of degradation, as well as other unstructured material. × 24,000.

FIGURE 2 A cytolysome enclosing a mitochondrion whose outer membrane is intact. A small lipid inclusion (*L*) is found in the cytoplasm. × 32,000.

FIGURE 3 A cytolysome containing a large number of dense bodies (*DB*). Though the enclosed mitochondrion has been deformed, cristae are clearly evident in the central region. × 32,000.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or 200 electron microscope. A detailed analysis of all the cytological changes in the interscapular fat pad, as well as an analysis of its lipid composition, will be reported at a later date.

The structures shown in the accompanying figures are typical of the bodies observed in brown adipose cells in this study. They are morphologically similar to the cytolysome of Novikoff and Essner, and the polymorphic dense body of Ashford and Porter. Their appearance in tissues actively mobilizing lipid may be described as membrane-enclosed vacuoles that contain a variety of adielectronic bodies and, in many instances, degenerating mitochondria (Figs. 1 to 3). These structures do not appear in any particular location within the cell, but are randomly distributed in the cytoplasm. They occur more frequently in cells nearly devoid of lipid, and have not been observed in brown adipose cells of control animals. The mitochondria enclosed within these cytolysomes show degrees of disorganization that vary from vacuole to vacuole. In some instances, distinct cristae from one or more mitochondria are clearly visible in vacuoles which also contain unstructured adielectronic material (Fig. 3); in other cases, only fragments of mitochondrial membranes and cristae remain. These cytolysomes do not contain other identifiable cell components such as the endoplasmic reticulum and RNP granules described by Ashford and Porter.

The occurrence of cytolysomes in the brown adipose cells just described raises two questions: (*a*) the function of such structures in cells which are not undergoing cytolysis, but rather are metabolically active, and (*b*) the origin and fate of these bodies.

There is a little reason to think that the cells in this study are undergoing cytolysis (as may have been the case in the previous studies). However, it seems most probable that the metabolism of these cells has been reoriented so that the rate of lipolysis is significantly greater than the rate of lipogenesis. The mobilization of lipid, which has been elicited by the experimental conditions, may induce an increased amount of hydrolytic enzymes that act preferentially upon the stored triglyceride to convert it into its component fatty acids. Upon the mobilization of nearly all the lipid these enzymes may remain in the cytoplasm. It seems possible that cytolysomes may arise at places in the cell where the enzymes have been localized, a membrane automatically forming at these sites (see Ashford and Porter) which protects the cytoplasm of the cell from their action. If a membrane forms *de novo* at such a site, it would not be surprising if mitochondria were accidentally caught in the enclosed vacuole and subsequently subjected to degradation by the enzymes inside. This is not unlike the concept of Ashford and Porter who suggest that the vacuoles arise at "foci of physiologic autolysis"; however, in this case, it is suggested that the membrane-enclosed vacuole is formed first, and that lysis of structural cell components occurs after they have been fortuitously trapped. The formation of cytolysomes might be regarded as a cellular process by which agents which may threaten the integrity of internal cell structure are effectively isolated from the internal environment. The formation of cytolysomes, then, may come about either as a prelude to cytolysis or as a result of an acute reorientation of normal metabolic processes within the cell.

Novikoff and Essner have suggested a definite mechanism for the formation of cytolysomes in the liver, namely, a dilatation and pinching off of portions of the Golgi cisternae. Although the development of the cytolysomes in brown adipose cells has not been fully documented at this time, it seems unlikely that they arise from Golgi cisternae as suggested by these investigators. The brown adipose cells are unique in the near absence of cytoplasmic membrane systems, and such structures as the Golgi complex and endoplasmic reticulum are infrequently encountered (3). The paucity of endoplasmic reticulum and Golgi complex forces us to conclude that there must be another source, at least in this tissue, for the development of the cytolysome.

The present observations have not revealed the complete life history of these structures in metabolically active brown adipose cells. However, a study of brown adipose cells from animals removed from the cold room and returned to a normal laboratory regime may reveal their ultimate fate.

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