AN ATYPICAL MITOCHONDRIAL FORM IN NORMAL RAT LIVER

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Morphologically atypical mitochondria have been reported by many investigators. Those possessing a cupped shape have been found in presumably normal cells of endocrine organs of widely differing species. Christensen and Chapman (1) observed them in interstitial cells of the albino rat testis and report that they have also seen them in Sertoli cells of the same tissue. Through personal communications, they report observations of cup-shaped mitochondria by Fawcett in opossum Leydig cells, and by Ito in nurse cells of *Drosophila* testis. Two reports of similarly shaped mitochondria have been made in conjunction with spermatogenesis, specifically in conjunction with the formation of the nebenkern of the grasshopper spermatozoa (2) and the nebenkern homologue in the scorpion (3). It thus appears that cup-shaped mitochondria are found relatively frequently in testicular tissue. DeRobertis and Sabatini (4) report a complex mitochondrial change in the adrenal cortex of the normal hamster, which they believe to be a degenerating form. These mitochondria bear a striking resemblance to those described by Christensen and Chapman (1). Munger (5) has shown that Beta cells of the islets in embryonic mouse pancreas sometimes have cup-shaped mitochondria.

MATERIALS AND METHODS

Although many adult rats have been used in the course of histochemical studies in this laboratory, the observations presented here are restricted to five adjacent liver cells of an otherwise normal single specimen. Prior to sacrifice by the administration of ether, this normal adult male rat had been maintained under standard laboratory conditions. It was 6 months old and weighed 380 gms. Liver tissue was removed and fixed in 5 per cent glutaraldehyde as described by Sabatini *et al.* (6). It was then subjected to the Gomori procedure for the demonstration of acid phosphatase as modified for the electron microscope by Holt and Hicks (7). The tissue was post-fixed for 1 hour in 1 per cent OsO_4 buffered with Veronal-acetate to pH 7.4, dehydrated, and embedded in Vestopal. Serial sections were cut for examination in the RCA EMU-3F electron microscope.

RESULTS AND DISCUSSION

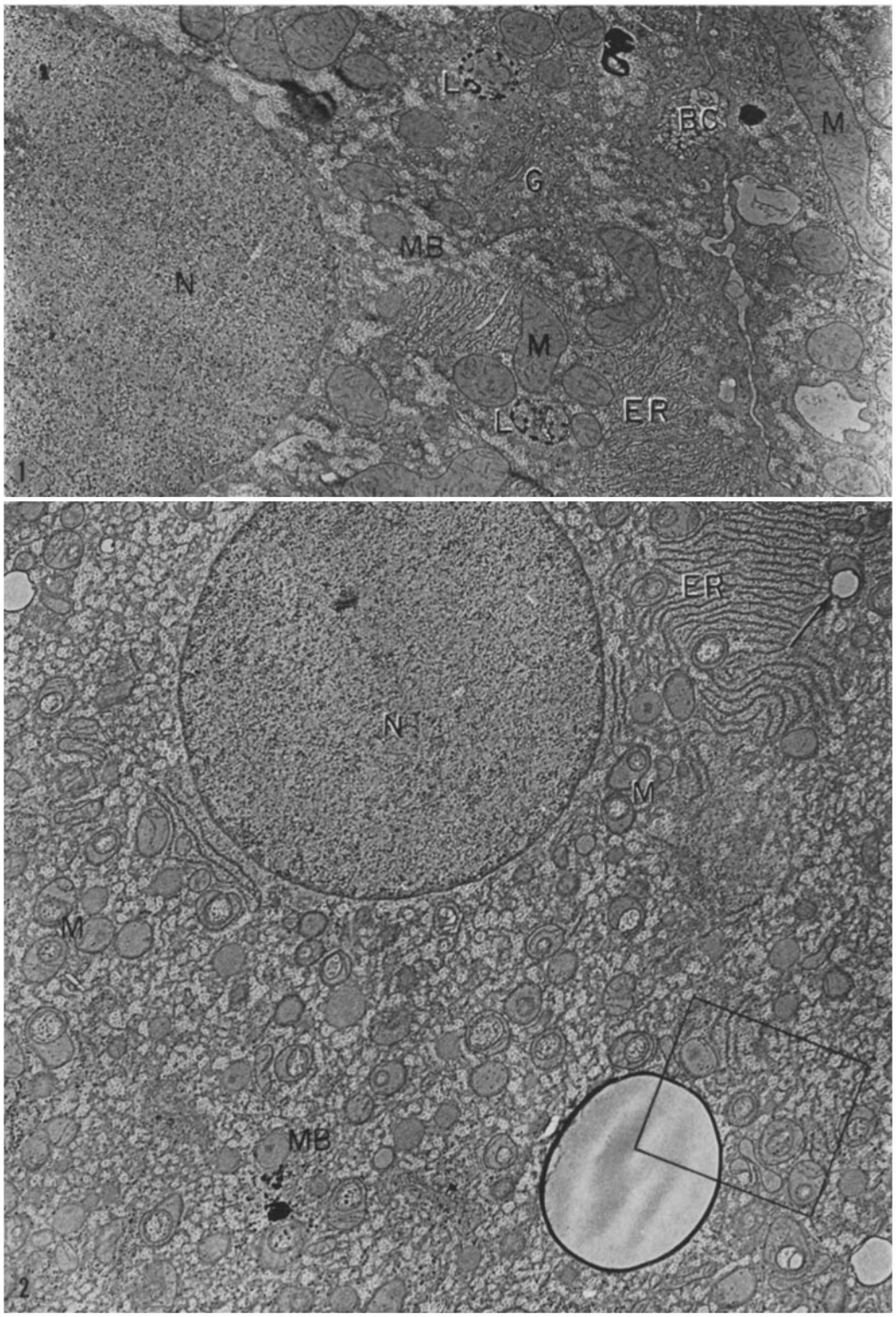
Since all aspects of the phosphatase reaction are being investigated further, observations will be restricted to the morphology of the mitochondria. Most of the cells in the liver contained normal

Explanation of Figures

BC, bile canaliculus
ER, endoplasmic reticulum
G, Golgi apparatus
L, lysosome
M, mitochondrion
MB, microbody
N, nucleus

The tissue represented by these micrographs has been treated for acid phosphatase activity; all aspects of the reaction product are being investigated further and will not be discussed here.

FIGURES 1 and 2 Both were taken from the same section of tissue. Fig. 1 shows normal cellular organelles; the variations in mitochondrial size and shape are normal for adult rat liver. The mitochondria in Fig. 2 are greatly modified and appear to have engulfed a small amount of cytoplasm which is located at one end of the mitochondrion. The engulfed cytoplasm communicates with the external cytoplasm through a small pore. A high percentage of the total population of mitochondria shows this modification. The arrow indicates a mitochondrion in close association with a lipid droplet. The area enclosed within the rectangle at the lower right indicates the position of the serial sections in Figs. 3 to 7. Fig. 1, × 13,000; Fig. 2, × 10,500.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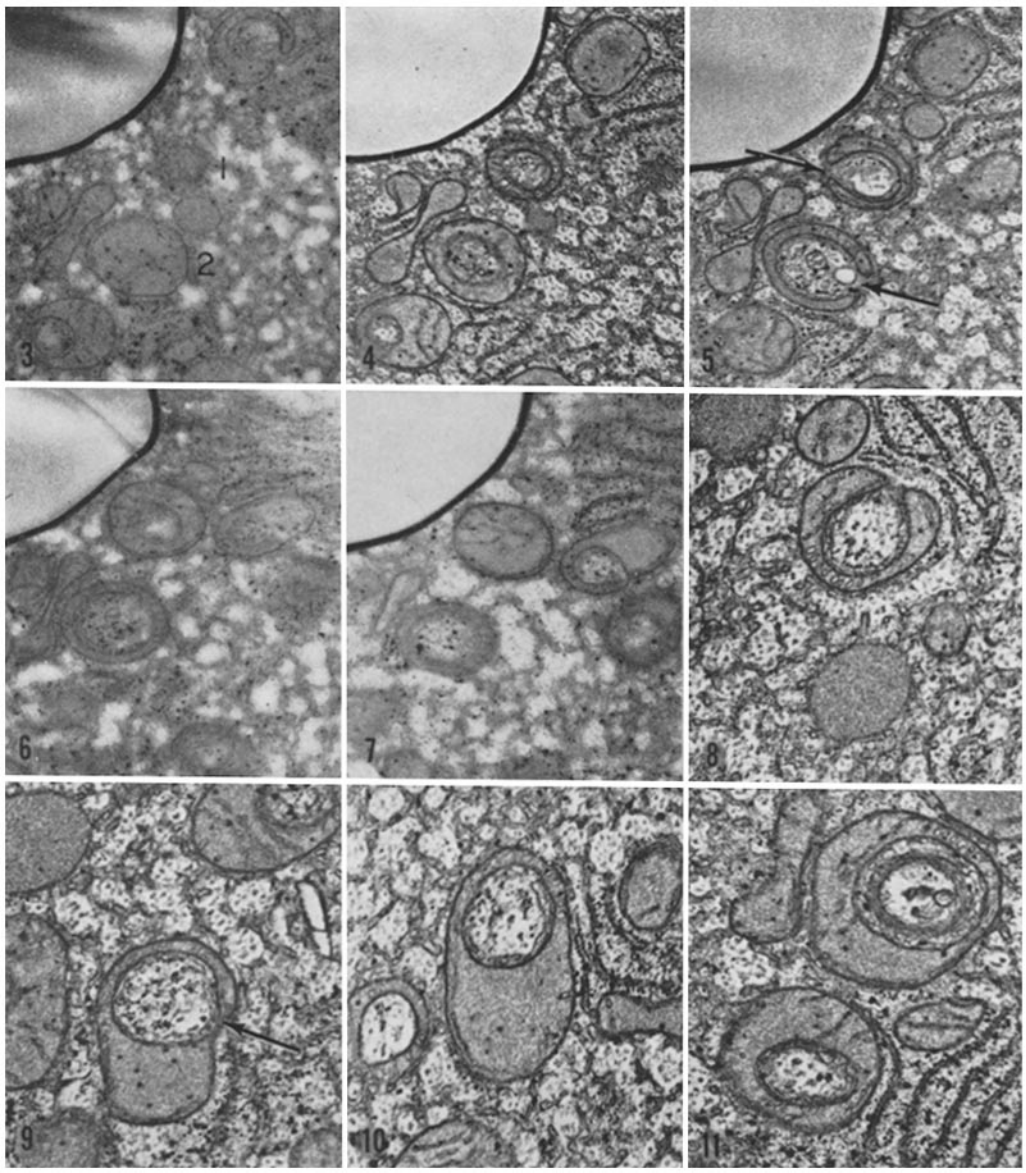

FIGURES 3 to 7 Serial sections in which mitochondria numbered *1* and *2* can be traced, showing that the engulfed cytoplasm is completely enclosed except for a small pore (arrows) shown in Fig. 5. × 13,000.

FIGURE 8 This cross-section of the mitochondrion passes directly through the pore and shows the restricted area of cytoplasmic communication. Note the regular arrangement of the cristae. × 26,500.

FIGURE 9 A longitudinal section of a mitochondrion which passes through the pore, clearly locating the position of the pore (arrow) adjacent to the thick end of a mitochondrion. × 26,500.

FIGURE 10 Another longitudinal section of a mitochondrion; at right angles to Fig. 9. No pore is present. × 26,500.

FIGURE 11 Two mitochondria showing the lamellar nature of the engulfed cytoplasm. Figs. 8, 9, and 10 also show a similar lamellation but to a lesser extent. × 26,500.

mitochondria as shown in Fig. 1. The external membranes and cristae appeared as expected; variations in length and diameter were normal for liver mitochondria. In marked contrast, however, the mitochondria in a few cells in the same section of tissue appeared greatly modified. These mitochondria comprised a high percentage of the total mitochondrial population of the cells in which they occurred, and their morphology varied markedly from that which is considered normal for this tissue. They appeared to have engulfed a small portion of cytoplasm (Fig. 2). By analysis of serial sections it was determined that this cytoplasm was confluent with the external cytoplasm only through a small pore (Figs. 3 to 9). The engulfed cytoplasm did not seem greatly modified, but under close observation several concentric layers of membranes were usually seen adjacent to the outer mitochondrial membrane, facing the engulfed cytoplasm (Figs. 8 to 11). In all cases the engulfed cytoplasm was displaced to one end of the mitochondrion (Figs. 9 and 10). The cristae had lost their regular arrangement in the thick portion of the mitochondria, were usually few in number, and were sometimes oriented parallel to the outer mitochondrial membrane. The mitochondrial granules remained, however (Figs. 9 and 10). In the thin portion of the mitochondrion surrounding the engulfed cytoplasm there was frequently found an orderly array of short cristae (Figs. 8 and 11).

We believe that this is the first report of mitochondria modified precisely in the above described manner. All the cells containing these altered mitochondria were adjacent to one another and were surrounded by what appeared to be normal liver cells containing normal mitochondria. An adequate explanation of the presence of these mitochondria is not immediately apparent; however, Kessling and Tobé (8) have produced altered liver mitochondria through prolonged administration of 15 per cent alcohol, a few of which resemble the mitochondria described here. The possibility therefore exists that the present findings may be the result of some noxious agent acting on a restricted area and affecting the mitochondria in a very uniform manner. The source of such an agent is not clear in this case; it may have been an unknown contaminant of the food, water, or air supply.

Occasionally a mitochondrion was found in close association with what was presumed to be a lipid droplet from which the lipid has been removed during dehydration (Fig. 2). Palade and Schedlowsky (9) and Palade (10) have shown that, under experimental conditions when guinea pigs are subjected to starvation, an increasing number of the mitochondria in the acinar cells of the pancreas become so closely associated with lipid droplets that they nearly surround them. Mitochondria so modified also resemble the altered mitochondria in this study. The lipid droplets have the same close relationship with the mitochondria as the engulfed cytoplasm. Palade's work shows clearly that the observed change in structure and presumably in function is in response to the experimental conditions imposed upon the animals, a response which would seem difficult to ascribe to the present study for two reasons: the animal used here was assumed to be normal; and of more importance, in Palade's work a certain number of the mitochondria in all of the acinar cells were involved, whereas in the present findings a high percentage of the mitochondria of only a few cells were modified. It is true, however, that from a functional point of view the shape of the mitochondrion has a greatly increased surface area and the enclosed cytoplasm is in a more confined relationship with the surface mitochondrial membrane. This condition may facilitate exchange of metabolic intermediates between the enclosed cytoplasm and the interior of the mitochondria. It should also be noted that the precise morphological uniformity observed in this study is not apparent in studies where a change in mitochondrial structure has been explained on the basis of a change in functional requirements.

Typical microbodies (11) were observed in both the normal cells and in the cells with altered mitochondria (Figs. 1 and 2). These organelles have a single limiting membrane and a homogeneous matrix with a more dense central area. Microbodies have been associated with the formation of both mitochondria (11) and lysosomes (12). According to more recent analysis by Baudhuin and Beaufay (13) they represent a separate organelle rich in uricase. It has been stated that the number of microbodies increases in regeneration and various pathological conditions in liver (11). Since a number of these microbodies are observed in the cells containing the altered mitochondria, this may indicate the beginning of a pathological condition, although we are unaware of any which is accompanied by a similar alteration of the mitochondria.

SUMMARY

During the course of an electron histochemical study of acid phosphatase activity, a unique mitochondrial form was observed in the normal adult rat liver. These mitochondria comprised a high percentage of the total mitochondrial population of the cells in which they occurred, and their morphology varied markedly from that which is considered normal for this tissue. Possible reasons for their existence are discussed.

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