THE REVERSAL OF MITOCHONDRIAL MEMBRANE

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ABSTRACT

An electron microscope study of mitochondria in hamster liver and kidney cells has revealed that at some points the outer membrane of these organelles is continuous with the inner membrane. Also, at such points the discontinuous components of the membrane pairs have free endings. The outer and the inner membranes of a mitochondrion, therefore, may not be two different and distinct entities, as has been conventionally assumed, but may rather be a part of the same unit. Such a morphological structure would make the intramitochondrial substance accessible to the cytoplasmic substance through the intermembrane channel. This structure would also facilitate the swelling of a mitochondrion either by an unfolding of the cristae, or a sliding of the two membranes, or by both these processes occurring simultaneously.

INTRODUCTION

Palade (11) and Sjöstrand (14) were the first to describe the fine structure of mitochondria with the aid of an electron microscope. They showed that a mitochondrion is bounded by two membranes and contains a system of double membranes running almost across it throughout its length. Palade believed that this latter system, which was lamellar in structure, was continuous with the inner mitochondrial membrane and represented folds in it. He called these intramitochondrial convolutions "cristae mitochondriales." The outer membrane which is exposed to the cytoplasm is supposed to be a single continuous structure without any folds or invaginations in it, enclosing, more or less like a shell, the mitochondrial substance. Such a structure will therefore interpose a mechanical barrier to the passage of material either from the cytoplasm inwards or from the mitochondria outwards, except by the process of permeation through the membrane. Studies on isolated mitochondria in media of various tonicities have indicated that the mitochondrial membrane is semipermeable (2, 8). On the basis of experiments measuring the permeability of highly lipid-soluble penetrants, the available surface area has been shown to remain constant during a swelling of mitochondria to approximately seven times their volume in an isotonic medium (16). It appears unlikely that a mitochondrion having the above-mentioned structure could increase its volume to such an extent without either a thinning out or a rupturing of the outer membrane. It is the purpose of this report to show that mitochondria may possess a structure which permits their swelling without either of the above two processes occurring. It is suggested that the outer and the inner membranes of mitochondria are not two different and distinct entities but integral parts of what is essentially a single unit.

MATERIALS AND METHOD

Pieces of tissues were excised from the anterior lobe of the liver and from the cortex of the kidney of 2- to 4-day-old hamsters under light ether anesthesia. The tissues were minced into approximately 1 cubic millimeter pieces in a pool of cold fixative and allowed to

stand for 1 hour at 4 to 5°C. The fixative used was 2 per cent buffered osmium tetroxide containing sucrose (1). The tissues were then rinsed momentarily in distilled water and in 10 per cent neutral isotonic formalin, and postfixed in the latter (9) for another hour. Dehydration was carried out in the cold in graded concentrations of alcohol which contained 0.2 per cent $MgCl_2 \cdot 6H_2O$ (19). The tissues were then embedded in the mixture B of Epon 812, described by Finck (5). The embedding procedure was modified by the use of the final mixture at all stages of impregnation. The resin was cured at 40°C overnight and at 60°C for the following 24 hours. Samples of hamster liver were also embedded in a 4:1 mixture of butyl and methyl methacrylates containing 0.5 per cent $\alpha\alpha$-azodi-iso-butyronitrile (13) as the initiator.

Electron microscope studies on cellular fractions of a few transplantable human tumors, obtained by differential centrifugation (18), were carried out earlier to check the purity of the fractions.[1] 0.88 M sucrose was used as the suspension medium. The mitochondrial pellet was cut into small pieces, fixed in Palade's fixative (10), and embedded in methacrylate containing 2 per cent benzoyl peroxide as the catalyst. The polymerization was carried out in a 80°C oven.

[1] Studies done by Dr. Helene W. Toolan on transplantable human tumors, HEp 1, HEp 3, HS 1, and A-42 (17).

Sections of the tissue were cut with glass knives on a Porter-Blum ultramicrotome, mounted on uncoated 300-mesh copper grids for epon-embedded tissues, and on 200-mesh, collodion-coated grids for methacrylate-embedded tissues, and stained with lead hydroxide (20), lead subacetate, or lead acetate with a subsequent exposure to ammonia vapors (3).

Sections were examined in a Siemens Elmiskop I electron microscope. Selected areas of the sections were micrographed at either 20,000 or 40,000 instrument magnification and enlarged photographically as desired.

OBSERVATIONS

The structure of mitochondria in the hepatic cells of the hamster is essentially the same as that observed by a number of investigators in other animal tissues. The mitochondria are bounded by two membranes, the inner one of which is continuous with the cristae. Each of these membranes measures 75 A in thickness and can be resolved into three components, each 25 A thick (Fig. 2, double arrows). The mitochondrial membrane thus has a structure quite similar to the "unit membrane structure" described by Robertson (12). The spacing between the double membranes varies from 100 to 150 A, and both of these are equally electron-opaque or osmiophilic.

At certain points in the limiting structure of

FIGURES 1 TO 7

Electron micrographs of mitochondria which show the accepted structure, a system of double membranes, the inner one of which is continuous with the cristae. At certain points along the surface of the mitochondria, a continuity between the outer and the inner membranes is observed. This has been referred to as a reversal of the membrane. At such points, the other components of the double membrane system have free endings. Tissues embedded in Epon.

FIGURES 1 TO 6

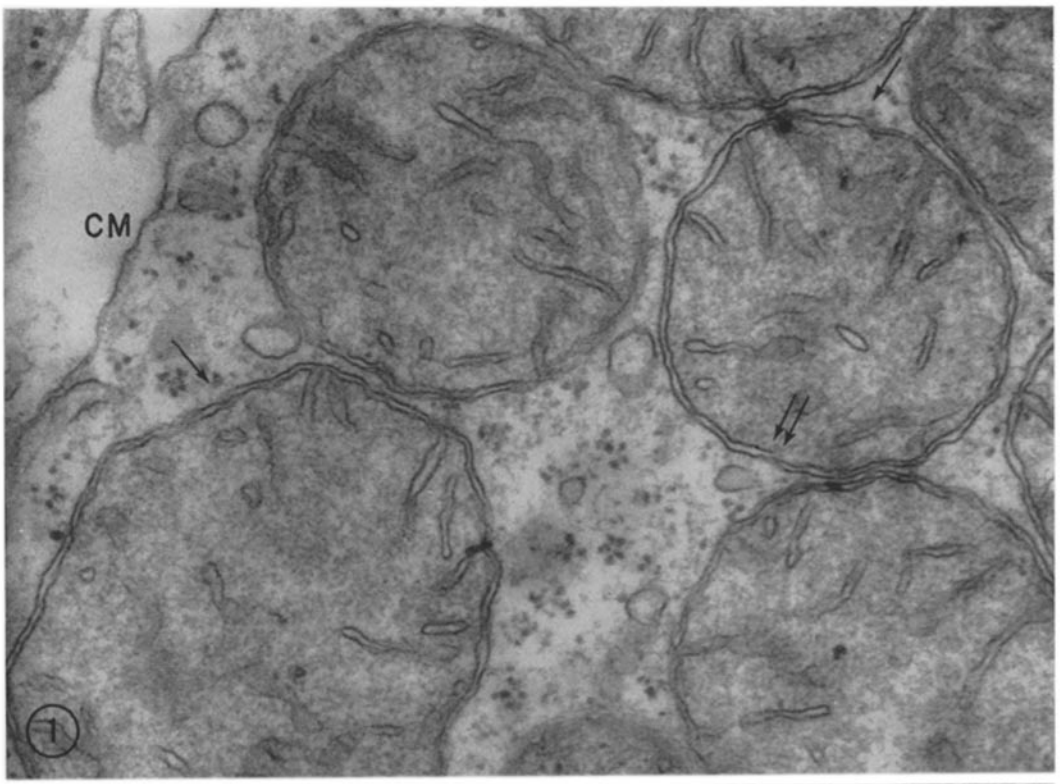
Electron micrographs of mitochondria in hepatic cells of hamster.

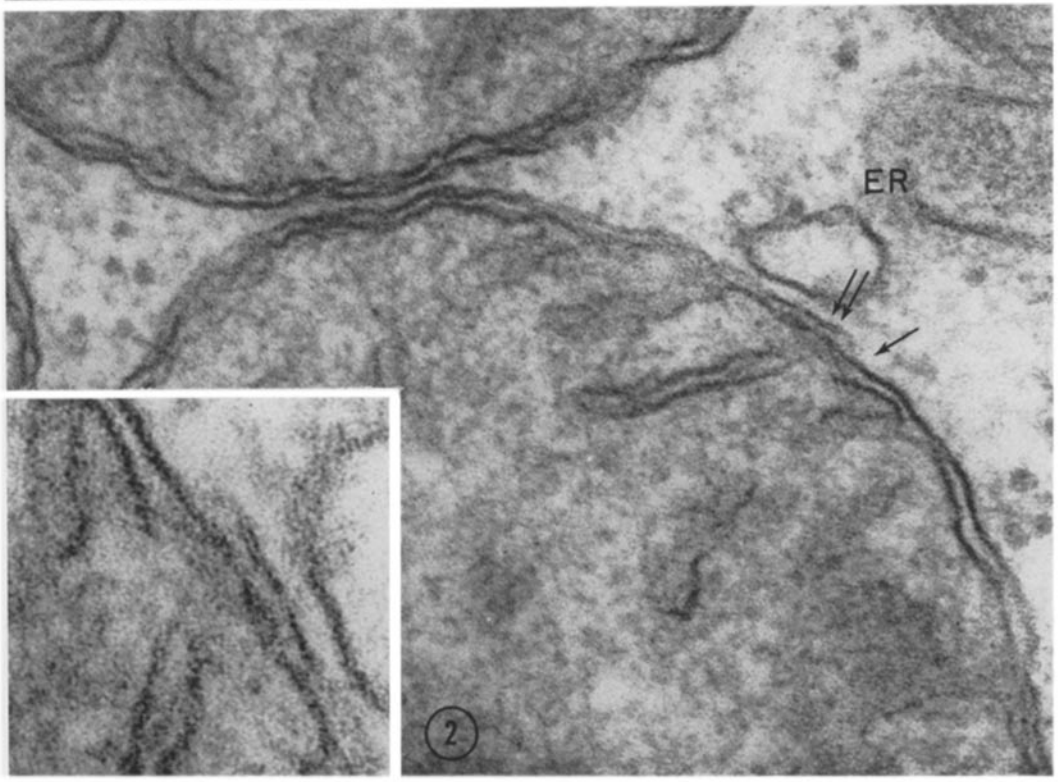
FIGURE 1

Two mitochondria in the same cell show the reversal of the membrane (arrows). At the double arrow, the inner membrane has an abrupt ending. The cell membrane (*CM*) is observed at the upper left corner of the micrograph. Magnification 60,000.

FIGURE 2

A segment of a mitochondrion which shows the reversal of the membrane (arrow). The double arrow points to the outer membrane which is observed to be resolved into three components. *ER*, endoplasmic reticulum. Magnification 160,000. The inset shows the resolved components clearly. Magnification 320,000.





mitochondria, the outer membrane is continuous with the inner membrane, as if a reversal of the membrane has occurred (Figs. 1 to 7). Also, at such points, the discontinuous parts of the double membrane system have free endings (Figs. 1 to 7). These free-ending membranes occasionally bend in the plane of the section and appear diffuse in the electron micrographs (Figs. 1 to 4). They also bend away from the other component of the double membrane system, increasing their 100 A separation (Figs. 4 and 6). Figs. 1 (double arrow) and 5 show rare instances where the free-ending membranes have abrupt terminations. The mitochondrion in Fig. 4 shows the reversal of the membrane at two adjacent points. At the point shown by the single arrow, the outer membrane is observed to be in direct and immediate continuity with the crista.

In the tubule cells of the hamster kidney, which have elongated mitochondria, instead of the nearly spherical ones seen in the hepatic cells, similar mitochondrial structures have been observed (Fig. 7).

The direct continuity between the outer and the inner membranes of a mitochondrion, together with the free endings of the other components of the double membrane system at these points, make the intermembrane channel open into the cytoplasmic matrix, as well as into the mitochondrial matrix (Figs. 1 to 7). Thus there is a direct continuity between the cytoplasmic and the mitochondrial matrices (Fig. 4).

It is of importance to note that similar mitochondrial structure has also been observed in methacrylate embeddings of hamster liver. Tandler (15) has observed the reversal of the mitochondrial membrane in the striated duct cells of the human submaxillary gland.

Electron micrographs of mitochondria in a pellet obtained by differential centrifugation reveal considerable artefactitious damage as observed in Fig. 8. The mitochondria are greatly swollen and are bounded by what is essentially a single membrane, with the inner one occasionally observed to be localized along a very small portion of the mitochondrial surface. The complete absence of the electron-opacity of the mitochondrial matrix indicates a loss of the mitochondrial substance. At the single arrow in Fig. 8, the crista appears to have an opening into the external milieu, while at the double arrow, the two membranes seem to overlap.

Fig. 9 shows diagrammatically the accepted structure of a mitochondrion on the left and the observed structure in the center. On the right is the diagram of a mitochondrion having a single reversal around a circumference (see Discussion for interpretation).

DISCUSSION

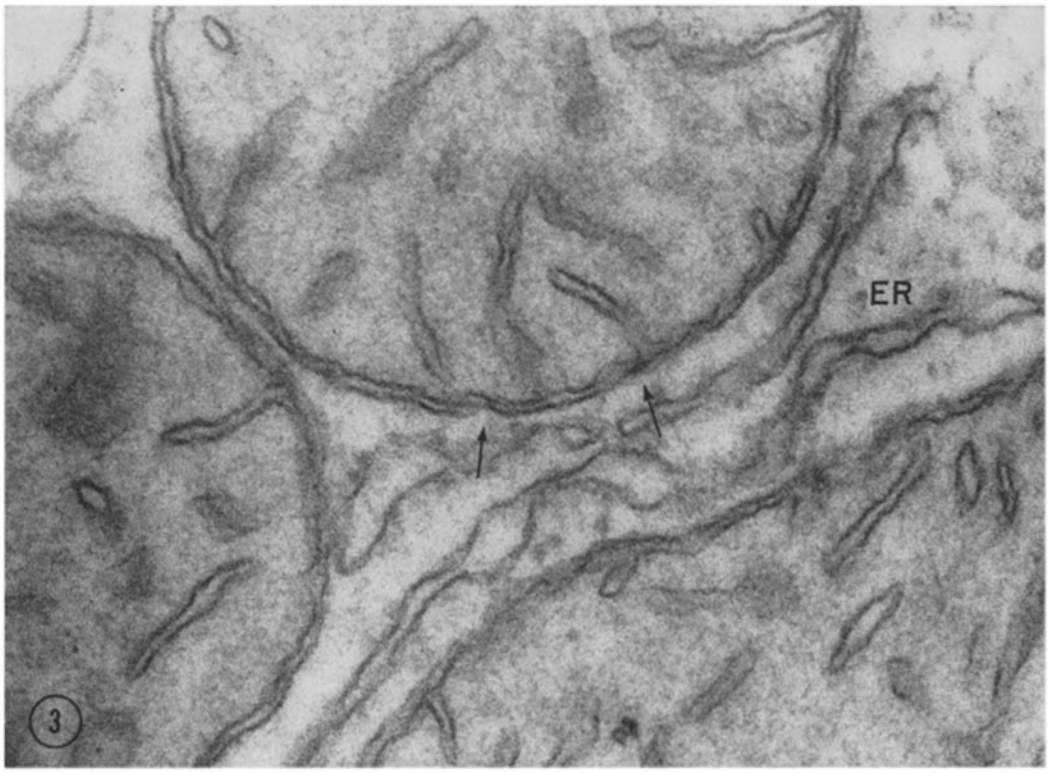
Studies relating to the physiological and biochemical characteristics of isolated mitochondria have given rise to a number of speculations regarding the mechanism of swelling of these organelles on the basis of the structure described by earlier investigators. Electron microscope studies on isolated mitochondria have suggested that the outer membrane becomes stretched during swelling,

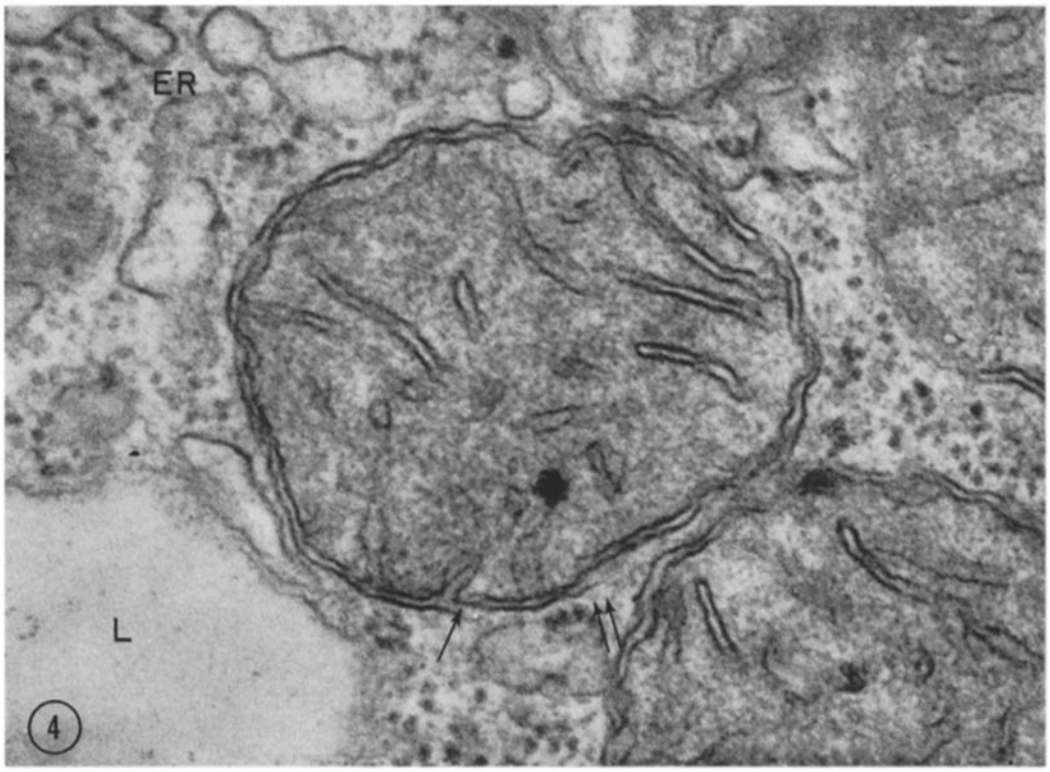
FIGURE 3

A mitochondrion showing the reversal of the membrane at the arrow in the center. The arrow on the right points to a possible occurrence of this phenomenon. *ER*, endoplasmic reticulum. Magnification 110,000.

FIGURE 4

A mitochondrion in which the reversal of the membrane is observed at two adjacent points. The single arrow points to a direct and immediate continuity of the outer membrane with a crista, and the double arrow points to the bending of the discontinuous components of the double membrane system away from the central continuous membrane. Such observations cannot be explained on the basis of a fold in the mitochondrial surface in the thickness of the section. In the space limited by the two arrows, the mitochondrial and the cytoplasmic matrices appear continuous through the intermembrane channel. In the cytoplasm are observed segments of endoplasmic reticulum (*ER*) and free RNP granules. On the lower left is a portion of a lipid body (*L*). Magnification 100,000.





because no folds have been observed in it in the normal state (21). On the other hand, some investigators have assumed that the membrane may break up and that a transfer of internal material and of the broken-up inner membrane to the inner surface of the outer membrane may take place (6 and 7).

The reversal of the mitochondrial membrane had not been observed and reported by previous investigators. The success of the present author in observing this structure lies in the fact that he scanned sections of tissues at an instrument magnification of 20,000 in search of very small cytoplasmic inclusions, when any "irregularity" in cytoplasmic structures could become fairly apparent. The liver tissue itself, containing a large number of mitochondria, provided a good system for observation. The fact that the reversal of the membrane has been observed in different samples of hamster liver and in two other tissues strongly suggests the reality of this structure.

The frequency of occurrence of the phenomenon of reversal in mitochondria of hamster liver cells appears to be fairly significant. It should, however, be pointed out that statistical data of this nature could not be accurate, since one is liable to miss, while scanning at 20,000 instrument magnification, some mitochondria, the number of which is again difficult to determine. On the other hand, one could count the same mitochondrion twice. Taking these considerations into account, a count of 10 to 15 mitochondria (out of 120 to 170 in one square of a 200-mesh grid) exhibiting the reversal of membrane appears significant.

The observed continuity between the outer and the inner mitochondrial membranes suggests that they may not be two different and distinct entities as has been conventionally thought (Fig. 9, left). If it is assumed that there is a single reversal which extends completely around a circumference of a mitochondrion, a longitudinal section of the organelle could be diagramatically represented as in Fig. 9, right. It is seen that the mitochondrial envelope consists of three different membranes, AA′, BB′, and CC′. It is also apparent that the membranes AA′ and BB′ are not physically held in position. They could, however, be held by molecules bridging the intermembrane channel but not made visible in the electron microscope. During the swelling of a mitochondrion by any mechanism, the membrane components AA′ and BB′ will always form a double membrane system. If during the swelling, the third component CC′ breaks up at a few points and is replaced by parts of AA′ and BB′, the profile of an extremely swollen mitochondrion will appear to be bounded by a single membrane, as observed in the electron micrographs of Watson and Siekevitz (21) and also in that described above. If, on the other hand, two or more reversals, each extending along a circumference, cross one another, a membrane which forms the outer envelope in one reversal system could form the inner envelope in another system. In such case, the mitochondrial envelope would be a part of a single membrane unit. If the reversals are localized in small areas, the envelope could again be visualized as a single-membrane unit. A mitochondrion could, therefore, have either a single reversal along a circumference or two or more reversals. The latter could either extend

FIGURE 5

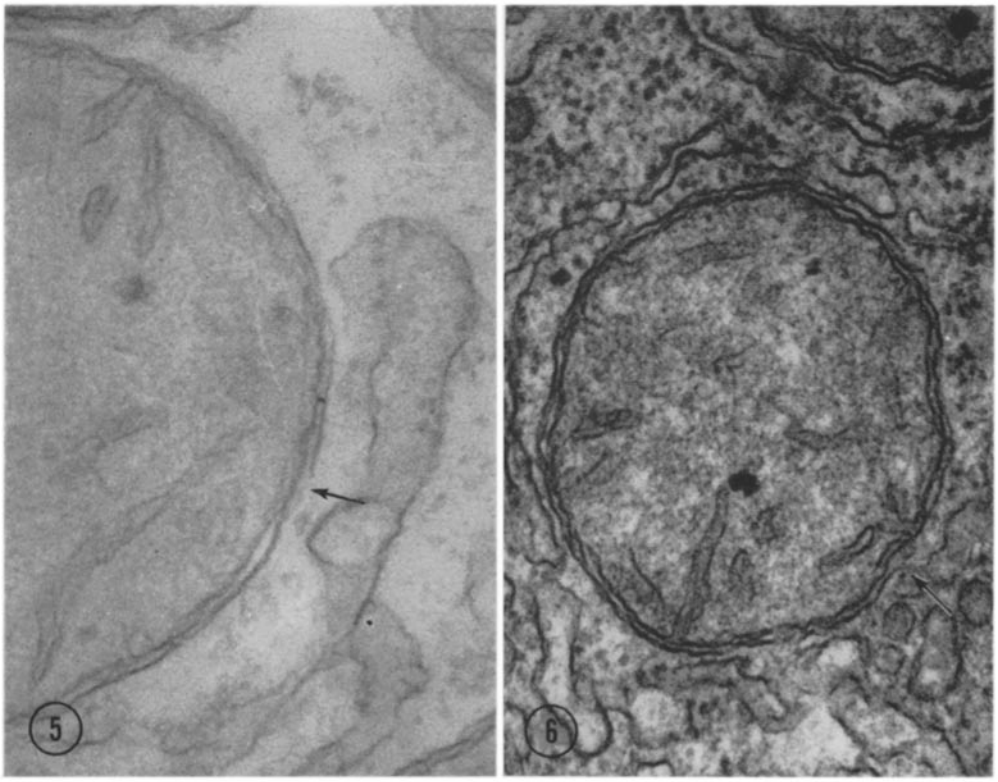
A mitochondrion in which the outer membrane shows a clear and abrupt ending (arrow). Magnification 100,000.

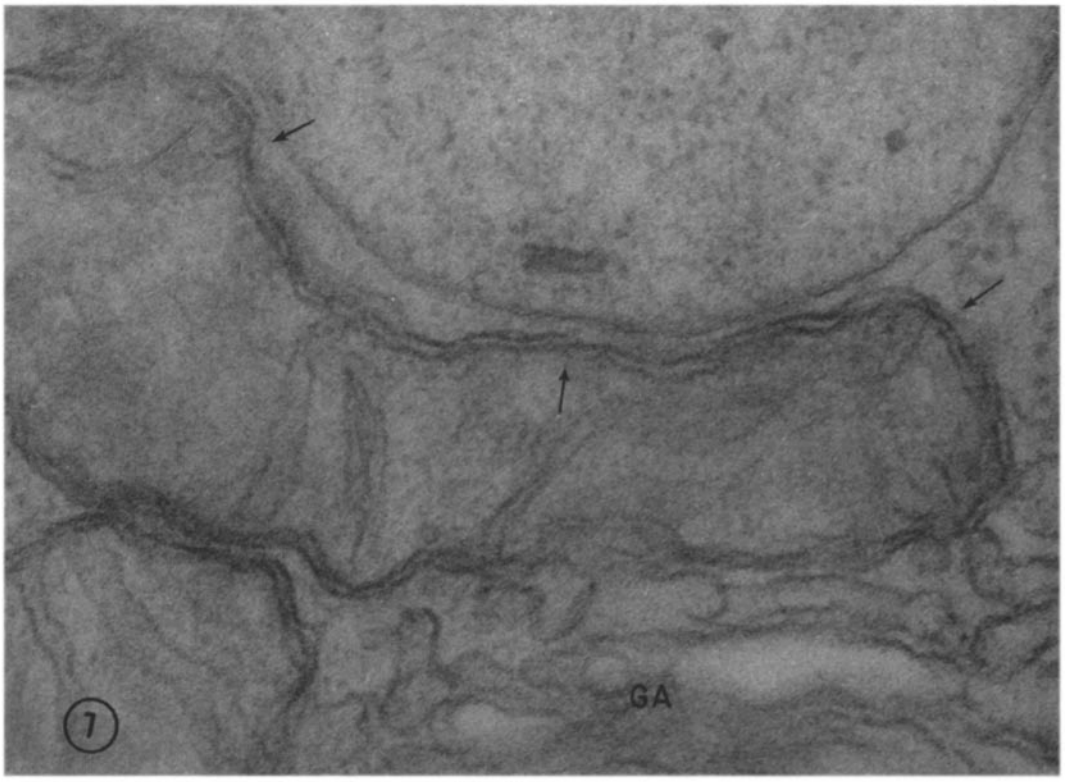
FIGURE 6

A mitochondrion showing the outer and the inner discontinuous membranes bending away from the central continuous membrane (arrow). Magnification 80,000.

FIGURE 7

An elongated mitochondrion in a tubule cell of hamster kidney. The arrow in the center points to the reversal of the membrane, while the two other arrows point to possible occurrences of the same phenomenon. A portion of the Golgi apparatus (*GA*) and a segment of a secretory vesicle are observed at the lower and upper central regions of the electron micrograph respectively. Magnification 120,000.





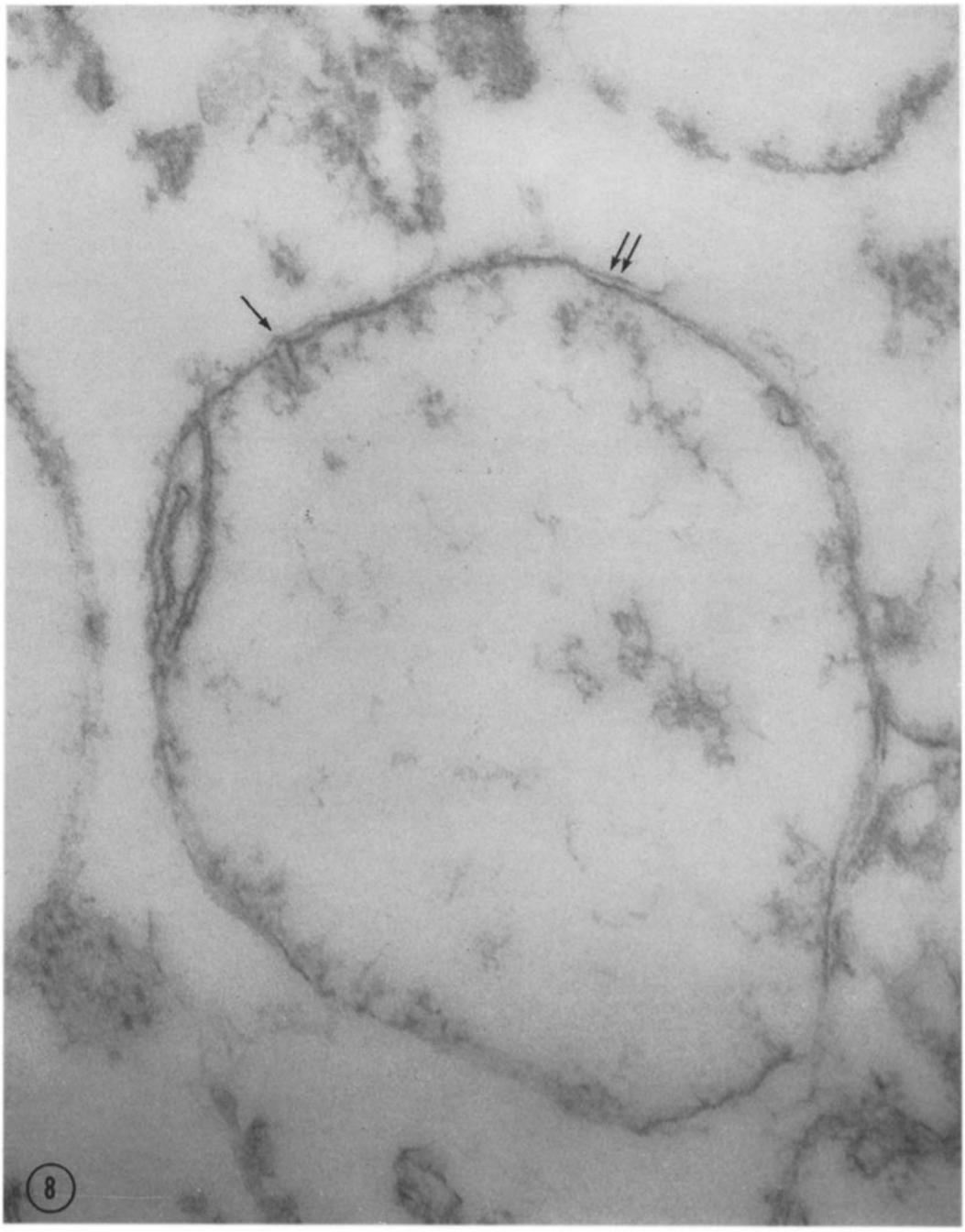


FIGURE 8

A mitochondrion in a pellet obtained by differential centrifugation of a transplantable human tumor. There is a loss of the mitochondrial substance as evidenced by an absence of electron-opacity. At the arrow, the crista appears to be open to the external milieu, and at the double arrow, there appears to be an overlapping of the two membranes. Magnification 140,000.

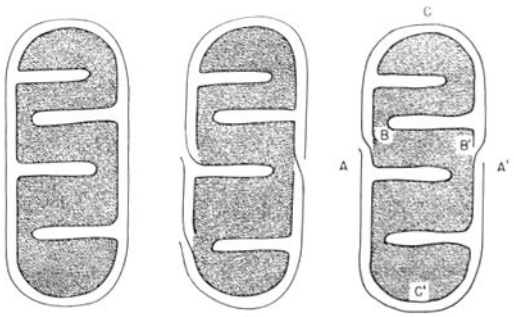


FIGURE 9

A diagram of the accepted membrane configuration of a mitochondrion on the left and of the observed and proposed structure in the center. On the accepted model, the inner membrane is continuous with the cristae, and the outer one is more or less like a shell enclosing the inner membrane system and the mitochondrial substance. On the observed model, the inner and the outer membranes are continuous at certain points, leaving the other components of the double membrane system free. Thus cristae are also continuous with the outer membrane. The outer and the inner membranes are therefore a part of a single membrane system. The diagram on the right shows a section of a mitochondrion with a reversal of the membranes extending along a circumference. See text for an interpretation of this configuration.

along two or more circumferences or be localized in small areas. Since in the case of a single reversal, the three membrane systems are not physically held together, such a structure is not thought to be a true one. A normal mitochondrion is, therefore, conceived of as one having the essential features described by Palade but exhibiting the reversal at a few points. The number of these reversals and the extent to which they lie on the mitochondrial surface probably depend on the physiological state of the organelle.

It could be argued that the observed reversal of the mitochondrial membrane and the free endings of the other components could be a result of a slight bending of the double membrane system in the thickness of the section, as shown in Fig. 10. An electron beam passing through the section along the direction BA′ will form a darker image in the electron microscope than that formed by adjacent beams passing through points *A* and *C*. In the electron micrographs this greater density will give an impression of a continuity between the outer and the inner mitochondrial membranes. At the same time, the portions *AD* and *B′D′* of the outer and the inner membranes respectively will appear to fade away towards the center of the membrane system. Such instances were encountered during these observations and interpreted accordingly. But the bending of the outer and the inner membranes away from the central continuous membrane at points of the reversal of a mitochondrial membrane, and their possession of free ends in the cytoplasmic and mitochondrial matrices (Figs. 4 and 6) cannot be conceived of as being due to a fold within the thickness of the section. Similarly, the observation of abrupt endings in mitochondrial membranes (Figs. 1, double arrow, and 5) cannot be explained on the same basis, unless the membranes happen to bend at right angles, which is highly improbable.

It has been mentioned that the observed free endings of the membranes make the cytoplasmic and the mitochondrial matrices continuous through the intermembrane channel. This does not mean that there is a free passage of material between the cytoplasm and the mitochondria through the channel 100 A or more in width. It is known that mitochondria behave selectively during the transport of material to and from the cytoplasm (4). The situation here may be analogous to that in nuclei which possess pores 500 A or more in diameter.

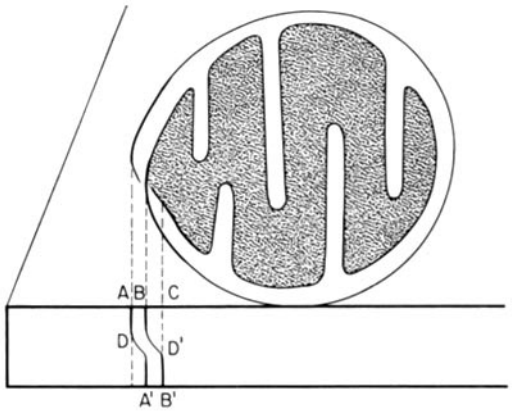


FIGURE 10

A diagram showing a bend in the surface of a mitochondrion in the thickness of a section. In the electron microscope one would therefore observe an apparent continuity between the outer and the inner membranes. Also, in such a case, the apparently free and discontinuous outer and inner membranes will appear to fade away towards the central continuous membrane. See text for interpretation.

It has been stated above that the outer and the inner membranes of a mitochondrion are a part of a single membrane system. When the cristae unfold, the outer as well as the inner mitochondrial surface increases in area. The existence of free endings of the two membranes also suggests that the two surfaces could slide relative to one another. The swelling of a mitochondrion will therefore occur as a result of two processes: (*a*) an unfolding of the cristae, and (*b*) a relative sliding of the two membranes. Although the opening of the crista to the external milieu and the overlapping of the membranes of the mitochondrion in Fig. 8 support this mechanism of swelling, an electron microscope study of isolated mitochondria in different stages of swelling is still needed to confirm the above hypothesis.

Although this structure of mitochondria has been observed in different samples of hamster liver and also in two other tissues, observations of this configuration in a number of tissues will be required to establish the universality of this structure.

In the past, attempts have been made to interpret the physiological findings on isolated and osmotically swollen or shrunken mitochondria on the basis of the accepted structure. It is hoped that the present mitochondrial model with a simple mechanism for swelling or shrinking will provide simpler explanations in understanding the behavior of mitochondria under different physiological conditions.

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