

## VARIATIONS IN THE STRUCTURE OF MITOCHONDRIA

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Mitochondria have been studied extensively by light microscopists, who are in general agreement that these organelles represent formed structures present in all cells at some stage of their life history. They are identifiable by their tinctorial properties, especially by their vital staining with Janus green, and by their size, shape, number, and disposition within cells. They are visible in living cells, especially with the aid of phase microscopy. They are by no means static, but move, grow, divide and recombine, and, in general, change in appearance from moment to moment. In size, they range from elongated objects several micra in length down to the limit of resolution with ordinary microscopy.

With the application of electron microscopy to tissues, and particularly to tissue sections, mitochondria were quickly identified by their characteristic sizes, shapes, and cellular distributions. In the earliest published micrographs, the organelles appeared merely as ovoid or filamentous objects of considerable density. After microtomy was developed so that sections 500 to 1000 Å in thickness were available, membranous internal structures were detectable, and with the thicknesses of sections now current (100 to 200 Å) these mitochondrial membranes have been clearly resolved in a large number of different animal and plant cells. The internal configuration of mitochondria has been discussed by Palade (1953) and by Sjöstrand and Hanzon (1954) among others. The outer wall of the organelle is bounded by a smooth, osmiophilic membrane, and is separated from a similar inner membrane by a space approximately 100 Å in width. The inner membrane frequently turns toward the interior of the mitochondrion, then reflects upon itself to return toward the point of separation and to resume its course parallel to the outer membrane. These inward-directed folds thus appear in section as double lines. The folds, in the third dimension, may extend over considerable distances so that they have plate-like or lamellar contours. The terminology ordinarily employed refers to an outer membrane, and an inner membrane with its internal folds, lamellae, or cristae (Palade, 1953). The internal folds are not necessarily lamellar in shape, but may be villous or tubular in form. This appearance is regularly encountered in the mitochondria of the adrenal cortex (Palade, 1953; Lever, 1955). Between the internal folds an amorphous, homogeneous

material of varying density, the matrix, is located. The matrix may be scant in amount and density, or it may exhibit such an excessive density that the limiting membranes are visible only with difficulty. Similarly, the space between the external and internal membranes, which is also continuous with the space between the twin lines of the cristae or internal folds, is ordinarily less dense than the membranes themselves. On occasion, however, the intermembranous density is so great as to obscure the double lines of the mitochondrial wall. Both the internal matrix and the intermembranous material sometimes exhibit areas more tenuous than others, so that frank vacuolar appearances are by no means uncommon. Likewise, dark granular aggregates may often be encountered apparently embedded in the matrix (Palade, 1953).

Light microscopists have long known, and electron microscopists have confirmed, the considerable pleomorphism of mitochondria. The characteristic size and shape of mitochondria in one region of a cell sometimes are quite different from the appearances of the organelles in other regions of the same cell. In neurons, for example, the mitochondria located in the perikaryon are usually slightly elongated structures of moderate size. In axons, however, the mitochondria may be greatly elongated. Filamentous mitochondria occur normally in the basal parts of tubular or glandular epithelia, while the apical mitochondria are characteristically smaller. Mitochondria smaller than the limit of resolution of light microscopes (in the order of  $0.1 \mu$ ) have been seen in small non-myelinated nerve fibers.

Even greater variability in mitochondrial appearance is encountered when different cell types are compared. These differences are apparent even in closely related cells. A good example of such close relationship, with contrasting mitochondrial structure, is encountered in the human placenta. The cytotrophoblastic cells of Langhans' are the germinal elements of the chorionic coating of the placental villi. These cells divide, their cell membranes break down, and their cytoplasm fuses into a syncytium which forms the external coat of the villi. Yet, the mitochondria of the cytotrophoblasts are large, with simple cristae and little matrix, whereas those of the syncytial trophoblast are small and exhibit a dense intermembranous component (Wislocki and Dempsey, 1955 *a*). Indeed, the differing characteristics of mitochondria from diverse locations are so striking that a fair guess as to the tissue of origin can be made from the appearance of the mitochondria alone. Generalizations are perhaps premature, but it appears at present that mitochondria from tissues having high oxidative rates, such as kidney, brain, and muscle, have many internal membranes or cristae whereas the organelles from more sluggishly respiring cells have fewer.

The variation in mitochondrial structure which accompanies altered physiological states would seem to be an attractive field for investigation. The literature of cytology, as studied by light microscopy, is replete with examples of

such correlations. With the electron microscope, a few instances have been noted but only in rather casual experiments. Weiss (1953), repeating Bensley's experiment in which mice were maintained on a sugar and water diet, confirmed the fact that the mitochondria of pancreatic acini are rapidly depleted in number. After refeeding such depleted animals, a normal number of mitochondria is promptly restored. Similar depletion and repletion in hepatic mitochondria by starvation and refeeding were described by Fawcett (1955). Mitochondria in the thyroid gland decrease in number and size after hypophysectomy, and increase in states in which thyrotropic hormone activity is elevated (Dempsey and Peterson, 1955). In the adrenal cortex, Lever (1955) has described changes in mitochondria which are related to the synthesis of adrenal lipide and which are induced by adrenocorticotrophic hormone.

Mitochondria have long been known to undergo changes in aging cells. Cowdry (1918) first drew attention to the characteristic decline in number and final disappearance of mitochondria from aging cells. Similar changes have been described for other cells, and in addition, Payne (1946) has described a process in the pituitary and adrenal whereby with increasing age, mitochondria become swollen and converted into pigment granules. Weiss and Lansing (1953) confirmed these changes, utilizing the electron microscope. Similar pigment granules develop in aging neurons, and these have been related through transitional forms with mitochondria by Hess (1955).

In the guinea pig, the gestation period lasts for 68 days and the fetuses are remarkably mature at birth. This prolonged gestation period provides an opportunity to study the fetal membranes in a situation wherein they have persisted longer than their accustomed life span in closely related rodents. Dempsey (1953) reported that the mitochondria of the yolk-sac epithelium were of a normal configuration during the early and middle portions of pregnancy, but that they become swollen and disorganized toward term. Concurrently, densely osmiophilic bodies, presumably pigment, appeared in these "aged" cells. In the rat and rabbit, on the contrary, the gestation period is short, the fetus is immature at birth, and the mitochondria of the yolk-sac are normal at term (Wislocki and Dempsey, 1955 *b*).

In the mare's placenta, the yolk-sac is a transient structure which develops and flourishes during the early part of pregnancy, only to decline and disappear at later stages (Amoroso, 1952). At the stage the yolk-sac is shrinking, some of its cells and their contained mitochondria are vacuolated, detached from their basement membrane, and possess swollen, vacuolated, and otherwise abnormal mitochondria (Fig. 1). Such changes, occurring as they do in moribund cells adjacent to normal cells, can hardly be ascribed to fixation artefacts but rather must be attributed to the aging and death of the affected cell.

An interesting experimental tool for studying mitochondria would appear

to be forthcoming from exposure of cells to agents which are toxic to the organelles. In the course of studying organs from rats in which silver deposits had occurred after the administration of silver nitrate in the drinking water, Dempsey and Wislocki (1955) noted the segregation within cells of the silver in aggregated clumps, surrounded by a membrane circumscribing the deposit and walling it off from the adjacent cytoplasm. These aggregates frequently were of a size, shape, and distribution such as to suggest their derivation from mitochondria. Moreover, transition forms were encountered in which granules of silver were located on the internal membranes and in the matrix of frank mitochondria. Where these granules became aggregated into clumps, the internal membranes were disorganized but still recognizable (Figs. 2 and 3). Concurrently, Weiss (1955) studying the effect of intravitaly administered neutral red upon pancreatic cells, noted that this and other dyes were segregated within mitochondria and caused swelling, vacuolation, and disruption of their internal membranes similar in many ways to the disorganization induced by silver.

The origin of mitochondria is questionable. They are reduced in number at cell division, and a full complement is restored later. In tissue cultures observed by phase microscopy, elongated mitochondria have been seen to break in two, as well as to recombine. Such observations lead to the assumption that mitochondria divide by fission, and that new mitochondria arise from previously existing mitochondria. This concept, similar as it is to the doctrine that all cells come from previously existing cells, is peculiarly attractive, and some morphological support for it has been forthcoming from experiments such as Fawcett's (1955) in which depleted liver cells, rapidly forming new mitochondria, occasionally exhibited forms which could be attributed to the process of fission. Nevertheless, there seems to be adequate reason to question this attractive hypothesis.

In repeating and confirming Bensley's experiment, Weiss (1953) observed that pancreatic acinar cells were greatly depleted in their mitochondrial complement after maintenance of rats on a sugar and water diet. In these depleted cells, only a few mitochondrial profiles were encountered in sections; these were ellipsoidal bodies with a dense matrix. Upon refeeding, new mitochondria rapidly put in their appearance in large numbers. The new mitochondria contained little matrix at first, so that they were easily differentiated from the previously existing ones which were dense. The new mitochondria gradually darkened as they acquired matrix substance. During this time of neoformation of mitochondria, the older denser organelles remained unchanged. It therefore appears unlikely that the older structures divided to form new ones, as they should have become unidentifiable in the process.

Lever (1955) has recently described changes in the mitochondria of the adrenal cortex in different functional states. Organelles from normal, control

animals were conventional in structure in that their limiting membranes were continuous. After treatment with large doses of adrenocorticotrophic hormone, cellular enlargement and multiplication of mitochondria occurred. In such cells, open forms were commonly encountered, that is, the outer limiting membrane recurved to combine with the inner one, thus leaving an opening or pore through which the interior of the mitochondrion was continuous with the external cytoplasm. Moreover, such open mitochondria often exhibited internal membranes similar but for location with the microtubules associated with the Golgi complex. The matrix of these incomplete, presumably newly formed, mitochondria was less dense than that observed in the adrenals from hypophysectomized animals or in those from normal controls. A fuller account of these phenomena will be presented elsewhere in the proceedings of this symposium.

Another situation in which rapid proliferation of mitochondria occurs is in the cleavage stages of blastocysts. Unfertilized ova from ovarian follicles, in rabbits and guinea pigs, contain relatively few small, ovoid, dense mitochondria. On the contrary, the mitochondria of blastocysts are more numerous, larger, pleomorphic, and less dense.

The kidneys of new-born rats are incompletely differentiated. During the first few days after birth the brush border of the proximal convoluted tubules becomes more complex, the basal invaginations of the cell membrane increase greatly in complexity, and the mitochondria increase in size and number. At these times, the mitochondrial matrix is less dense than in adulthood, and open forms, with incomplete limiting membranes, are occasionally encountered (Clark, personal communication). Somewhat similar changes may be observed in the mitochondria of differentiating heart muscle (Muir, unpublished). Myoblasts contain relatively few, small mitochondria. As myofibrils form, the mitochondria enlarge and their internal membranes become more complex. Again, occasional forms are seen suggesting open mitochondria. Parallel observations have also been made on differentiating neural cells (Luse and de Lorenzo, unpublished).

A final instance should be noted in which mitochondria are altered in cells, the metabolism of which has been changed. Luse and Smith (unpublished data) have infected splenic cells in mice by injecting massive doses of salivary gland virus intraperitoneally. They have also studied human fibroblasts grown in tissue culture after infection with virus. In both instances, the cells enlarge and exhibit an increased number of mitochondria, with frequent "open" forms, at times just prior to the appearance of recognizable viral bodies within the cell. In late stages, after the development of large inclusions, the mitochondria are greatly depleted in number. In the intermediate stages, spherical bodies, presumably related to the virus, appear within membranous vacuoles, in the membranous tubules and vesicles of the Golgi com-

plex, and within bodies indistinguishable from mitochondria. These observations indicate a relationship between all of the membranous intracytoplasmic organelles, and further support the hypothesis that mitochondria may be formed *de novo* from previously existing membranous structures.

## SUMMARY

A characteristic internal structure, consisting of a double-layered outer wall enclosing a matrix-filled space through which pass double-layered membranous folds, would appear to comprise as satisfactory a definition of mitochondria for electron microscopy as their intravital affinity for Janus green affords for light microscopy. Relying for identification upon this characteristic internal structure, mitochondria appear to be pleomorphic structures which vary in size, shape, complexity, and density. They are labile also in that their number may increase or decrease under controlled conditions. The possibility therefore exists that these organelles are constantly being formed and destroyed, perhaps by their participation in metabolic processes.

The problem of the origin of mitochondria is in an unsatisfactory state. New organelles unquestionably are formed in particular physiological states. The possibility that new bodies are produced by fission of ones already present does not seem adequate. On the other hand, the possible fabrication of new mitochondria out of intracellular membranes, although an attractive hypothesis, has not been adequately substantiated.

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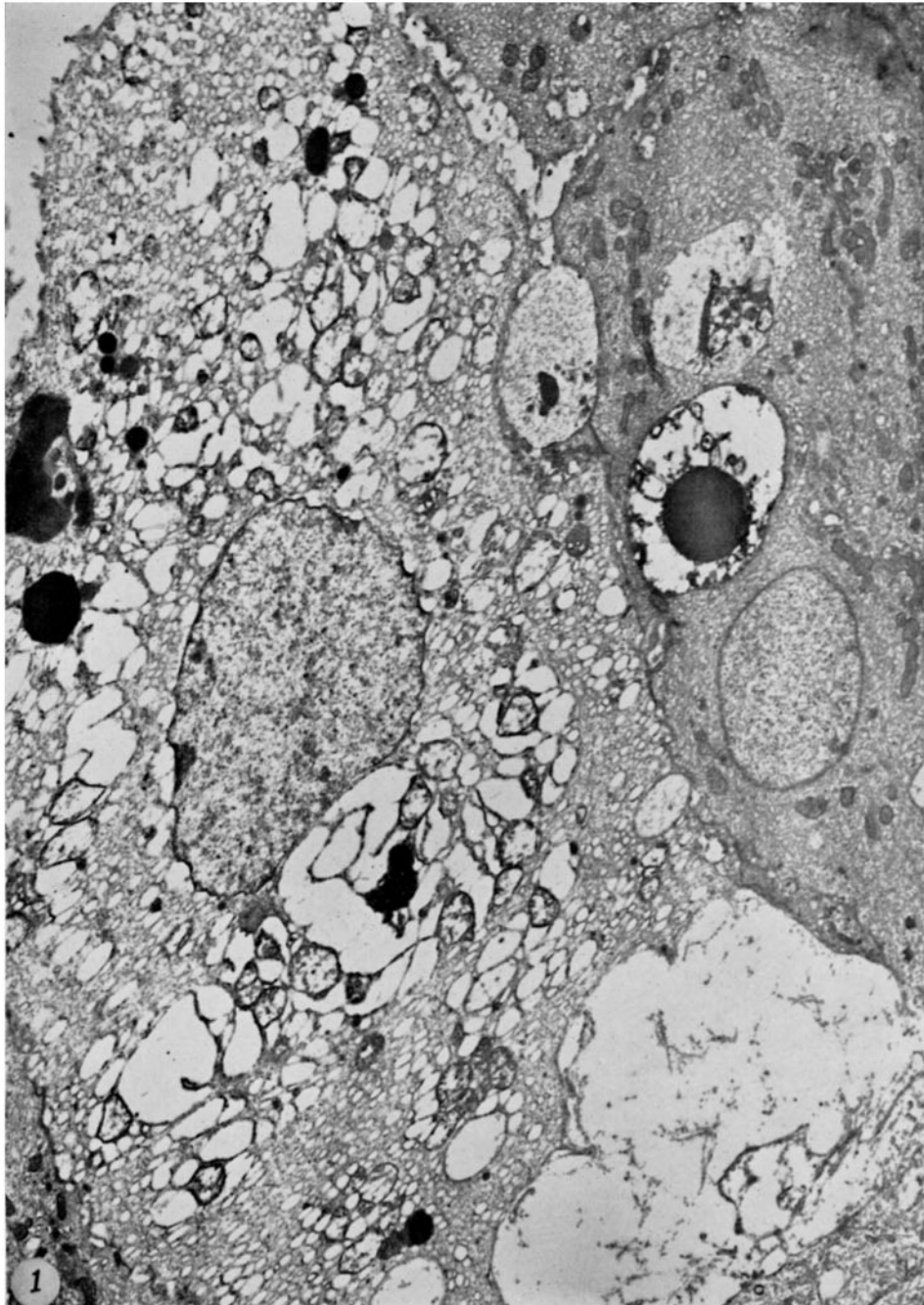
PLATES

## EXPLANATION OF PLATES

## PLATE 96

FIG. 1. Section through two adjacent cells from the yolk-sac of a mare 5 months pregnant. At this stage the yolk-sac is degenerating and becoming vestigial. The cell on the left exhibits vacuolation and has swollen, distorted mitochondria. The one on the right, however, has normal appearing mitochondria. The degenerative changes would appear to be real, therefore, and not a result of faulty fixation. Palade's fixative.  $\times 6000$ .



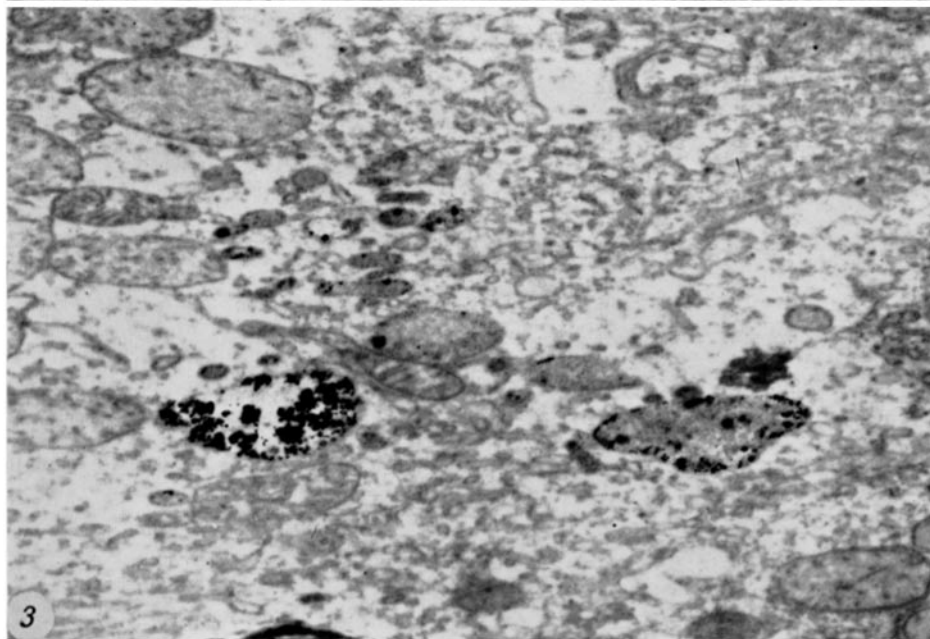
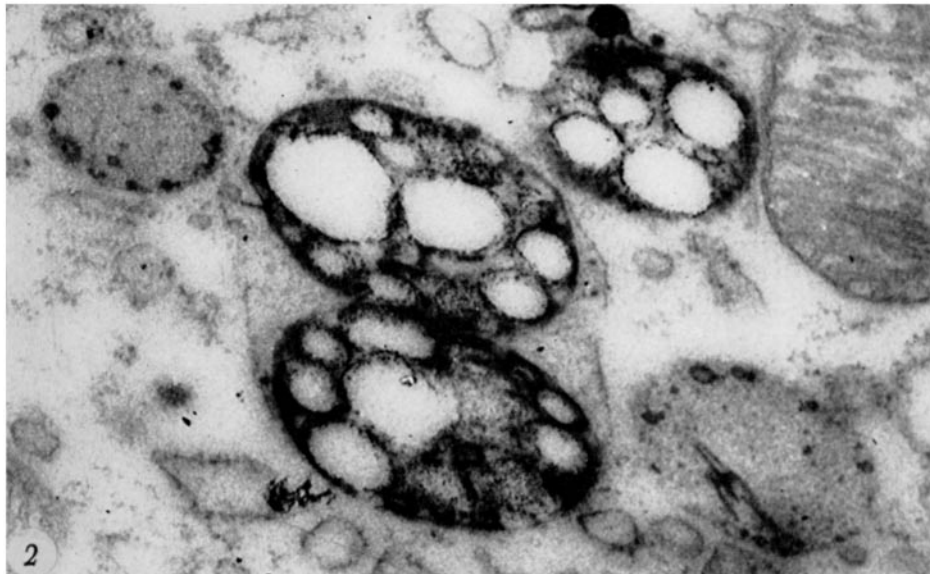


(Dempsey: Variations in structure of mitochondria)

PLATE 97

FIG. 2. Section through a portion of a cell from the chorioid plexus of a rat which had received  $\text{AgNO}_3$  in its drinking water for 12 months. Inclusions containing dense silver deposits may be seen in the center of the field. At the upper left and lower right are bodies containing argyrophilic granules. These are interpreted as deformed mitochondria. The one at the lower right exhibits a few disorganized internal membranes. Palade's fixative.  $\times 57,000$ .

FIG. 3. Section from the liver of a rat which had received  $\text{AgNO}_3$  in its drinking water for 12 months. Argyrophilic inclusions, similar to those illustrated above, are present in the hepatic cells. Palade's fixative.  $\times 15,000$ .



(Dempsey: Variations in structure of mitochondria)