THE INTERNAL ORGANIZATION OF MITOCHONDRIA*

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Plates 113 and 114

In our description of the mitochondrion of *Paramecium aurelia* and *Paramecium bursaria* (4), it was stated that "the undifferentiated mitochondrion in *Paramecium* is interpreted to consist of a compact mass of twisted tubules, the walls of which are made up of at least two kinds of substances (based on density differences in the photographs)." Since then more evidence confirming this aspect of mitochondrial structure in *Paramecium aurelia* has been gathered by us, and this basic type structure has been recognized in sections of mitochondria of other Protozoa in our and other laboratories. Furthermore, sections of mitochondria of Metazoa as well as plants show, in addition to plates and folds, a range of structure that includes tubules very similar to those of the protozoan mitochondrion, indicating that a rigid concept of mitochondrial structure that excludes this type is incorrect.

Materials and Methods

The materials were two kinds of ciliate Protozoa as indicated below, rat kidney, and the ovotestis of *Helix*. These were prepared by fixation in 1 per cent OsO_4, pH 7.4, sectioned in methacrylate by a glass blade at a thickness of 0.025 μ, and mounted on carbon membranes. The EMU-2 and the EMU-3A models were used.

RESULTS

Paramecium aurelia.—The section (Fig. 1) is evidence, in addition to that presented before (4), demonstrating that in this cell the mitochondrion is a mass of tubules. Again, the clear circles and ovals (transversely sectioned tubules) are bounded by an electron-dense material. These in turn are surrounded by a less electron-dense material that forms the continuum of the mitochondrion. Several of the tubules (at points indicated by the arrows) open to the cytoplasm.

Euplotes patella.—The mitochondrion of this hypotrichous ciliate (Fig. 2) presents the same aspect in section as that of *Paramecium*. Here again the two kinds of material are clearly evident—the electron-dense material immediately surrounding the clear lumens, and the less dense material between and

* Work performed under the auspices of the United States Atomic Energy Commission.

among these circles and ovals. All dimensions are similar to those in *Paramecium aurelia*.

The rat.—In the rat kidney (Fig. 3) the numerous mitochondria also are seen to consist of two materials—the electron-dense which at times is seen to form circles, ovals, and very short parallel lines, and the less dense material in and around the more dense. From these sections the mitochondria are best interpreted as consisting mostly of tubules. These tubules are of the same general dimensions as those seen in the protozoan mitochondria.

Helix aspersa.—In the oviduct of the snail (Fig. 4), the mitochondria in section are seen to be constructed on the same general plan as those described above. The interior consists of the two kinds of materials, and the tubular arrangement of the more dense material is evident.

However, in the same organ but in the male portion (Fig. 5), the mitochondria in section give a different picture. While there are two materials, a dense and less dense, the dense material is obviously not arranged in a twisted tubular mass. When visible, it is arranged in parallel pairs of lines extending the length of the long axis of the mitochondrion. These may represent sections of plates, and in the regions of mitochondria where none is visible the section may be in the plane of the plate.

DISCUSSION

The demonstration of at least two kinds of electron-dense material within all the mitochondria sectioned is unequivocal. The specific chemical nature or biochemical function of the materials is open to conjecture. A conclusion that seems justified is that the material that immediately surrounds the lumen of the mitochondrial tubule reduces OsO_4 more effectively, and, therefore, must have a higher content of easily oxidized materials than the other material. There is, in addition, the substance that occupies the lumen of the mitochondrial tubule, which has little affinity for Os and appears clear in these photographs. These are the only empty spaces that should be seen. It is our opinion that the success of fixation, sectioning, and photographing of the mitochondrion may be judged partially by the absence of empty spaces about the tubules (or homologous structures, as discussed below), and the presence of the less dense material constituting the continuum.

It is equally clear from the series of photographs shown here that mitochondria from several phyla (Protozoa, Mollusca, Chordata) exhibit tubular organization. Also, in addition to our demonstration here in *Paramecium aurelia* and *Euplotes patella*, we see evidence of the presence of mitochondrial tubules in Protozoa in *Paramecium bursaria* (4), in *Paramecium multimicronuleatum* (7), in *Pelomyxa* (unpublished observations in this laboratory), and in *Amoeba* (our interpretation of unpublished micrographs of Marie Greider).

However, not all investigators interpret sections like these as evidence for tubular elements. Sedar and Porter (7) use the term "microvillus" to describe structures that in section appear just like the internal elements we present, and they state that the elements terminate freely in the mitochondrial matrix. In the figure presented as evidence for this concept, we see no blind endings of elements and, therefore, no indication of this kind of organization. On the contrary, the figure supports the tubular organization proposed by us, although it fails to exhibit the continuum of less dense material surrounding the tubules.

The authors state further that the lumen of each element is continuous externally with an interspace existing between an inner and an outer mitochondrial membrane. Of the two instances pointed out, one can be interpreted as the tubule's turning at right angles at the edge of the mitochondrion (an L instead of a T), and not as an internal projection of an internal membrane. Consideration must be given to the role of both peripheral and internal branching (fusion?) of tubules, as well as to the possibility that the external membrane is a cytoplasmic component about the whole mitochondrion, which itself is membraneless. Neither membrane nor interspace continuity is demonstrated. For proof of this concept of the structure of the edge of the mitochondrion and its relation to the internal elements, more evidence is necessary.

In the oviduct of the snail, and, at times, in the kidney of the rat, mitochondria comprise mostly tubules. But in the same organ of the snail, there are mitochondria whose structure can be interpreted differently; and the demonstrations of plate-like structures in the mitochondria of the mammalian kidney, e.g., the evidence of Sjöstrand and Rhodin (8), are unquestionable. Therefore, in these organs there are at least two types of internal organization observed in sections.

However, the differences may not be essential.[1] The dense material lining the tubule and that making up the plate are similar in appearance, and the origin of one from the other can be easily imagined. As we have pointed out before, the mitochondrial tubule may be a basic cellular structure that differentiates into many things in different kinds of cells, including mitochondrial structures that no longer retain their tubular appearance. (However, even anatomically identical mitochondria may differ in other ways; in view of the nucleolar origin of at least some of them (2), enzymatic differences between mitochondria of the same cell may be expected.)

When the mitochondrion is thus viewed as a dynamic organelle consisting of tubules, but capable of differentiation and change, the reconciliation of apparent differences is possible. However, a more elementary unit than the tubule (perhaps a sphere) may yet be found in mitochondrial genesis. Which one of the various states is more elementary cannot, of course, be recognized now, but at present the nearly ubiquitous phylogenetic occurrence of the

[1] For example, according to one criterion, homologous chromosomes are different; by another, heterologous chromosomes are alike. On mitochondria, see Ritchie and Hazeltine (5).

tubule, and its presence in the early differentiation of finally lamellated structures (for example, the tubular proplastid that develops into the lamellated plastid (3) and the filamentous mitochondria that develop into the lamellated spermatid nebenkern (1, 9)), suggest that, of known structures, the tubule is the basic one.

It is important that the extent to which these relations exist be described. To accomplish this it will be necessary to recognize the extent to which mitochondria differ in structure and the extent to which apparently differently constructed mitochondria are similar, as well as the extent to which other structures in the cell are similar to the mitochondrial elements.

SUMMARY

Sections of mitochondria in *Paramecium* and *Euplotes* present a consistent pattern. The mitochondrion in these cells can be conceived of as a twisted mass of closely compacted tubules. Two general kinds of substances can be recognized: the electron-dense that borders the lumen of the tubule, and the less dense that forms the continuum. In sections of mitochondria in rat kidney and snail oviduct, tubular internal organization can be recognized. In the same organs, mitochondria with lamellar internal structure can be demonstrated. The thesis is developed that the mitochondrion is a structure capable of differentiation and change, and that developmental continuity among the different kinds may exist. Mitochondria that appear to be different may be quite similar basically; mitochondria that appear to be similar in structure may be different in other ways. The tubule is proposed as the most basic of the presently recognized mitochondrial structures.

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PLATES

EXPLANATION OF PLATES

PLATE 113

FIG. 1. A section including the pellicle of *Paramecium aurelia* showing sections of mitochondria composed of tubules. The arrows designate points at which the lumen of the tubule appears to communicate with the cytoplasm. × 42,000.

FIG. 2. A section of *Euplotes patella* demonstrating the tubular structure of the mitochondria. The section includes the bases of several ciliary groupings called membranelles. Also shown are ciliary rootlets extending across the field (see Roth, 6). × 30,000.

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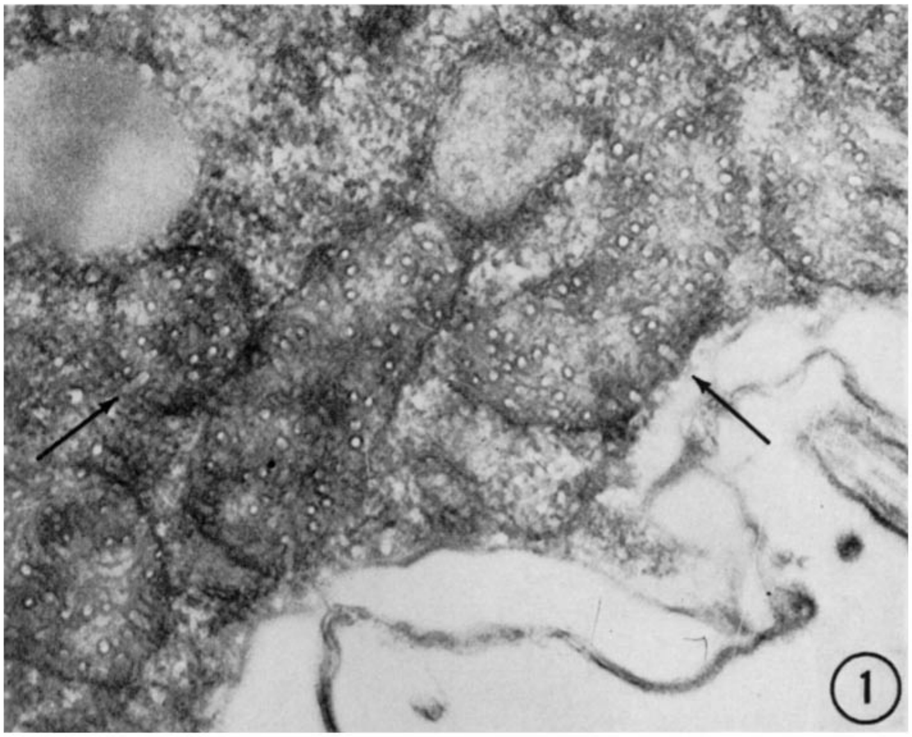

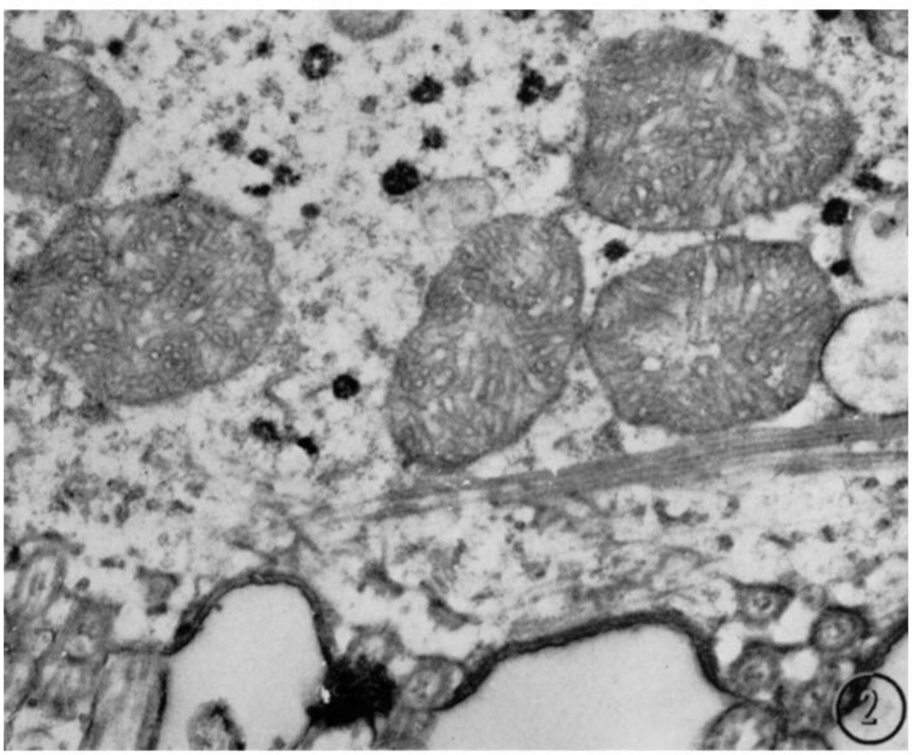

(Powers *et al.*: Structure of mitochondria)

PLATE 114

FIG. 3. A section through the convoluted tubule of the rat kidney showing numerous mitochondria consisting of structures that may be considered tubules, as well as those that may be interpreted as lamellae. × 27,000.

FIG. 4. A section through the oviduct of *Helix aspersa*. × 30,000.

FIG. 5. A section through the male portion of the ovotestis of *Helix* showing the typical organization of mitochondria in these cells. This section is from the same organism as that from which Fig. 4 was taken. × 49,000.

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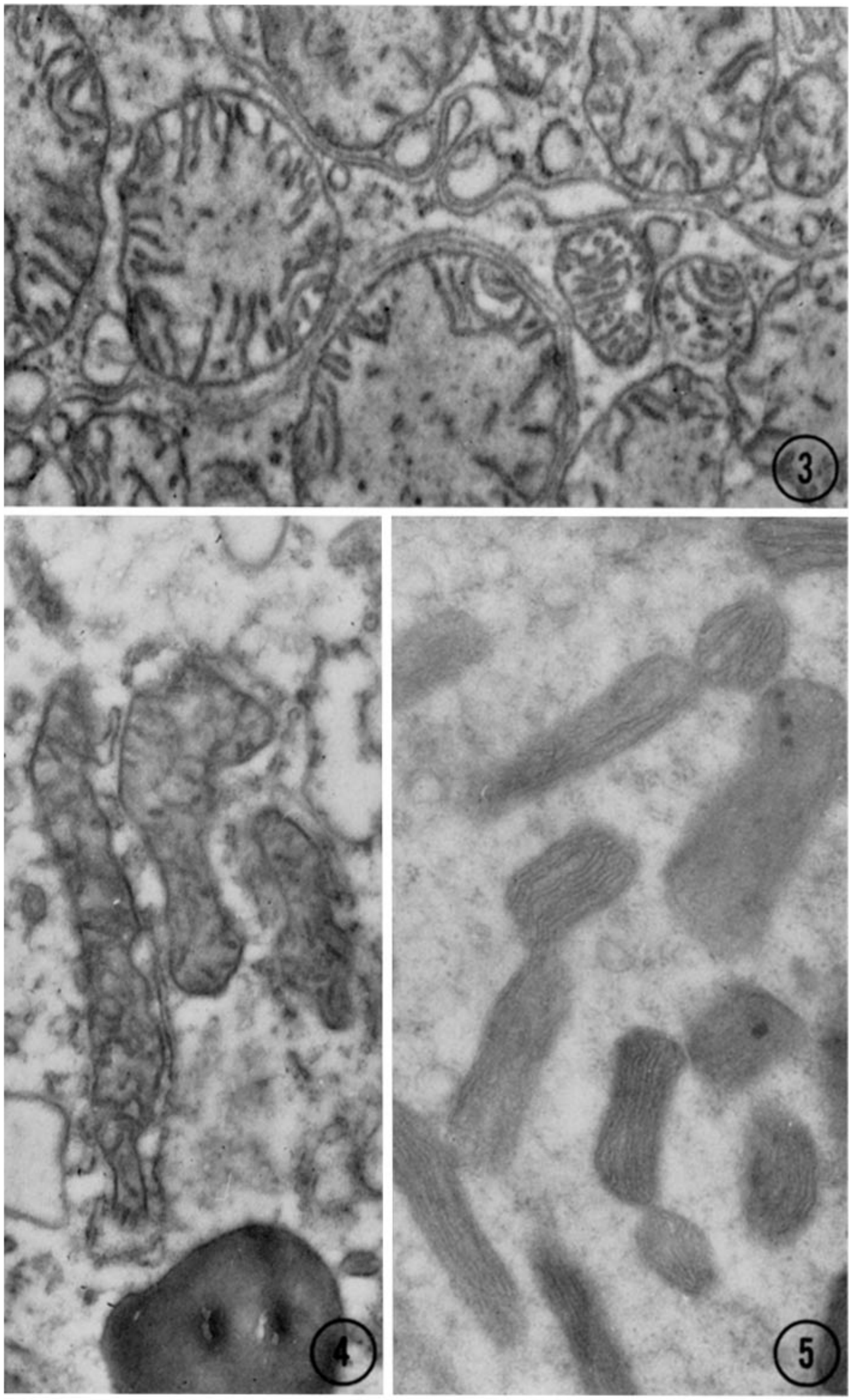

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