

## MITOCHONDRIA OF PROTOZOA

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PLATES 109 AND 110

In recent years mitochondrial fine structure has received a good deal of attention from electron microscopists. Palade (7, 8) has shown that the mitochondria of plant and a wide variety of animal cell types are very similar in possessing an internal system of ridges (cristae mitochondriales) that project perpendicularly from the inner surface of the mitochondrial membrane into the interior. However, Sjöstrand (15) has proposed that the internal structure of the mitochondrion is comprised of "double membranes" which are not necessarily continuous with the limiting membrane of the mitochondrion.

Since Palade's observations upon the mitochondria did not extend to protozoa except for the chlorophyll-bearing flagellates (18), it is of interest to examine mitochondrial structure in certain representatives of the protozoa in order to compare it with mitochondrial structure in the metazoa. The following report is largely drawn from observations of (a) Sedar and Porter (13) on *Paramecium multimicronucleatum*; (b) Rudzinska and Porter (12) on *Tetrahymena pyriformis* and *Tokophrya infusionum*; and (c) Pappas (9) on *Amoeba proteus*. The papers of these investigators should be consulted for details concerning the method of preparation of the various organisms for examination with the electron microscope.

*Mitochondria of Paramecium multimicronucleatum.*—The endoplasm of *Paramecium* has proportionately a greater number of mitochondria than the cortex (Fig. 1). In an oblique section of the organism, the mitochondria appear as oval, rod-shaped, or circular profiles (Fig 1.). At higher magnifications it can be seen that they are similar in structure to that described in a number of animal cell types (7, 8, 15, 16, 13); but noticeably different in finer details (Fig. 2). The elements comparable to the "cristae mitochondriales" or "double membranes" of most mammalian somatic cells are seen to be microvilli or finger-like projections protruding into the interior of the mitochondrion from the inner surface of the limiting membrane of the organelle. The approximate diameters of the microvilli (35  $m\mu$ ) are greater than the thickness reported for corresponding structures in mammalian somatic cells. Evidence for the finger-like form of the microvillus is indicated by (a) the appearance of oval, elongated, and circular profiles in each mitochondrial section, (b) the constant

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occurrence of oval profiles, and (c) analysis of serial sections in which the oval profiles of the microvilli can be traced from section to section (Figs. 3 and 4). In some instances the microvilli were observed to branch (Fig. 2), but generally this was not the case. They appear to follow a winding path through the mitochondrial matrix, rarely making a second contact with the inner surface of the mitochondrial membrane. This membrane is seen in many places to (Fig. 2) consist of two closely apposed membranes, an outer mitochondrial membrane and an inner mitochondrial membrane. It has been shown recently that the lumen of a microvillus is continuous with the space separating the inner and outer limiting membranes (13). The double nature of the mitochondrial membrane has also been reported in mammalian cell types (8, 15, 16, 11).

*Mitochondria of Amoeba proteus.*—Mitochondrial structure in *Amoeba proteus* is essentially the same as that described above for *Paramecium multimicro-nucleatum*. An unusually high proportion of both elongated and oval profiles of microvilli can be observed in the mitochondria of this organism (Fig. 5) and, if there is any significant difference, it is in the number of microvilli. They seem extraordinarily numerous here. Examples of this sort can also occasionally be found among the mitochondria of *Paramecium*. The diameters of the microvilli range from 30 to 40  $\mu$ .

*Mitochondria of Tokophrya infusionum.*—An examination of sections through *Tokophrya* reveals that the mitochondria are not confined to any special region but are scattered at random throughout the cytoplasm in relatively small numbers (Fig. 6). When examined at higher magnifications they are observed to contain elongate, oval, and circular profiles of microvilli, similar to these encountered in other protozoa. Some of the elongated profiles have been observed to make a second contact with the inner mitochondrial membrane; some appear to branch (Fig. 7). The average diameter of microvilli is 28  $\mu$  in *Tokophrya*.

*Mitochondria of Tetrahymena pyriformis.*—Electron micrographs of *Tetrahymena* show that the mitochondria are distributed throughout the whole cytoplasm. This may be seen in Fig. 9. Striking is the great abundance of mitochondria found in all planes of sections through the organism. The shape of the mitochondria is oval and quite frequently very elongated. The internal structure shows many elongated, oval, and circular profiles representing microvilli (Fig. 8). The diameters of these elements are about 22  $\mu$ , very close to the thickness of cristae mitochondriales in mammalian cells (20  $\mu$ ).

One of us (M. A. R.) is convinced after study of a great number of micrographs that some of the elongated profiles represent cristae.

#### DISCUSSION

Other reports of mitochondrial structure in protozoa, as revealed by electron microscopy, have been recorded. The unpublished observations of Sedar and

Hirschfield (14) indicate that mitochondrial differentiation in *Blepharisma undulans* closely resembles that in *Paramecium*. Although no description was offered to the mitochondria of *Paramecium caudatum* by Ornstein and Pollister (6), their micrograph (8 d) shows elements within the mitochondrial matrix that correspond to the microvilli described by Rudzinska and Porter (12) in *Tokophrya infusioformis* and *Tetrahymena pyriformis* and by Sedar and Porter (13) in the mitochondrion of *Paramecium multimicronucleatum*. Similar intramitochondrial structures were described by Hamilton, Gettner, and Stock in *Paramecium* (5). Other investigators have described the occasional appearance of a "plicate structure" within the mitochondria of *Paramecium* (17). Powers, Ehret, and Roth (10) have chosen to interpret mitochondria from *Paramecium* as an osmiophilic continuum interrupted with tubular spaces without evidence of a distinct mitochondrial limiting membrane. A possible explanation of the divergent opinions of these investigators was offered in another publication (13) and need not be repeated here.

The mitochondria in *Euglena gracilis* and *Poteriochromonas stipitata*, both representative of the Mastigophora, were observed to possess a limiting membrane and a system of internal ridges (cristae mitochondriales) similar to those described for higher animals (18). In *Trichomonas muris*, another flagellate, two different structural patterns have been described: (a) a varying number of projections from the external limiting membrane that come in contact with an internal membrane dividing the mitochondrion into a series of compartments, and (b) vesiculated spheres with an electron-dense outer area (1). Since the embedding matrix was removed from the sections of the organism before examination with the electron microscope, it is possible that some structural alteration may have occurred.

It is clear from these several studies that representatives of a number of classes of the protozoa Sarcodina (*Amoeba*), Ciliata (*Paramecium*, *Tetrahymena*, *Blepharisma*), and Suctoria (*Tokophrya*) show similarities in mitochondrial fine structure. The device common to all for increasing the intramitochondrial surface area appears to be microvilli. Only *Euglena* and *Poteriochromonas* among the forms studied show any departure from this pattern. Considering that *Euglena* and *Poteriochromonas* belong to phytoflagellates, a group which combines organisms with typical plant characteristics on one hand and affinities with animals on the other hand we may come to the conclusion that microvilli are the prevailing and characteristic structure for protozoan mitochondria.

Protozoa are not exclusive in possessing finger-like intramitochondrial projections. Similar structures have been reported in metazoan tissues including adrenal cortex and kidney (8) and liver (4). In the mitochondria of the Malpighian tubules of the insects both tubular and double membrane structures have been observed (3, 2).

## SUMMARY

A study of thin sections of *Paramecium multimicronucleatum*, *Tetrahymena pyriformis*, *Tokophrya infusionum*, and *Amoeba proteus* shows that the mitochondria in all these protozoa are similar in certain aspects of their fine structure to that described in metazoan cells. As in higher organisms the mitochondrion is surrounded by a double limiting membrane and contains protrusions directed inward from the innermost of the double membranes.

There are, however, some differences. In a majority of higher organisms the internal structure of mitochondria consists of ridges or cristae mitochondriales and in a few instances only of finger-like projections, or microvilli. In all protozoa described here and elsewhere microvilli represent the dominant structure. They are characteristic therefore of protozoan mitochondria.

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PLATES

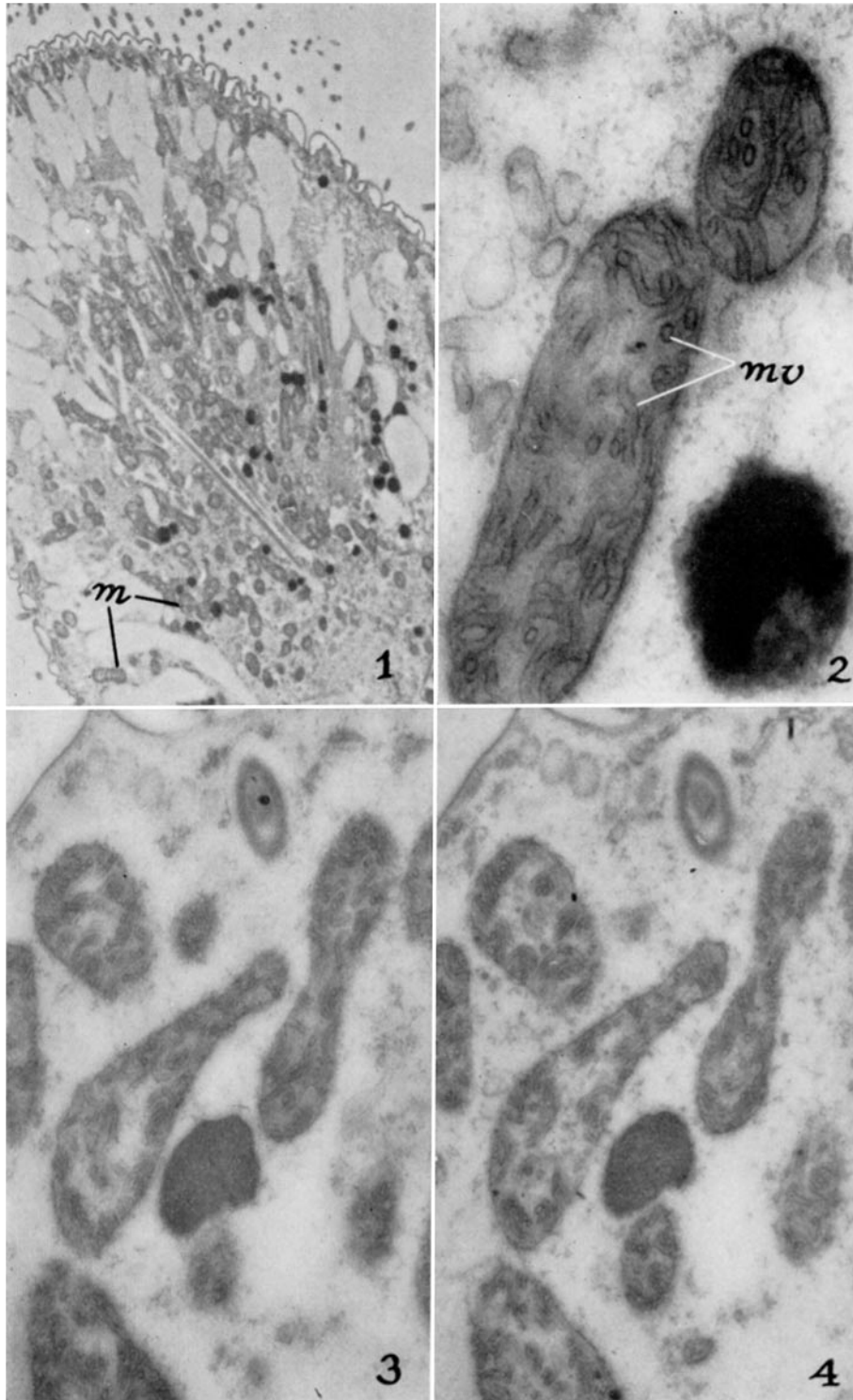
## EXPLANATION OF PLATES

## PLATE 109

FIG. 1. A low power micrograph of an oblique section through a portion of *Paramecium multimicronucleatum* showing the cortex surrounding the endoplasm of the organism. The cortex is covered externally by the pellicle. Below the pellicle, the surface of the organism is molded into ridges. Cilia are seen in places protruding from depressions in the organism's surface. Other cortical components visible in the micrograph include the carrot-shaped trichocysts, a few mitochondrial profiles (*m*), and lipide bodies embedded in the cytoplasmic matrix. Internally the endoplasm contains a greater number of mitochondrial profiles (*m*), some lipide bodies, and portions of internally discharged trichocyst shafts.  $\times 4,500$ .

FIG. 2. An electron micrograph depicting portions of two mitochondria located in the cortex of *Paramecium multimicronucleatum*. The limiting membrane is observed to be double in a number of regions consisting of an outer and inner mitochondrial membrane. Comparable elements to the cristae mitochondriales in most mammalian somatic cells appear as microvilli (*mv*) projecting into the interior of the organelle from the inner surface of the mitochondrial membrane. In addition to the elongated profiles of the microvilli, a number of oval profiles are also visible, representing end views of the finger-like projections. The diameter of the microvilli is approximately 325 Å. Other structures seen in the micrograph include vesicles of the endoplasmic reticulum and the small particulate component.  $\times 52,800$ .

FIGS. 3 and 4. Electron micrographs of two serial sections through portions of a number of mitochondria in the cortex of *Paramecium multimicronucleatum*. In places, some of the oval profiles of the microvilli can be followed from one section to the next, supporting the conclusion that these elements are villi and not ridges that are found in mitochondria of most mammalian cell types. In other regions the oval profiles disappear from one section to the next. This behavior can be accounted for if the sinuous course of the microvilli within the mitochondrion, as well as possible distortion introduced in the thin-sectioning process, is kept in mind.  $\times 26,400$ .



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PLATE 110

FIG. 5. An electron micrograph of mitochondria from *Amoeba proteus* depicting a similarity in internal fine structure to that of *Paramecium*. Note the unusually high proportion of elongated and oval profiles of the microvilli packed within the mitochondrion. The approximate diameter of the microvilli here is  $35\text{ m}\mu$ .  $\times 29,500$ .

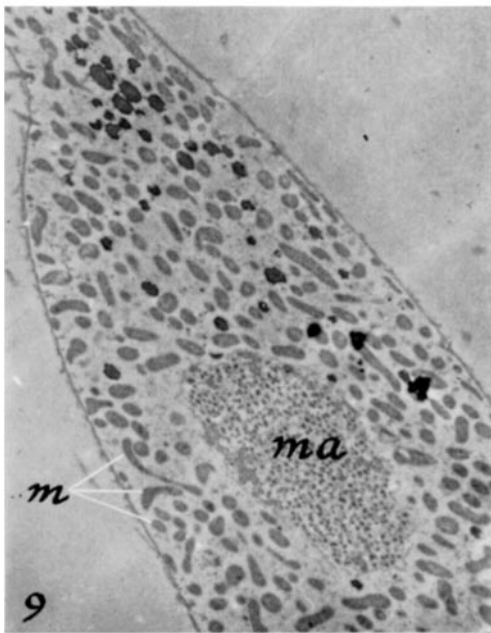
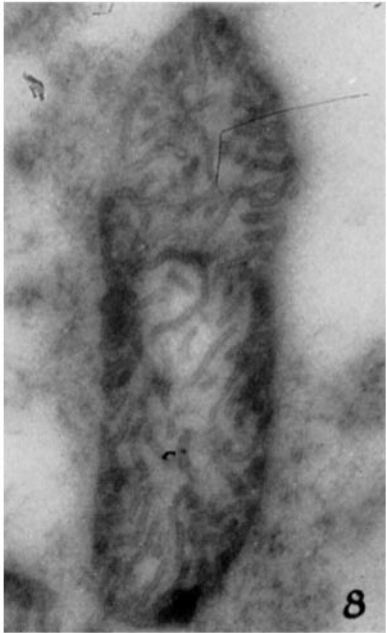
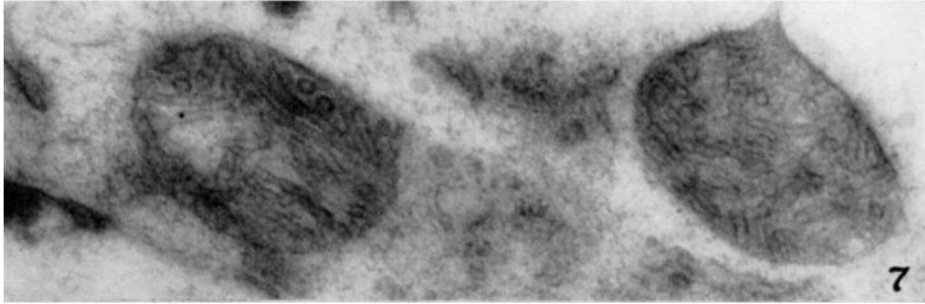
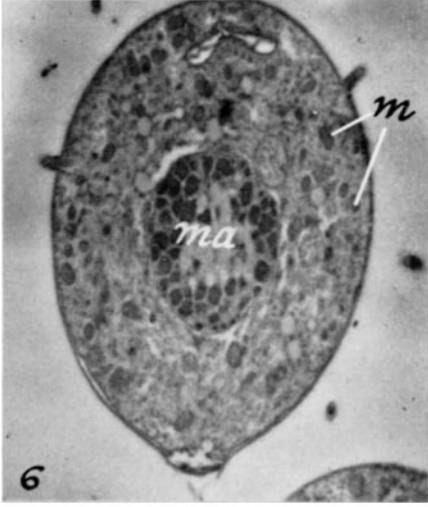
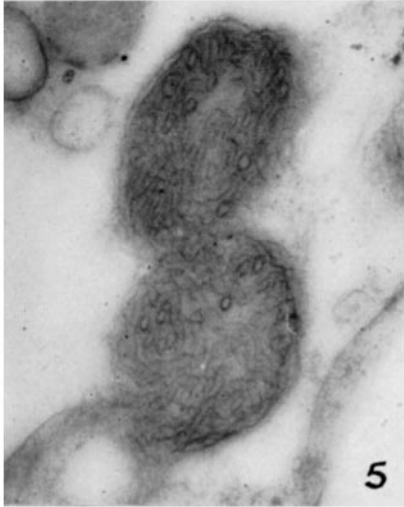
FIG. 6. Low power electron micrograph representing section through whole *Tokophrya infusioenum*. Mitochondria (*m*) appear as dense oval profiles scarcely scattered through the cytoplasm. In the center of the body is the macronucleus (*ma*). The body is covered by a pellicle, below which the plasma membrane may be seen. The two protrusions represent oblique sections through the proximal part of tentacles.  $\times 3150$ .

FIG. 7. High power electron micrograph of mitochondria of *Tokophrya infusioenum*; circular, oval, and elongated profiles represent sections through microvilli. Some of the elongated profiles appear to branch. The diameter of microvilli is about  $30\text{ m}\mu$ .  $\times 34,440$ .

FIG. 8. High magnification of mitochondrion in *Tetrahymena pyriformis*. Many of the elongated, circular, and oval images represent sections through microvilli. The diameter of the microvilli is about  $21\text{ m}\mu$ .  $\times 40,740$ .

FIG. 9. Low power electron micrograph of sections through *Tetrahymena pyriformis*. Characteristic is the abundance of mitochondria (*m*) which are more or less evenly distributed throughout the cytoplasm. Some of the mitochondria appear to be very long. A few lipides may be noticed in the cytoplasm. In the center of the body is the macronucleus (*ma*).  $\times 3,500$ .





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