

Mitochondria

I. Fine Structure of the Complex Patterns in the Mitochondria of *Pelomyxa carolinensis* Wilson (*Chaos chaos* L.)*

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PLATES 52 TO 54

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ABSTRACT

Some of the mitochondria in the free-living giant ameba *Pelomyxa carolinensis* (*Chaos chaos*) exhibit unusual and strikingly complex morphological patterns. A study of serial sections of these mitochondria reveals that the patterns are formed by the organization and packing of minute villi (*crisetae mitochondriales*). The form of the individual villus is a regular soft zigzag (or wave) with a bulbous enlargement at each point of inflection ("elbow") on the wave. The pattern of the mitochondria may become increasingly complex as a result of branching and fusing of the wavy villi. Densely packed fibrillar material is sometimes present in the stroma of the mitochondria.

INTRODUCTION

In the original communications on the fine structure of mitochondria (1, 2), Palade describes the existence of two limiting membranes and observes that the inner of these is infolded. Subsequent investigations have confirmed these findings (3-5). In most cells the infoldings of the inner membrane are described as ridges (*crisetae mitochondriales*), while in protozoa (6), and in some metazoan cells (1, 2), they have been identified as tubular projections or villi. Generally seen in a random array, the infoldings have occasionally been observed in parallel configurations.

Some mitochondria in the giant ameba *Pelomyxa carolinensis* (*Chaos chaos*) have distinctive configurations of striking complexity and order (7). The present report describes these complex formations more fully on the basis of serial sections examined in the electron microscope.

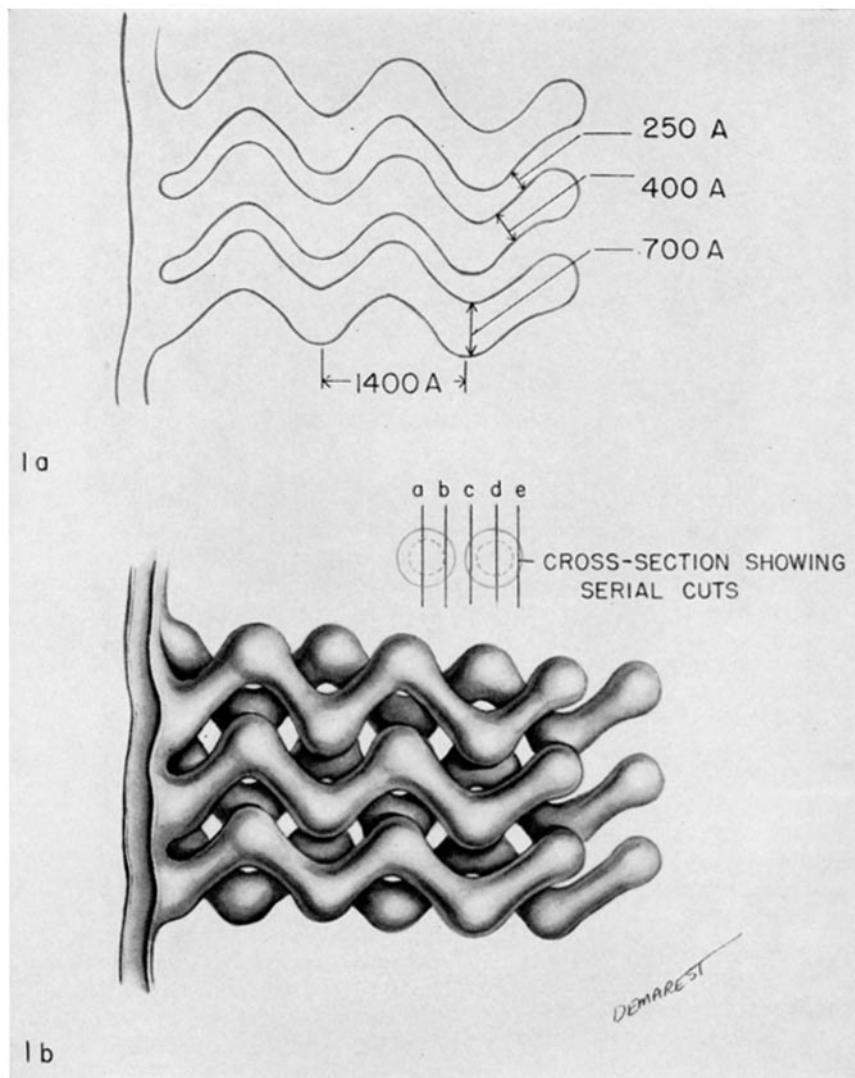
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Materials and Methods

Specimens of *Pelomyxa carolinensis* (*Chaos chaos*) were obtained from Carolina Biological Supply Co., Elon College, North Carolina. The amebas were washed in spring water several times and then lightly packed into a pellet by centrifugation. The pellet was fixed in 1 per cent OsO₄ in veronal acetate buffer at pH 8.6 with 0.01 per cent CaCl₂ added. On frequent occasions, individual amebas were fixed in 1 per cent OsO₄ in a 1/1400 molar veronal acetate solution at pH 8.6, a procedure that somewhat reduced distortion and extraction.

The fixation time was 5 to 10 minutes at 4°C. The pellet was dehydrated in alcohol and embedded in *n*-butyl methacrylate with, on occasion, the addition of 10 per cent methyl methacrylate to produce a harder polymerized block. In many instances, single amebas were fixed, dehydrated, and embedded in individual capsules. Thin sections were cut on a Porter-Blum microtome and collected on formvar-coated 200-mesh specimen grids. Serial sections were collected on formvar-coated Sjöstrand grids. Subsequent to drying, the grids supporting the sections were covered with a thin coat of carbon in a vacuum evaporator, since sections "sandwiched" between formvar and carbon were found to be more stable than "non-sandwiched" specimens in the electron beam (8). Sections were then examined in an RCA-EMU-3C electron microscope.



TEXT-FIG. 1 *a*. An outline drawing of three regular wavy tubules formed by the infolding of the inner mitochondrial membrane. The waves of these villi are in phase. The "wave length" of the villi is about 1400 A. The distance between adjacent villi is 250 A. The thickness of a villus varies from about 400 A, along the "shaft," to 700 A at the bulbous apices.

b. A three-dimensional representation of the serial sections in Figs. 5 *a* to *e*. The two rows of villi are out of phase with each other. The letters indicate where the longitudinal sections were cut. *a* and *d* represent longitudinal sections through the core (dotted outline) of the first and second rows of villi. The rest of the letters represent more superficial cuts which only pass through the bulbous enlargements (solid outline) of the villi. The latter appear as circular profiles in the sections.

RESULTS

Several configurations assumed by the inner mitochondrial membrane in *P. carolinensis* are visible in Fig. 1, which shows a section through four mitochondria. The configurations appear only in one end of each mitochondrion, while the other end contains a few irregularly shaped

villi and densely packed fibrillar material in the stroma (*F* in Figs. 1, 3, and 4). The filaments of fibrillar material are about 4 to 5 μ in diameter.

The patterns formed by the projection of the inner mitochondrial membrane can be extremely complex. The mitochondrion *B* in Fig. 1 shows wavy projections branching at several places

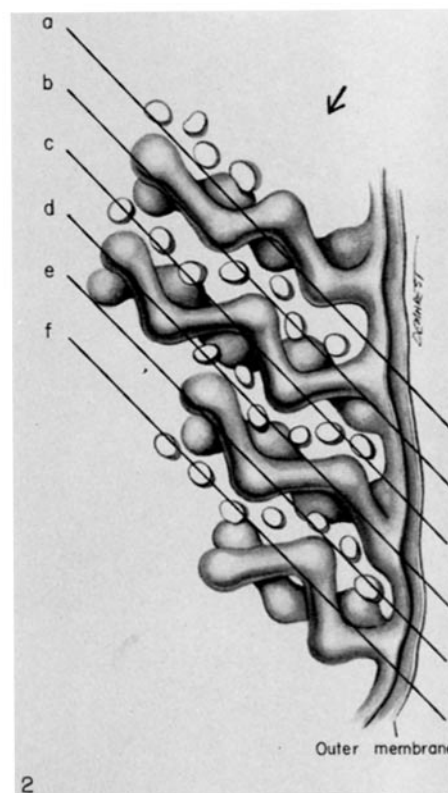
(arrows). Profiles of wavy projections are the most frequently encountered patterns. In the region of the wavy patterns, the stromal area is confined to the 250 Å space between villi (see Text-fig. 1 *a*). The width of the projections varies from 400 Å along the "shaft" to 700 Å at the bulbous elbows. The largest amount of space in the region of the configurations is the intramembranous area, *i.e.* the space between the inner and outer mitochondrial membranes.

A study of serial sections completes a three-dimensional picture of the configurations. In the series shown in Figs. 5 *a* through 5 *f*, the wavy projections appear to be tubular or finger-like. Longitudinal sections through four villi, the waves of which are in phase, form a pattern in Figs. 5 *a* and *b*. Regularly arrayed circular profiles of these villi, observable in Figs. 5 *b* and *c*, occur when the section goes through the edge of the bulbous protuberances. Fig. 5 *d* shows a second row of four villi cut longitudinally. The regular wavy pattern of this row of villi is out of phase with the row of villi seen in Fig. 5 *a*. The circular profiles in Fig. 5 *c* represent a longitudinal superficial cut through the bulbous elbow enlargements of the second row of wavy villi.

In Figs. 5 *e* and *f*, the wavy profiles of the second row of villi have largely disappeared, and only circular profiles resulting from superficial cuts of the bulbous enlargements remain.

Figs. 5 *a* through *f* represent a progressing series of longitudinal profiles through two rows of villi, one out of phase with the other. Text-fig. 1 *b* sums up the evidence in this series in a diagrammatic three-dimensional model.

The series in Figs. 6 *a* through *f*, shows circular to dumb-bell-shaped (*C*), and thin, elongate profiles (*E*). The complete reversal from the former (*C*) to the latter (*E*) occurs from one section to the next, indicating that rows of wavy villi are intersected by thin (300 Å) elongate villi. Text-fig. 2 presents a three-dimensional diagram showing the alternating arrangement of regular wavy villi and circular profiles. The rows of regular wavy villi are lined up so that adjacent rows are out of phase with each other. Sections cut through the regular villi produce the profiles varying from circular to dumb-bell-shaped (*C*). Perpendicular to these villi are found less regular thin villi which, because of the section orientation, are always seen as elongate profiles (*E*). Alternation of dumb-bell profiles (*C*) with elongate profiles (*E*) from one section to the next is due to the thickness and orientation of the section



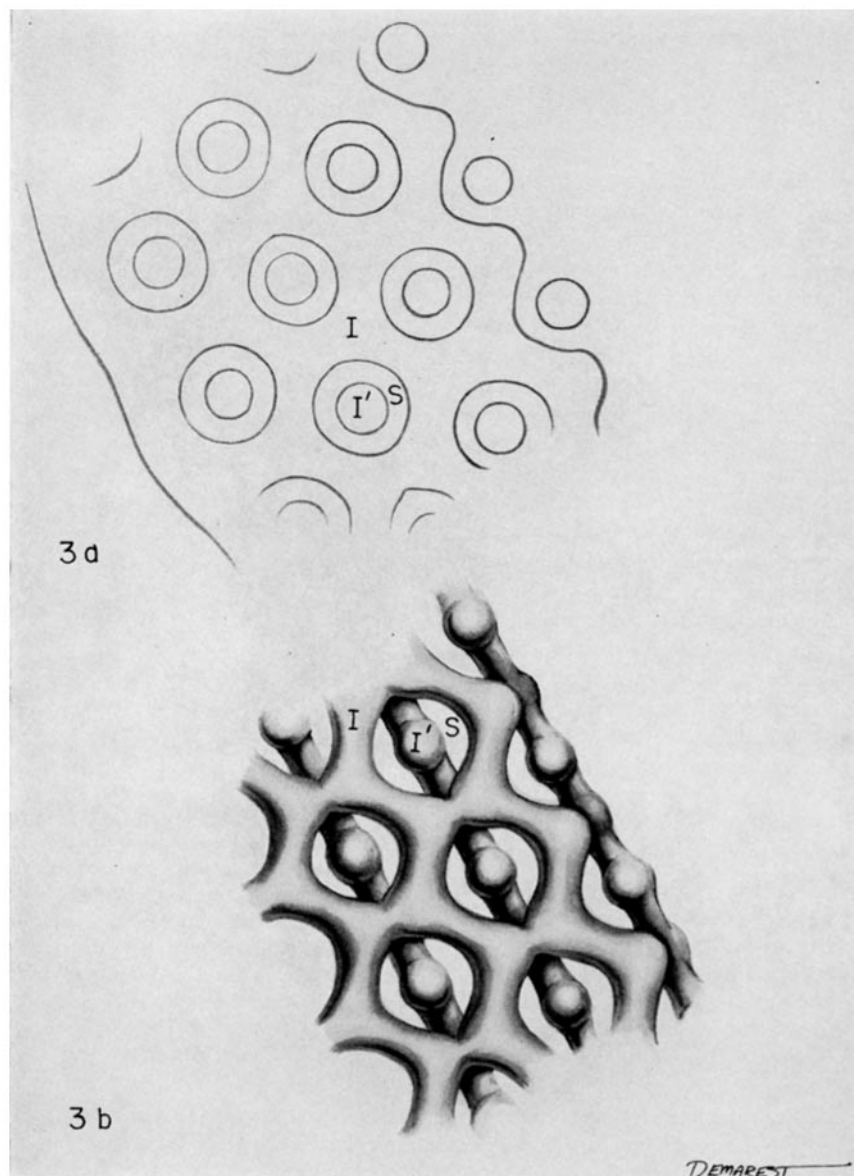
TEXT-FIG. 2. A three-dimensional reconstruction of the patterned areas of the serial sections in Figs. 6 *a* to *f*. The arrow indicates a direction normal to the planes of the serial sections. The adjacent rows of regular wavy villi are lined up so that they are out of phase with each other. Dispersed between these villi are the elongate and smaller villi which in this plane are shown as oval forms. The letters *a* to *f* represent the sections in Figs. 6 *a* to *f*.

(shown diagrammatically in Text-fig. 2). The "irregular" villi are diagrammed in Text-fig. 2 as circular profiles.

Since a single section includes a whole wavy villus, the serial sections in Figs. 6 *a* through *f* are about 70 to 80 μ thick. The serial sections are thinner in Figs. 5 *a* through *f* (about 40 μ), since longitudinal sections do not include the whole villus (see Text-fig. 1 *a*).

It is consistent with the facts to postulate that the unit structure in the various configurations is a regular wavy villus with a slight bulbous enlargement at the apex of each wave.

Concentric circular profiles are seen in the mitochondrion *A* in Fig. 1, and even more perfectly formed circular profiles ("doughnuts") are found in Fig. 2. The relatively capacious areas



TEXT-FIG. 3 *a*. An outline drawing of the concentric circular profiles found in the mitochondrion in Fig. 2. The areas *I* and *I'* represent spaces between the two mitochondrial membranes, whereas the very small area *S* represents stroma, which is enclosed by both membranes. The circular profiles or "doughnuts" are in hexagonal array. Centers of adjacent inner circles (*I'*) are separated by distances of 1400 Å. This is the same as the "wave length" of the villus (see Text-fig. 1 *a*).

b. A three-dimensional illustration showing the above relationships. The large structure *I* is formed by the fusion of out-of-phase villi. The larger outer circle (*S*) is completed by the fusion of the apposing peaks of the villi. The inner circle (*I'*) is formed by the wave crests of the deeper rows of villi. These villi are oriented at right angles to planes defined by the fused villi.

I and *I'* are the intramembranous regions, whereas only a very small area within the configurations is stromal (*S*).

Fig. 2 shows clearly that the "doughnuts" are arrayed hexagonally, and not randomly. The

large area between the two membranes (*I*) is probably formed by the fusion of out-of-phase villi as diagrammed in Text-fig. 3. The larger outer circle is formed by the fusion of apposing apical protuberances. The inner circle is a section

through the elbows of deeper rows of villi in which the plane of zigzag is oriented at right angles to the plane of the section, and fitted into the circular spaces formed by the fused villi. The distance from the center of the inner circle I' to any adjacent inner circle is about 1400 Å (see Text-figs. 1 *a* and 3), which is equal to the "wave length" of the villus.

The patterns formed by the wavy villus can become increasingly complex as a result of this fusing and branching. Profiles of sections through mitochondria in which some villi are fused are shown in Figs. 2 to 4. Mitochondrial profiles *C* and *D* in Fig. 1 are probably formed by unfused villi that may have some simple branchings.

DISCUSSION

Membranous structures in the stroma of the mitochondria clearly appear to be infoldings of the inner mitochondrial membrane. The general model of mitochondrial structure as proposed by Palade (1, 2), fits the structure of the mitochondria described here. On the other hand, we have found little evidence in favor of the alternate model proposed by Sjöstrand (9).

Regular arrangements of the infoldings of the inner mitochondrial membrane have been observed in some cell types, *e.g.* brown fat (10) and spermatids during spermatogenesis (11, 12). However, the degree of complexity in the mitochondria of the large amoeba *P. carolinensis* appears, to date, to be unique, although not all of these cells nor all of their mitochondria show these patterns (7).

The configurations exist because certain relationships in the structure of these mitochondria are constant. The fundamental unit of the configurations in *P. carolinensis* is the regular wavy tubular projection having a slight bulbous enlargement at the apex or elbow of each wave. In most cells, both the distance between the two mitochondrial membranes and the thickness of the infoldings are invariable (13). These relationships hold true in *P. carolinensis* mitochondria. Further, the packed villi have a constant distance of about 250 Å between them. This separation may reflect an important feature of the packing, in that it may contain components not visualized in the electron micrographs. Perhaps if the distance between villi becomes less than 250 Å, the villi fuse (as in Fig. 2).

The complex structure of these mitochondria affords a large membrane surface area. The mitochondria contain a small stromal and a large

intramembranous volume (*i.e.* space between the two membranes). Since the 250 Å space between the villi is less than the thickness of a villus (see Text-figs. 1 and 3), the intramembranous volume in the regions of the patterns is much greater than the stromal or intermembranous volume.

The significance of these configurations in amoeba is obscure. In tissue such as muscle, high oxidative capacity has been correlated with large numbers of inner mitochondrial membrane infoldings. Buvat and Lance showed recently (14) that the villi or cristae in plant cell mitochondria are most numerous during the period of most active photosynthesis. The finding of Siekevitz and Watson (15) that cytochrome oxidase and succinoxidase are located on the mitochondrial membranes, further supports the concept that oxidative phosphorylation activity is proportional to membrane area. The patterns of the mitochondria of *P. carolinensis* provide a large membrane area which may be correlated with a high capacity for oxidative processes.

We should keep in mind, however, that increase in infoldings also means increase in intramembranous volume and hence a proportionate decrease in stromal (or matrix) volume. Therefore, packing of cristae represents a local concentration of the enzymes associated with the membranes. The mitochondrial structure of *P. carolinensis* is an example of extremely tight packing.

Almost all profiles show that one end of the mitochondrion consists of a large stromal area containing only a few irregularly shaped villi. Having observed these "empty" ends in mitochondria in brown adipose tissue, Napolitano and Fawcett have suggested (10) that they may be newly formed "growing tips" that have not yet developed an internal structure. At the same time, the existence of a large stromal space at one end, and concomitant packing of infoldings at the other end, may indicate a more efficient system for mitochondrial activity.

The fibrous inclusions in the stroma of the amoeba mitochondria resemble more closely the crystalline inclusions described recently by Napolitano and Fawcett (10) than they do the dense bodies more commonly found in mitochondria (9, 13). Dense bodies were also seen by Dalton and Felix (5) in *P. carolinensis*. It may be noted that the fibrillar arrays found in the matrix of these mitochondria resemble the fibrillar component of the nuclear envelope of this species as described by Pappas (7). A possible relationship

of mitochondrial and nuclear fibrillar material is discussed by Brandt and Pappas (16).

BIBLIOGRAPHY

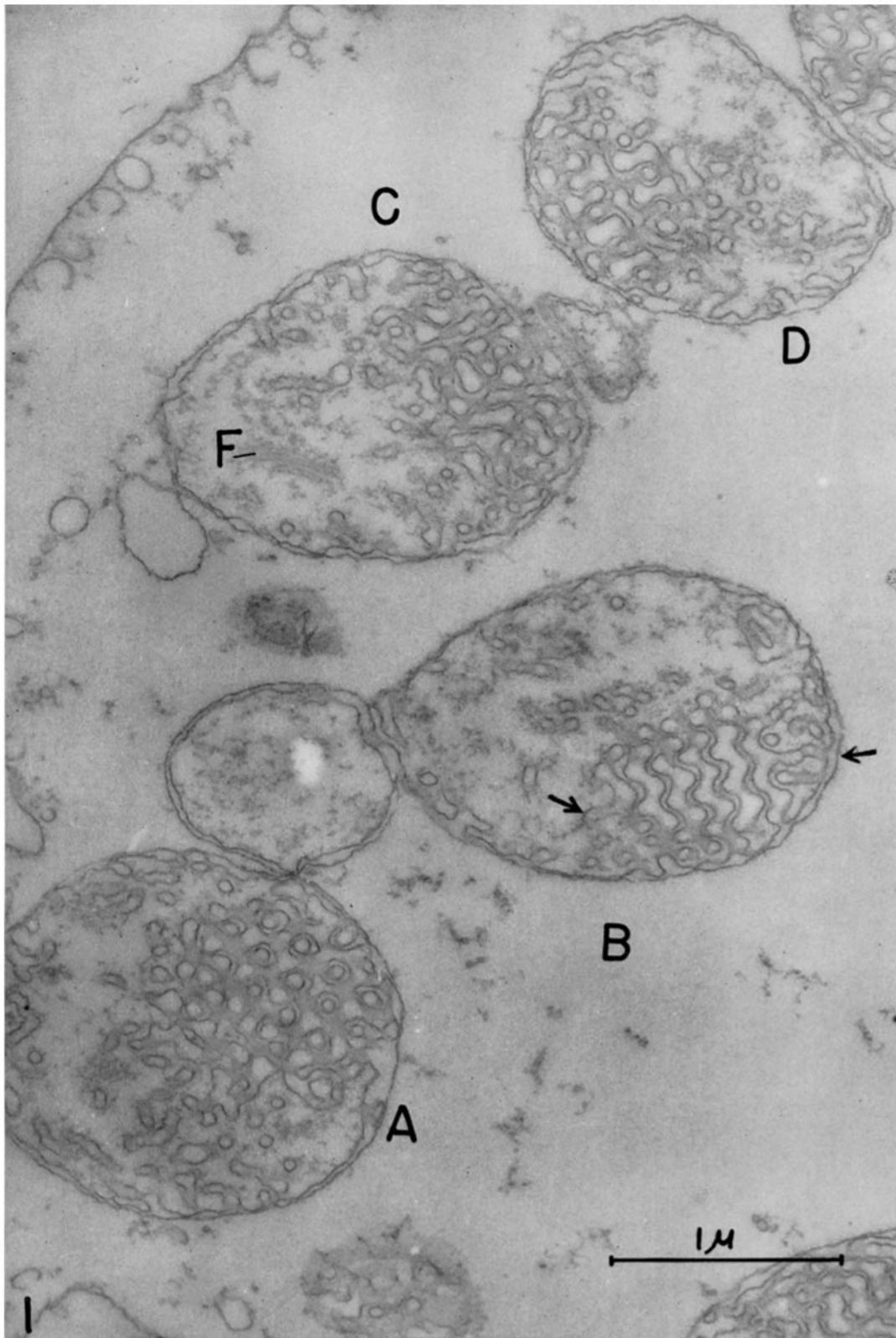
1. Palade, G. E., The fine structure of mitochondria, *Anat. Rec.*, 1952, **114**, 427.
2. Palade, G. E., An electron microscope study of the mitochondrial structure, *J. Histochem. and Cytochem.*, 1953, **1**, 188.
3. Low, F. N., Mitochondrial structure, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 337.
4. Dempsey, E. W., Variation in the structure of mitochondria, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 305.
5. Dalton, A. J., and Felix, M. D., Electron microscopy of mitochondria and the Golgi complex, *Symp. Soc. Exp. Biol.*, 1957, **10**, 148.
6. Sedar, A. W., and Rudzinska, M. A., Mitochondria of protozoa, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 331.
7. Pappas, G. D., Electron microscope studies on amebas, *Ann. New York Acad. Sc.*, 1959, **78**, 448.
8. Watson, M. L., Carbon films and specimen stability, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 31.
9. Sjöstrand, F. S., The ultra-structure of cells as revealed by the electron microscope, *Internat. Rev. Cytol.*, 1956, **5**, 455.
10. Napolitano, L., and Fawcett, D. W., The fine structure of brown adipose tissue in the newborn mouse and rat, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 685.
11. Kaye, J. S., Changes in the fine structure of mitochondria during spermatogenesis, *J. Morphol.*, 1958, **102**, 347.
12. André, M. J., Découverte chez le scorpion *Euscorpis flavicaudis* d'une nouvelle ultrastructure mitochondriale, *Compt. rend. Acad. sc.*, 1958, **247**, 1232.
13. Palade, G. E., Electron microscopy of mitochondria and other cytoplasmic structures, *in* Enzymes: Units of Biological Structure and Function, New York, Academic Press, Inc., 1956.
14. Buvat, R., and Lance, A., Evolution des infrastructures de mitochondries au cours de la différenciation cellulaire, *Comp. rend. Acad. sc.*, 1958, **247**, 1130.
15. Siekevitz, P., and Watson, M. L., Cytochemical studies of mitochondria. II. Enzymes associated with a mitochondrial membrane fraction, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 653.
16. Brandt, P. W., and Pappas, G. D., Mitochondria. II. The nuclear-mitochondrial relationship in the ameba *Pelomyxa carolinensis* Wilson (*Chaos chaos* L.). *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 91.

EXPLANATION OF PLATES

Figs. 1 through 6 are electron micrographs of sections of mitochondria of the ameba *Pelomyxa carolinensis* (*Chaos chaos*).

PLATE 52

FIG. 1. Electron micrograph of a section of *Pelomyxa carolinensis* showing patterns in the mitochondria formed by the infoldings of the inner membrane. These patterns are found only at one end of the mitochondrion. The unit structure in the formation of various patterns is a regular wavy villus which has a slight bulbous enlargement at the apex of each wave (see Text-fig. 1). The patterns formed by the wavy villi can become more complex as a result of fusing and branching (at arrows in mitochondrion B). Fibrillar material F (in mitochondrion C) is sometimes found in the stromal area. $\times 37,000$.



(Pappas and Brandt: Mitochondria. I)

PLATE 52 *a*

FIG. 2. Concentric circular profiles are seen in the mitochondrion. The areas I and I' are the intramembranous regions (between the two membranes) while the very small area (S) is stromal, *i.e.* within both membranes. The circular profiles or "doughnuts" are in hexagonal array. The large area (I) is probably formed by the fusion of out-of-phase villi as shown in Text-fig. 3. The distance from the center of the inner circle (I') to any adjacent inner circle is about 1400 Å (see Text-figs. 1 and 3). This distance corresponds to the "wave length" of the villus. $\times 80,000$.

FIGS. 3 and 4. Mitochondria showing patterns probably formed by the fusion of villi. Fibrillar arrays (F) may be found in the stromal area. In Fig. 3, the fibril packing is shown in longitudinal section. Fig. 4 shows the fibrillar material in cross-section. $\times 48,000$.

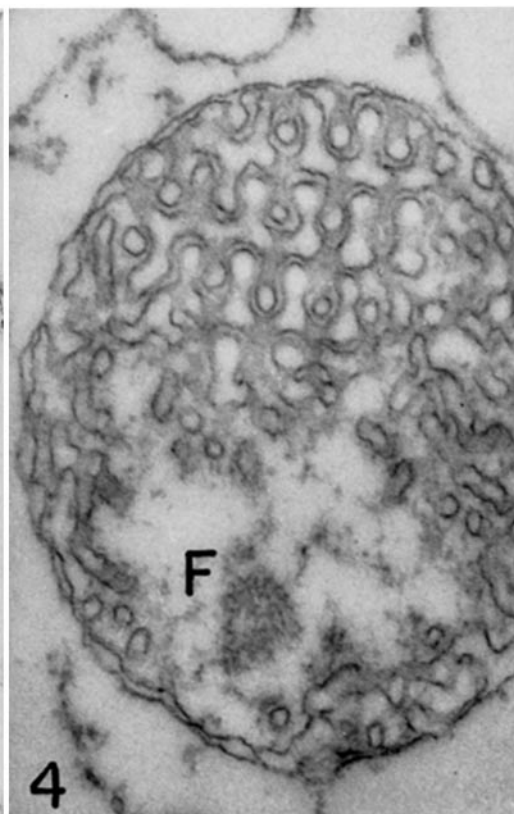
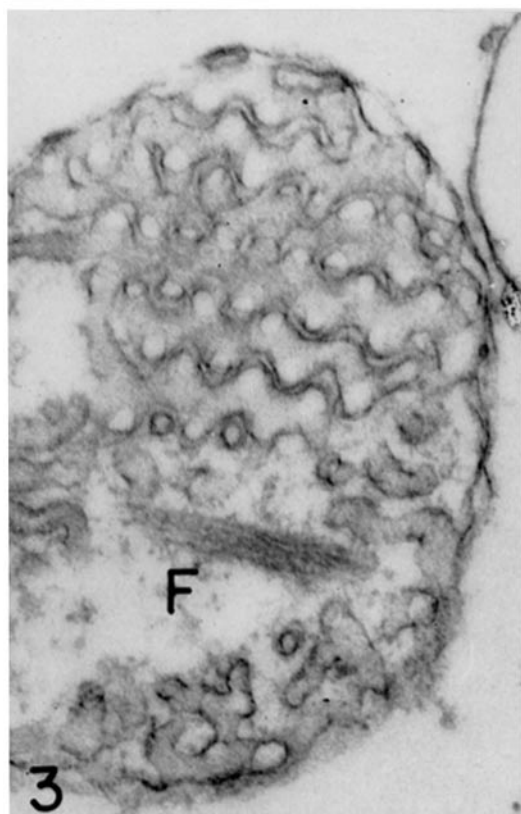


PLATE 53

FIGS. 5 *a* to *f*. Serial sections through the patterned area of a mitochondrion. Fig. 5 *a* is a longitudinal section through four wavy villi which are in phase. Fig. 5 *d* shows a second row of four villi cut longitudinally. The regular wavy pattern of this row of villi is out of phase with the row of villi seen in Fig. 5 *a* (see Text-fig. 1 *b*). Circular profiles found in the other sections in the area of the wavy patterns are formed when the longitudinal section cuts through edges of the bulbous portions of the wavy villi (see Text-fig. 1 *b*). $\times 38,000$.

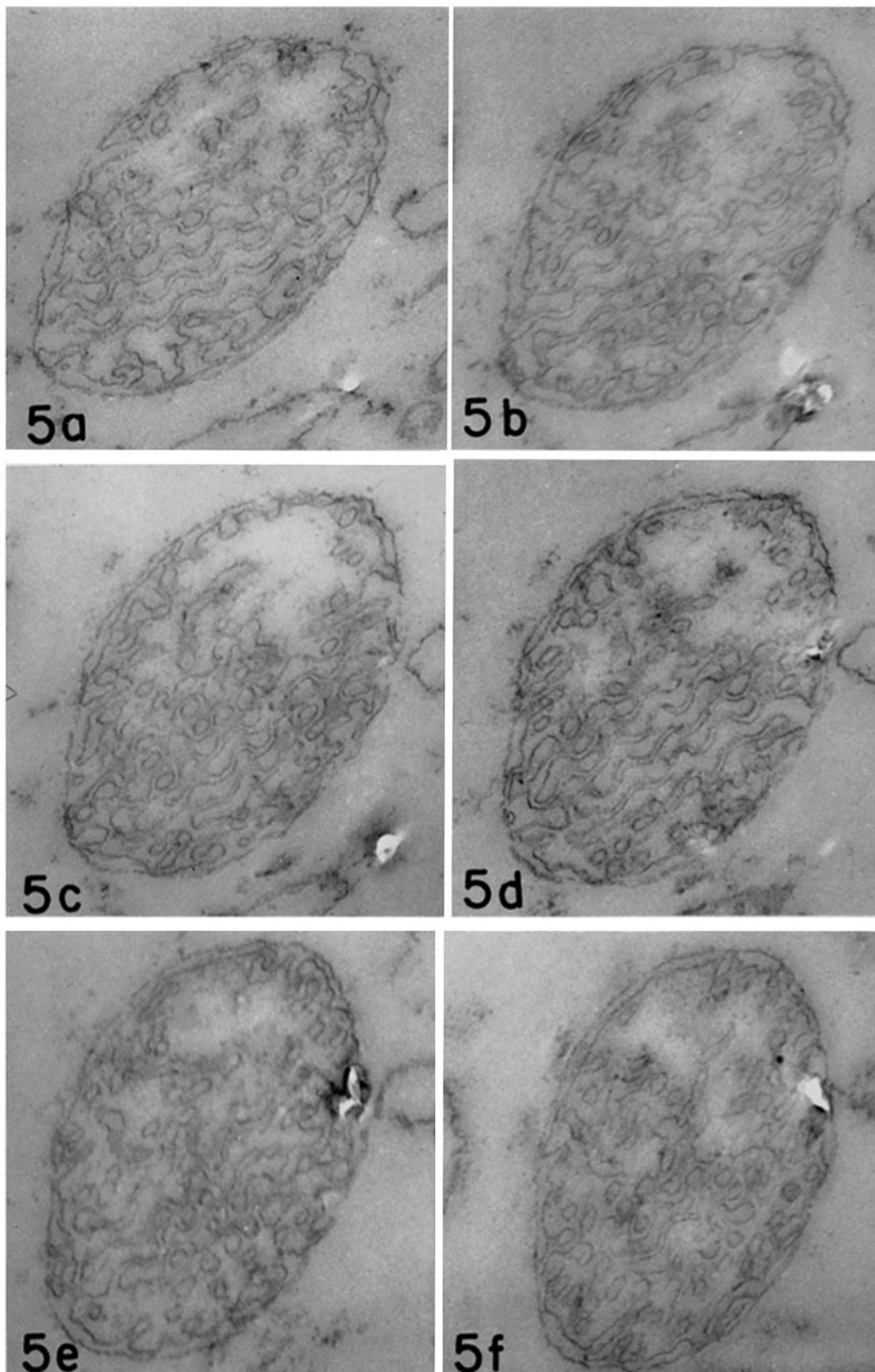
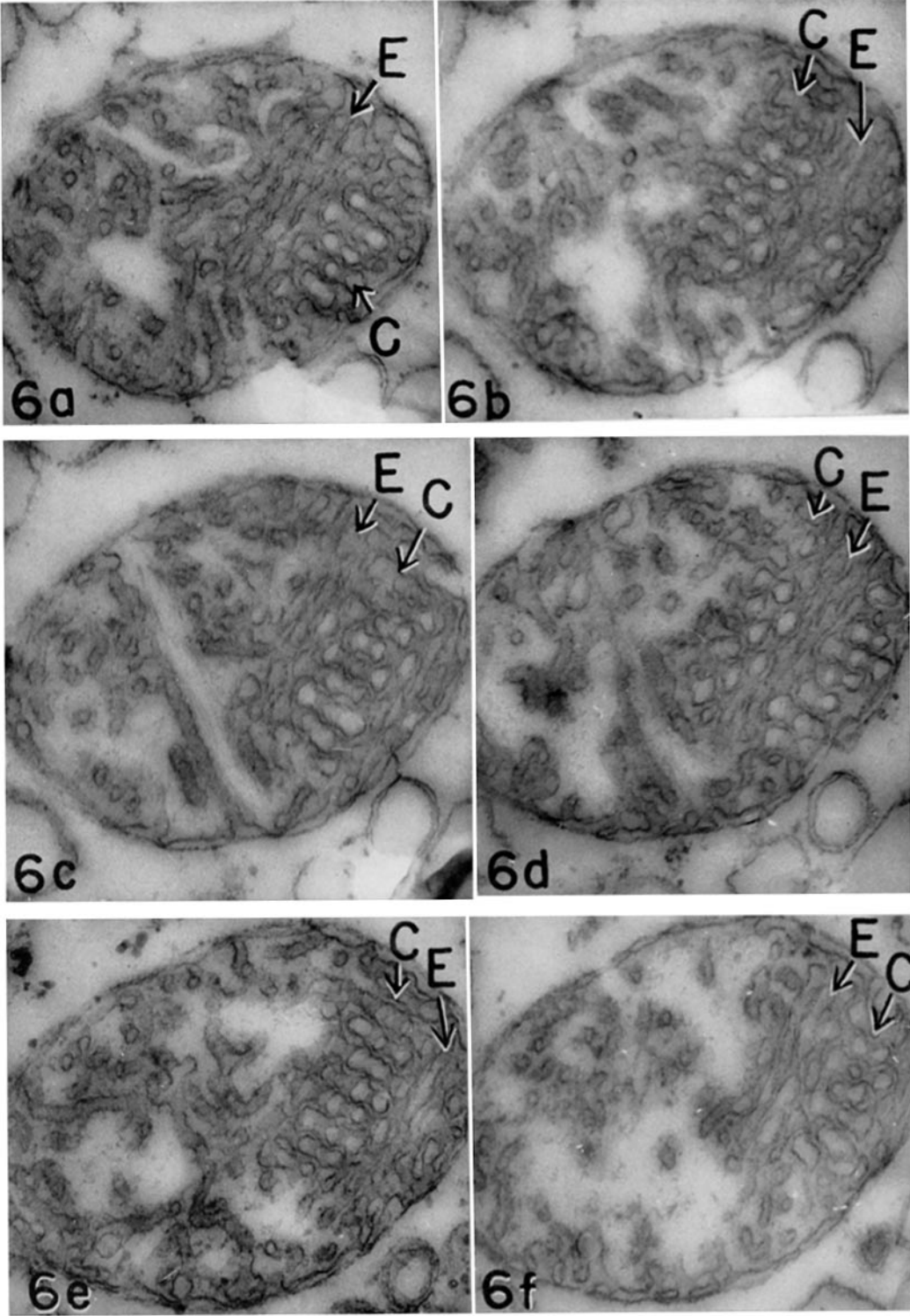


PLATE 54

FIGS. 6 *a* to *f*. A series of serial sections through a mitochondrion. From one section to the next there is a reversal of circular or dumb-bell profiles (*C*) to thin elongate ones (*E*). These patterns are interpreted as indicating rows of wavy villi (*C*) intersected by thin elongate villi (*E*). Text-fig. 2 shows the arrangement of the regular wavy villi alternating with elongate villi in a three-dimensional reconstruction of these sections. $\times 33,000$.



(Pappas and Brandt: Mitochondria. I)