

# THE FATE OF MITOCHONDRIA DURING AGING IN *TETRAHYMENA PYRIFORMIS*

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## ABSTRACT

During the growth cycle of *Tetrahymena pyriformis* the mitochondria undergo changes in position, number, and structure. Ciliates in the logarithmic growth phase possess elongated mitochondria which are aligned along the plasma membrane and are closely associated with the kinetosomes and kinetodesmata. Mitochondria appear to divide across the long axis at this time, resulting in two or more products. Throughout this phase of growth mitochondrial divisions keep pace with cytokinesis so that the population of mitochondria remains at essentially the minimal level. As the ciliates enter the stationary growth phase the mitochondria increase in number, become oval to spherical in shape, and some migrate into the cytoplasm. Intramitochondrial masses of various configurations appear at this time. Some of the mitochondria lying in the cytoplasm become incorporated into vacuoles. Within these vacuoles either a single mitochondrion appears or several mitochondria may be seen along with other cytoplasmic structures. Later in the stationary growth phase the contained mitochondria are dense and the tubules are more compact than normal. Various stages in disorganization of the mitochondria are observed in a single large vacuole. Cytochemical tests reveal the presence of acid phosphatase, suggesting that hydrolysis of the vacuolar contents occurs. Lipid droplets increase in number during the middle and late stationary phase of growth. These events are interpreted as being associated with the normal process of aging in *T. pyriformis*.

## INTRODUCTION

Protozoa, like other microorganisms, when grown in a closed system with an adequate food supply, pass through the usual growth cycle of lag, logarithmic, and stationary phases, during which they undergo certain physiological and morphological changes (14, 18). These phases may be compared to youth, maturity, and old age in metazoan cells. Since the ciliated protozoan, *Tetrahymena pyriformis*, can be cultured axenically in a precisely controlled environment (8) it should be possible to follow the behavior and structure of the mitochondria as the organisms pass through their growth cycle.

Very little is known about the fate of mitochondria during aging in either metazoan or protozoan cells. Hess (15) and Duncan *et al.* (7) attempted to show that senility pigment arose from mitochondria in the neurons of mammalian cells. Rudzinska (28), in her work on the protozoa, has studied the fate of the mitochondria in aging *Tokophrya infusionum*. She suggests that the accumulated pigment granules that appear in old cells may arise from mitochondria. Earlier observations by the authors (3, 9) demonstrated that certain structural changes in the mitochondria occurred during the growth cycle. The pur-

pose of this investigation is to explore more fully these and other changes as they occur in aging *Tetrahymena pyriformis*.

#### MATERIALS AND METHODS

Two strains of *Tetrahymena pyriformis* were used in this investigation. Most of the observations were made on the classical amiconucleate strain E; the micronucleate strain WH<sub>6</sub> was used only in the studies of mitochondrial distribution. The cells were cultured axenically in 500-ml Erlenmeyer flasks in 2 per cent proteose-peptone (Difco Laboratories, Detroit) supplemented with 0.5 per cent glucose, liver extract, and a mineral salt mixture. In order to provide an ample supply of oxygen for maximal growth, 100 ml of media was used in each flask. The initial inoculation consisted approximately of 200,000 cells in the logarithmic growth phase, and the incubation temperature was 25°C. Cells in logarithmic and stationary growth phases were withdrawn at appropriate periods and concentrated by centrifugation into a soft pellet which was then fixed for 30 minutes in 1 per cent OsO<sub>4</sub> buffered with 0.14 M Veronal acetate (pH 7.4) containing 45 mg of sucrose per ml. The ciliates were then dehydrated with graded ethanols and embedded in either methacrylate (with 2 per cent Luperco CDB, 20 per cent methyl methacrylate and 80 per cent n-butyl methacrylate or Epon 812 according to the method of Luft (21). The blocks were polymerized for several days at graded temperatures (35° to 60°C). The embedded pellets were sectioned with a Porter-Blum microtome and examined in an RCA EMU 3E.

For the identification of acid phosphatase in the electron microscope, cells were fixed in 7 per cent cold glutaraldehyde and buffered with cacodylate at pH 7.0 for 1 hour. They were then incubated for 20 minutes at 37°C in the glycerophosphate medium of Gomori (12). Following incubation the cells were postfixed for 20 minutes in 1 per cent osmium

tetroxide containing 7.5 per cent sucrose (17). Dehydrating and embedding procedures were then followed as described above. Appropriate controls were maintained.

For light microscopy, whole cells were fixed in formol-calcium and stained with Oil Red O according to the method of Lillie (19) and Nile blue sulfate following Cain's technique (4) for the localization of lipid. Photographs were taken on 4 x 5 inch (X1200) Kodak M plates with a Spencer photomicrograph camera.

#### OBSERVATIONS

Variations in the location of mitochondria during the various growth phases were made with strain WH<sub>6</sub> embedded in methacrylate, which provided the best sections of whole cells. All other observations were made with strain E embedded in Epon 812.

Ciliates taken in logarithmic growth (48 to 72 hours' incubation) show mitochondria closely associated with the plasma membrane and the ciliary apparatus; very few are found in other parts of the cytoplasm (Fig. 1). Most of the mitochondria are elongated (Fig. 2) and some form complete rings as seen in tangential sections (Fig. 3). These "doughnut-shaped" mitochondria lie flattened against the plasma membrane. Apparently the ends of the elongated mitochondria fuse to form rings. Stages that appear to be in division are common at this phase of the growth cycle. Most of the profiles suggest fission into two relatively equal daughter mitochondria (Fig. 4 C, D, E, F); a few show two cleavages resulting in three fragments (Fig. 4 B). Mitochondria with extensions of the outer membrane (Fig. 4 G) are probably a result of remnants

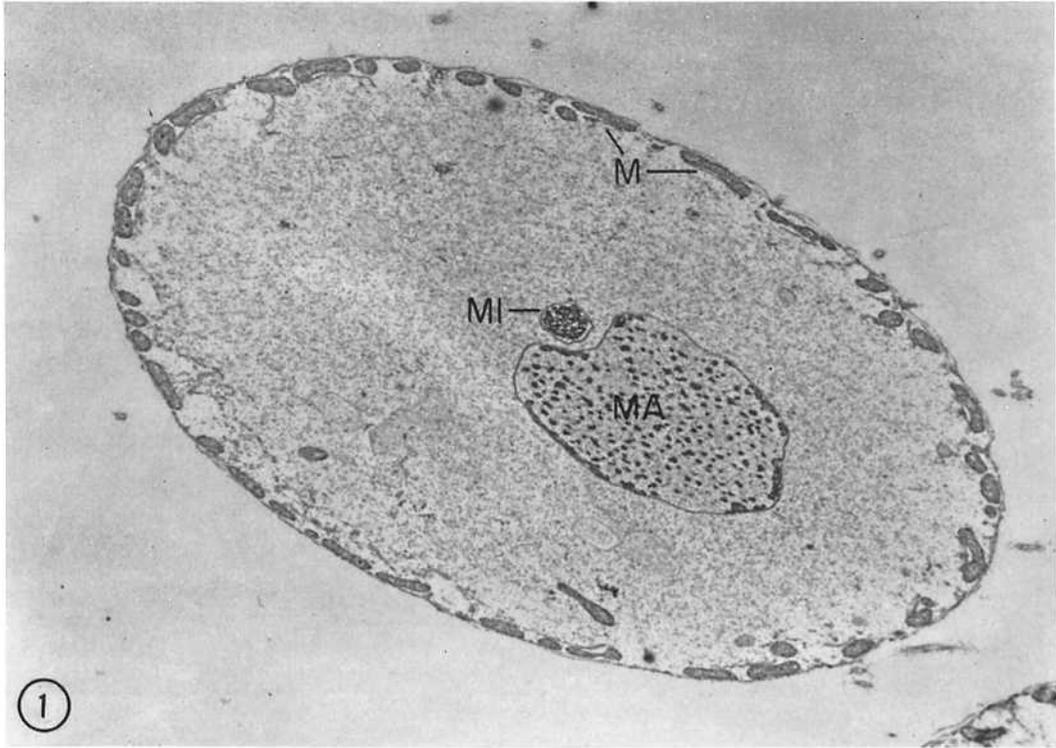
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Figs. 1 and 5 are of *T. pyriformis* of strain WH<sub>6</sub>, all other figures are of strain E. Figs. 1 to 4 are of ciliates in logarithmic growth (48 to 72 hours following inoculation).

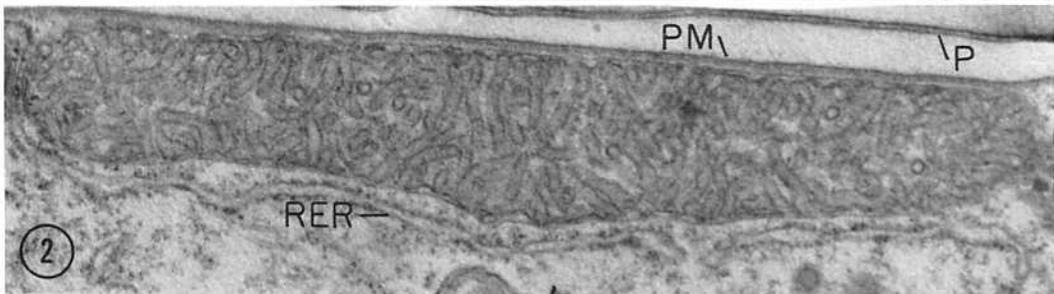
FIGURE 1 Longitudinal section of a whole ciliate showing the elongated, peripherally located mitochondria (*M*). Section is taken through the macronucleus (*MA*) and the micronucleus (*MI*). X 2400.

FIGURE 2 An elongated mitochondrion is closely associated with the plasma membrane (*PM*) and the rough endoplasmic reticulum (*RER*). Part of the pellicle (*P*) shows at the top of the micrograph. X 32,000.

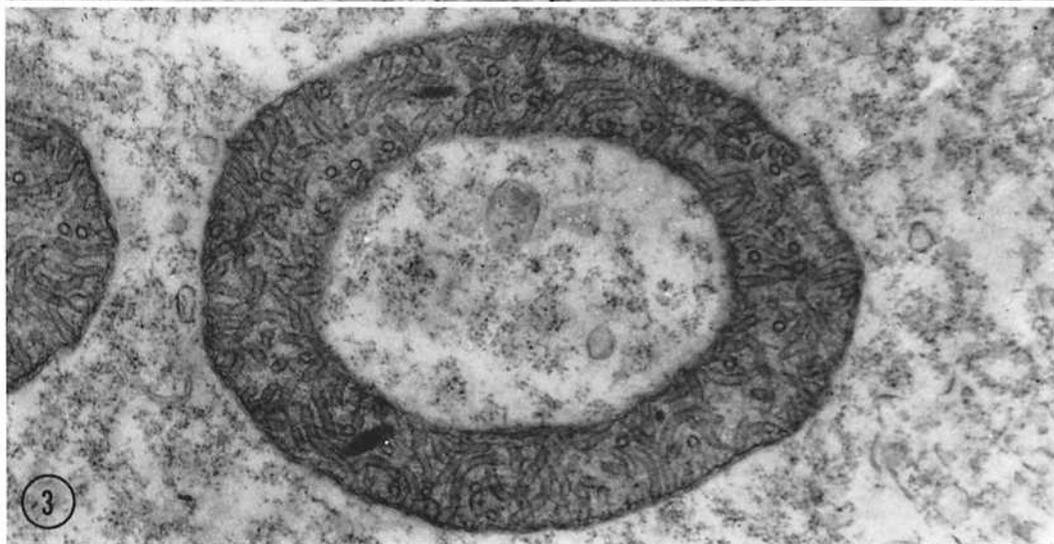
FIGURE 3 Occasionally in tangential sections the elongated mitochondria take on the shape of a doughnut, as shown in this micrograph. X 21,000.



①



②



③

following division. Smooth endoplasmic reticulum appears in some sections (Fig. 4 *D, E*). Otherwise the cytoplasm seems to be without granules other than the numerous free ribosomes (Figs. 1, 4 *A*).

When ciliates enter the stationary growth phase (5 days' incubation) striking changes occur in both the distribution and the structure of the mitochondria (Fig. 5). Almost all of the mitochondria are oval in shape and many are randomly distributed throughout the cytoplasm, occasionally accumulating near the macronucleus (Fig. 6). The number of them has also increased. The mitochondrial tubules show no change when compared to those in the logarithmic phase of growth (compare Figs. 2 and 3 with Fig. 7).

The most dramatic alterations in mitochondrial structure occur during the next 5 days of growth, and are interpreted as being associated with aging. The first noticeable changes occur within the mitochondria. Following the 5th day of incubation electron-opaque masses appear within many of the mitochondria. Some masses are spindle-shaped (Fig. 10) but most of them are spherical (Figs. 8 and 9). In some mitochondria the tubules are parallel in orientation (Figs. 9 and 17), resembling those in liver cell mitochondria of thyrotoxic rats as seen by Greenwalt, Foster, and Lehninger (13), or in the stacked cristae in liver cells of mice fed on a diet deficient in the essential fatty acids as described by Wilson and Leduc (31). Whether or not these unusual mitochondria in *T. pyriformis* are the same ones that later undergo degeneration is impossible to determine from static micrographs.

Some mitochondria lying in the cytoplasm are enveloped by rough endoplasmic reticulum (Fig. 11) during the early stationary phase of growth (6 days of incubation). This type of association is common during logarithmic growth when the mitochondria are elongated and located near the plasma membrane (Figs. 2, 4 *A*), but it is observed in only a few mitochondria at this stage of

growth when they are oval or spherical in shape and are located in the cytoplasm. The proximity of the rough endoplasmic reticulum suggests rapid enzyme synthesis, which is understandable during logarithmic growth when protein synthesis is maximal, but just what function such enzymes serve at this stage of growth is not clear. They may be hydrolytic enzymes which subsequently function in the lysis of the vacuolar contents (see below).

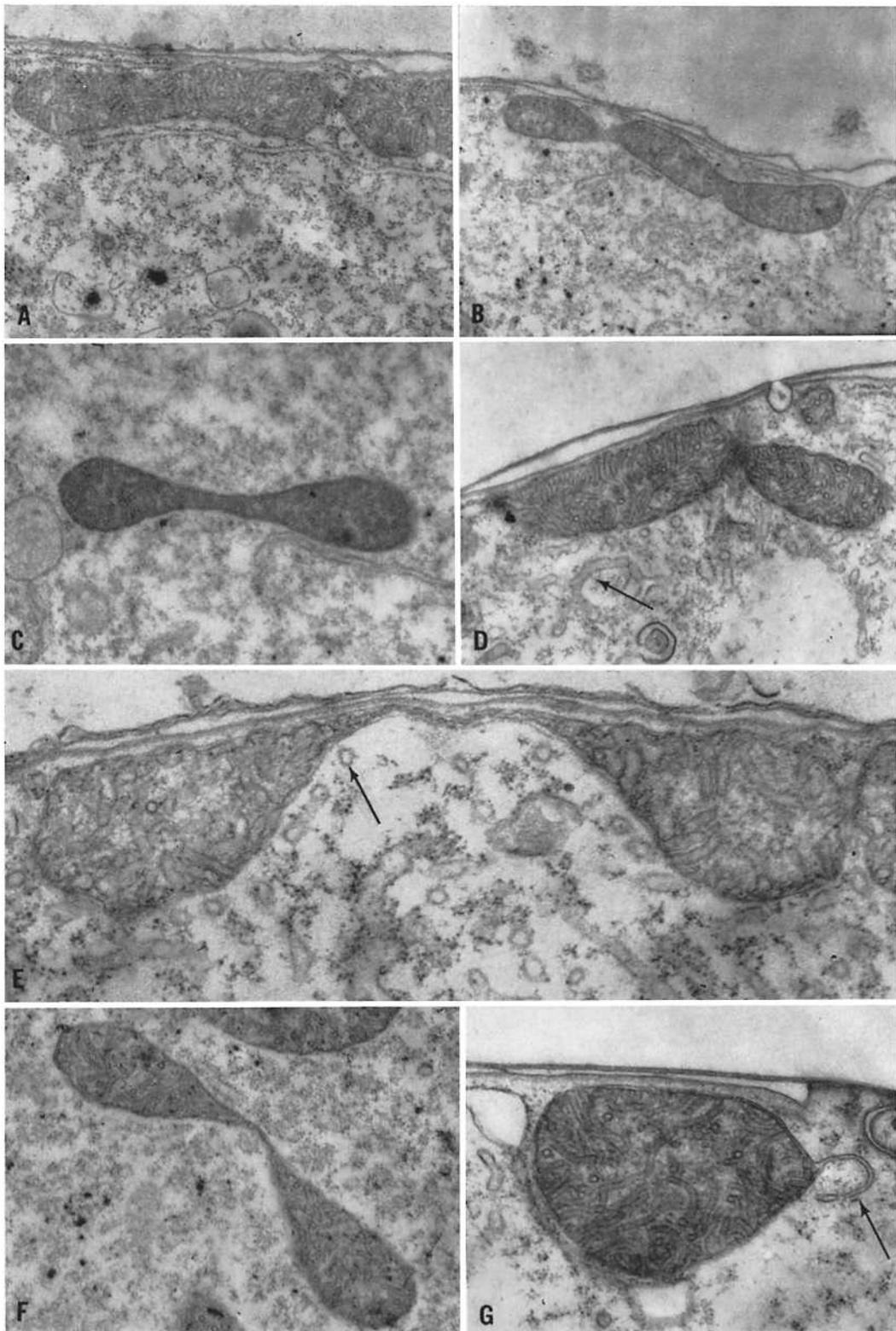
From the 6th to the 9th day of incubation many single mitochondria are encapsulated in a double-membraned vacuole, the membranes of which usually adhere closely to the outer mitochondrial membrane (Figs. 12, 13, and 17). Small vacuoles can be seen lying near the surface of the larger vacuole (Fig. 12) and may serve as a source of its membrane. The mitochondrial tubules in different vacuoles show varying densities which suggests sequential stages of degeneration (compare Figs. 12, 13, and 14). The tubules are diminished in diameter and are more compact than in normal mitochondria during the stationary phase. When cells at this stage of growth are tested for the presence of acid phosphatase, such single encapsulated mitochondria, along with other cytoplasmic structures, react positively, indicating the presence of hydrolases (Fig. 20). The mitochondrion usually contains a dense mass. The ultimate fate of these vacuoles has not been determined.

At this time completely opaque granules appear in the cytoplasm which seem to be without membranes (Figs. 15 and 17). Whether these granules represent a continuation of mitochondrial degeneration or are unrelated to this process cannot be determined from static micrographs. Occasionally their profiles are irregular (Fig. 15), which suggests that they may be recently formed lipid droplets. Others are stellate (Fig. 16), about which more will be described later.

With advancing age (11 days of incubation) larger vacuoles appear in the cytoplasm which contain two or more mitochondria together with

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FIGURE 4 Mitochondria profiles which appear to be in various stages of division. They usually divide into two daughter mitochondria (*C, D, E, F*), although some form three progeny (*B*). Occasionally fragments of the outer membrane remain following division, as shown in *G* (arrow). Smooth endoplasmic reticulum appears in some sections (*D* and *E*, arrows). *A*,  $\times 13,000$ ; *B*,  $\times 8400$ ; *C*,  $\times 12,000$ ; *D*,  $\times 15,000$ ; *E*,  $\times 27,000$ ; *F*,  $\times 23,000$ ; *G*,  $\times 22,000$ .



varying amounts of coiled endoplasmic reticulum and other undetermined cytoplasmic structures (Figs. 17 and 18). The mitochondria within these large vacuoles seem to follow the same course of degeneration as the small vacuoles containing only a single mitochondrion. That is, they become dense and the tubules decrease in diameter and are more compact. Different stages in mitochondrial degeneration can frequently be observed within one vacuole; some mitochondria retain their structure and therefore can be easily recognized whereas others show various stages of disorganization (Fig. 18). The endoplasmic reticulum is smooth surfaced and highly coiled. Small vacuoles of varying density can also be seen within these large vacuoles.

In order to identify these large vacuoles, cells were tested for the presence of acid phosphatase. Micrographs of such cells show positively reacting vacuoles similar in size and location to those described above (Fig. 21). This observation indicates that these vacuoles, like those containing only a single mitochondrion, contain hydrolytic enzymes and that digestion of the vacuolar contents is taking place.

Along with the vacuoles just described are others which show no organized mitochondria but do contain numerous small vacuoles and coiled endoplasmic reticulum (Fig. 17). Still others seem to contain little or no structured material and their membrane is broken, suggesting that their contents are being deposited into the cytoplasm (Fig. 19). Numerous coiled membrane fragments (Fig. 13) are seen in the cytoplasm of cells in the late stationary growth phase which may be remnants of broken vacuoles. If it is assumed that the vacuolar contents are undergoing digestion, these vacuoles could represent the final stages of degradation. Here again, such speculation is based on static micrographs which cannot

be taken as proof that such a sequence of events actually takes place.

The number of mitochondria varies with different phases of the growth cycle, as revealed by counts of 75 low magnification micrographs of cross-sectioned material. No effort was made to estimate the actual number of mitochondria per cell. It was assumed that counts of profiles per section would reflect the relative increase or decrease in total numbers. Ciliates that have been permitted to starve for 48 hours in distilled water show the lowest number of mitochondria; about 50 profiles can be counted in cross-section. These mitochondria appear to be normal in all respects. About half of them are closely associated with the kinetosomes and plasma membrane, and the remainder are distributed throughout the cytoplasm. When such cells are placed in a rich medium, within 5 hours the mitochondria located in the cytoplasm move to the plasma membrane and begin to elongate in preparation for division. During the next 2 to 3 days of logarithmic growth the mitochondrial divisions keep pace with cytokinesis of the ciliates, remaining at about the minimal number, 50. From the 4th through the 10th day of incubation the number of mitochondria gradually increases until there are approximately 100, twice the number seen at the beginning of logarithmic growth. This number includes those in various stages of degeneration.

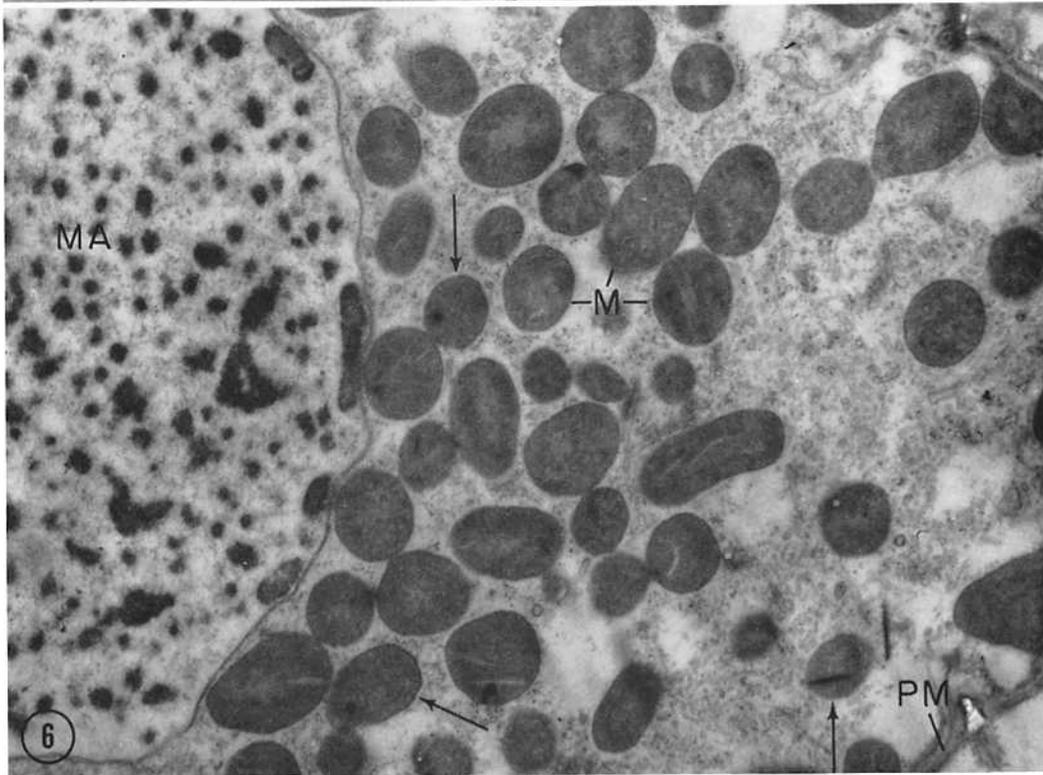
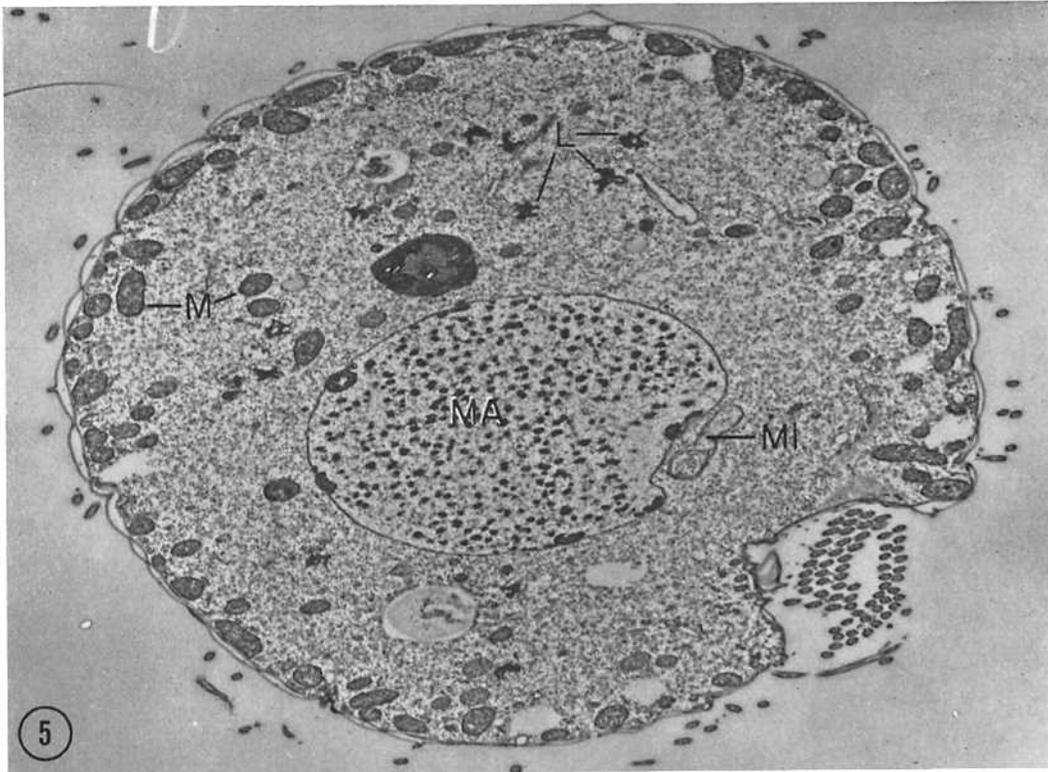
It would appear that a minimal number of mitochondria is essential for normal metabolism of the ciliate which is maintained even under severe starvation. When the cells are actively dividing, the minimal number also seems adequate to take care of the activities of the ciliate during the early part of logarithmic growth. However, later on, even while the cells are dividing at their maximal rate, the mitochondria gradually increase in

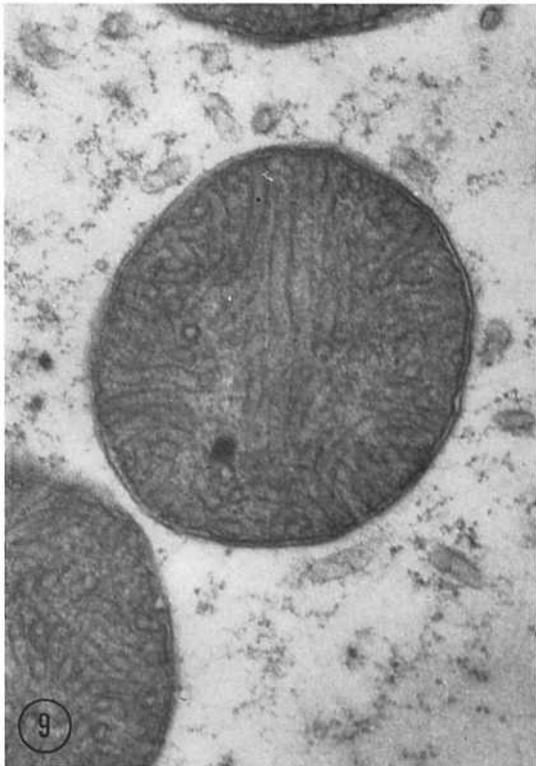
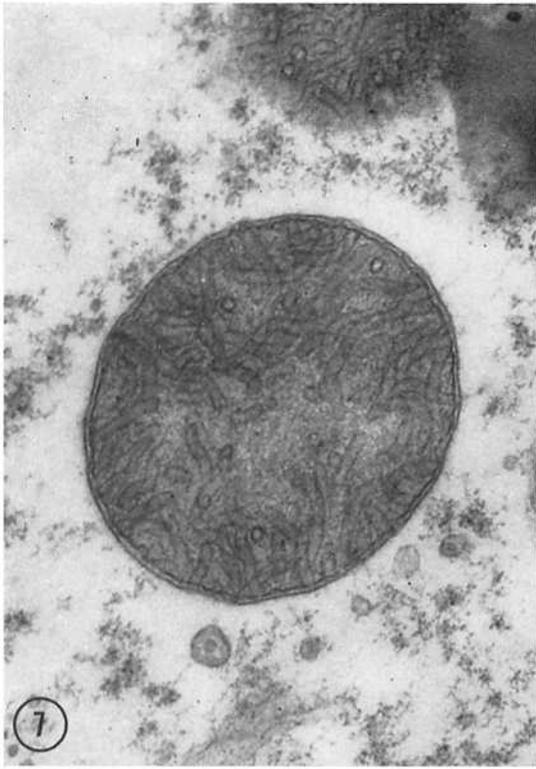
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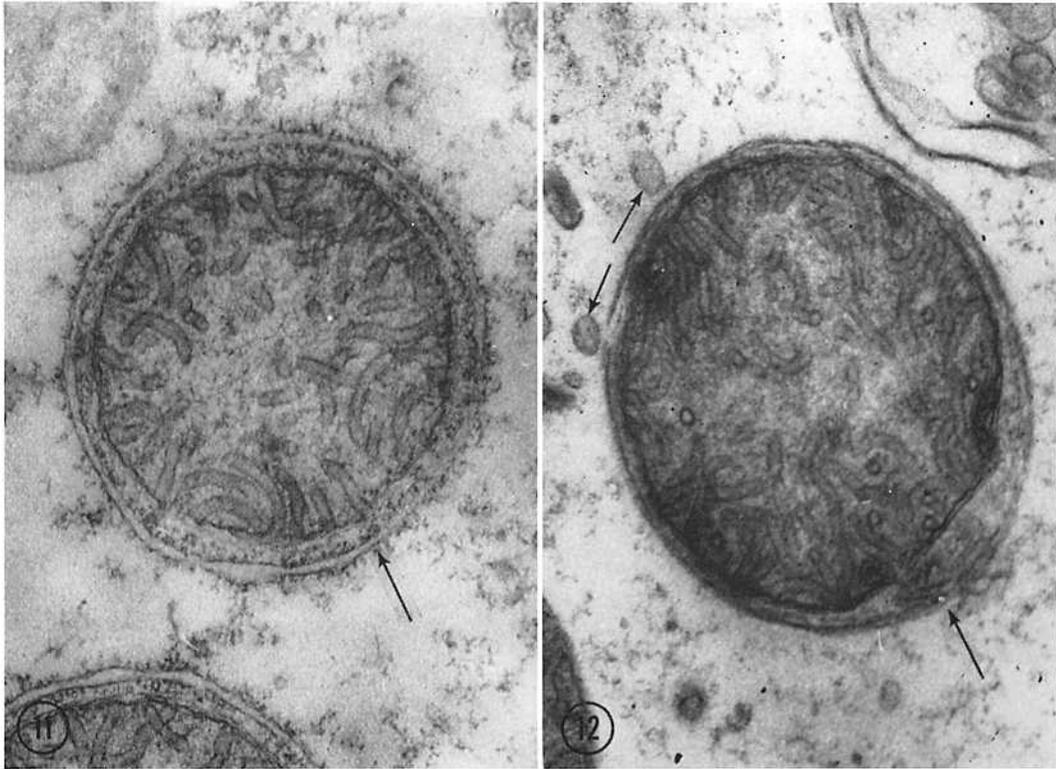
Figs. 5 to 10 are of ciliates in stationary growth phase (5 days following inoculation).

**FIGURE 5** Longitudinal section of a whole ciliate showing the oval-shaped mitochondria (*M*) distributed in the cytoplasm as well as at the plasma membrane. Stellate lipid droplets (*L*) appear at this phase of growth. The macronucleus (*MA*) and part of the micronucleus (*MI*) are included in the section.  $\times 3000$ .

**FIGURE 6** The mitochondria (*M*) in this cell are located in the cytoplasm near the macronucleus (*MA*). Several mitochondria contain an intramitochondrial mass (arrows). Only a few remain near the plasma membrane (*PM*).  $\times 8400$ .







Figs. 11 to 19 are of ciliates in stationary growth phase (6 to 10 days after inoculation).

FIGURE 11 Mitochondrion enveloped by rough endoplasmic reticulum (arrow) (6 days).  $\times 36,000$ .

FIGURE 12 Mitochondrion encapsulated in a double-membraned vacuole (long arrow). The peripheral tubules are more dense than in a normal mitochondrion. Small vacuoles (short arrows) lie near the membrane of the large vacuole (6 days).  $\times 41,000$ .

number, many of which undergo degeneration as already described.

Since Hogg and Wagner (16) have shown that during the stationary growth phase (7 days of incubation at 25°C) ciliates of this same strain of *T. pyriformis* contain 15 to 20 per cent lipid, an effort was made to localize this material in young

and old cells. In an earlier report (10) it was demonstrated in electron micrographs that cells (strain WH<sub>6</sub>) of approximately the same age (4 days of incubation at 35°C) contained stellate bodies which were identified as lipid particles, although no cytochemical tests were carried out. In the present study similar stellate bodies are

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FIGURE 7 A typical mitochondrion in stationary growth phase.  $\times 30,000$ .

FIGURE 8 Mitochondrion with a spherical intramitochondrial mass.  $\times 29,000$ .

FIGURE 9 Mitochondrion with parallel oriented tubules as well as a small, spherical intramitochondrial mass.  $\times 29,000$ .

FIGURE 10 Mitochondrion with both a spindle- and a spherical-shaped intramitochondrial mass.  $\times 29,000$ .

found in cells in the stationary growth phase (Figs. 5 and 16). The lipid droplets vary considerably in shape, changing from bodies with essentially regular surfaces (Fig. 15) to those that are stellate (Fig. 16). Since young cells contain fewer lipid droplets (see below) than old cells, only an occasional section shows lipid granules in cells in the logarithmic growth phase (Fig. 1). The distribution of lipid droplets within ciliates can best be demonstrated with the light microscope following appropriate staining.

The tests described earlier were employed to specifically identify lipid. This was done by treating whole cells, at various stages in the growth cycle, with Oil Red O (for neutral lipid) and examining them under the light microscope. Cells in logarithmic growth contain a few lipid droplets in the oral region (Fig. 22), demonstrating that ciliates at this phase contain a small amount of lipid. Parallel tests with cells in the stationary phase show numerous lipid droplets (Fig. 23) which correspond in size and distribution to the dense stellate granules observed in the electron micrographs (Figs. 5, 16). The identification of lipid in cells in both the logarithmic and stationary phases was further confirmed with Nile blue sulfate which also stains neutral lipids. It would seem reasonable to suggest that the stellate granules seen in the electron micrographs are composed of lipid.

#### DISCUSSION

When cells can be examined during precise stages in both the growth and division cycles, one should be able to determine the origin and fate of the mitochondria. A serious effort by the authors to

discern whether mitochondria originate from the nuclear membrane, endoplasmic reticulum, or pinocytotic vacuoles, or arise *de novo* has yielded only negative results. The remaining source of mitochondria is pre-existing mitochondria; in other words, mitochondria divide. Evidence for this conclusion comes from several sources. For example, Luck (20), by following radioactive choline incorporation into mitochondrial lipid during logarithmic growth of *Neurospora crassa*, has shown that the total mitochondrial mass increases by the addition of new lipid to the existing mitochondrial structure and that the population of mitochondria increases by their division. Also, Bahr and Zeitler (2), employing quantitative electron microscopy, have demonstrated that two morphological groups of mitochondria appear in rat liver cells, a spherical one and an elongated one. They conclude that mitochondria increase their weight by linear growth and that such mitochondria undergo division which may or may not be symmetrical. These observations may have their parallel in *T. pyriformis* during the growth cycle where elongated mitochondria, present during logarithmic growth, show profiles that appear to be dividing. Cells in stationary growth, with oval or spherical mitochondria, rarely show profiles that could be interpreted as stages in mitochondrial division. The elongated mitochondria appear to separate into two oval-shaped daughter mitochondria of equal size, although some eccentric divisions have been observed. The products of division then appear to elongate for the next division. All stages can be observed in any particular cell in logarithmic growth. Obviously, it is not possible to select a series of mito-

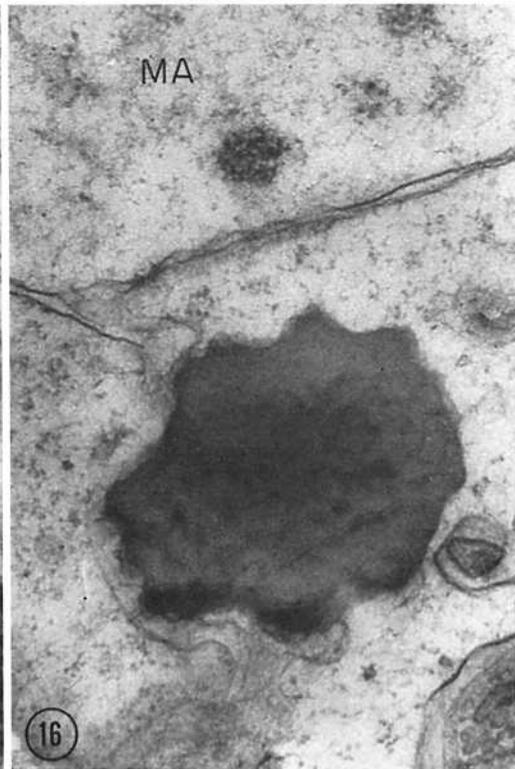
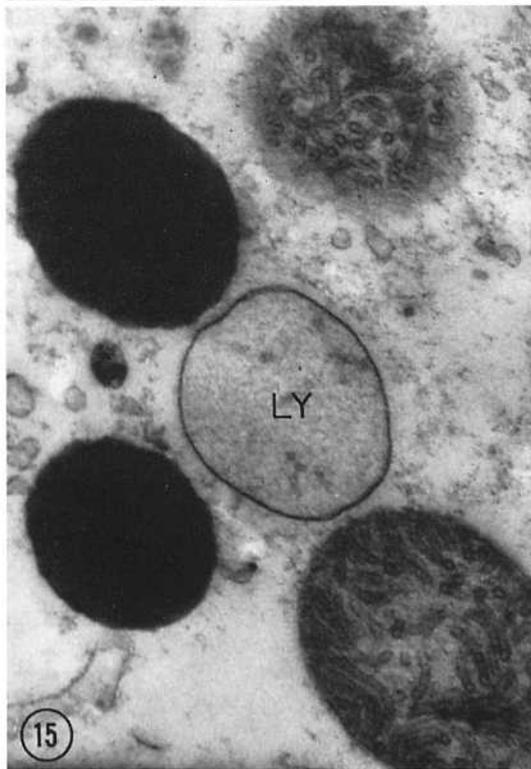
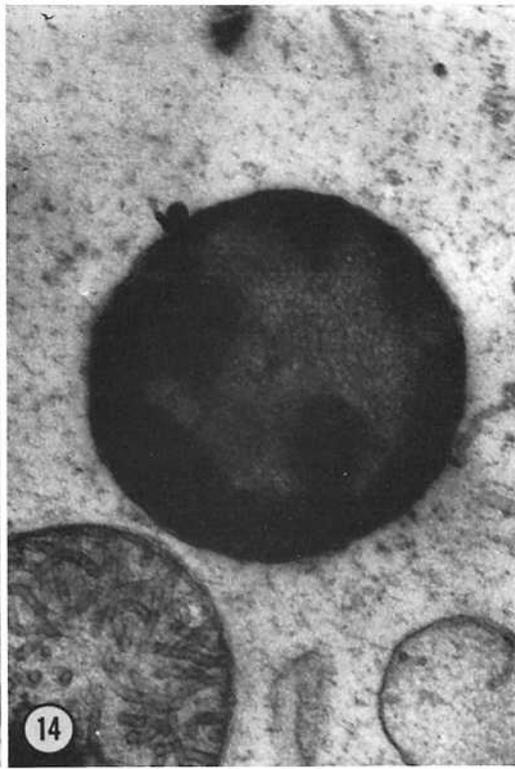
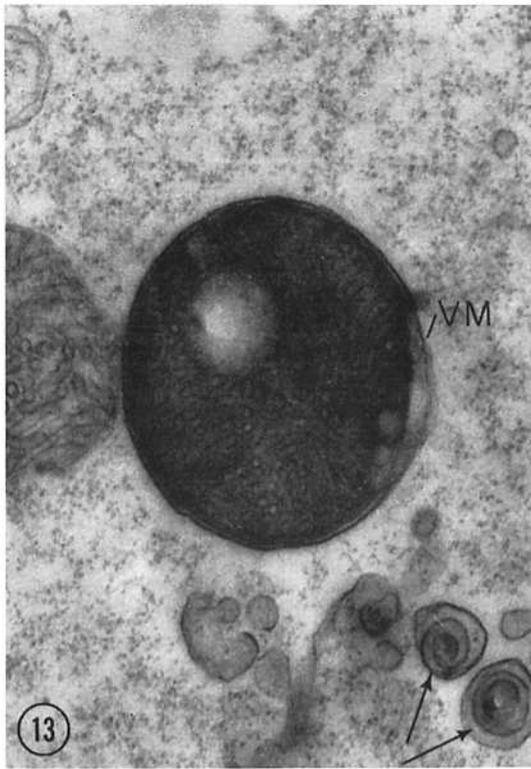
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FIGURE 13 This mitochondrion shows compacted tubules which are smaller in diameter than those in normal mitochondria in stationary growth phase (compare with Fig. 7). The vacuole membrane (*VM*) adheres closely to the mitochondrion which is quite dense. The unmarked arrows indicate coiled membrane fragments (6 days).  $\times 27,000$ .

FIGURE 14 The tubules are visible in parts of this mitochondrion. The outer membranes are not clearly demonstrated in this section (11 days).  $\times 23,000$ .

FIGURE 15 The two homogeneous black bodies shown here may be lipid droplets. Note that they are without membranes, and that their contours are irregular. The central vacuole may be a lysosome (*LY*) (11 days).  $\times 27,000$ .

FIGURE 16 A stellate lipid droplet lying near the macronucleus (*MA*). (11 days).  $\times 27,000$ .



chondrial profiles as positive proof for stages in division, but micrographs show profiles that are compatible with these events.

Among the protozoa some evidence exists for the formation of intramitochondrial material. Pappas and Brandt (26) describe fibrillar material in mitochondria of *Pelomyxa*, and Rudzinska (28, 29) finds pigment granules inside some mitochondria in aging *Tokophrya*. The so-called intramitochondrial mass has been seen by Roth and Minick (27) in *T. pyriformis* and by ourselves (3). It appears only in occasional mitochondria of young cells, but many contain it after the 5th day of culture, hence it apparently is related somehow to the aging process. Since its size (100 to 300 Å) is below the resolution of the light microscope, cytochemical techniques with the light microscope have not revealed its composition. Because the intramitochondrial mass remains following treatment with ribonuclease, it seems reasonably certain that the mass is not composed of RNA (10). The present study gives no hint as to its chemical composition in *T. pyriformis*.

It is generally agreed that mitochondria are most frequently localized in regions of the cell where the rate of metabolism is highest (22). This is strikingly portrayed in mammalian skeletal muscle where the association between the fibers and mitochondria is intimate (24). In *T. pyriformis* the mitochondria lie in close contact with the plasma membrane, the kinetodesmata, and the kinetosomes, during logarithmic growth, when the cells are most active both metabolically and physically. At this time the ciliary apparatus

functions maximally, as reflected by the rapid swimming of the ciliate. The proximity of most of the mitochondria to the structures involved in movement suggests rapid metabolic activity in these regions of the cell. Once the cells pass into the stationary phase of growth they swim more slowly. This reduced activity coincides with the migration of some of the mitochondria away from the plasma membrane to become distributed throughout the cytoplasm. Sufficient mitochondria remain in juxtaposition to the kinetosomes and kinetodesmata to maintain movement at a minimal level. The mitochondria that move into the internal cytoplasm are randomly distributed and show no apparent association with specific organelles.

During late logarithmic and early stationary growth the mitochondria continue to divide at the same rate, whereas cell division slows down. This results in a surplus of mitochondria, some of which apparently degenerate. The mitochondria become encapsulated within vacuoles, some of which contain a single mitochondrion and others, two or more, along with coiled smooth endoplasmic reticulum as well as other cytoplasmic structures. The mitochondria, when first enveloped, are only slightly more dense than normally, but later they become opaque and show little or no structure. Since these vacuoles contain acid phosphatase, it may be assumed that they are lysosomes as defined by de Duve (6).

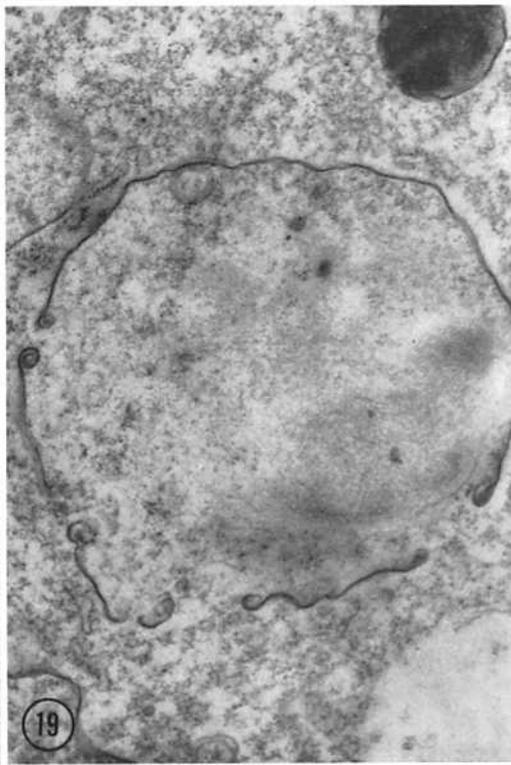
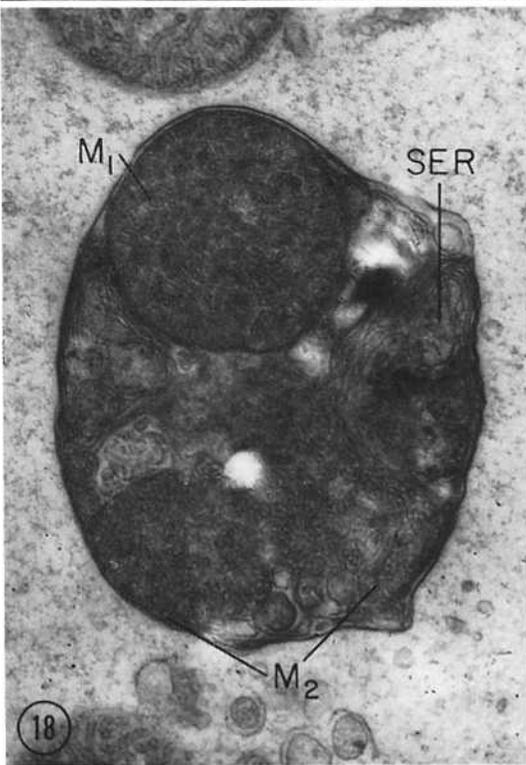
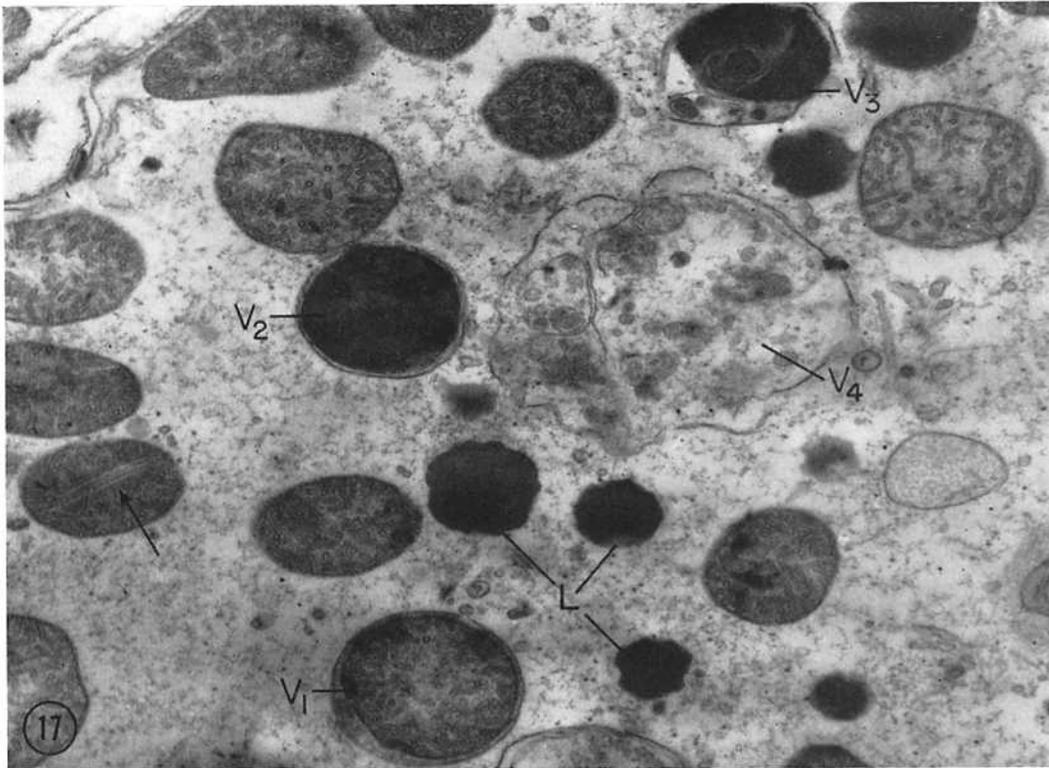
These vacuoles resemble the lysosomes described by Ashford and Porter (1) in the cells of rat liver perfused with glucagon. These authors suggest

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FIGURE 17 A low power micrograph showing lipid droplets (*L*) and autolytic vacuoles in various stages of degeneration. A single mitochondrion, essentially normal, is enveloped in a vacuole (*V*<sub>1</sub>). Another, also within a vacuole, is extremely dense (*V*<sub>2</sub>). A larger vacuole (*V*<sub>3</sub>) contains two dense mitochondria and other cytoplasmic structures. A still larger vacuole (*V*<sub>4</sub>) contains unidentified fragments and smooth endoplasmic reticulum. The membrane is ruptured, suggesting that this vacuole may be discharging its contents into the cytoplasm. The arrow indicates a mitochondrion with parallel oriented tubules. (11 days). × 18,000.

FIGURE 18 This large vacuole contains one intact dense mitochondrion (*M*<sub>1</sub>) and other mitochondria in stages of degeneration (*M*<sub>2</sub>), as well as smooth endoplasmic reticulum (*SER*). (11 days). × 27,000.

FIGURE 19 A low power micrograph showing a large vacuole containing no organized material. The vacuole membrane is broken and the ends coiled. Such coiled fragments are frequently seen in the cytoplasm (11 days). × 27,000.



that these lysosomes are derived from cytoplasmic structures, including mitochondria, which are ultimately destroyed by hydrolysis. Cytochemical tests were not made, however. Novikoff and Essner (23) find similar degenerating mitochondria encapsulated in vacuoles in rat liver cells treated with Triton WR-1339 which they call cytolysosomes. Using cytochemical techniques they demonstrated that these vacuoles contain acid phosphatase and suggest that hydrolysis is going on within the vacuole. Both Ashford and Porter, and Novikoff and Essner agree that this degenerating process results in the ultimate destruction of mitochondria.

The vacuoles seen in *T. pyriformis* are remarkably similar to those reported in rat liver cells by both Ashford and Porter and Novikoff and Essner. The difference in interpretation is that in the ciliate the vacuoles appear during the normal aging process whereas in the rat liver cells they appear only under stress and are said to be associated with pathological conditions.

Lipid is one of many particulates that has been reported to come from mitochondria under various conditions in metazoan cells, in both intact animals and tissue culture (22). The mere close association of lipid droplets with mitochondria is no proof that the latter have transformed into the former as has been shown by Palade in cortisone-treated rat liver and pancreas cells (25). There seems to be no unequivocal evidence to support the theory that mitochondria are trans-

formed into lipid. On the other hand, there is evidence that some cell products accumulate in mitochondria. For example, Ward (30) has shown that yolk platelets form inside the mitochondria of *Rana pipiens* oocytes, and Favard and Carasso (11) have demonstrated that yolk platelets in the eggs of the snail *Planorbis corneus* are deposited within the mitochondria. Rudzinska (29) suggests that mitochondria might well give rise to pigment granules in aging *Tokophrya*, a statement based on the close association of mitochondria with pigment granules and the increase of the latter with advancing age as the mitochondria decrease in number. Moreover, she refers to the pigment granules as lipid-like, although this assumption was not verified cytochemically. In *T. pyriformis* there can be little doubt that lipid droplets accompany the aging process. Ciliates in logarithmic growth show very few lipid droplets in both light and electron micrographs. There is a gradual increase in the number of lipid droplets, beginning on about the 5th day of incubation and continuing into the late stationary growth phase. Fifteen-day old ciliates are heavily laden with lipid droplets. The droplets are randomly distributed throughout the cytoplasm and seem not to be associated with any other organelle. Although they resemble the single, degenerate mitochondrion, particularly an extremely dense one (Fig. 17), no convincing evidence exists that the droplet originates from the mitochondrion. The degenerating mitochondria are always en-

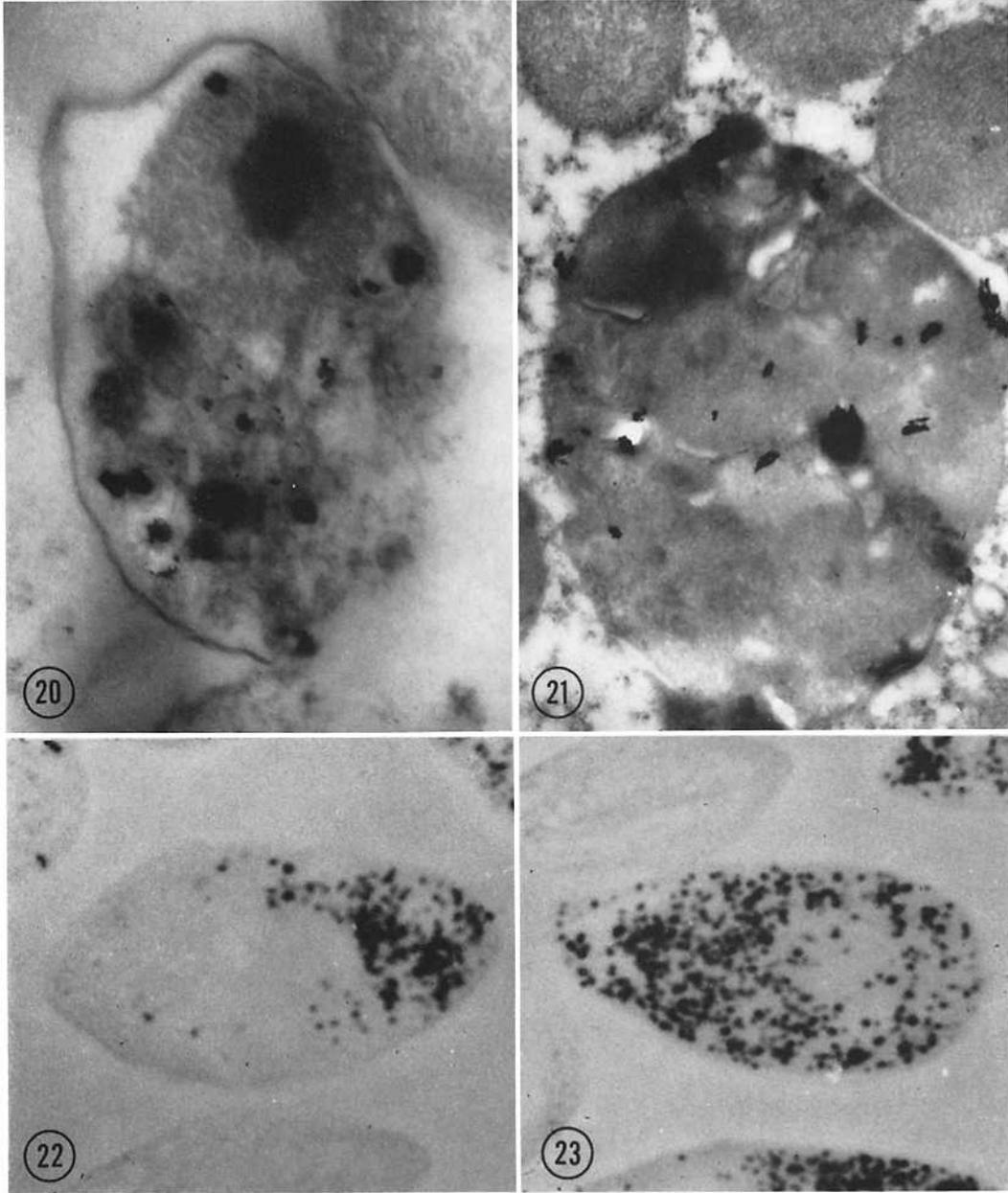
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FIGURE 20 This vacuole is from a cell tested for the presence of acid phosphatase. The vacuole has reacted positively. Note that it contains a single mitochondrion together with other unidentified cytoplasmic structures. The mitochondrion contains a large dense mass. The ruptured membrane is probably a result of poor fixation or embedding. A normal mitochondrion is seen at the upper right. (11 days).  $\times 30,000$ .

FIGURE 21 This large vacuole from a cell tested for the presence of acid phosphatase shows a positive reaction. It contains numerous mitochondria in different stages of degeneration. Note the normal mitochondria at the top of the micrograph (11 days).  $\times 17,000$ .

FIGURE 22 This photomicrograph is of a whole cell in logarithmic growth stained with Oil Red O. Note that the oil droplets are located in the anterior region of the cell.  $\times 1200$ .

FIGURE 23 Same as Fig. 22, except that this is of an 11-day old ciliate. Note that the lipid droplets are randomly distributed throughout the cell and that they are more numerous than in a cell in the logarithmic growth phase.  $\times 1200$ .



veloped in a vacuole, whereas the lipid droplets are without membranes. Nothing about the origin of the droplets has been gained from this morphological study. However, when ciliates are placed in distilled water the lipid droplets gradually disappear, suggesting that they function as the source of energy during starvation.

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