

ON CELL ORGANELLES IN TETRAHYMENA. WITH SPECIAL REFERENCE TO MITOCHONDRIA AND PEROXISOMES

by

JYTTE R. NILSSON

Institute of General Zoology, University of Copenhagen,
Universitetsparken 15, DK-2100 Copenhagen Ø

Keywords: Tetrahymena pyriformis, cell organelles, change in organelle composition, structure and function, mitochondria, peroxisomes

In the ciliate, *Tetrahymena pyriformis*, the population of cell organelles shows variation during the culture cycle and when the cells are subjected to various treatments. New cell constituents may appear and the fine structure of mitochondria and peroxisomes may change. Such dynamic structural changes are described and reviewed in relation to physiological and biochemical studies on *Tetrahymena*. The cell organelles discussed are about 1 μm in diameter or smaller; reference is also made to the nucleolar organization which is the most sensitive indicator of the physiological state of the cells. The presumed distribution of the various cell organelles in subcellular fractions of *Tetrahymena* is mentioned, since dependent on the pretreatment of the cells, especially the mitochondrial and peroxisomal fractions may be contaminated to varying degrees with other cell organelles.

The purpose of the review is to demonstrate that a correlation exists between the structure and function of cell organelles, especially mitochondria and peroxisomes, and that the overall fine structure of a cell reflects its physiological state.

1. INTRODUCTION

With different growth conditions the population of cell organelles varies in *Tetrahymena* and structural changes may occur in some organelles. The purpose of the present review is to demonstrate that there is a correlation between the fine structure of a cell and its physiological

state. The condition for making such a correlation is that the growth characteristics of the cell population are determined before and after withdrawal of samples for electron microscopy. The structural analysis of the cell organelles will be based mainly on the author's own work* on *Tetrahymena* grown under standard conditions, as outlined below, whereas the correlation between structure and function of the cell organelles will be discussed mainly with refer-

* Part of this work was carried out at The Biological Institute of the Carlsberg Foundation, Tagensvej 16, DK-2200 Copenhagen N, Denmark.

Figure 1a-i. Presentation of the various cytoplasmic constituents of *Tetrahymena pyriformis*; some are permanent structures whereas others appear under certain conditions as discussed in the text.

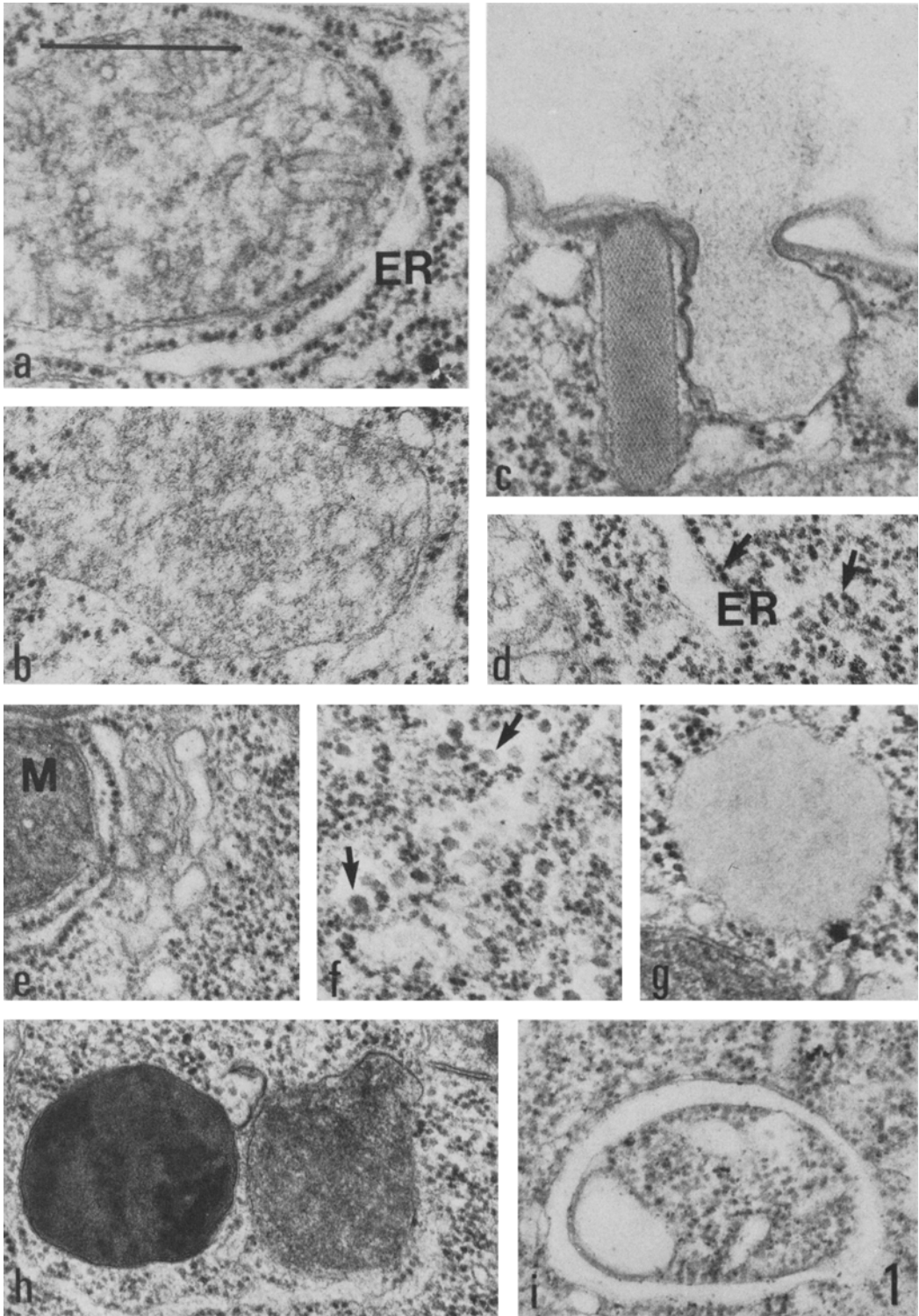
The permanent organelles are: mitochondria associated with rough endoplasmic reticulum (Figure a), peroxisomes (Figure b), mucocysts, shown in the intact and extruding form (figure c), ribosomes (arrows) associated with endoplasmic reticulum or free in the cytoplasm (Figure d), and golgi complexes closely associated with mitochondria (Figure e). Structures not permanently present are: glycogen particles (arrows) which are larger than ribosomes (Figure f), lipid droplets (Figure g), dense granules of varying electron density (Figure h), and autophagic vacuoles (Figure i). Mitochondria (M), rough endoplasmic reticulum (ER). (Bar = 0.5 μm).

ence to biochemical investigations. After a general presentation of the cell organelles (section 2) the structural changes of *Tetrahymena* during the culture cycle (section 3) and in response to various treatments (section 4) will be discussed. The presumed distribution of the cell organelles in subcellular fractions of *Tetrahymena* will be mentioned since, dependent on the physiological state of the cells, the mitochondrial and peroxisomal fractions may be contaminated to varying degrees with other cell organelles.

2. THE CELL ORGANELLES

The cell organelles to be discussed are all of a size about 1 μm in diameter, or smaller; food vacuoles and lysosomes in *Tetrahymena* have been reviewed recently (97). A brief characterization of the various organelles (Figure 1) will be given in the following. THE MITOCHONDRIA (Figure 1a) are composed of a double membrane system of which the inner membrane, enclosing the matrix, forms the typical tubular structures. Mitochondria are autonomous organelles with independent DNA (110, 137, 131, 136, 111, 14, 41, 13) and RNA species (133, 134, 18, 46, 135, 13); they are capable of protein synthesis (2, 146, 13). Replication of mitochondrial DNA occurs throughout the cell cycle (111, 13), although synchronous replication is induced when 24-h starved cells are transferred to fresh growth medium (38). Mitochondria divide by binary fission (60) and they are sensitive to treatment with ethidium bromide (16, 81, 58, 77). The organelles may play a role in the intracellular control of ions (56, 101), thus isolated mitochondria accumulate calcium at the expense of energy (22); the electron dense core found in mitochondria under unfavourable conditions (117, 32, 36, 33, 92, 101) may represent accumulated inorganic material (101). For reviews on the respira-

tion of *Tetrahymena* and the respiratory components of its mitochondria see J. F. RYLEY (118), W. F. DANFORTH (29), and D. LLOYD (77). THE PEROXISOMES (Figure 1b) are limited by a single membrane enclosing a finely granulated content. The organelle was first characterized biochemically (54, 7, 31, 86) and later morphologically (6, 83, 85, 143, 88, 92). Peroxisomes are non-phosphorylating, respiratory organelles (30) containing enzymes which produce and utilize hydrogen peroxide, and enzymes of the glyoxylate bypass of the tricarboxylate cycle (54, 86, 53, 83, 30, 85); enzymatic activity of the organelles has also been demonstrated cytochemically (130, 62). Peroxisomes are apparently formed by sequestration from the endoplasmic reticulum (92) as found in liver cells (30, 108) where, however, biochemical studies (63, 64) indicate that the marker enzyme, catalase, is synthesized outside the peroxisomes but that completion of the molecule occurs within the organelles; disconnection of forming peroxisomes from the endoplasmic reticulum during homogenization could explain these findings. THE MUCOCYSTS (Figure 1c) are cortical organelles; they are shown in the intact and extruding form. Mucocysts have been studied extensively both structurally (140, 3, 143, 144, 122, 123, 120, 92) and chemically (1, 50); however, the biogenesis of the organelles has not been revealed in detail. *Tetrahymena* contains about 1000 mucocysts; the extruded mucocyst material binds the cationic dye, alcian blue (90, 92, 100, 138), and the possible role of this material in nutrient uptake has been discussed (92, 97). The mucocysts extrude their contents in response to a variety of stimuli and the discharged material is an annoying contaminant in cell homogenates; these organelles will not be discussed further. THE RIBOSOMES (Figure 1d) are found free in the cytoplasm or bound to the endoplasmic reticu-



lum, depending on their associated messenger RNAs. The quantity of ribosomes varies with the physiological state of the cells (66). THE GOLGI COMPLEXES (Figure 1e) are inconspicuous organelles closely associated with mitochondria (45, 43, 92, 44, 97). The forming face is a differentiated area of the rough endoplasmic reticulum associated with mitochondria, and the Golgi complex shows a distinct polarity with increasing membrane thickness towards the maturing face. The Golgi complexes in *Tetrahymena* probably play the universal role of packaging hydrolytic enzymes in primary lysosomes, and also of modifying membranes, from the type of the endoplasmic reticulum to the type of the plasma membrane, during formation of membrane renewal vesicles for the plasma and food vacuolar membranes (92). The early description of Golgi complexes in conjugating *Tetrahymena* (34) may represent stacks of reserve membrane vesicles, typical of these cells (42). During cell fractionations, Golgi complexes may sediment with mitochondria due to their close association with this organelle, alternatively they may sediment with the microsome fraction, unless they become disrupted, as found for liver cells (51) where portions of the Golgi complex are found in both fractions. In *Tetrahymena* the finding of phosphocholine transferase activity (129) in the mitochondrial fraction (see Table I) indicates sedimentation of Golgi complexes in this fraction, whereas the finding (142) that the food vacuolar membrane shows closer similarity to membranes of the microsome fraction than to that of isolated cilia, representing the plasma membrane, may indicate sedimentation of Golgi complexes, or parts of these, in the microsome fraction (92, 97). GLYCOGEN PARTICLES (Figure 1f) are larger than ribosomes and stain less intensely with routine contrasting methods. Native glycogen from *Tetrahymena* has been characterized (5) and the presence in situ of α - and β -particles has been demonstrated (132). *Tetrahymena* may contain large amounts of glycogen (118, 80, 73, 119, 72, 71, 127). LIPID DROPLETS (Figure 1g) are usually of low electron density; they represent stores of triacylglycerol and are abundant in cells from the stationary growth phase (4, 139, 72, 71, 12). SMALL ELECTRON DENSE GRANULES (Figure 1h) have contents of varying electron density. They

are limited by a membrane which in structure resembles that of the plasma, and food vacuolar, membrane (101). The granules appear in the stationary growth phase and their presumed role in ion-regulation will be discussed. THE AUTO-PHAGIC VACUOLES (Figure 1i), the last type of organelles to be discussed, are small vacuoles enclosing portions of cytoplasm (32, 72, 88, 89, 33, 92) destined for digestion; thus, they are a kind of lysosomes.

Not all these cell organelles are found in *Tetrahymena* in all stages of growth; furthermore, those organelles permanently present may change in structure as will be outlined in the following. Although the cell nucleus is not considered in this review, reference will be made to the nucleolar organization because it is the most sensitive indicator of the physiological state of *Tetrahymena*; the direct relationship between the nucleolar structure and the production of ribosomes has been reported repeatedly (15, 103, 121, 66, 92, 93, 67).

3. THE CULTURE CYCLE

The growth pattern of *Tetrahymena pyriformis* GL-8 (11) under standard conditions is shown in Figure 2. The growth medium is 2% proteose peptone enriched with 0.1% liver extract and inorganic salts (112); the cultures are aerated and shaken at 28 °C. After inoculation (200 cells/ml) follows a short lag period before the cells enter the log. phase, i.e. exponential cell multiplication (generation time less than 3 h), up to a cell density of about 150,000 cells/ml; at this transition stage the cells enter the early stationary phase of growth for additionally 2–3 cell doublings at a decreased rate up to a cell density of 800,000–1,000,000 cells/ml which marks the true stationary phase. This high cell density is maintained for about 36 h after which the onset of cell deaths marks the decline phase. The transition point, or entry into the early stationary phase, is associated light microscopically with the appearance of small, refractile granules which increase in number with time, initially they are found in the anterior end of the cells and later distributed throughout the cells (102), as indicated in Figure 3. The appearance of the granules is invariably correlated with an increased cell generation time, thus they repre-

sent a sensitive light microscopical marker for the physiological state of the cells. Since the fine structure of *Tetrahymena* may vary with the fixation procedure (89, 92) it is important to mention that all illustrations are of cells fixed in glutaraldehyde followed by osmium tetroxide (92).

The fine structure of *Tetrahymena* in different phases of the culture cycle is shown in Figure 4. In the log. phase (Figure 4a), the mitochondria and peroxisomes are of low electron density, i.e. they are of the 'light' type (89, 92); the nucleus contains numerous small nucleoli and ribosomes are abundant as typical in rapidly growing cells. At the transition (Figure 4b) into the early stationary phase, the mitochondria are of higher electron density, i.e. they are of the 'dense' type (89, 92), whereas the peroxisomes remain of the light type; the nucleoli are enlarged and lipid droplets (not shown) appear. In the early stationary phase (Figure 4c) where the rate of proliferation has decreased, mitochondria as well as peroxisomes are of the dense type; the nucleoli form small fusion bodies and the cells contain glycogen particles, lipid droplets, and small dense granules. In the stationary phase (Figure 4d) where cell proliferation has ceased, the

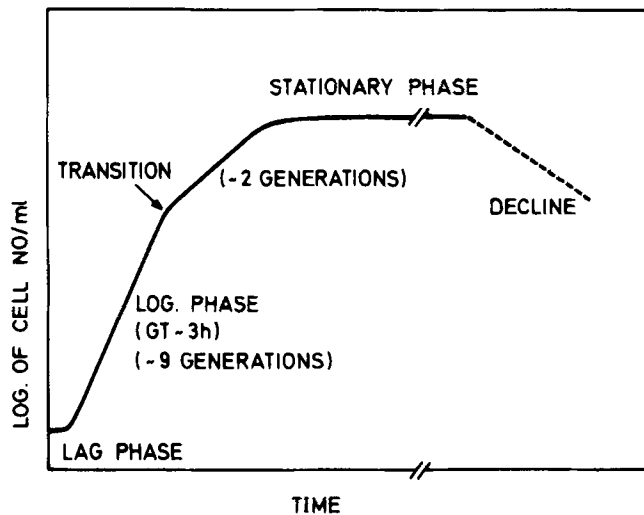


Figure 2. Typical growth pattern of a culture of *Tetrahymena pyriformis*.

The various growth phases of the culture cycle are indicated on the curve, representing cell density plotted against time; the phases are defined according to the rate of increase, or decrease, in the relative number of cells per ml. Note the transition point marking the abrupt alteration in the rate of cell proliferation.

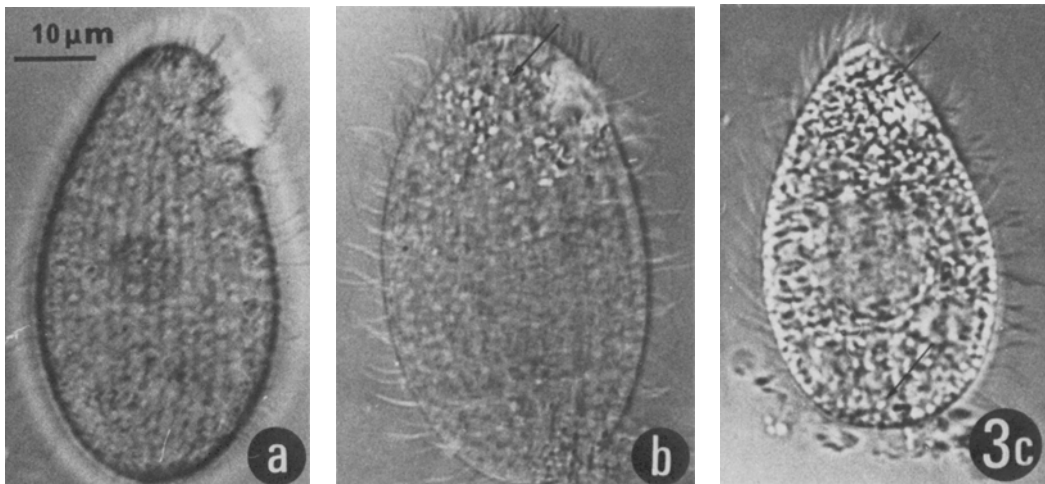
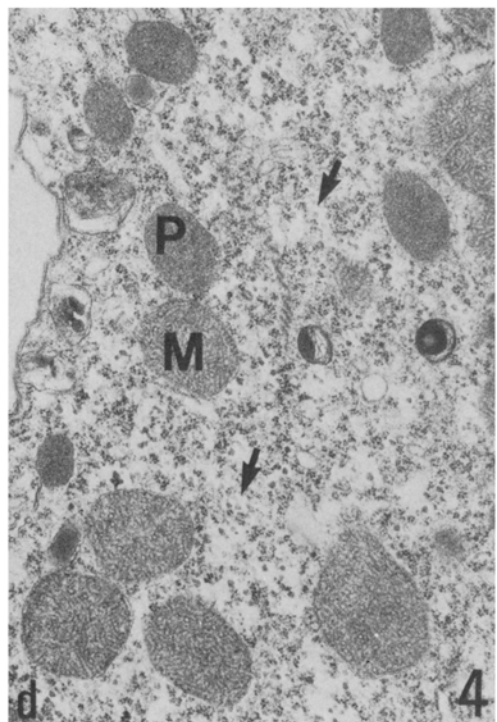
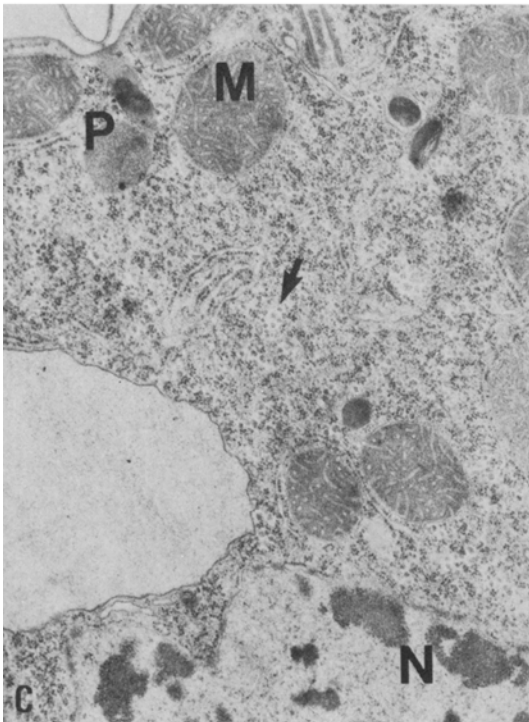
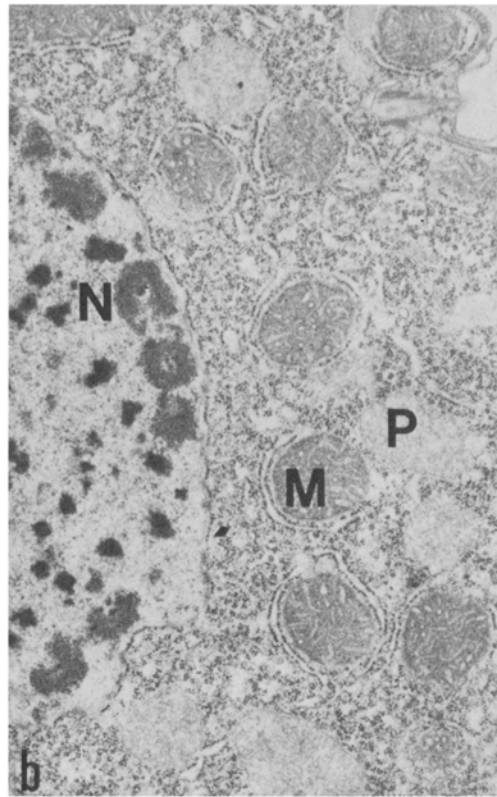
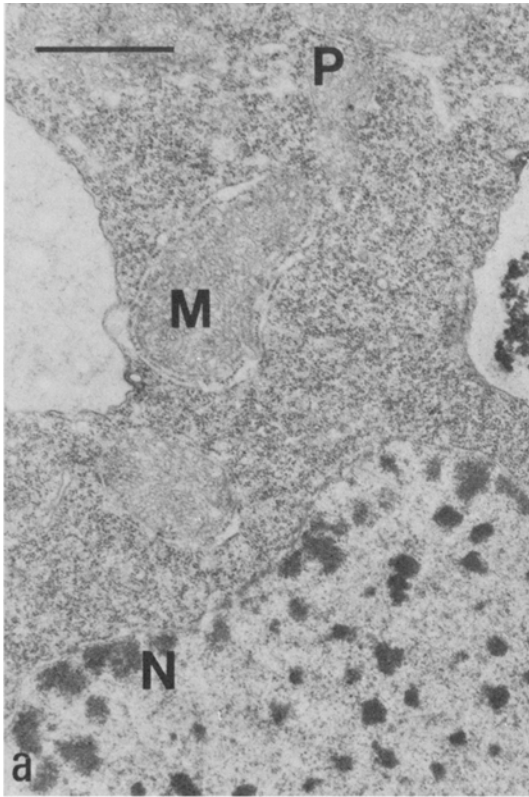


Figure 3a-c. Light micrographs illustrating the cyclic appearance of small, refractile granules in *Tetrahymena* fixed briefly in osmium tetroxide. Granules are absent in rapidly multiplying (log. phase) cells (Figure a); however, they appear in cells from the early stationary phase of growth, initially in the anterior region of the cell (Figure b), and as the granules continue to accumulate they become distributed throughout the cells in the stationary phase of growth (Figure c). Reproduced from (92).



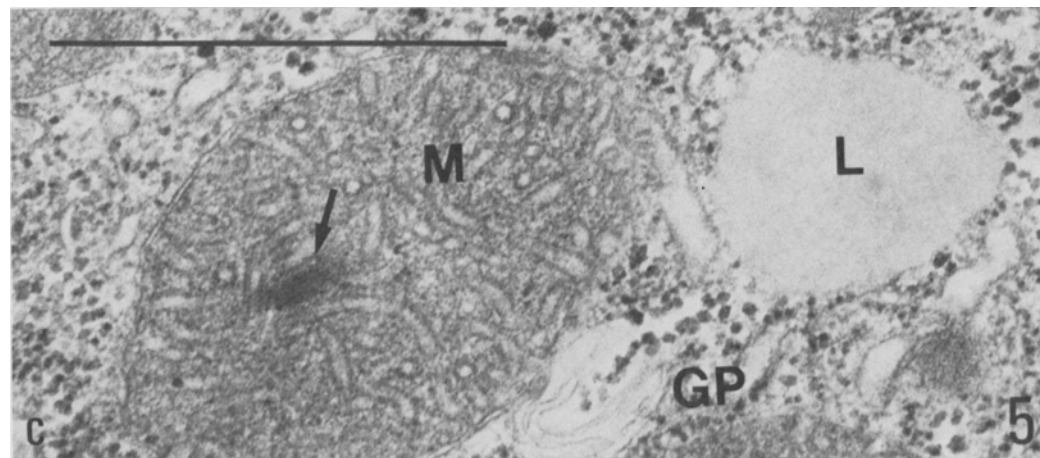
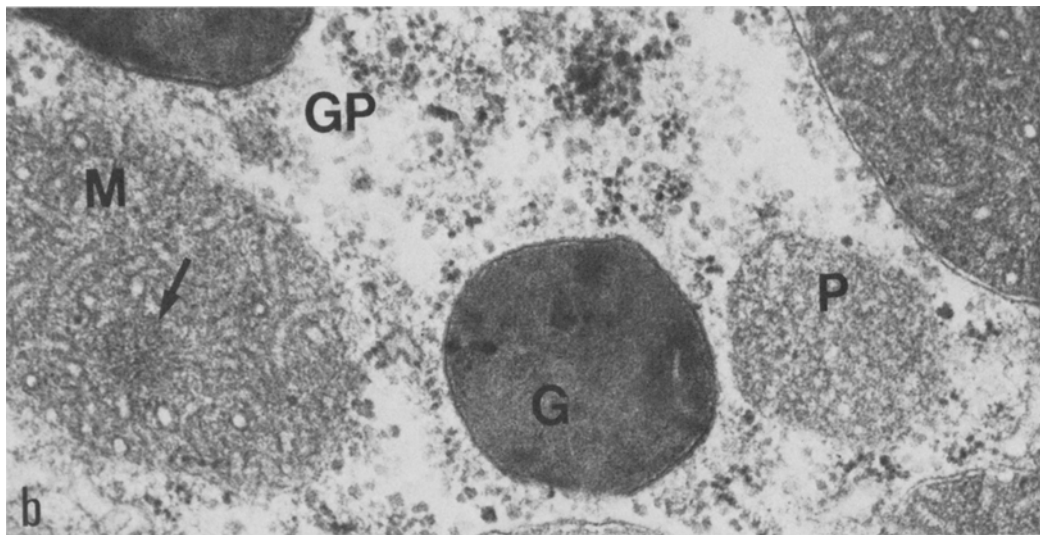
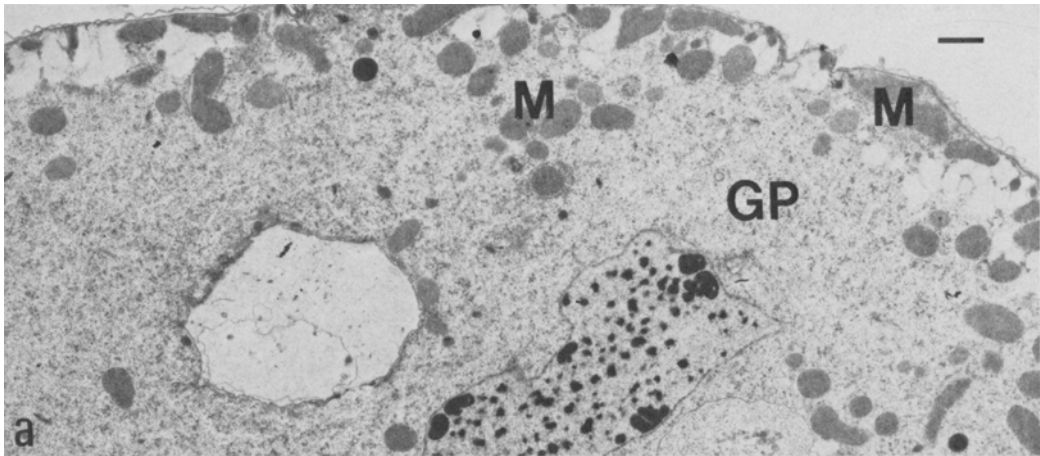


Figure 4a-d. Sections of *Tetrahymena* in different phases of growth.

In the log. phase (Figure a) mitochondria and peroxisomes are of the light type, ribosomes are abundant, and the nucleoli are small. At the transition stage (about 150,000 cells/ml) the mitochondria are dense and the peroxisomes remain light type; the nucleoli are slightly enlarged (Figure b). In the early stationary phase (280,000 cells/ml) the mitochondria and peroxisomes are dense type (Figure c), glycogen particles (arrow) and small dense granules are seen; the nucleoli have fused. In the stationary phase (no cell proliferation) the mitochondria and peroxisomes are dense (Figure d) and glycogen particles (arrows) form islands in the cytoplasm. Mitochondria (M), peroxisomes (P), nucleoli (N). (Bar = 1 μ m).

Figure 5a-c. Sections of *Tetrahymena* late in the stationary phase of growth.

The typical feature is the large amount of glycogen, confining larger organelles to islands in the cytoplasm (Figure a); the nucleoli have formed fusion bodies. Mitochondria and peroxisomes are dense type (Figure b) and the former organelles may have a dense core (arrow); lipid droplets (Figure c) and dense granules (Figure d) are present and there are few ribosomes. Mitochondria (M), peroxisomes (P), dense granules (G), and glycogen particles (GP). (Bar = 1 μ m).

mitochondria and peroxisomes remain of the dense type and there is a progressive accumulation of glycogen particles, forming small islands in the cytoplasm, and fusion of nucleoli (not shown). Late in the stationary phase (Figures 5b, 5c) lipid droplets and small dense granules are numerous, whereas there are few ribosomes and sparse endoplasmic reticulum; the mitochondria may contain a dense core (Figure 5c) and they are confined to the cortical region and to small islands due to large amounts of glycogen particles (Figure 5a). Glycogen may comprise more than 25% of the weight of *Tetrahymena* (118, 80, 124, 73, 119, 71). Thus, from the transition point and onwards marked changes occur in the population of cell constituents and in the structure of mitochondria, peroxisomes, and nucleoli.

The structural changes observed in *Tetrahymena*, as it passes through the culture cycle, is a reflection of a changing cellular physiology. Thus, the rate of oxygen consumption is decreased in stationary phase cells (109, 55, 29, 49, 78, 71), as compared to log. phase cells;

furthermore, stationary phase cells have a high capacity for glyconeogenesis, i.e. the conversion of lipid into glycogen (54, 73, 53, 83, 71, 127), which morphologically correlates with the presence of glycogen particles. Considered in relation to the physiology of the cells, the fine structure of mitochondria and peroxisomes indicates that the dense type of mitochondria correlates with a low activity of the organelle in oxidative phosphorylation, and likewise that the dense type of peroxisomes correlates with activity of the glyconeogenic enzymes in this organelle (92). In cells from the log. and stationary phase of growth both organelles appear in the corresponding type, light or dense, respectively (Figures 6a, 6c, 6d); however, this relationship is not observed in the transition stage (Figure 6b). How may structure and function be related to this point of the culture cycle? In *Tetrahymena* the enzymes of the glyoxylate bypass of the tricarboxylate cycle are distributed between mitochondria and peroxisomes (86) which means that substrate must pass from mitochondria to the peroxisomes and

Figure 6a-d. Structural relation of mitochondria and peroxisomes in *Tetrahymena* in various phases of the culture cycle.

In the log. phase (Figure a) both organelles are of the light type, typical of rapidly multiplying cells. At the transition stage (160,000 cells/ml) the mitochondria have changed to the dense type, whereas the peroxisomes remain light (Figure b). In the early stationary phase both mitochondria and peroxisomes are of the dense type (Figure c); glycogen particles are present. Late in the stationary phase (Figure d) mitochondria and peroxisomes remain dense, glycogen is abundant and ribosomes are few in number. Mitochondria (M), peroxisomes (P), glycogen particles (arrows), ribosomes (R), dense granules (G). (Bar = 0.5 μ m).

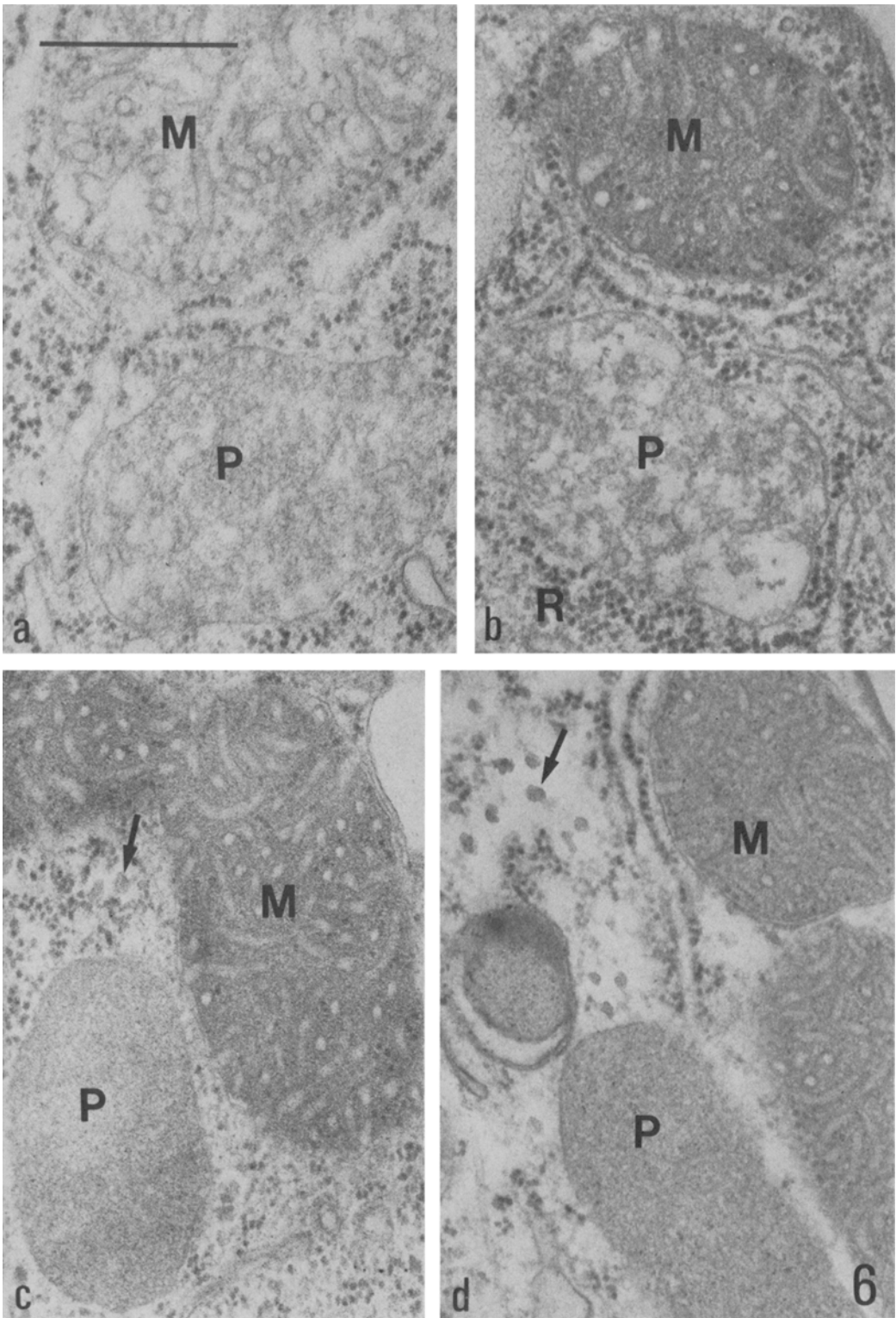


Table I

Mitochondrial and peroxisomal enzymes with indication of their activity: constant (C), or maximal in logarithmic (L) or in stationary (S) growth phase

Mitochondria		Peroxisomes	
(S)	citrate synthase (53)	(S)	isocitrate lyase (53, 79)
	aconitase	(S)	malate synthase (53, 79)
	succinate dehydrogenase	(C)	isocitrate dehydrogenase (79)
	acetyl-CoA synthase		acetyl-CoA synthase
(C)	fumarase (79)		D-amino acid oxidase
	malate dehydrogenase		glyoxylate oxidase
(S)	lactate dehydrogenase (113)		L- α -hydroxy acid oxidase
(L)	aspartate transaminase (113)	(L)	glycolate oxidase (79)
(C)	glutamate dehydrogenase (113)	(C)	catalase (78, 79)
(L)	succinate oxidase (78)		
(L)	cytochrome c oxido-reductase (78)		
(S)	phosphocholine transferase (129)		

General information from references: 7, 31, 86, 53, 83, 30, 78, 10, 71, 113, 79, 76; studies on levels of enzyme activity are indicated in parantheses.

back again (86, 83). The signal for activation of the glyoxylate bypass enzymes in peroxisomes is thus likely to come from the mitochondria, in which case a structural change, as a reflection of changed function, would be expected to occur first in mitochondria and later in peroxisomes as actually observed in transition phase cells. The mutual exchange of substrate between the mitochondria and peroxisomes may require a close spatial relationship as that depicted in Figure 6; however, a 3-dimensional relationship cannot be deduced readily from a 2-dimensional plane and no connecting structure has been observed between the organelles.

The glyoxylate bypass enzymes in peroxisomes are isocitrate lyase and malate synthase (86); they are present but inactive in cells in the log. phase of growth (53). Activation of the enzymes occurs even in the presence of inhibitors of RNA and protein synthesis (68, 53, 71, 37, 127), which indicates that peroxisomes may change from the light to the dense type without involvement of protein synthesis. Inactivation of the enzyme is, however, dependent on protein synthesis (70, 75) and concomitantly the activity of an extralysosomal neutral protease increases. This protease has been proposed (75) to be involved in the modification of the peroxisomes which presumably means a change from the dense to the light type of the organelle as would

occur during the lag period after inoculation of a new culture with stationary phase cells. That organelles already present are modified is likely since there is no evidence for autophagic removal of peroxisomes during conditions where they change from the dense to the light type (89, 92).

The activity of the mitochondrial and peroxisomal enzymes may vary during the culture cycle (78, 69, 71, 79, 127). Enzymes from the two organelles in *Tetrahymena* are listed in Table I; for some of the enzymes it is indicated whether maximal activity is found in the log. or the stationary phase of growth, or whether their activity is constant. Apart from functioning in the glyoxylate bypass cycle, mitochondria are active in the tricarboxylate cycle (141), and peroxisomes are active in various oxidations leading to the production of hydrogen peroxide which is utilized by catalase, the marker enzyme for peroxisomes. It has been proposed (92) that the light and dense type of mitochondria represent high and low respiratory capacity of the organelle, respectively, and that the light and dense type of peroxisomes represent major activity in oxidation and in glyconeogenesis, respectively. This is in agreement with the levels of enzyme activity in the two organelles as far as information is available; future information on the maximal activity of other enzymes may reveal further evidence for this postulate. Inci-

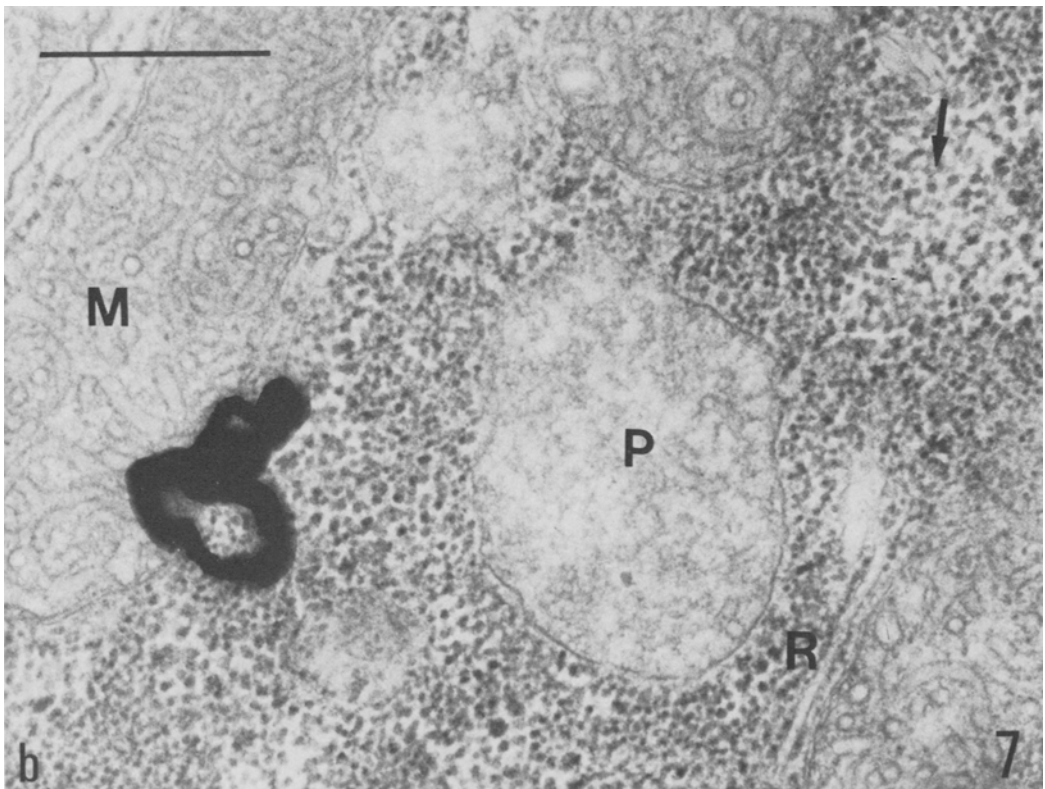
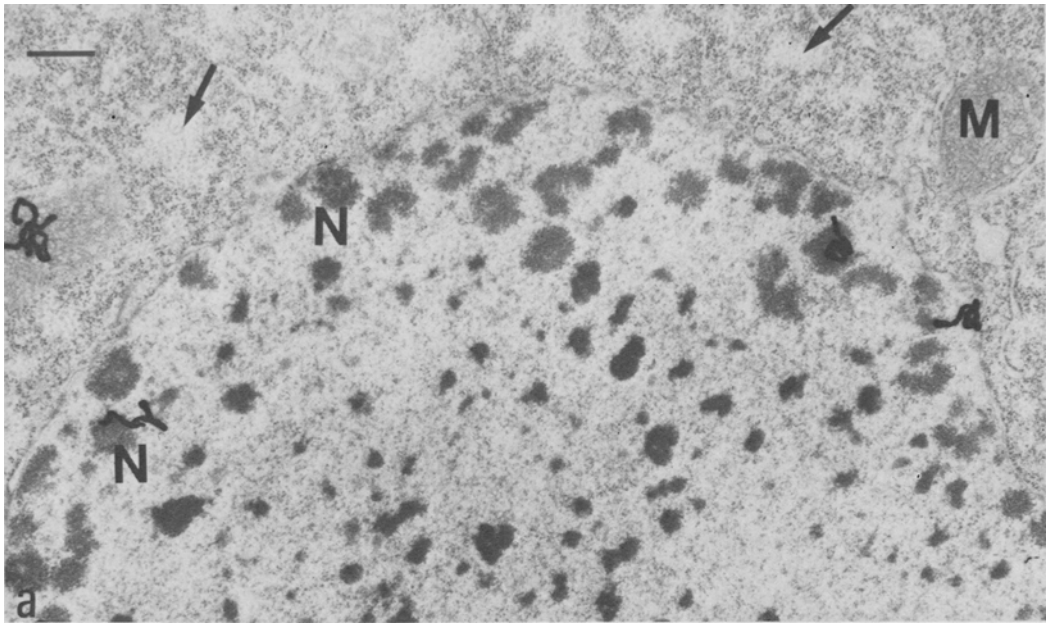


Figure 7a-b. Supplementation of growth medium with glucose.

Glycogen particles form islands in cytoplasm (Figure a) although other features, such as light type mitochondria and peroxisomes (Figure b), abundant ribosomes, and small nucleoli (Figure a) indicate that the cells are in the log. phase of growth. (Cell density = 80,000 cells/ml; generation time = 3 h). Autoradiograms of tritiated thymidine labelled cells; the curly configurations (silver grains) above mitochondria and nucleoli indicate their activity in DNA synthesis. Mitochondria (M), peroxisomes (P), glycogen particles (arrows), ribosomes (R), nucleoli (N). (Bar = 0.5 μ m).

dentally, peroxisomes from stationary phase, or starved, cells have an increased median bouyant density as compared with peroxisomes from log. phase cells (78), whereas the density of mitochondria is unchanged; however, an increased electron density does not necessarily imply an increase in intrinsic mass, it may merely reflect an increased binding capacity for the heavy metals used in routine contrasting for electron microscopy.

The presence of glycogen particles in *Tetrahymena* grown in proteose peptone medium is usually a clear indication of activity in glyconeogenesis and of a decreased rate of cell proliferation. However, if glucose is added to the medium, although *Tetrahymena* does not need an extra carbon source in this medium (59), then glycogen particles are found in cells which otherwise show the typical fine structure of log. phase cells (Figure 7) and which have an unchanged generation time of 3 h. The explanation is that excess glucose may be converted directly into glycogen (72, 57, 27, 113, 76, 127) and that exogenous glucose has been found to depress the activity of the glyoxylate bypass enzymes (53, 113, 79). Thus, the presence of glycogen particles in *Tetrahymena* is not an unambiguous indication of a prolonged cell generation time and of activity in glyconeogenesis.

The presence of lipid droplets in *Tetrahymena* is invariably associated with a decreased rate of cell proliferation. The droplets appear in the early stationary phase of growth and whenever the cells are subjected to treatments that interfere with cell multiplication, as will be discussed below (section 4). The number of lipid droplets increases as the cells pass into the stationary phase but decreases late in this phase (39, 40, 4, 36, 139, 72, 26, 71, 92). They are generally believed to represent stores of reserve energy, a question which was investigated (12) after labelling the triacylglycerol in cells which had just ceased proliferation in the stationary phase. The labelling pattern showed a 'first in first out' kinetics and 88% of the labelled triacylglycerol was turned over after 90 min; 18% only was recovered as CO₂, 3% as glycogen, and 76% as phospholipids (12), an indication that at this stage of growth the major part of the triacylglycerol goes into membrane synthesis. A demand

for membrane synthesis at this stage may seem strange since cell proliferation has ceased; however, membrane is involved in the formation of dense granules and autophagic vacuoles. The latter organelles are prominent in starved cells (Figure 9) as will be discussed below (section 4) but they also appear in cells in the late stationary phase (32, 36, 92, 101), either as a result of partial starvation due to partial exhaustion of the medium or because worn-out organelles must be removed, such organelles could be mitochondria having accumulated so much calcium that their respiratory capacity is lost.

The small, dense granules also appear in cells in the stationary phase. Together with lipid droplets (Figures 5b, 5c) they represent the light microscopically visible, refractile granules (Figure 3). Analysis of isolated and in situ granules has revealed the presence of calcium, magnesium, potassium, and phosphorus in an organic matrix (115, 116, 87, 23); other ions added to the growth medium, such as strontium and lead, may also accumulate in the granules (24, 98). The amount of inorganic material varies in the granules (23, 24), as does the electron density of their contents (101), thus indicating that the granules are part of an ion-regulating system in *Tetrahymena* and that they act as waste disposals (56, 23, 24, 101). Since the granules appear in cells in the normal growth medium, their role in ion-regulation may be illustrated for calcium (Figure 8). A low intracellular concentration of calcium may be established in rapidly multiplying cells because there is an equilibrium between entry and output of the ion across the plasma membrane, on one side, and maintenance of a certain calcium pool and a constant flow of ions into metabolism, on the other side. When cell proliferation ceases in the stationary phase, less calcium is drawn into metabolism and excess ions enter the pathway of granule formation, assuming constancy of the other parameters; the amount of the ion accumulated and bound in the granules may correspond to 2 M-calcium (101). The limiting membrane of the dense granules resembles the plasma and food vacuolar membrane in thickness and polarity (101) as mentioned earlier, and the apparently gradual accumulation of ions in the granules could be mediated by special ATPases. Interesting in this respect is that *Tetrahymena* has a calcium-activated ATP-

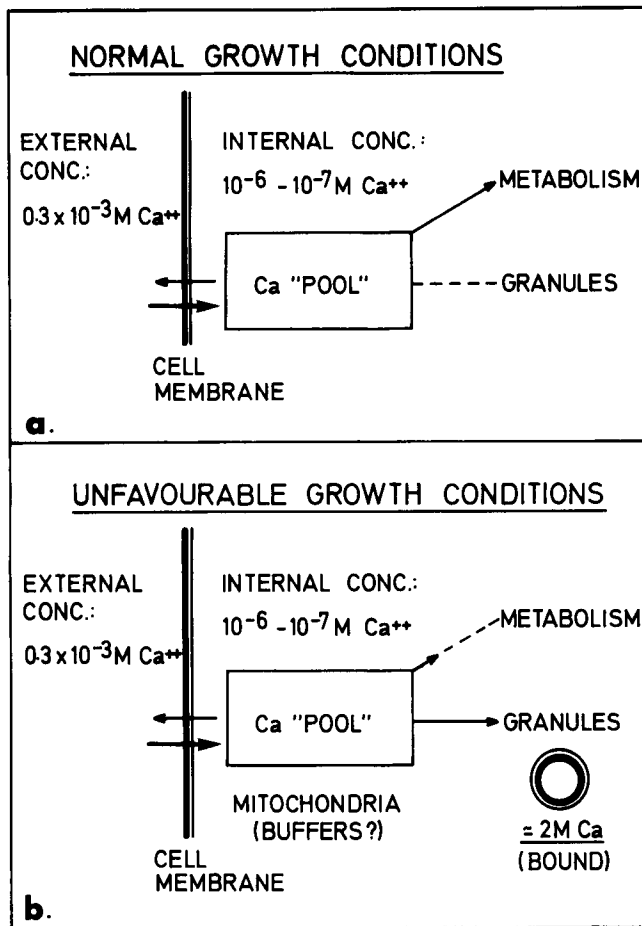


Figure 8. Model of the ion-regulating system in Tetrahymena illustrated for calcium.

To establish the low internal (cytoplasmic) concentration in a high external (medium) concentration of calcium, an efficient regulation is necessary. The model is based on the assumption of: 1) a constant influx, as well as outflux, of calcium across the cell (plasma) membrane and 2) maintenance of a compartmentalized calcium «pool» and a certain flow of ions into metabolism (Figure a); this situation is valid for rapidly growing cells which do not contain refractile granules (see Figure 3). Under unfavourable conditions with decrease, or cessation, of cell proliferation less calcium is drawn into metabolism (Figure b) and the excess ions enter the pathway of granule formation for storage in a bound form. Before granule formation is activated, mitochondria may act as a buffer system in the initial removal of excess calcium. The model may be extended so as to apply to removal of other unwanted ions from the cytoplasm. Modified version of previous model (92).

ase, the activity of which increases in the stationary phase (19, 20) where granule formation occurs, although this ATPase was obtained from the cytosol fraction. The dense granules with their varying content of inorganic material may contaminate the mitochondria and peroxisome fractions.

The dynamic change in the population of cell organelles in Tetrahymena during the culture cycle is summarized in Table II. Not only the

composition of cell constituents but also their number change in the various growth phases. Recognition of such changes is important in the preparation of cell homogenates because, dependent on the culture stage, the mitochondria and peroxisome fractions may be contaminated differently. The presence of autophagic vacuoles may be revealed by their hydrolases, whereas the detection of dense granules is more questionable.

Table II

Changes in population of cell constituents during the culture cycle

Cell constituent	Log. phase	Transition	Early stationary phase	Stationary phase	Late stationary phase
Mitochondria	light type	dense type	dense type	dense type	dense type («cores»)
Peroxisomes	light type	light type	dense type	dense type	dense type
Golgi complexes	present	present	present	present	present
Glycogen particles	absent*)	absent*)	present	abundant	present
Lipid droplets	absent	few	many	abundant	present
Dense granules	absent	absent	present	abundant	abundant
Autophagic vacuoles	absent	absent	absent	few	present

Nucleolar organization	numerous small nucleoli	numerous slightly enlarged nucleoli	early fusion of nucleoli	nucleolar fusion bodies	large nucleolar aggregates

*) Unless medium supplemented with glucose.

4. RESPONSE TO VARIOUS TREATMENTS

Structural changes are also observed in exponentially multiplying cells subjected to treatments interfering with cell proliferation. Some of the changes resemble those observed in the stationary phase of growth but others may relate to the specific action of a particular inhibitor.

Starvation is a common physiological condition which most cells experience. In *Tetrahymena* it can be induced abruptly by transfer of the cells to an inorganic salt medium (47, 112). Within 1 h the mitochondria and peroxisomes change to the dense type (Figure 9b) (89, 92) which is in agreement with a decrease in the rate of respiration to 50% (72) and 30% (47) after 1 h and 2 h, respectively, and cellular activity in glyconeogenesis (71). Starvation also causes fusion of nucleoli (Figure 9a) (72, 103, 92) and the cells contain lipid droplets, glycogen particles, small dense granules, and autophagic vacuoles (72, 89, 92). Two types of autophagic vacuoles occur and they can be defined according to the integrity of their cytoplasmic contents, i.e. identifiable or not, the latter owing to advanced digestion (88); the former type is most frequent during early starvation. Autophagy is a mode by which the cells may sustain a basic metabolism

in the absence of exogenous nutrients (89, 71, 92) and *Tetrahymena* can be starved for several days at the expense of cellular RNA (65, 21, 61) and protein (28, 68, 84). Mitochondria, peroxisomes, and general cytoplasm (Figure 9b) may be enclosed in autophagic vacuoles (72, 88, 89, 92) during starvation. The factors determining the selection of cell components to be digested are as yet unknown; however, early during starvation mitochondria seem to be preferentially enclosed (89) causing a decrease in the number of the organelles in the central cytoplasm but apparently leaving a constant population of mitochondria in the cortical region. Typically mitochondria enclosed in autophagic vacuoles have an electron-dense core; however, this feature cannot be a general criterion for removal of the organelle because most mitochondria in starved cells may have such a dense core (92).

The inhibitor, 2,4-dinitrophenol (DNP), is an uncoupler of oxidative phosphorylation. It has a dose-dependent effect on the rate of food vacuole formation in *Tetrahymena* and no vacuoles are formed in 0.5 mM-DNP (92). At this concentration the cellular rate of oxygen consumption is reduced to 30% after a 1-h treatment (47), a similar response is found for isolated mitochondria (25). After a 1-h exposure to 0.5 mM-DNP,

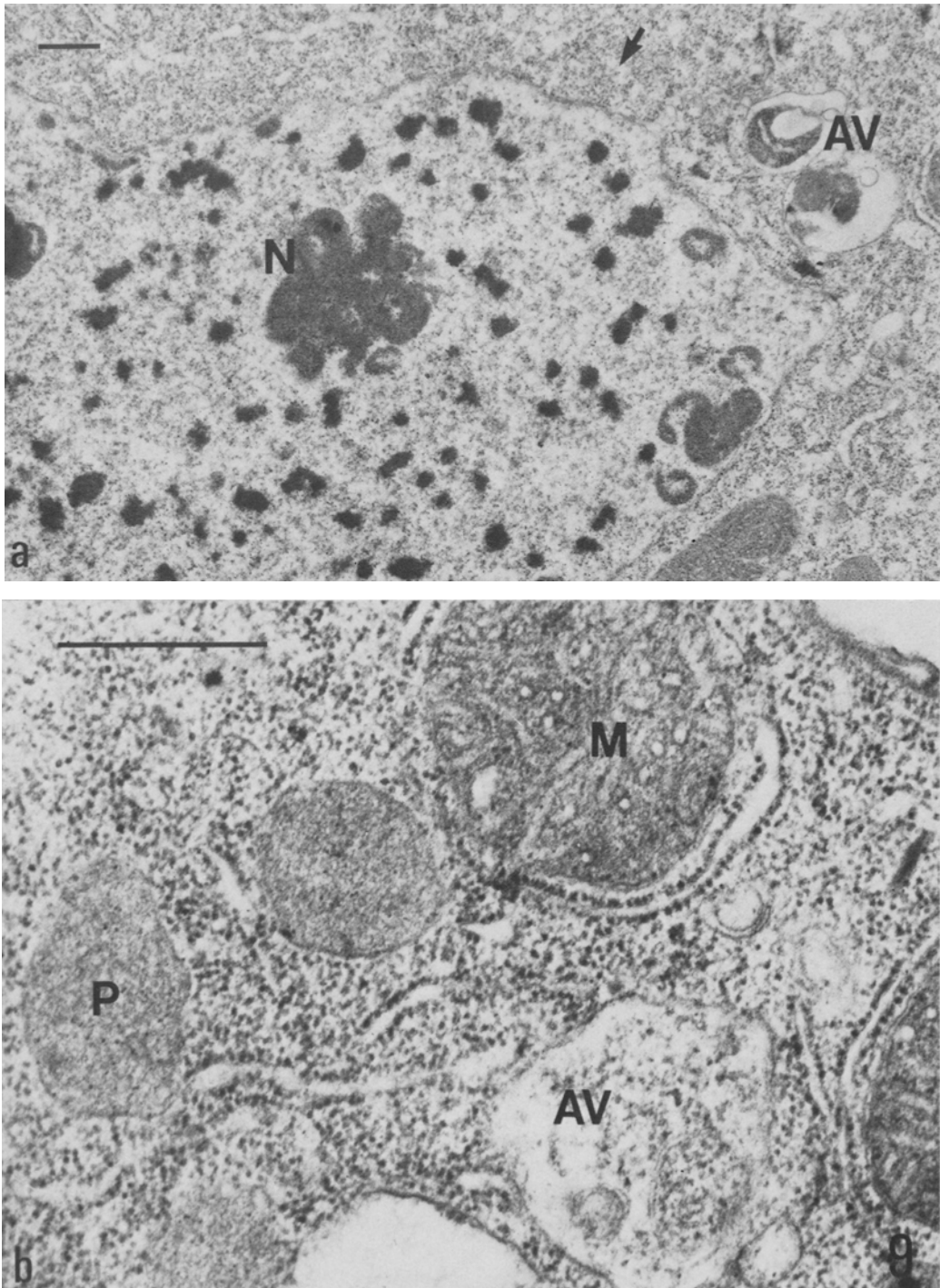


Figure 9a-b. The effect of starvation on Tetrahymena from the log. phase of growth.

Within 1 h of starvation mitochondria and peroxisomes have changed into the dense type and autophagic vacuoles are present (Figure b). Starvation causes progressive nucleolar changes and large fusion bodies are seen after 3 h (Figure a) at which time several autophagic vacuoles and glycogen particles (arrow) are present. Mitochondria (M), peroxisomes (P), autophagic vacuoles (AV), nucleoli (N). (Bar = 0.5 μ m).

the mitochondria appear very dense with contracted regions whereas the peroxisomes appear as an extremely light type (Figure 10a); no lipid droplets or glycogen particles are seen and the nucleoli appear »frozen« (Figure 10b), i.e. very dense and contracted (92). The cellular content of ATP is very low and cell motility has almost ceased after a 1-h treatment (unpublished results); however, the treatment is reversible although recovery is slow and involves additional and rather conspicuous changes (92).

Dimethyl sulphoxide (DMSO) is used as a solvent for water insoluble compounds or as a cryoprotective reagent. It also has a dose-dependent effect on the rate of food vacuole formation in *Tetrahymena* (91, 92); cessation of vacuole formation occurs in 7.5% DMSO. After 1 h in this concentration, the mitochondria and peroxisomes have changed into their dense type (Figure 11a), typical of starved and stationary phase cells; furthermore, lipid droplets, glycogen particles, autophagic vacuoles, and various nucleolar changes are found (94, 95). Apart from the variable nucleolar changes, these effects resemble those observed during starvation; however, additional changes such as helical patterns of polyribosomes (95) and the appearance of fibrous bundles in the nucleus (99) may be ascribed to a specific action of DMSO, whereas the former effects reflect a decrease in the rates of cell proliferation (94), oxygen consumption (128), and synthesis of RNA (94) and protein (99). The cells recover from the 1-h treatment; however, although the rate of RNA synthesis has returned to the control level 1 h after removal of DMSO (94), only mitochondria have reversed into the light type after 3 h (Figure 11b), the peroxisomes remain dense. Modification of the peroxisomes seems to be a slow process.

Cytochalasin B (CCB) interferes with microfilaments. This inhibitor also has a dose-dependent effect on food vacuole formation in *Tetrahymena* (107, 52, 114) and phagocytosis is completely inhibited in $40 \mu\text{g}\cdot\text{ml}^{-1}$. However, at this concentration both mitochondria and peroxisomes remain of the light type (92, 96). The rate of cell proliferation is somewhat affected, as also reflected by slightly enlarged nucleoli and a slightly reduced rate of RNA synthesis (96). Although phagocytosis is inhibited in CCB the cells show considerable uptake of compounds

normally internalized by phagotrophy (114). These apparently conflicting findings may be explained by the presence of a new type of small vacuoles in CCB-treated cells (96); such vacuoles are not found in untreated cells and they may represent pinocytic vacuoles formed in the oral region. Thus, inhibition of food vacuole formation may not necessarily imply starvation and concomitant changes in the structure of mitochondria and peroxisomes. The small digestive vacuoles, formed in the presence of CCB, may sediment with mitochondria and peroxisomes in cell fractionations.

Addition of 0.8% glycerol to the growth medium has a slight effect on the rate of cell proliferation without causing marked structural changes. However, in the early stationary phase mitochondria change into the dense whereas the peroxisomes appear as an extremely light type (Figure 12), although the nucleoli show advanced changes (compare Figures 12a and 4 c). Furthermore, glycogen particles are present which is not in agreement with the structure of peroxisomes; however, glycerol may enter the pyruvate to glucose pathway and be converted into glycogen without involvement of the glyoxylate bypass (9, 113, 76) which explains the apparent discrepancy in these experiments.

Heat synchronization of cell divisions in *Tetrahymena* (125, 148) involves a series of temporal elevation of the growth temperature to a sublethal level (34°C). This »multi-heat shock« treatment (148) of log. phase cultures initially causes an arrest of cell division but not of cell growth (148) in the asynchronous culture and at the end of the treatment the cells divide with a high degree of synchrony; this technique has been used extensively for studies of cell cycle events (82, 148). In synchronized cells, the mitochondria and peroxisomes are both of the dense type (92) in agreement with a decreased rate of oxygen consumption (48) and activity in glyconeogenesis (124, 71); the latter function is also indicated by the presence of glycogen particles in the cells (92). The nucleoli are somewhat enlarged (104) in accordance with a decreased rate of RNA synthesis (8, 148, 146); incidentally, gross nucleolar changes are seen when cultures of high cell density are subjected to the heat synchronization technique (35). When the »one heat shock per generation«

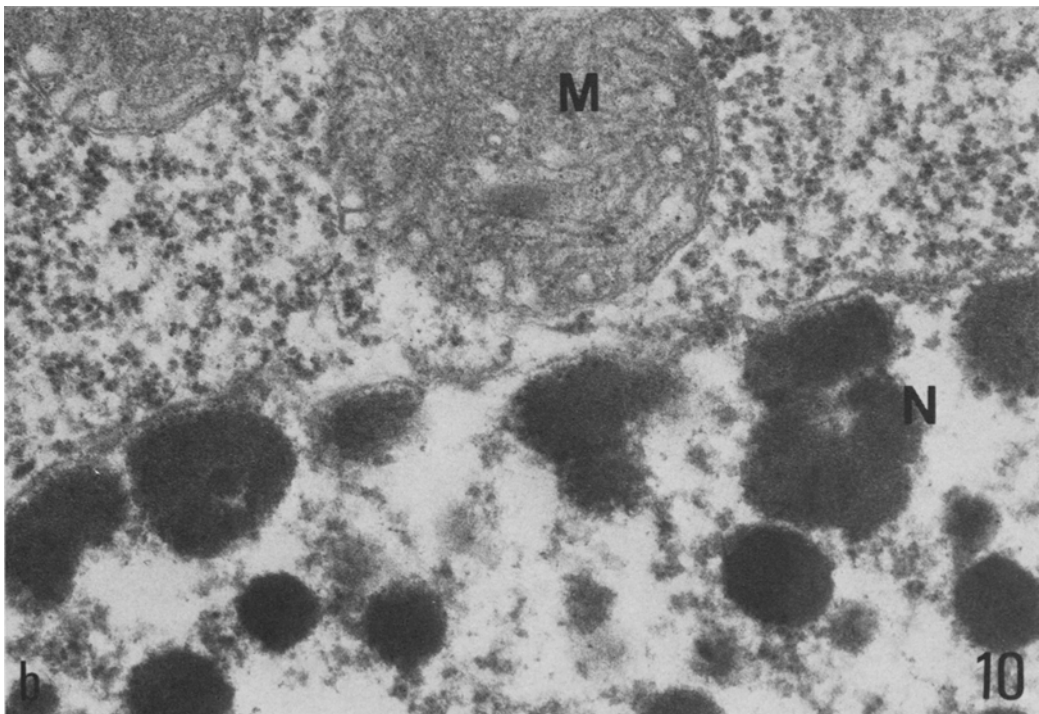
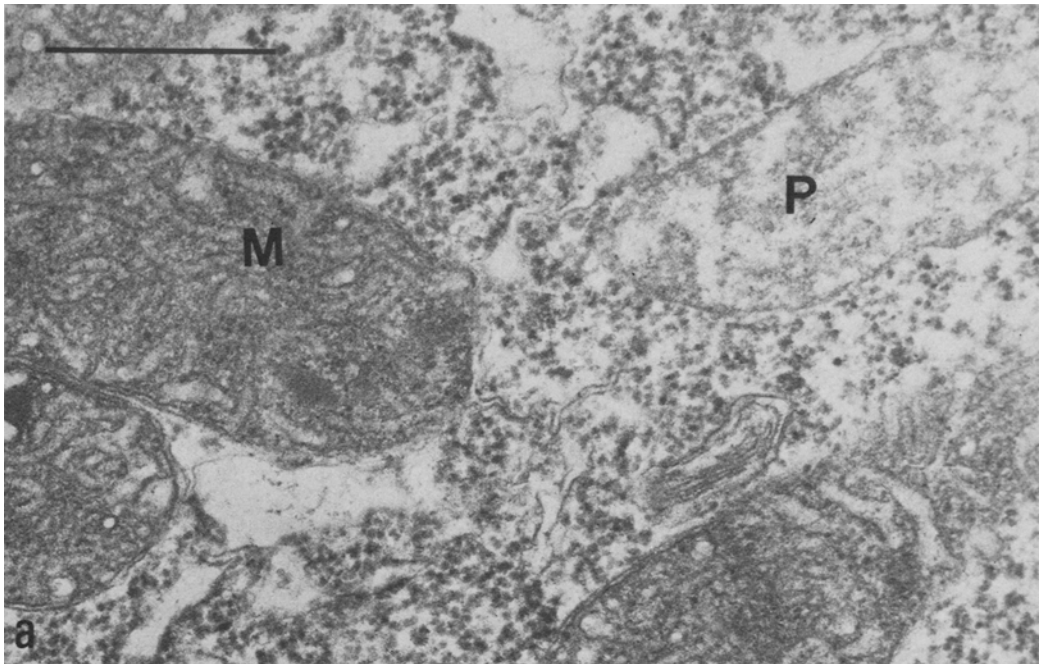


Figure 10a-c. The effect of a 1-h exposure to 0.5 mM-2,4-dinitrophenol of Tetrahymena from log. phase cultures.

The mitochondria are extremely dense with »contracted« regions (both figures) and the peroxisomes are an extremely light type (Figure a); the nucleoli are very dense and appear »frozen« (Figure b). For structural changes during recovery from the treatment see (92) from which these figures are reproduced. Mitochondria (M), peroxisome (P), nucleoli (N). (Bar = 0.5 μ m).

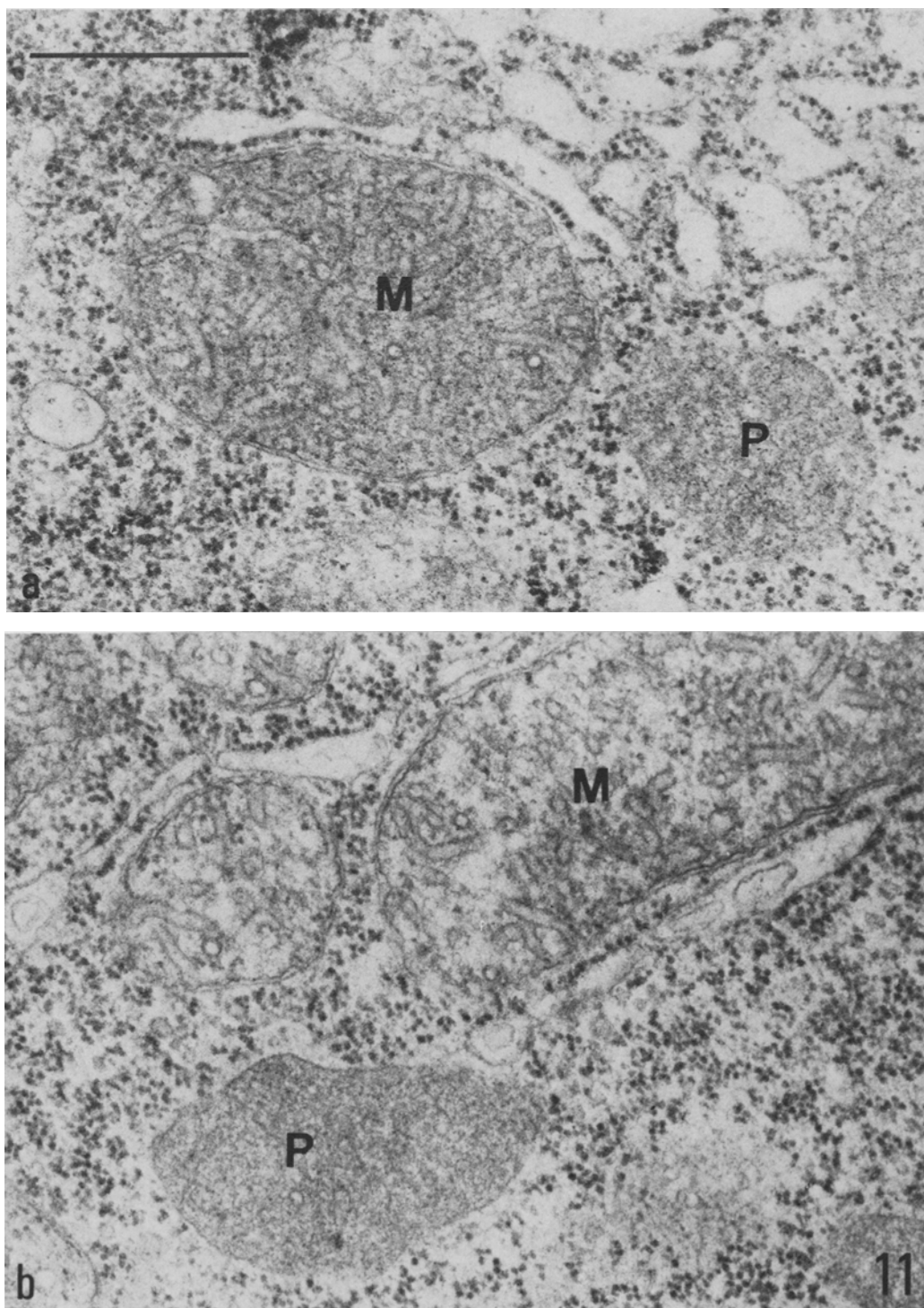


Figure 11a-b. Effect of a 1-h exposure to 7.5% dimethyl sulphoxide of *Tetrahymena* from log. phase cultures.

The mitochondria and peroxisomes have changed into the dense type (Figure a). Recovery from the treatment is slow and 3 h after removal of the reagent, the mitochondria have changed into the light type, whereas the peroxisomes are still of the dense type (Figure b). Mitochondria (M), peroxisomes (P). (Bar = 0.5 μ m).

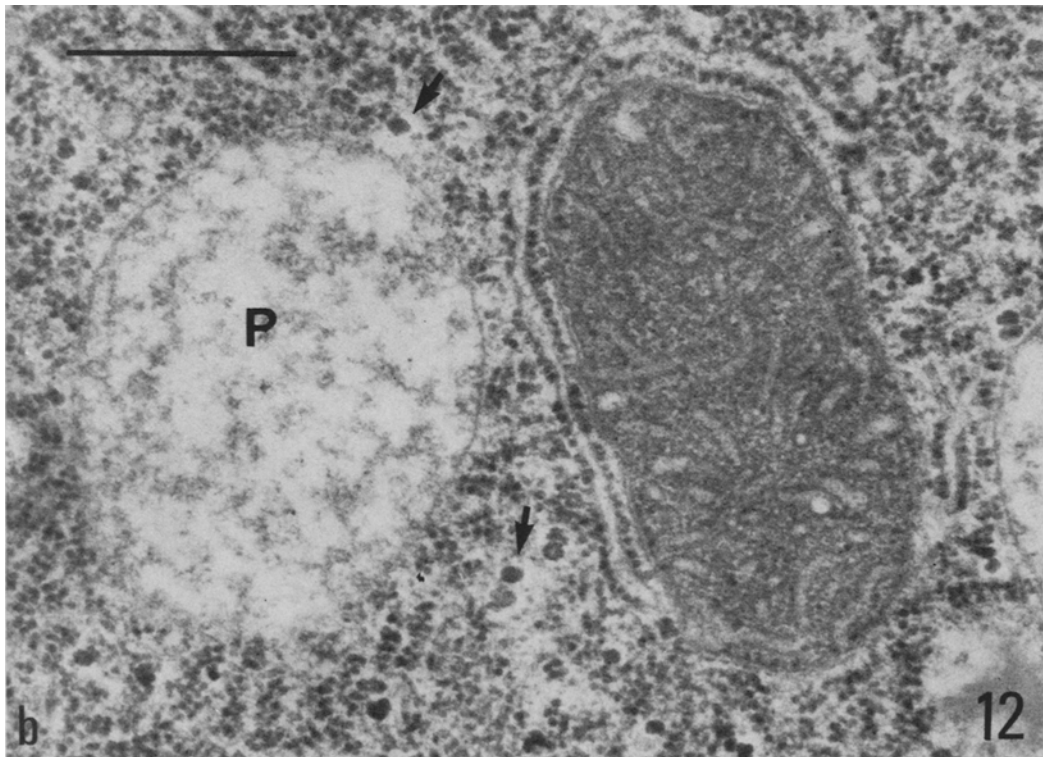
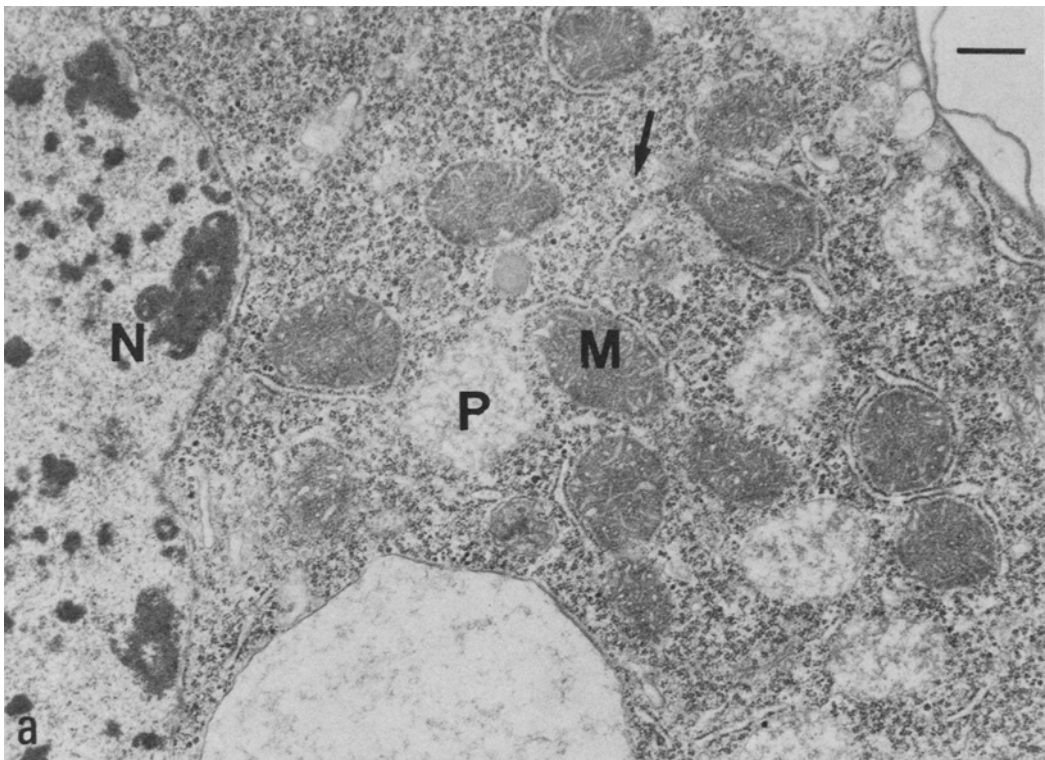


Figure 12a–b. Addition for 5 h of glycerol (0.8%) to the growth medium.

In the log. phase of growth little structural change is observed (slightly prolonged generation time); however, here in the early stationary phase of growth, as also indicated by the nucleolar changes (Figure a; compare with Figure 4c), the mitochondria are dense but the peroxisomes are extremely light in structure (Figure b) and glycogen particles (arrows) are present. Mitochondria (M), peroxisomes (P), nucleoli (N).

(Bar = 0.5 μ m).

method (147) is used to synchronize the cells, mitochondria and peroxisomes remain unchanged (106) and no nucleolar changes are observed (105). During heat synchronization the cells are exposed to the sublethal temperature for 20–30 min (148) spaced with intervals at the normal growth temperature; however, if the cells are exposed continuously to the high temperature different structural changes are observed (74, 92). Thus, after a 3-h treatment, the mitochondria of the dense type appear with enlarged tubuli (92) and the peroxisomes appear as an extremely light type (74, 92); in addition, autophagic vacuoles and large nucleolar fusion bodies are seen (74, 92). Macromolecular synthesis is largely inhibited in these cells (148).

The relationship between the structure of mitochondria and peroxisomes in Tetrahymena is summarized in Table III. In most instances the organelles appear as the corresponding type, either light or dense, in agreement with the rate of cellular respiration and activity in glyconeoge-

nesis as discussed in the individual cases. When this correlation is not seen, plausible explanations for finding the peroxisomes in the light type have been presented; however, in the case of the DNP-treated cells, and cells exposed to sublethal temperature, the situation seems more obscure. The extremely light peroxisomes may have an increased rate of oxidation, i.e. they take over as respiratory organelles (30), in order to protect the cells against oxygen toxicity during treatments which affect grossly the mitochondrial activity (92).

5. CONCLUDING REMARKS

An attempt has been made to illustrate that the physiological state of Tetrahymena is reflected in the overall fine structure of the cells. The present review elucidates the dynamic changes which the population of cell organelles undergoes during the different phases of the culture cycle and in response to various treatments of

Table III

Interrelationship of structural types of mitochondria and peroxisomes

Condition	Mitochondria	Peroxisomes	Reference
Logarithmic phase (control)	light	light	(89, 92)
Transition stage	dense	light	
Stationary phase	dense	dense	(92)
Starvation (1 h)	dense	dense	(89, 92)
0.5 mM-DNP (1 h)	extremely dense	extremely light	(92)
7.5% DMSO (1 h)	dense	dense	(92, 95)
40 μ g CCB/ml (1 h)	light	light	(92, 96)
0.8% glycerol (5 h) (early stationary phase)	dense	extremely light	
Heat synchronization 1.	dense	dense	(92)
Heat synchronization 2.	light	light	(106)
Sublethal temperature (3 h)	dense with enlarged tubuli	extremely light	(74, 92)

DNP = 2,4 dinitrophenol; DMSO = dimethyl sulphoxide; CCB = cytochalasin B; heat synchronization: 1. = »multi-heat shocks« (125) and 2. = »1 shock per generation« (147).

rapidly growing cells. Moreover, evidence has been presented for the proposal that the fine structure of mitochondria and peroxisomes reflects the energized state of the organelles. Indirect support from other investigators for this proposal is given by the observations that on exposure of Tetrahymena to phenol, which reduces the rate of oxygen consumption, the mitochondria change into a more dense form (126), and that the giant peroxisomes in chemostatically grown cells with a high rate of glyconeogenesis, are dense in structure (62). Although the overall impression may be that the observed fine structure of the cell organelles seems to reflect their functional state, much more research is needed on the subject before this relationship is fully understood.

ACKNOWLEDGEMENTS

My sincere thanks to Dr. MIKLOS MÜLLER for asking me to review this topic in the Symposium on Cell Organelles held during the VI International Congress on Protozoology in Warsaw, July 1981. Furthermore, I am most grateful to the Editors of Carlsberg Research Communications for publishing this review on the grounds that much of the basic work was carried out during the 10 years I worked at the Biological Institute of the Carlsberg Foundation. The continued support of the Carlsberg Foundation is gratefully acknowledged. Dr. CICILY CHAPMAN-ANDRESEN and Professor CHRISTIAN OVERGAARD NIELSEN are thanked for critical reading of the manuscript.

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