THE EFFECTS OF ENUCLEATION ON THE CYTOPLASMIC MEMBRANES OF *AMOEBA PROTEUS*

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ABSTRACT

The dependence of cytoplasmic membranes upon the nucleus was studied by examining enucleated amebae with the electron microscope at intervals up to 1 wk after enucleation. Amebae were cut into two approximately equal parts, and the fine structure of the enucleated portions was compared with that of the nucleated parts and starved whole cells which had been maintained under the same conditions. Golgi bodies were diminished in size 1 day after enucleation and were not detected in cells enucleated for more than 2 days. The endoplasmic reticulum of enucleated cells appeared to increase in amount and underwent changes in its morphology. The sparsely scattered short tubules of granular endoplasmic reticulum present in unmanipulated amebae from stock cultures were replaced in 1–3-day enucleates by long narrow cisternae. In 3–7-day enucleates, similar cisternae of granular endoplasmic reticulum encircled areas of cytoplasm partially or completely. It was estimated that in most cases hundreds of these areas encircled by two rough membranes were formed per enucleated cell. The number of ribosomes studding the surface of the endoplasmic reticulum decreased progressively with time after enucleation. In contrast, the membranes of nucleated parts and starved whole cells did not undergo these changes. The possible identification of membrane-encircled areas as cytolysomes and their mode of formation are considered. Implications of the observations regarding nuclear regulation of the form of the Golgi apparatus and the endoplasmic reticulum are discussed.

INTRODUCTION

In recent years, evidence has accumulated that chloroplasts and mitochondria are DNA-containing, self-reproducing organelles, and that they are independent of the cell nucleus to a considerable extent (10, 14, 15). There is little information, however, concerning the degree of independence of other membranous organelles, such as the Golgi apparatus and endoplasmic reticulum. Knowledge of the extent to which the form and activity of these membranes are dependent upon the nucleus is, therefore, fundamental to an understanding of the mechanisms of their production and growth.

Amoeba proteus is a favorable subject for experimental investigation of this question because it may be cut into nucleated and enucleated parts, and the latter will survive for 1–3 wk (23). The fate of the cytoplasmic organelles may then be followed by electron microscope examination of cells at different intervals after enucleation. In addition, some chemical properties of nucleated and enucleated ameba fragments have already been studied (2, 4) and may be correlated with observations of fine structure.

The results of the present study indicate that in enucleated cells the endoplasmic reticulum undergoes changes in its extent and morphology while the Golgi apparatus disappears within 2 days after enucleation.

MATERIALS AND METHODS

Cultures

Stock cultures of *Amoeba proteus* were maintained in Prescott's medium (23) with daily feedings of washed *Tetrahymena*. All cultures used in the experiments to be described did not receive food organisms for 2 days preceding the experiment. Cells from such a culture provide a norm for comparison with the experimental animals. They will be referred to as unmanipulated amebae.

Enucleation

Several hundred amebae were removed from a stock culture and were allowed to attach to an agar substrate in a Petri dish partially filled with medium. Cells were cut into two approximately equal parts with either a glass needle controlled with a micromanipulator or the sharp tip of a braking pipette. Enucleated portions were distinguished (and separated) from the nucleated parts by the tendency of the enculeates to round up, by their sluggish movements, and by their failure to attach firmly to the substrate. About 100 cells were cut and separated in 1 hr. Several hundred whole amebae which were to serve as controls were removed from the stock culture at the same time as cells were taken for cutting. These three groups of cells, i.e. nucleated parts, enucleated parts, and whole cells, were maintained separately in medium without food organisms at 21 °C for 1, 2, 3, 5, or 7 days. About 20–30 enucleates usually remained alive at the end of this time. Cells were not studied beyond 1 wk because few viable enucleates remained.

Processing for Electron Microscopy

The organisms were fixed for 30 min at room temperature in 1 ml of Karnovsky's glutaraldehyde-formaldehyde mixture at pH 7.3 in 0.1 M cacodylate buffer (13) contained within a 10 ml capillary bottom centrifuge tube. Amebae were then placed in distilled water for 16–24 hr. They were postfixed for 15 min in 1% OsO_4 buffered with 0.1 M cacodylate, and rinsed with distilled water for 10 min. Cells were collected by centrifugation between steps. Dilutions of the Karnovsky mixture, glutaraldehyde fixatives, or buffered osmium tetroxide were tried but were not used in this study because they resulted in apparent vesiculation of the endoplasmic reticulum and/or varying degrees of extraction of the cytoplasmic matrix. Overnight washing in distilled water was utilized because it rid the amebae of many crystals contained in vacuoles (20). If allowed to remain, these crystals were not penetrated by the embedding medium, and resulted in holes in the sections.

In order to prevent dispersion and loss of the small numbers of cells during subsequent processing, we embedded the organisms in a small cube of agar by methods described previously (9). The cube was then dehydrated in a graded series of ethanols, immersed in propylene oxide, and embedded in Araldite. Sections showing silver to pale gold interference colors were cut with a Porter-Blum MT-1 microtome equipped with a diamond knife. The sections were mounted on uncoated copper grids and stained with lead citrate (27). Micrographs were obtained with a Philips EM-200 electron microscope operated at 60 kv.

RESULTS

Summary of Terms Used to Designate Amebae

UNMANIPULATED: These are cells removed at random from a stock culture and fixed immediately. They were taken as a norm for comparison with experimental organisms.

EXPERIMENTAL: These organisms include nucleates, enucleates, and starved whole cells.

NUCLEATES AND ENUCLEATES: These are the nucleated and enucleated parts, respectively, of cut amebae maintained separately at 21°C without food organisms for 1–7 days before preparation for electron microscopy.

STARVED WHOLE CELLS: These are cells removed from the stock culture at the same time as cells were obtained for cutting. They were maintained for 1–7 days under the same conditions as nucleates and enucleates but were not subjected to cutting.

Unmanipulated Amebae

Amebae taken at random from the stock cultures display a cytoplasmic fine structure similar to that described previously (e.g., references 20 and 21). Endoplasmic reticulum is not abundant (Fig. 1). Sparse short tubules of the granular variety and clumps of agranular tubules and vesicles (Fig. 2) are scattered throughout the cytoplasm. Multiple small Golgi bodies (Figs. 2 and 8) consist of a stack of six to eight curved, centrally flattened cisternae and accompanying smooth vesicles. Occasionally, larger vacuoles located at the concave pole contain a filamentous material resembling the ameba surface coat (Fig. 8). Some agranular membranes have an appearance that resembles coated vesicles (25) owing to the presence of a small amount of associated dense material. Locations of such membranes include the following: the expanded ends of both Golgi cisternae (Fig. 8) and tubules of agranular endoplasmic reticulum (Fig. 2), and also smooth vesicles

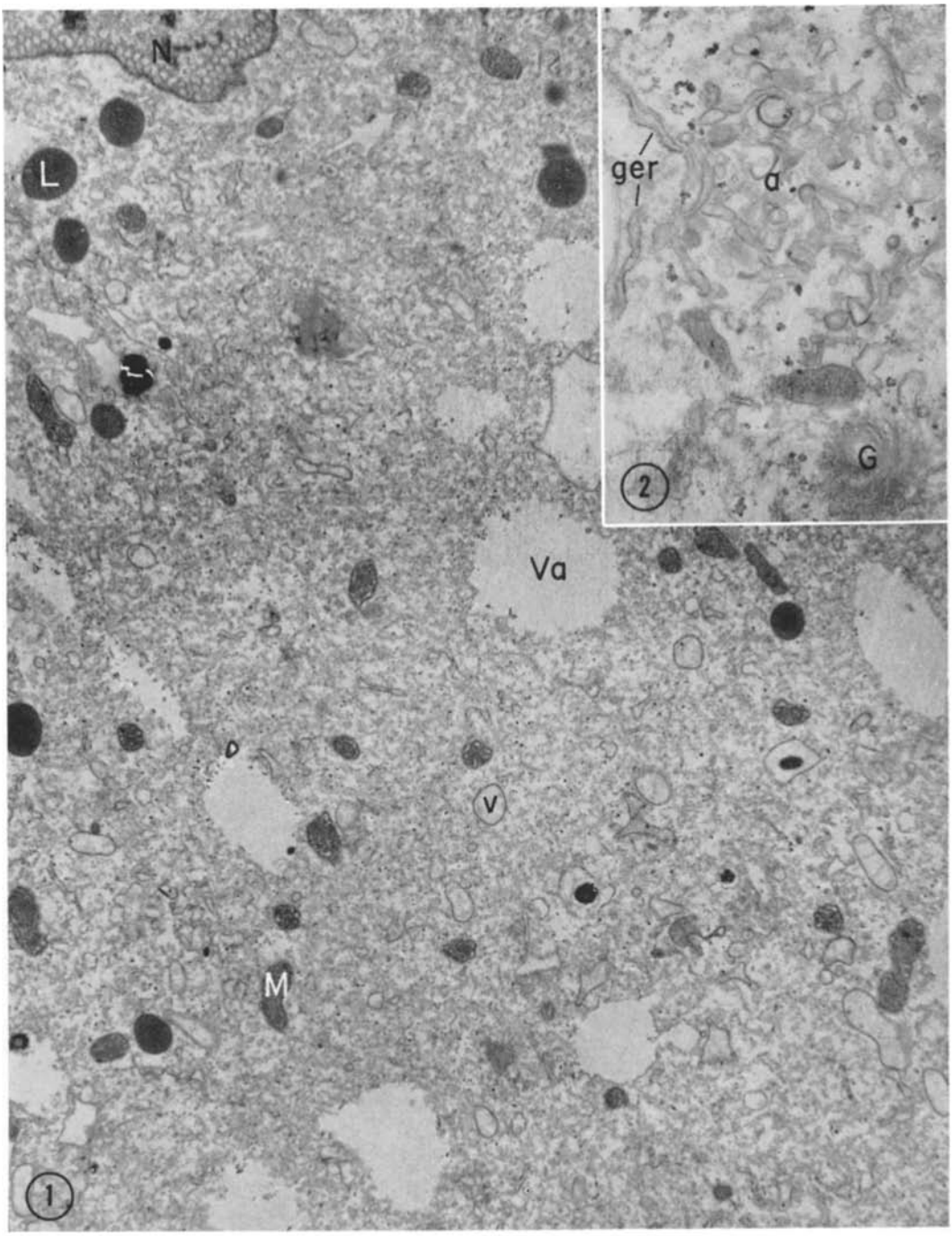


FIGURE 1 Low-power electron micrograph of a portion of an unmanipulated ameba from a stock culture. Mitochondria (*M*), homogeneous dense lipid droplets (*L*), vesicles possibly of pinocytotic origin (*V*), and larger vacuoles (*Va*) are scattered throughout a coarse granular cytoplasmic matrix. Elements of the endoplasmic reticulum are sparse. A small part of the nucleus (*N*) is visible. The large vacuoles resemble those that contain crystals (20) if prolonged washing in distilled water is omitted. × 6500.

FIGURE 2 Some of the membranous cytoplasmic organelles in an unmanipulated ameba. Several short tubules of granular endoplasmic reticulum (*ger*), a clump of agranular tubules and vesicles (*a*), and one of the multiple Golgi bodies (*G*) are present. Some of the agranular membranes appear more dense than others and resemble coated vesicles. × 20,000.

associated with Golgi bodies and clumps of agranular reticulum (Fig. 2).

Additional components of the cytoplasm (Figs. 1 and 2) are mitochondria with tubular cristae, irregular vesicles and vacuoles of varying size, homogeneous dense lipid droplets, food vacuoles, and short filaments. Some vacuoles contain the bacterium-like infective organisms characteristic of *A. proteus* (24) (Figs. 4, 7, and 16; enucleated cells). Variable numbers of the irregular 150–400-A dense granules found in many free-living amebae (21) are present in the coarsely granular cytoplasmic matrix. A filamentous surface coat is located external to the plasma membrane.

Experimental Organisms

The major fine structural features of cut amebae and starved whole cells will be surveyed and compared initially. A more detailed description of changes in the Golgi apparatus and endoplasmic reticulum of the enucleated cells will follow.

General Observations

1–2 days after cutting, the appearance of the nucleates and the starved whole cells is similar to that of unmanipulated cells, but in the enucleates the concentration of endoplasmic reticulum appears increased, and its morphology is altered (Figs. 3 and 12). In contrast to the scattered short tubules of the unmanipulated cells, the granular endoplasmic reticulum forms longer cisternae, and some areas of cytoplasm are partially or completely encircled by a cisterna. Groups of agranular membranes, now more vesicular than tubular, are both larger and more frequently encountered. By 3–7 days after enucleation, these changes in the endoplasmic reticulum are still more pronounced (Fig. 4). Many areas of cytoplasm are demarcated by two membranes studded with patches of ribosomes while much of the intervening cytoplasm is occupied by agranular vesicles. The portions of cytoplasm enclosed by membranes in this fashion morphologically resemble cytolysomes or autophagic vacuoles.

Golgi bodies are reduced in size in cells enucleated for 1 day and are only rarely encountered in 2-day enucleates. They have not been observed in cells enucleated for more than 2 days.

Comparable membrane changes do not take place in either the nucleated parts of cut cells or in starved whole organisms. The cytoplasmic membranes of these two groups closely resemble those of unmanipulated cells: that is, the granular endoplasmic reticulum consists of scattered short tubules, the agranular reticulum is represented by small clumps of tubules and vesicles, and Golgi bodies persist for at least 7 days (Fig. 5). The encirclement of portions of cytoplasm by cisternae of endoplasmic reticulum has not been observed in nucleates or starved whole amebae in this study, although the presence of cytolysomes has been reported in whole amebae following pinocytosis (11).

The number of lipid droplets diminishes progressively in nucleates and starved whole cells so that very few remain at the end of 7 days (Fig. 5), but large numbers persist in enucleates (Fig. 6). Lipid droplets and the membranous organelles are sometimes aggregated in the center of the enucleates and are separated from the cell surface by a rim of cytoplasm containing few membranes (Fig. 7). This concentration may accentuate the appearance of abundant lipid and membranes in enucleated cells if the field viewed includes only the center of the cell, but it is estimated that the absolute amount is greater in the enucleates than in nucleates or in starved whole amebae.

Black matrix granules and food vacuoles become progressively less numerous with time in all of the experimental cells. They are almost completely absent by 7 days (Fig. 5).

Golgi Apparatus

The Golgi bodies in unmanipulated cells (Fig. 8) contain six to eight cisternae which range in diameter from 1 to 2 μ and have an average extent of 1.2 μ. After 1 day of enucleation, the diameter of the Golgi cisternae is reduced to 0.4–0.6 μ (Fig. 10), roughly one-half that of the unmanipulated cells. In addition, the number of cisternae per Golgi body is diminished so that stacks of only two to four cisternae are common (Fig. 11). By 2 days after enucleation, Golgi bodies are rare, and no Golgi apparatus has been identified in several hundred micrographs of cells enucleated for more than 2 days. Golgi bodies in nucleates (Fig. 9) and in starved whole cells (Fig. 5) possess the usual six to eight cisternae, and they persist for at least 7 days.

Endoplasmic Reticulum

The granular endoplasmic reticulum of unmanipulated cells, as noted above, consists almost

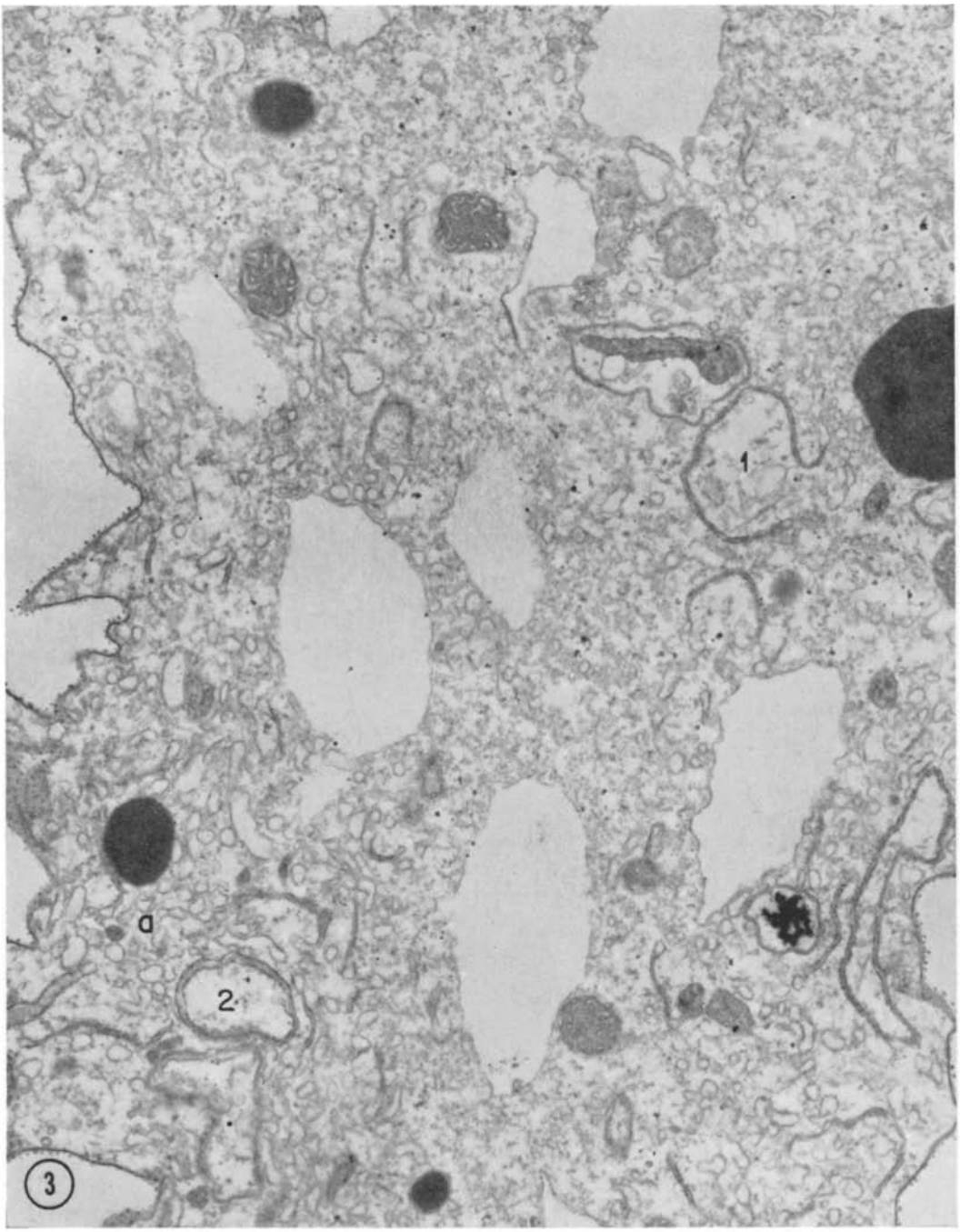


FIGURE 3 2 day enucleate. The concentration of endoplasmic reticulum appears greater than in unmanipulated cells, nucleates, or controls. Long cisternae of granular endoplasmic reticulum are present. Some cisternae partially (*1*) or completely (*2*) encircle areas of cytoplasm. Agranular vesicles (*a*) are moderately abundant. Some lipid droplets are present. × 12,500.

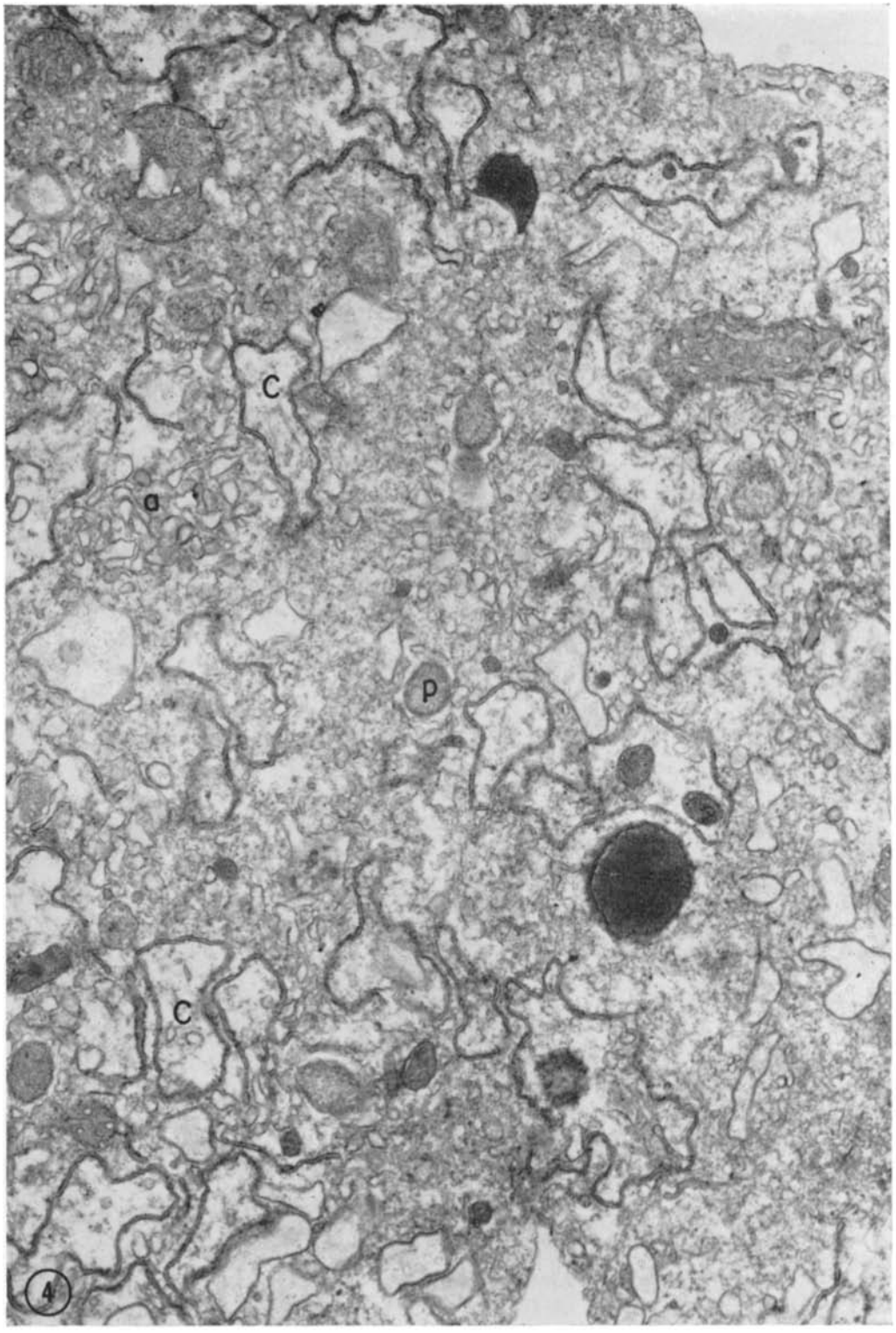
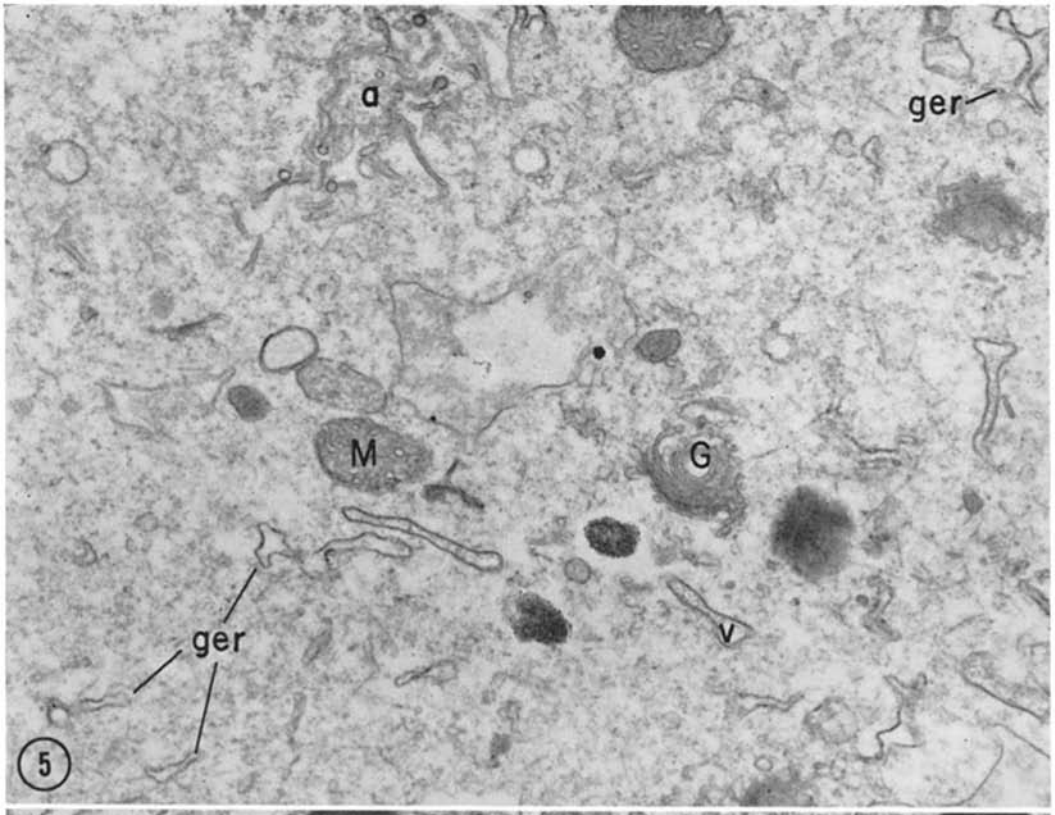


FIGURE 4 3 day enucleate. Many areas of cytoplasm are encircled by cisternae of granular endoplasmic reticulum (*C*). Agranular vesicles (*a*) occupy much of the intervening cytoplasm. Golgi bodies are absent. *p*, bacterium-like infective organism. × 16,000.



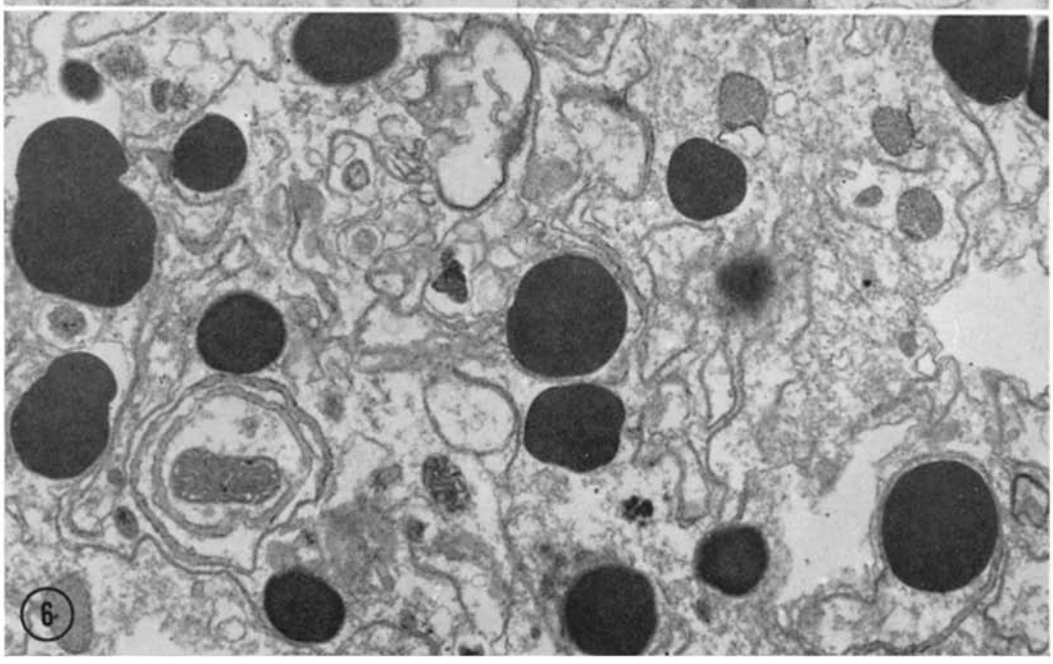


FIGURE 5 Whole ameba starved for 7 days. The cytoplasmic membranes are similar to those of unmanipulated amebae (Figs. 1 and 2) and have not undergone the changes observed in enucleated cells (Figs. 3, 4, and 6). The granular endoplasmic reticulum (*ger*) is represented by short tubules. Scattered clumps of agranular tubules and vesicles (*a*) are present, and Golgi bodies (*G*) persist. Lipid droplets are scarce (see Fig. 6) as are black matrix granules. *M*, mitochondria; *v*, vesicles possibly of pinocytotic origin. × 16,000.

FIGURE 6 7 day enucleate. Lipid droplets are present in large numbers (see Fig. 5, whole cell). × 10,000.

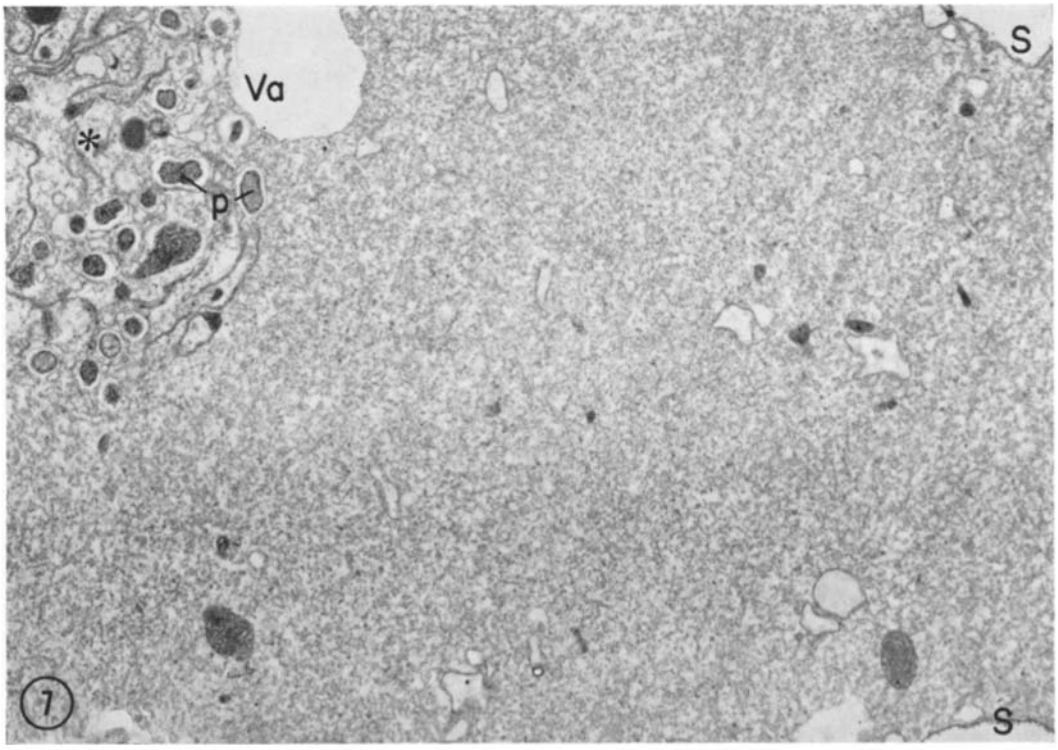


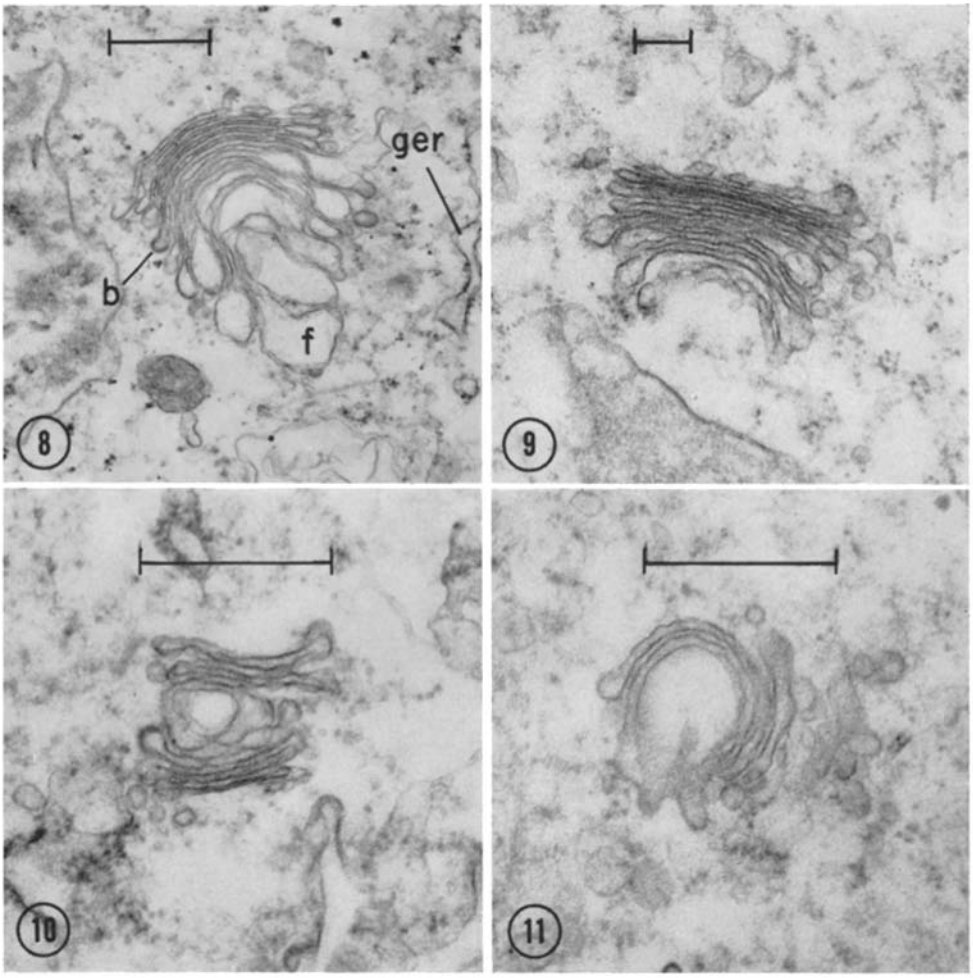
FIGURE 7 5 day enucleate. Lipid droplets and the bacterium-like parasites (*p*) are aggregated in the center of the enucleate (*), separated from the cell surface (*S*) by a rim of cytoplasm containing few membranous organelles. *Va*, vacuole. × 7000.

entirely of short tubules 500–2000 A wide and usually less than 1 μ long in any given section. Only rarely are cisternae up to 2–3 μ long encountered. In contrast, cells enucleated for only 1 day may contain extensive cisternae of granular endoplasmic reticulum that reach a length of 8–10 μ while pursuing a sinuous course through the cytoplasm (Fig. 12). The lumen of the granular endoplasmic reticulum is about 250 A wide, and its width is less variable than that of the unmanipulated cells. From 1 to 3 days after enucleation, many similar cisternae of granular endoplasmic reticulum partially encircle portions of cytoplasm 1–2 μ in diameter (Fig. 13). By 3–7 days after enucleation, many areas are completely encircled within the plane of section (Fig. 14). It is possible, however, that there is an opening to the general cytoplasm in another plane and that these structures are in reality cup-shaped rather than complete spheres.

15–20 encircled areas may often be counted within a single low-power field (Fig. 4). The total number in a single enucleated cell appears, therefore, to reach the hundreds. Most of these areas contain well-preserved cytoplasmic components such as mitochondria (Fig. 14), but a few, particularly in the older enucleates, contain material that appears to be degenerating (Fig. 15).

Some 1- to 3-day enucleates contain end-to-end alignments of tubules and short cisternae about 500–2000 A wide (Fig. 16). These configurations suggest a possible intermediate stage in the formation of the long cisternae from short tubules.

As the time after enucleation increases, the number of ribosomes studding the granular endoplasmic reticulum progressively decreases. The long cisternae of the 1–2-day enucleates possess ribosomes along most of their length (Fig. 12). The cisternae in 3–5-day enucleates, however, have smooth stretches of membrane up to several microns long interspersed with patches of ribosomes (Fig. 13). By 7 days of enucleation, the membranes are predominately smooth, but scattered groups of ribosomes can still be detected



FIGS. 8–11 Illustrations of changes in the Golgi apparatus in enucleates.

FIGS. 10 and 11 are twice the magnification of Fig. 8. The bar represents 0.5 μ.

FIGURE 8 Unmanipulated ameba. The Golgi apparatus is composed of a stack of six to eight centrally flattened cisternae, a few smooth vesicles, and vacuoles (*f*) containing filamentous material at the concave pole. Some membranes at the expanded ends of the cisternae appear coated (*b*). The cisternae are slightly more than 1 μ in diameter. *ger*, granular endoplasmic reticulum. × 24,000.

FIGURE 9 1 day nucleate. Morphology of the Golgi apparatus is similar to that of unmanipulated cells, except that large vacuoles are absent from the concave pole. Diameter of the cisternae is in excess of 2 μ. × 14,000.

FIGURE 10 1 day enucleate. The Golgi apparatus is smaller than that of unmanipulated cells, nucleates, or starved whole cells. The cisternae are about 0.5 μ in diameter. × 48,000.

FIGURE 11 1 day enucleate. A Golgi apparatus is composed of only two or three cisternae less than 0.5 μ in diameter. × 48,000.

(Fig. 14). Ribosomes which are free in the coarsely textured cytoplasmic matrix are difficult to distinguish from the multitude of other granules of varying sizes, but in general they also appear to be less numerous in older enucleates.

The lumenal content of the endoplasmic reticulum of unmanipulated cells is very finely granular and has a low electron opacity. The granular endoplasmic reticulum in enucleates of 2–3 days or more, however, contains a moderately dense material that, in favorable sections, displays a striated or rodlike organization (Figs. 14 and 17). Parallel striations are seen in tangential sections through cisternae (Fig. 17). They frequently appear to be arranged in pairs, and some indication of a linear periodicity is present. Cross-sections through cisternae (Fig. 14) often demostrate long lumenal rods. In some instances, the rods appear to have a lighter core. This unusual content has been detected in short cisternae (Fig. 3) as well as in those that partially (Fig. 13) or completely (Fig. 14) encircle areas of cytoplasm.

DISCUSSION

In a preliminary account of the fine structure of amebae enucleated for 5 days (3), a breakdown of vesicles and mitochondrial swelling was noted. These alterations have not been detected in the present study, but changes have been described in the endoplasmic reticulum and the Golgi apparatus of enucleated amebae. It is possible that differences in preparatory techniques may account for this discrepancy.

The cytoplasmic changes in enucleated cells do not appear to be merely the result of starvation, because comparable changes were not seen in starved whole amebae maintained under identical conditions. In addition, these alterations have not been observed in the nucleated parts of cut cells as would be expected if they were the result of injury incurred during the cutting process. It seems reasonable to assume, therefore, that they are a consequence of the removal of the nucleus. It is not known, however, whether the results are due to the loss of a nuclear contribution that directly affects the endoplasmic reticulum and the Golgi apparatus or the changes follow nuclear removal more indirectly; for example, as a result of some general metabolic alteration in the enucleate cytoplasm.

Golgi Apparatus

A Golgi apparatus has not been observed in cells enucleated for more than 2 days, but Golgi bodies persist in nucleates and controls for at least 7 days. The Golgi apparatus appears, then, to depend in some way upon the nucleus. The nucleus may be required to maintain the Golgi membranes in their characteristic form. Alternatively, if individual Golgi bodies have a life-span limited to a few days, participation of the nucleus may be needed for the production of new Golgi bodies. A similar dependence of *Acetabularia* dictyosomes upon the nucleus has been reported by Werz (28). Dictyosomes appeared to be destroyed in algae that were enucleated for 7–21 days and had ceased to grow, as well as in cells treated with actinomycin for 12 days.

The disappearance of Golgi bodies in amebae is preceded by a diminution in both the size of Golgi cisternae and the number of cisternae per Golgi body, but no information has been obtained regarding the fate of the Golgi membranes. These membranes may be dismantled or they may be transferred to other sites such as the endoplasmic reticulum. The addition of membranes derived from the Golgi apparatus would help to account for some of the apparent increase in the extent of the endoplasmic reticulum, but no direct evidence has been obtained to support such an exchange.

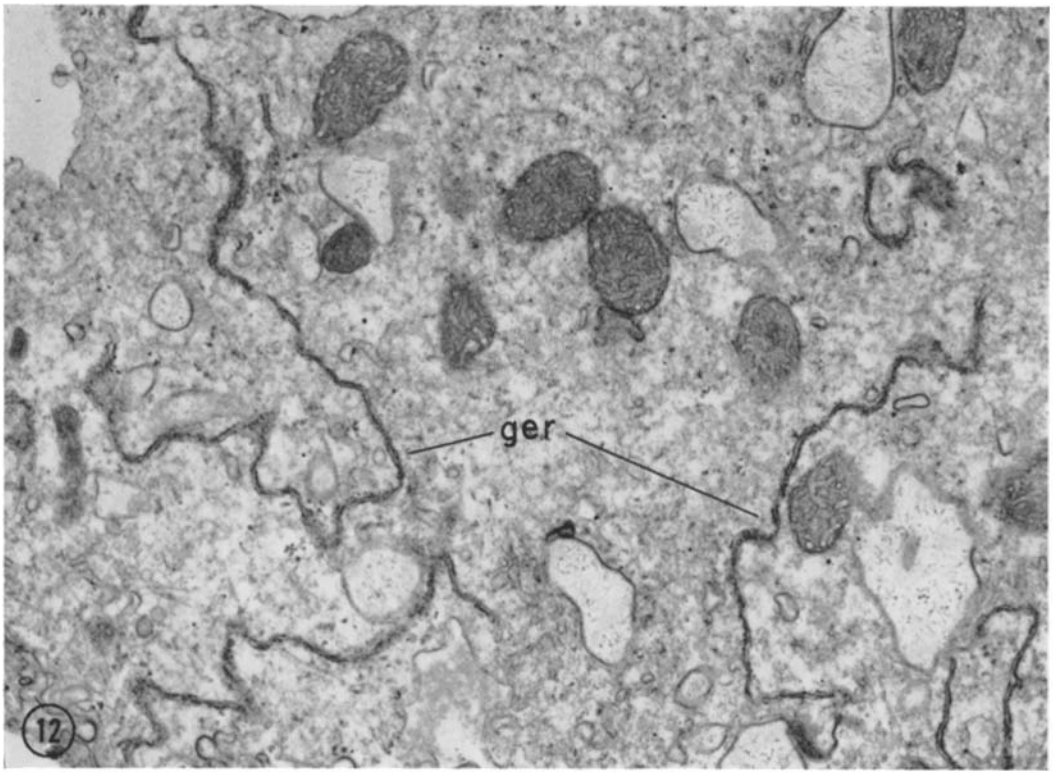
Endoplasmic Reticulum

The endoplasmic reticulum undergoes complex changes in the absence of the nucleus. The sparsely scattered short tubules of granular endoplasmic reticulum are replaced in early enucleates by long cisternae with a narrower lumen. It appears that these cisternae, or cistenae with a similar morphology, progressively encircle islands of cytoplasm. The following observations support this inference from static micrographs. First, in a series of enucleates of advancing ages, there is a trend in the configuration of the granular endoplasmic reticulum from long cisternae to incomplete circular cisternae and, finally, to complete circular cisternae. Second, ribosomes are identified on the surface of all three configurations, but their number decreases progressively from long cisternae through incomplete circles to full circles. Third, the content and size of the cisternal lumen is similar in all three forms.

The cisternae of granular endoplasmic reticulum progressively become largely agranular. This may be due to the release of ribosomes, the synthesis of new smooth membrane, or a decrease in the total number of ribosomes as their life-spans expire in an enucleated cell that is incapable of replacing them. The latter explanation is consistent with cytochemical studies (2) which have shown that the RNA content of the enucleate ameba cytoplasm is decreased by 60% after 10 days of enucleation. Since some ribosomes remain in 7-day enucleates (Fig. 17), however, the life-span of individual ribosomes in amebae must exceed 1 wk.

The means of formation of the long cisternae of granular endoplasmic reticulum is unclear. Some micrographs (Fig. 16) suggest that short tubules may coalesce end-to-end to form 500–2000 A wide cisternae. These cisternae may subsequently become flattened to a width of about 250 A. It appears unlikely, however, that the small amount of endoplasmic reticulum in the normal unmanipulated ameba is sufficient to account for the formation of the extensive reticulum of the enucleates solely by this means. Some membrane might be derived from other cellular membranes such as the Golgi apparatus, as noted above. At least the nuclear envelope can be excluded as a source of granular endoplasmic reticulum in this instance, although, in general, the two are in frequent continuity and have been regarded as specializations of the same basic organelle (22). Finally, enucleates are able to synthesize protein (16), and may, then, be capable of synthesizing new membranes, but this remains to be demonstrated.

The nucleus, then, seems to be important in maintaining the endoplasmic reticulum in its usual form in normal amebae, but how the changes in the enucleate are determined is com-



FIGS. 12–14 The apparent sequence of changes in the granular endoplasmic reticulum of enucleated cells.

FIGURE 12 1 day enucleate. The granular endoplasmic reticulum (*ger*) is composed of long cisternae of relatively constant 250 A width. × 16,000.

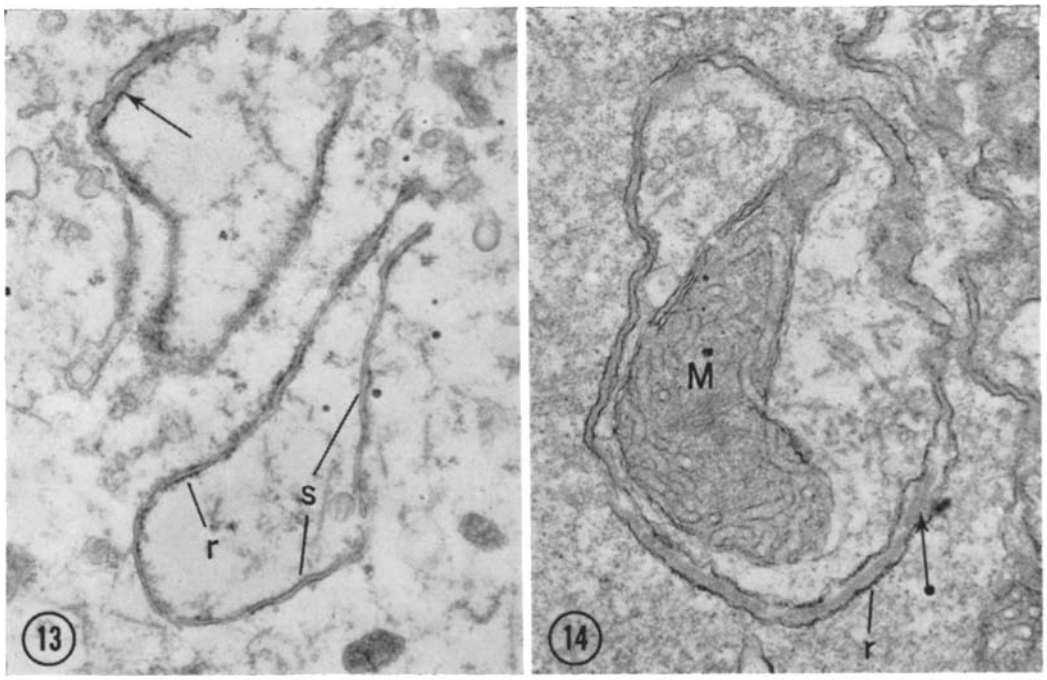


FIGURE 13 3 day enucleate. Cisternae of endoplasmic reticulum partially encircle areas of cytoplasm. Stretches of smooth membrane (*s*) are interspersed with patches of ribosomes (*r*). In some places the lumen contains a moderately dense, rodlike material (arrow). × 24,000.

FIGURE 14 7 day enucleate. A portion of cytoplasm is completely encircled, within the plane of section, by a cisterna of endoplasmic reticulum. The membranes are smooth for most of their extent, but patches of ribosomes are still present (*r*). The encircled area contains a fairly well preserved mitochondrion (*M*). Some rodlike material is present in the lumen of the cisterna (arrow). × 25,000.

pletely unknown. The possibility of a long-lived messenger derived from the nucleus seems remote. Alternatively, the tendency to undergo these changes might be a property of the cytoplasm itself, manifest when released from nuclear control.

The Nature of the Membrane-Encircled Areas of Cytoplasm

Membrane-encircled areas of cytoplasm containing various cytoplasmic constituents have been referred to as cytolysomes, cytosegresomes, or autophagic vacuoles. In many instances, associated acid phosphatase activity has been demonstrated cytochemically. (See review by de Duve and Wattiaux, reference 6.) The formation of cytolysomes in enucleated amebae might be expected in view of the chemical studies of Brachet (2, 4) who compared nucleated and enucleated ameba fragments with respect to the utilization of carbohydrate, lipid, and protein. Carbohydrate and lipid content declined in nucleated cells and was nearly exhausted after 1 wk of starvation, whereas protein remained nearly constant. In contrast, the behavior of enucleates was similar for about 3 days, but they then stopped using carbohydrate and lipid and began to catabolize protein. At the end of 1 week, the enucleates still contained much lipid and carbohydrate. Brachet (4) stated that the enucleated part "when it becomes unable to utilize its normal reserve stores, is the site of a kind of 'autophagy'." This information correlates with the following: (*a*) the formation of cytolysome-like structures that reach large numbers about 3 days after enucleation; (*b*) the continued abundance of lipid droplets in enucleates; and (*c*) the absence of membrane changes and the diminution of lipid in nucleates and starved controls.

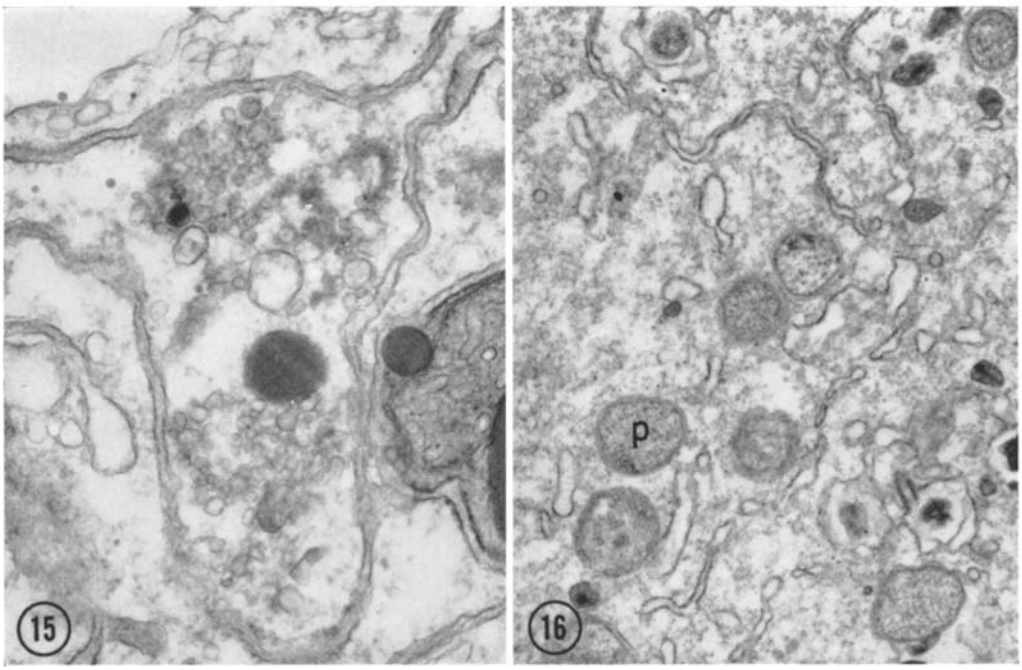


FIGURE 15 7 day enucleate. The contents of a membrane-encircled area appear to be degenerating. × 38,000.

FIGURE 16 Several tubules and short cisternae of granular endoplasmic reticulum are aligned end-to-end. *p*, bacterium-like parasitic organism. 3 day enucleate. × 11,000.

Interpretation of the nature of the membrane-encircled areas is complicated, however, by the additional findings of Brachet (2, 4) that, by 10 days after cutting, the acid phosphatase and esterase activities of the enucleates have fallen to one-fifth that of nucleated cells. One possible explanation that would account for both the chemical and ultrastructural observations is as follows. Perhaps the enucleated cells begin the formation of cytolysomes by the segregation of portions of cytoplasm within membranes but then are incapable of producing the enzymes necessary for the normal digestion of the contents. That is, it appears that the membrane changes can proceed in the absence of the nucleus, but transcription may be required for the production of the hydrolases. This conjecture is consistent with the observation that, although hundreds of segregated areas are formed per enucleate, the contents appear to be degenerating in only a few, even after 1 wk when the number of enucleates surviving has declined to less than 20–30% of those originally cut. In addition, it has been proposed (19) that cytolysomes bounded by two membranes are early stages in the formation of the more typical single-membrane–bounded variety from endoplasmic reticulum, and that one of the membranes subsequently degenerates. Cytolysomes limited by two membranes have in fact been observed in several studies (7, 8, 12, 19, 26), and it has been noted (8) that their contents often appear to be better preserved than those of the single-membrane–bounded type. In some instances, the inner of the two membranes was discontinuous, suggesting a stage in its disintegration. The absence of stages in the degeneration of one of the membranes in the present study might be a further indication of an "arrest" in the development of cytolysomes.

The membrane-encircled areas have, then, some morphological characteristics of cytolysomes, but the problem of their precise identification is not settled. Electron microscope cytochemical studies may help to resolve the question. In any event, the bounding membranes appear to be derived from the granular endoplasmic reticulum. Previous studies have led to a variety of opinions concerning the origin of the limiting membranes

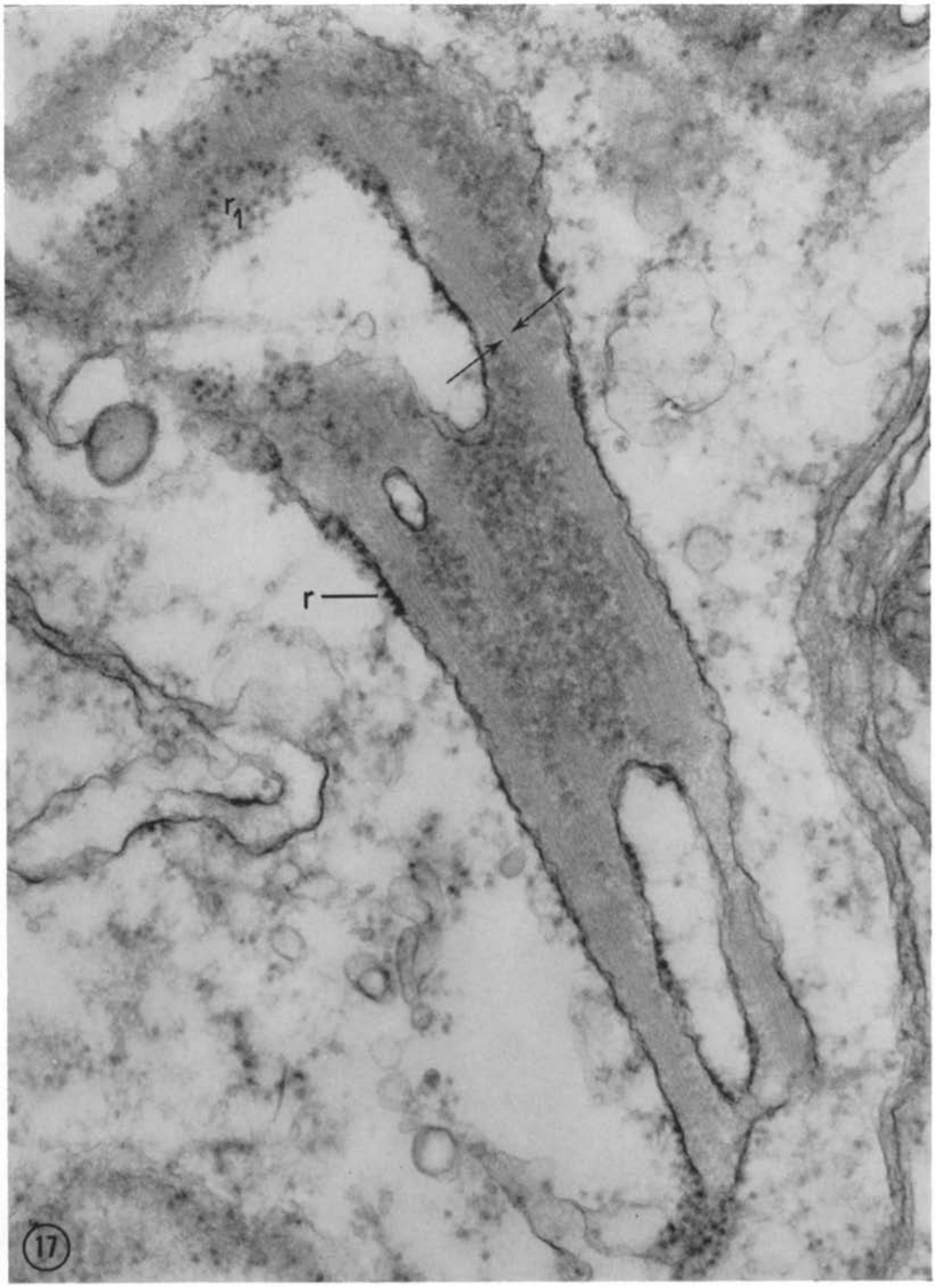


FIGURE 17 A tangential section through a cisterna of endoplasmic reticulum in a 7 day enucleate. Note the extent of the striated lumenal material and the ribosomes (r) on the membrane surface. The spirally arranged ribosomes (r_1) may represent polysomes. The lumenal striations frequently appear paired (arrows), suggesting that the material may be organized into rods or tubules. × 74,000.

of cytolysomes. Although some authors (1, 18) have speculated that the membrane is formed *de novo*, most investigators have maintained that it is derived from a preexisting cellular membrane. Interpretations have differed, however, concerning the specific membranous organelle involved; the endoplasmic reticulum (19), Golgi apparatus (5), lysosomes (17), and pinocytotic vesicles (6) have all been implicated. Most authors have suggested that segregation occurs by fusion of a series of sacs around a portion of cytoplasm or by invagination of a bud of cytoplasm into a single vesicle. In the present study, the membrane-enclosed areas do appear to be formed from the endoplasmic reticulum by a process of encirclement, possible fusion, and flattening similar to that suggested by Novikoff and Shin (19) for the formation of rat liver cytolysomes. In the ameba, however, the granular endoplasmic reticulum appears to be involved rather than the agranular variety as in hepatic cells.

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