ELECTRON MICROSCOPIC OBSERVATIONS OF AMOEBA PROTEUS IN GROWTH AND INANITION*

By ADOLPH I. COHEN, Ph.D.

(From the Department of Anatomy, Washington University School of Medicine, St. Louis)

Plates 276 to 280

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The common mononucleate ameba, *A. proteus*, has recently regained prominence as a research tool for studying nucleocytoplasmic relationships and for physiological studies pertaining to individual cell histories as opposed to population phenomena. These activities follow from recent technological advances permitting enucleation, nuclear transplantation, and physical and chemical measurements on single cells (1–3). On the other hand, despite an extensive literature on the biology of the ameba, relatively little is known of its biochemical specializations and cytological organization. For this reason a coordinated biochemical and cytological investigation has been undertaken. The biochemical findings have in part been published (4–6) and more recent findings are being prepared for publication. This report is devoted to cytological observations of amebae during growth and starvation, employing electron microscopy.

Previous electron microscopic studies on amebae have mainly been devoted to observations on the nuclear membrane of the interphase ameba (7–10), a brief note on intranuclear structure (11), and some studies on the structure of cell membranes (17). The present report further develops these observations on the normal interphase cell, and extends them to include phenomena relating to cell division and inanition.

METHODS

Amoeba proteus of the Chicago Biological Supply House strain were maintained in nonsterile cultures with *Tetrahymena gelii* as the sole food source supplied. The cultures were made without agar or rice grains, and the culture medium lacked carbon and nitrogenous components. The methods of culturing have been described elsewhere (6).

Cells in prophase were obtained by recognizing their characteristic rounded configuration with short, blunt pseudopodia, in a background of moderately fed amebae which are elongate and actively motile.

Starved cells were obtained from populations initiated from heavily fed and actively expanding (counted) populations and were maintained in carbon and nitrogen free medium with daily changes to fresh medium.

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All cells were cultured and maintained at 18°C.

Cells were transferred with a braking pipette to Dalton's chrome-osmic fixative (12) at a pH of 7.8. All conditions of fixation were rigorously standardized. It was recognized, however, that the quality of fixation of starved cells may differ from that carefully established for actively growing cells. The transfer to the fixative was made under microscopic observation, and the cells quickly stirred to prevent local dilution of fixative by the medium about the cells. Failure to stir resulted in blebs forming on the surface of the animals. After 5 minutes the cells were transferred to a small chamber cut in 2 per cent washed agar and additional warm (38°) agar employed to seal the chamber. A block of agar containing the cells was then removed and transferred to 30 per cent alcohol. The block was then dehydrated and embedded in a mixture of butyl and methyl methacrylates. After polymerization, thick sections for phase microscopy or thin sections for electron microscopy were cut on a Servall Porter-Blum microtome. Sections were examined in an EMU 2-E RCA electron microscope.

RESULTS

A. General Observations

1. Outer Membrane.—The outer membrane, as seen at the highest range of magnifications employed, is a thin, single layer of about 70 A width with fine filamentous fringes (about 350 A in length) on both of its surfaces (Fig. 9). Occasionally, villous extensions of the membrane of about 2750 x 275 A are evident (Fig. 2). Against the inner surface of this membrane, the granular cytoplasm may occur, or be separated from it by an optically empty space of highly variable width.

2. Mitochondria.—These organelles were identified by their structural similarity to mitochondria of higher forms and by their location. The contractile vacuole is known to be surrounded by a constellation of organelles capable of combining with Janus green B. The organelles designated herein as mitochondria similarly may be disposed around a particular vacuole. The presumptive mitochondria are, as judged from their appearance in cross-sections, spherical to ellipsoid bodies, about 2.5 by 1.5 micra. They have a wall consisting of a double membrane of about 110 A in thickness, the inner component of which penetrates the mitochondrial "core" as villi. The mitochondrial "core" is optically empty in well nourished animals. (Figs. 1, 2, and 8).

3. Golgi.—Accumulations of flattened vesicles or membranes of the type designated as Golgi in higher forms are found in amebae (Fig. 1).

4. Alpha Particles.—This organelle is classically described (13) as being somewhat smaller than mitochondria, not taking up Janus green B, and otherwise without distinguishing features. This probably corresponds to a dense, spherical to ellipsoid particle, slightly smaller than the mitochondria, which is found within a vacuole. In thin sections of starved animals (Fig. 10), some internal structure of the particle can be discerned.

5. Crystals.—The amebic crystals survive osmic fixation and subsequent dehydration and imbedding. They are visible in the phase microscope in thick sections but fall out of thin sections, leaving spaces, the cross-sections of which are irregular polygons.

6. Foamy Particles.—In both osmic- and formaldehyde-fixed amebae there occur, both in the nucleus and the cytoplasm, electron-dense particles which in section have a spongy structure. The smallest of these particles are notably concentrated in the nucleoli. In the cytoplasm the particles vary greatly in size and are found in or surrounding certain cytoplasmic vacuoles. They have been seen everywhere in the cell, but never outside. Their external dimensions are irregular. They surround crystal spaces and may be in them. Their appearance suggests that they may have been altered structurally by heating under the electron beam. (Figs. 3 and 4).

7. Vacuoles.—Vacuoles possess a membrane resembling the cell membrane except for a minimum of fringe material. The contractile vacuole possesses no distinguishing features except for the extraordinary numbers of surrounding mitochondria.

8. Other Cytoplasmic Entities.—In well fixed material there are found in the cytoplasm small particles and fine vesicles which may constitute what have been termed microsomes in cell fractionation studies. No dense particles organized on membranes have been observed. Oil droplets are numerous in well fed animals, as are food vacuoles, mostly containing easily recognizable remnants of *Tetrahymena* and rarely, bacteria. There are also numerous empty appearing vacuoles with no apparent associations.

9. Nucleus.—The interphase nuclear membrane, as previously defined and described by others, is a two component structure consisting of an inner, thick layer, and a thin, outer layer. The thin layer has a double membrane structure. Along the inside of the nuclear membrane are presumptive nucleoli which contain small, foamy particles imbedded in their matrix, which otherwise, in sections, has a granular background. The smallest foamy particles are of the order of 140 A and the background granulations, which may represent sectioned threads, are of the order of 90 A. Pappas (11) has described helical nucleoplasmic components in the central area. While we have observed threads of the same dimension (70 to 80 A), only occasionally have we seen a suggestion of coiling. The presumptive identification of the submembrane entities as nucleolar rather than chromosomal, is based on the impression that their total mass greatly exceeds that of the objects of the metaphase plate and that material clearly accumulating at the site of the metaphase plate can be identified before there is any obvious decrease in the submembranal masses (Fig. 5).

B. Nuclear Division

Nuclear division and accompanyine chromosomal movements have been described by Dawson *et al.* (16), based on light microscopy, and these authors discuss the older literature on the subject. Our electron microscopic observations of numerous dividing animals have resulted in the following findings. In early prophase the nucleolar material moves away from the nuclear membrane. Since, subsequently, one finds only a few nucleolar masses and these of low

density, it is suggested that there is a fusion of nucleoli and a loss of nucleolar substance. At the time of nucleolar migration evaginations of the nuclear membrane occur. These appear to pinch off in masses about the dimensions of mitochondria and, indeed, bearing some resemblance to these organelles (Figs. 5 and 6). In metaphase, one observes that the inner, thick membrane component has vanished completely while the outer, thin membrane is still complete and that the chromosomal material is aligned in a typical metaphase plate (Figs. 7 and 8). Intermediate stages have been observed with the inner nuclear membrane markedly reduced in thickness. We have also observed variability in the thickness of the inner nuclear membrane, relative to the outer, in random growing cells. Thus, a stage believed to be an early prophase showed a size ratio of 18:1 between the inner and outer membrane thicknesses. The same ratio measured on random interphase cells was of the order of 7:1 to 12:1. While the obliquity with which the section cuts the membrane can influence this ratio, we believe the variance to be real and worthy of detailed study in reference to the growth cycle. Anaphase phenomena have proven rather difficult to analyze. Our preliminary observations suggest that the outer nuclear membrane shatters during chromosomal separation and that, through extensive gaps, the nucleoplasm is for an interval in contact with the cytoplasm.

C. Starvation

Studies on starvation are somewhat handicapped by the variance in stored food in cells at the start of the experiment. On the other hand, work to be reported elsewhere showed that cell size did not influence cell survival time. It should also be recognized that materials may accumulate in starving cells which extract during fixation and dehydration. An example of this might be free amino acids. The first observation that can be unequivocally made is that after 2 weeks at 18°C. most of the round dense droplets, presumably oil droplets have vanished. The second unequivocal observation is that some *Tetrahymena* material either has not been digested, or that some digestive residua has not been expelled. These residua remain at least as long as 3 weeks and probably to the point of cell autolysis. The third definite observation is that the mitochondrial "core," originally optically empty, is now notably dense, suggesting lipide unmasking or, less likely, lipide synthesis (Fig. 10).

DISCUSSION

A recent monograph by Andresen (3) on the cytology of the giant ameba *Chaos chaos* supplies many references to *A. proteus*, and the electron microscopic observations herein may be compared with this excellent summary of light microscopy. The classical description of ameba cytoplasm as consisting of an ectoplasmic and endoplasmic component, is only supported in our electron microscopic observations by the existence of an optically empty space between

the cell membrane and the granular cytoplasm. This space, which increases in size when the fixative is not rapidly applied, may nevertheless represent a true structure. Even the increase in thickness may reflect a variety of physiological responses. In many sections, however, the granular cytoplasm abuts directly on the plasma membrane. The hair-like fringes on the plasma membrane, which are beyond the reach of light resolution may play a role in the prolonged resistance of this cell to exposure to distilled water. The hair-like processes may also correlate with the finding that the ameba surface gives a positive stain with methods generally used to demonstrate pectins (14). Lehmann's observations of globular components in the cell membrane and ectoplasmic space cannot be compared to these findings because of differences in fixation and other aspects of technique (17).

The most interesting observation concerning the mitochondria is the consistent appearance of a marked electron density, probably an osmophilia, during advanced starvation. This confirms the caution required in deciding whether optically empty areas are devoid of significant materials. Regarding the alpha particles and Golgi membranes, electron microscopy merely establishes the presence of entities which could correspond to these structures. The appearance of the presumptive alpha particle as an entity in a vacuole bears considerable resemblance to descriptions given of cytoplasmic inclusions present after viral infections (15).

The appearance of a vacuole surrounded by an inordinately large concentration of distinctive organelles served as the basis of recognition of the contractile vacuole and of the mitochondria. If this be valid, then it must be stated that the vacuolar membrane possesses no unusual characteristics, such as being thicker than other vacuole walls. Since this does not correspond to conclusions based on light microscopy (13), it is probable that the extra thickness observed in stained preparations represents a component of low density or osmiophilia or both.

The most interesting findings concern the nucleus. The observed variability in the thickness of the inner membrane component may represent a gradual reaccumulation of this material which has been shown to vanish during nuclear division. If the thickness of this layer can be related to cell growth, then its measurement may constitute a useful clock for nuclear maturation. The absence of any signs of cytoplasmic orientation outside the metaphase nucleus supports the idea that the nuclear division apparatus is largely intranuclear. Some nucleolar material remains at metaphase, but the bulk of the nucleolar material is no longer apparent (Figs. 5 and 7). Finally, the significance of the membrane evaginations in early prophase cannot be evaluated. The resemblance of the evaginated material to mitochondria is, at the present time, purely a descriptive finding relating mostly to external dimensions. The simplest hypothesis concerning the foamy bodies is that the extranuclear and intranuclear bodies are

qualitatively similar. The existence of the smallest foamy bodies in the nucleoli and mixed sizes elsewhere throughout the cell might then suggest formation of the material in the nucleoli, or the inability of larger masses to enter the nucleus. In any event, the nature of the material is unknown.

The description of ameba mitosis, based on light microscopy, by Dawson *et al.* (16) is essentially supported by these findings.

Studies on the starving animals were correlated with biochemical assays. The disappearance of oil droplets in 2 weeks corresponds to a period when extraction of lyophilized cells with fat solvents produces a minimum weight reduction. The persistence of vacuolar food remnants throughout starvation poses a difficulty for students of ameba biochemistry since it means that one can always be in doubt as to the contribution of food remnants to a particular measurement.

SUMMARY

Electron microscopic observations have been made on growing and dividing specimens of *Amoeba proteus* and also on starving animals. Structures presumably corresponding to the mitochondria, alpha particles, vacuoles, and Golgi material are described. A new entity, designated as a foamy particle, is noted.

Descriptions are given of the cytoplasmic and nuclear membranes. During division the inner, thick nuclear membrane component is seen to vanish and the outer membrane persist. Measurements suggest a gradual reappearance of the inner component with growth.

Starving animals show a loss of cytoplasmic granularity and an increase in the electron density of mitochondria, presumably due to lipide accumulation.

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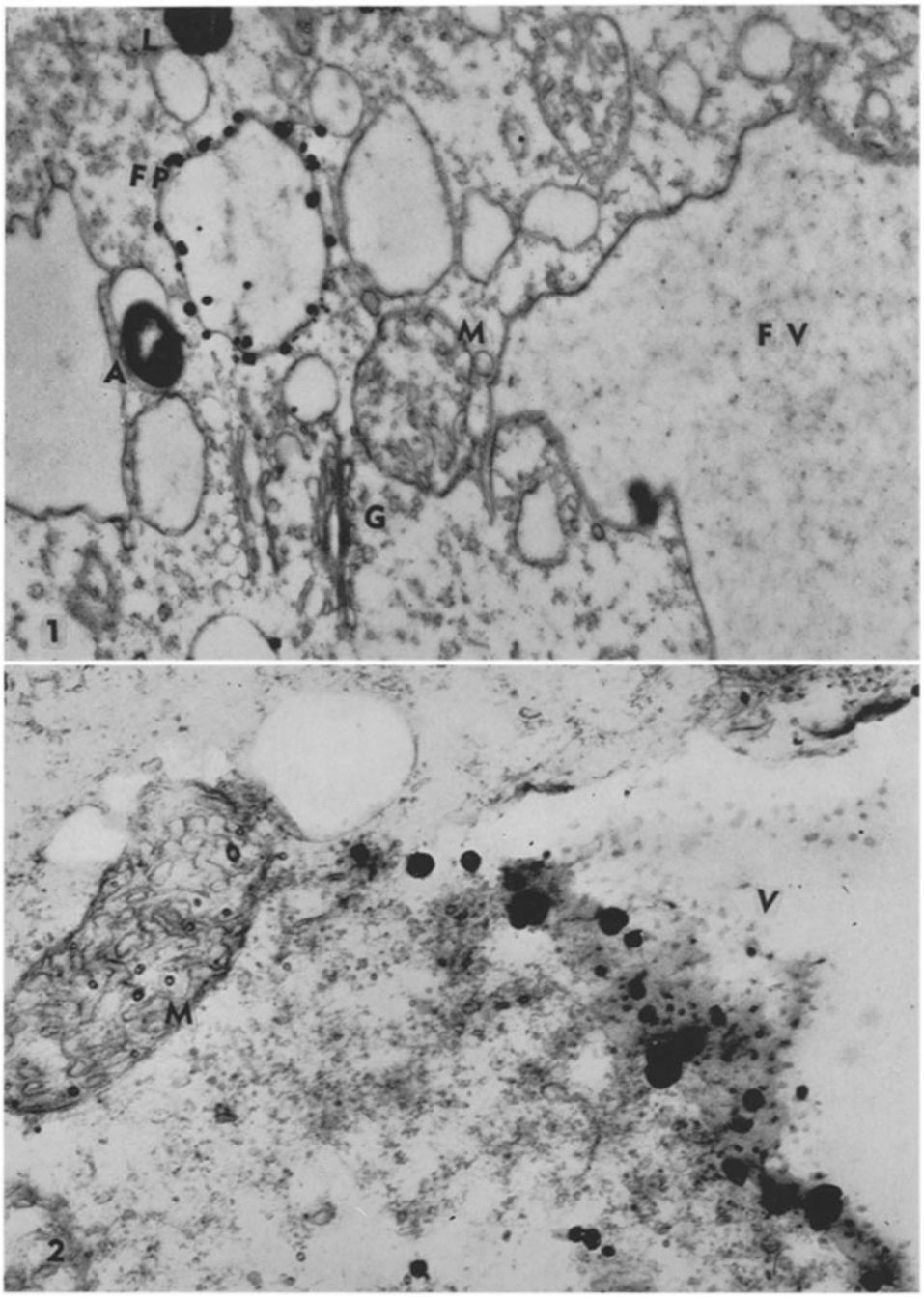
EXPLANATION OF PLATES

PLATE 276

FIG. 1. The figure shows a general cytoplasmic view with an alpha particle (*A*), a food vacuole (*FV*), a lipide drop (*L*), Golgi membranes (*G*), a mitochrondrion (*M*), and foamy particles (*FP*) arranged about a vacuole membrane and on both sides of it. The spongy internal structure of the foamy particles is not evident in this photograph. × 10,000.

FIG. 2. The figure shows an area where the cell membrane is villated (*V*). This is rare. A mitochondrion is also evident (*M*). × 30,000.

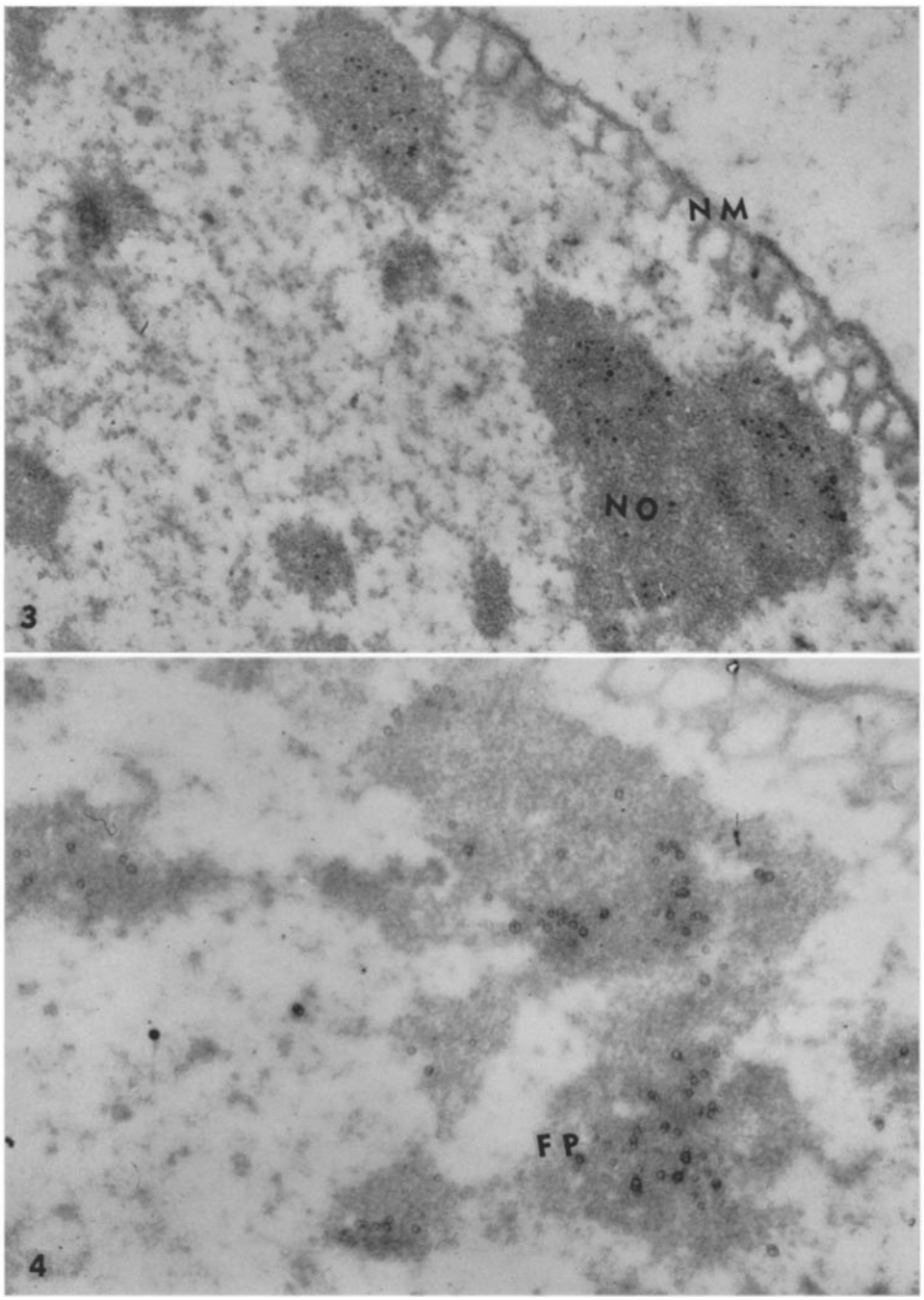


(Cohen: Observations of *Amoeba proteus*)

PLATE 277

FIG. 3. The figure shows a portion of a nucleus of a cell starved for 14 days. The granular material (*NO*) is nucleolar. The typical two-layered nuclear membrane (*NM*) is also seen. × 15,000.

FIG. 4. The figure is printed to show the foamy particles typically occurring in the nucleolar material. × 34,000.

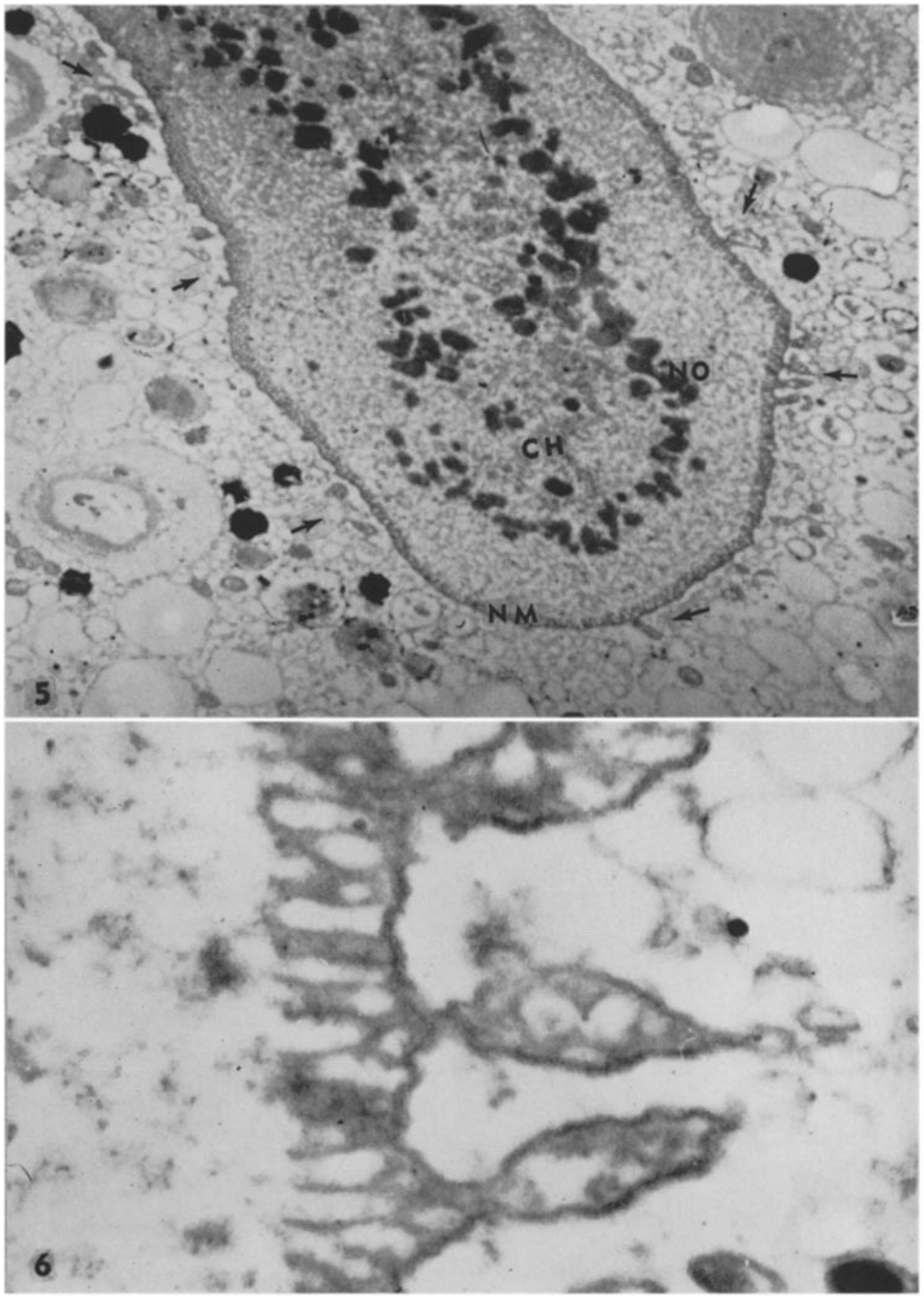


(Cohen: Observations of *Amoeba proteus*)

PLATE 278

FIG. 5. This low power view shows an early prophase with the nucleolar material (*NO*) moving away from its usual location under the nuclear membrane (*NM*). Some chromosomal material (*CH*) is evident in the central area. The arrows indicate evaginations of the nuclear membranes. × 3,600.

FIG. 6 shows a detailed view of an area of evaginating nuclear membranes. × 39,000.

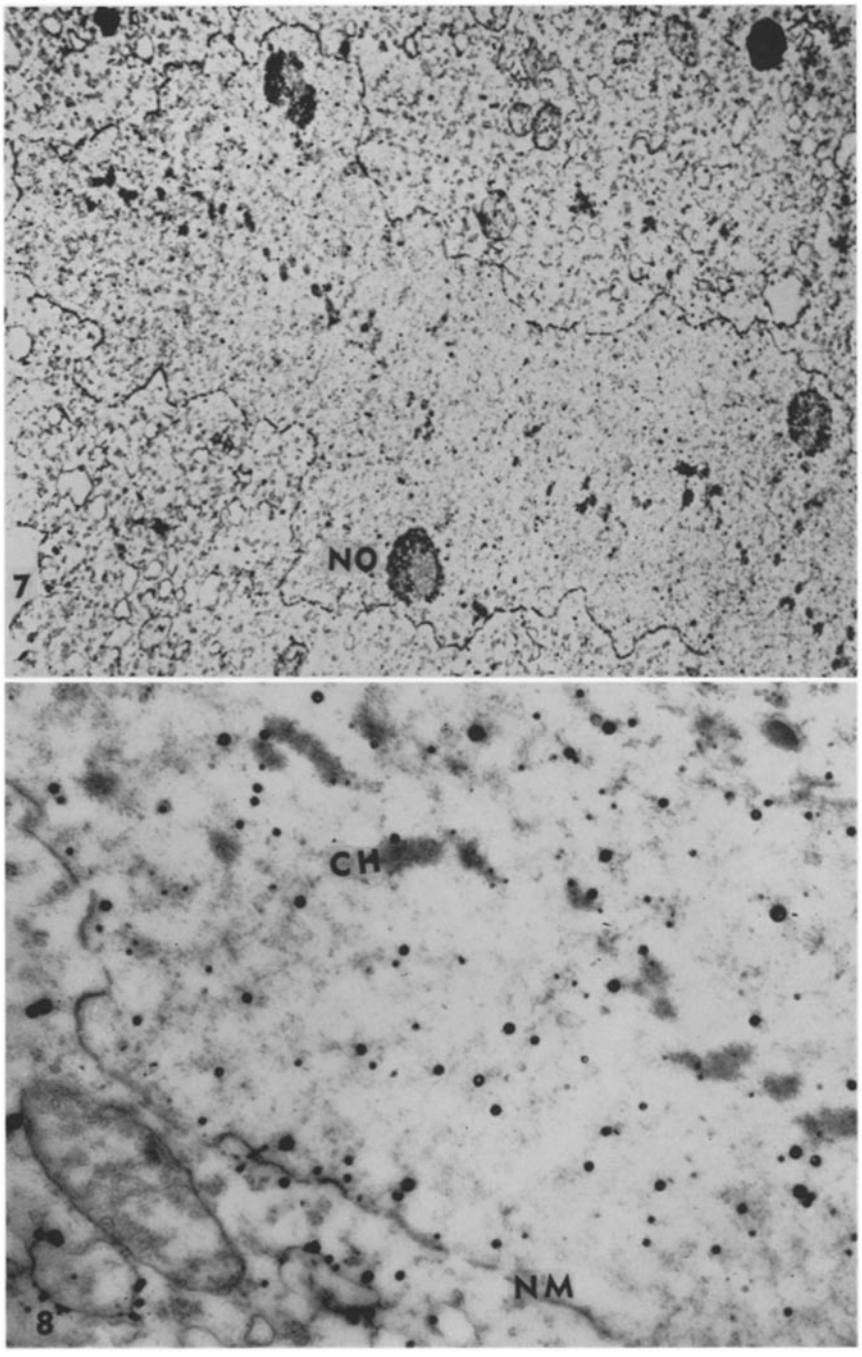


(Cohen: Observations of *Amoeba proteus*)

PLATE 279

FIG. 7. The figure shows a metaphase nucleus with the chromosomes aligned in a plane and with some nucleolar masses (*NO*) evident. Note the absence of the inner nuclear membrane component. × 3,600.

FIG. 8 is a view at higher magnification of another metaphase nucleus with the chromosomes (*CH*) and the remaining outer nuclear membrane (*NM*) evident. × 14,000.

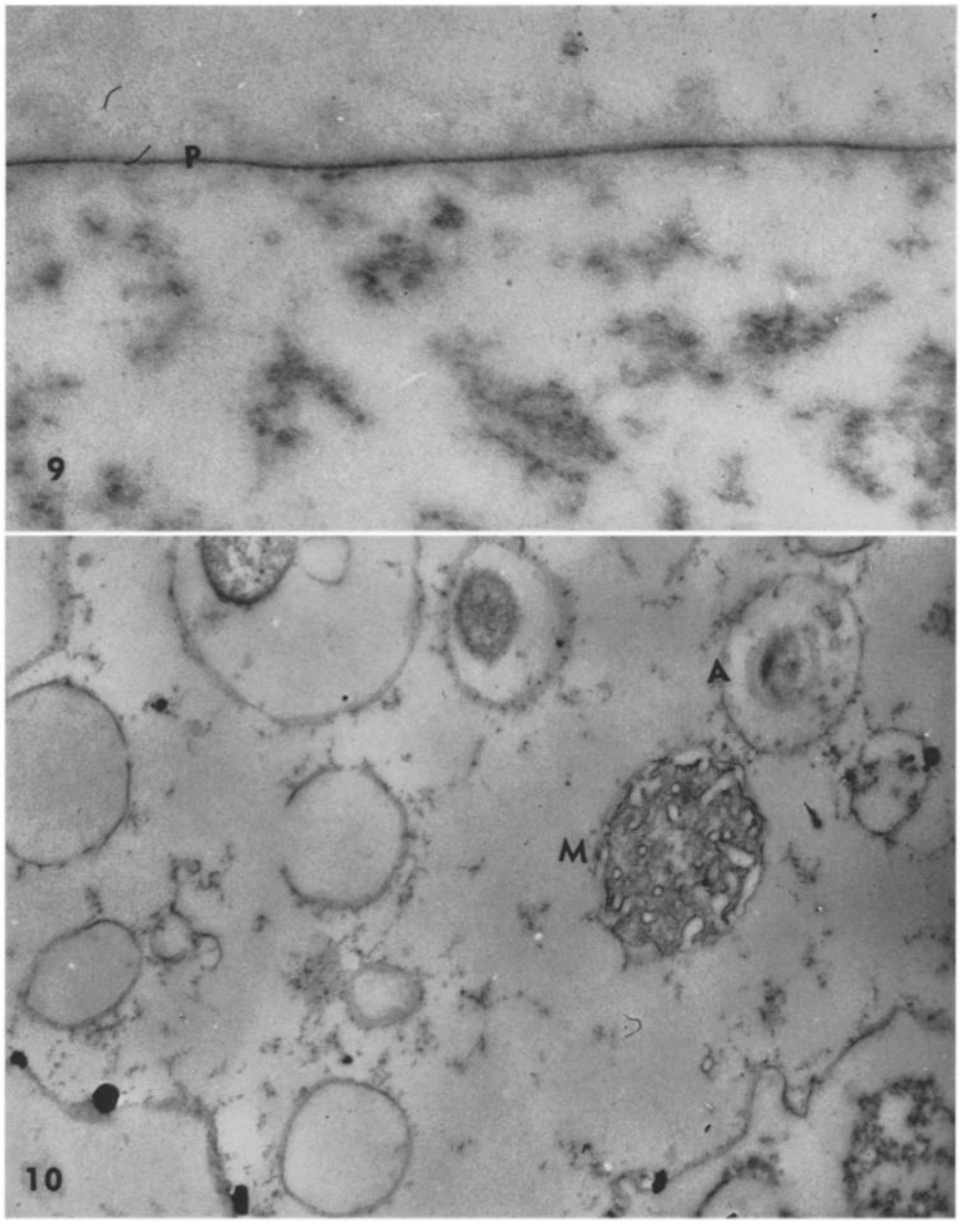


(Cohen: Observations of *Amoeba proteus*)

PLATE 280

FIG. 9 is a view of the plasma membrane (*P*) at high magnification. Note the singleness of the membrane and the fringe on both surfaces. × 65,000.

FIG. 10 is a view of the cytoplasm of a cell starved for 3 weeks. Note the degenerating mitochondrion (*M*) and a degenerating alpha particle (*A*). × 25,000.



(Cohen: Observations of *Amoeba proteus*)