THE FINE STRUCTURE OF *ACANTHAMOEBA CASTELLANII*

I. The Trophozoite

BLAIR BOWERS and EDWARD D. KORN

From the National Heart Institute, Laboratory of Biochemistry, the Section on Cellular Physiology, National Institutes of Health, Public Health Service, United States Department of Health, Education and Welfare, Bethesda, Maryland 20014

ABSTRACT

The fine structure of the trophozoite of *Acanthamoeba castellanii* (Neff strain) has been studied. Locomotor pseudopods, spikelike "acanthopodia," and microprojections from the cell surface are all formed by hyaline cytoplasm, which excludes formed elements of the cell and contains a fine fibrillar material. Golgi complex, smooth and rough forms of endoplasmic reticulum, digestive vacuoles, mitochondria, and the water-expulsion vesicle (contractile vacuole) are described. A canicular system opening into the water-expulsion vesicle contains tubules about 600 A in diameter that are lined with a filamentous material. The tubules are continuous with unlined vesicles or ampullae of larger diameter. Centrioles were not observed, but cytoplasmic microtubules radiate from a dense material similar to centriolar satellites and are frequently centered in the Golgi complex. Cytoplasmic reserve materials include both lipid and glycogen, each of which amounts to about 10% of the dry weight.

INTRODUCTION

Acanthamoeba castellanii,[1] a small, free-living soil ameba that is easily cultured under axenic conditions (29), has found increasing use in physiological and biochemical studies (20, 23, 27, 28, 30, 45, 49). With *Acanthamoeba*, in contrast to the extensively studied giant amebae, *Amoeba proteus* and *Pelomyxa carolinensis* (*Chaos chaos*), it is relatively easy to obtain good morphological preservation for electron microscopic observation. Consequently this small ameba is a very useful cell for studies of structural organization in the Rhizopoda, as well as for correlative studies of cell organelle structure and function.

The appearance of the strain of *Acanthamoeba* used in this study has been described by Neff (29) at the light microscope level. Other useful light microscopic descriptions of this species are those of Volkonsky (48), Singh (43), and Page (32).

Several comparative studies on ameba fine structure include observations on *Acanthamoeba* (or *Hartmanella rhysodes*). The most extensive description is Vickerman's comparison of the structure of *Acanthamoeba* with that of three other small amebae (47). Mercer (26) and Pappas

[1] The taxonomy of amebae is difficult; the ameba used in this study has for some years been referred to in the literature as *Acanthamoeba* sp. Recent taxonomic studies by Adam (1) and Page (32) indicate that the amebae called *Hartmanella rhysodes* and *Acanthamoeba castellanii* are identical with *Acanthamoeba* sp. We have adopted Page's definition of the species as *A. castellanii*.

(33) have considered *Acanthamoeba* briefly in relation to the structure of the giant amebae *A. proteus* and *P. carolinensis*. All of these descriptions were of cells fixed in potassium permanganate or osmium tetroxide.

We have reinvestigated the structure of the trophozoite of *Acanthamoeba castellanii*, Neff strain, as found under laboratory culture conditions, by taking advantage of the considerable improvement in cell preservation obtained by the use of aldehydes in combination with osmium tetroxide for fixation. This study provides a morphological background for physiological and biochemical studies being carried out in this laboratory and may be similarly useful to other investigators. The description of the growing cells also forms the background against which structural changes during encystment (to be reported in a subsequent publication) can be interpreted.

MATERIALS AND METHODS

Acanthamoeba was obtained from Dr. R. J. Neff and was cultured in axenic suspension cultures as described previously (22). The amebae were fixed for electron microscopy at 4, 7, 8, and 12 days after subculture. At 7 days the suspension cultures contained about 10^6 cells per milliliter. At a density of 3–4 × 10^6 cells per milliliter the growth reaches a plateau and the cells begin to encyst (31). Cultures for inoculum were carried in shallow stationary cultures in the same medium. Some of these stationary cultures were also examined 5, 7, 13, and 14 days after subculture.

For fixation the amebae were collected from the culture flasks by low speed centrifugation and obtained as a heavy suspension in growth medium. The suspension was diluted with a large volume of fixative, and the cells were spun down and resuspended in fresh fixative. In some cases the amebae were allowed to settle on an agar substrate before fixation. The fixing solution was 3% glutaraldehyde (biological grade; Fisher Scientific Company, Pittsburgh, Pa.) in 0.1 M NaH_2PO_4–Na_2HPO_4 buffer, pH 6.8, which contained 1 mM $CaCl_2$. The cells were fixed for 1 hr at room temperature in glutaraldehyde, rinsed in cold buffer (four changes over 1 hr), and postfixed in cold 1% osmium tetroxide in the same buffer. Dehydration was carried out through a graded series of cold ethanol solutions. The cells were pelleted by low speed centrifugation for each reagent change. For purposes of comparison, some cells were fixed in glutaraldehyde alone, osmium tetroxide alone, or potassium permanganate alone. None of these fixatives gave optimal fixation, but they were useful for corroborative observations.

Fixed, dehydrated cells were taken through propylene oxide and embedded in Epon 812 (25). Thin sections were picked up on bare copper grids and stained for 5 min in 1% aqueous uranyl acetate, and for 5 min in lead citrate (35). The sections were examined with an RCA EMU 3G electron microscope at 50 kV and photographed at magnifications of 2,500–40,000 diameters.

Glycogen was extracted from a lipid-free residue of amebae with 30% KOH for 3 hr at 90°C. The polysaccharide was precipitated from the supernatant solution by the addition of ethanol to a final concentration of 65%. The precipitate was collected by centrifugation, redissolved in water, and reprecipitated with ethanol. This procedure was repeated three times and the final precipitate was washed with ethanol until the washes were neutral. The polysaccharide was then dissolved in water, dialyzed against distilled water for 2 days at 2°C, and lyophilized. Carbohydrate was measured by the anthrone reaction (42). Infrared spectra were determined from fused KBr pellets with a Beckman IR-7 (Beckman Instruments, Inc., Fullerton, Calif.) spectrophotometer. After acid hydrolysis sugars were identified by paper chromatography by using the upper phase of a mixture of pyridine:ethyl acetate:water (1:3.6:1.15) as a solvent.

RESULTS

The Cell Surface

Acanthamoeba is one of the "naked" amebae, and its surface is devoid of any detectable material outside the plasma membrane (Figs. 1, 2). The membrane itself shows the usual trilaminar structure with an over-all dimension of 70–90 A. The membrane frequently appears to be asymmetric in that the outer dense line stains more intensely, or is slightly thicker, than the inner dense line (Fig. 2).

When moving on a substrate, the ameba has a broad "locomotor" pseudopodium, usually with filar projections (acanthopodia, reference 32), 1–2 μ in diameter, that extend from the ameba for several microns (Fig. 3). The locomotor pseudopodium and the acanthopodium are composed of hyaline cytoplasm, which seems to exclude physically the numerous vacuoles and particles that fill the interior of the ameboid cell. The cortex of rounded cells is also composed of hyaline cytoplasm (ectoplasm) (Figs. 1, 4). In *Acanthamoeba*, as in other amebae (7, 21, 47, 51), the hyaline region is seen with the electron microscope to contain only the smallest of the formed elements within the cell: glycogen particles, free ribosomes, and some small vesicles and tubules

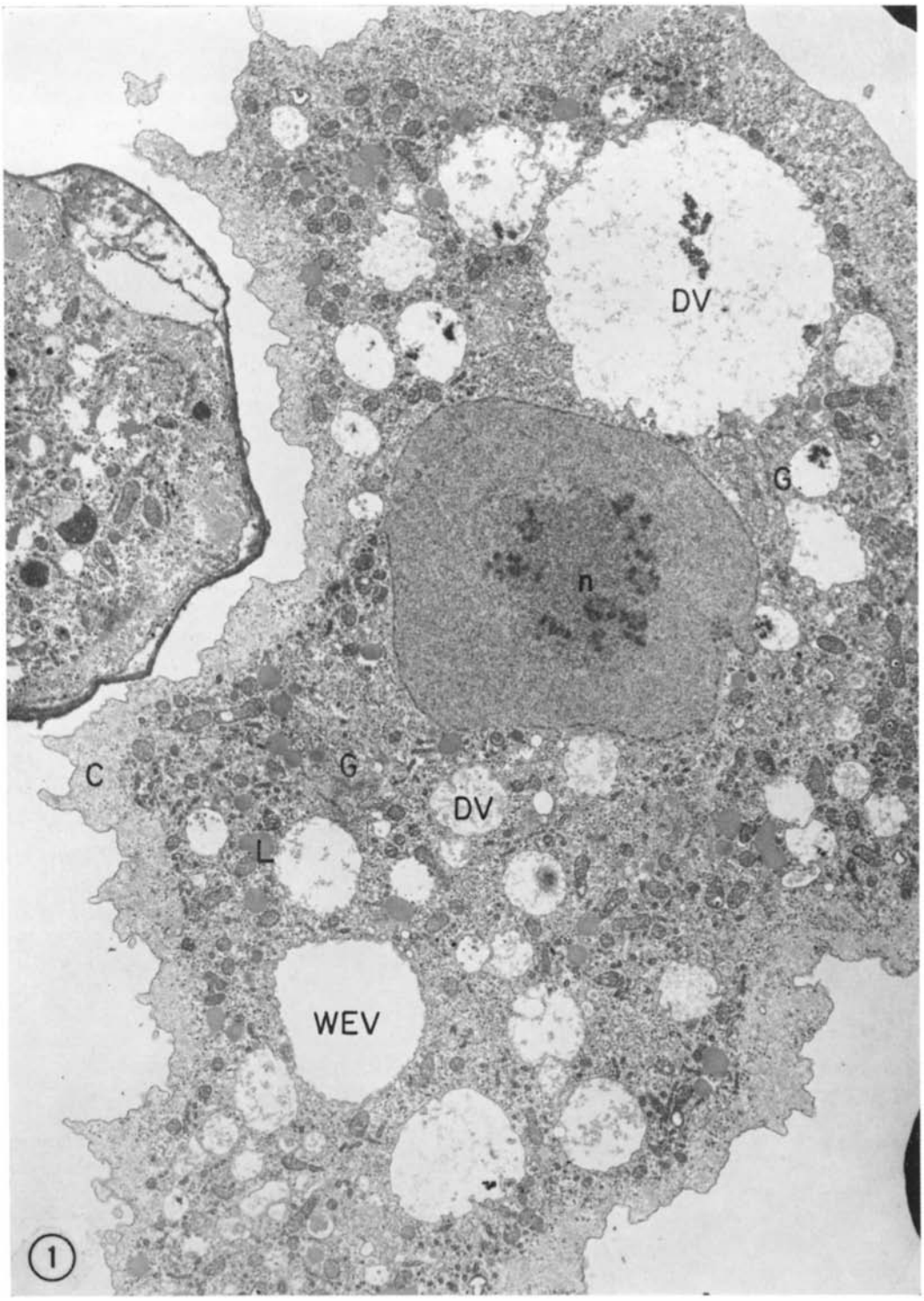

FIGURE 1 An ameboid cell from an agitated culture, 8 days after subculture. The cell shows a distinct cortex (*C*) from which larger formed elements of the cytoplasm are excluded. Numerous digestive vacuoles (*DV*) and one water-expulsion vesicle (*WEV*) are shown in this section. Golgi complex (*G*); lipid droplet, *L*; nucleolus, *n*. × 5900.

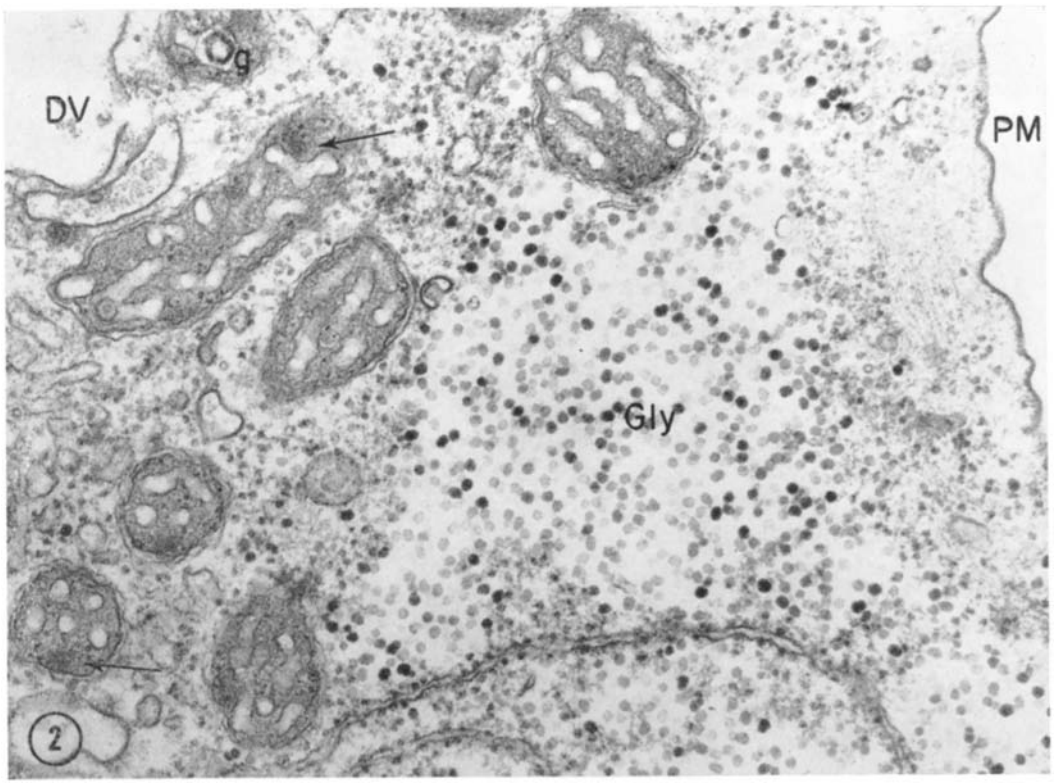

FIGURE 2 A portion of a cell from an agitated culture, 8 days after subculture. A very thin cortical region is present and contains fibrous material. Glycogen granules (*Gly*) are present in a "puddle" in the center of the micrograph. The plasma membrane (*PM*) is devoid of an external coat, but the outer dense line stains more intensely than the inner dense line. Note similarity of the PM and the membrane of the digestive vacuole (*DV*). Mitochondria, with tubular cristae, show ribosome-like granules and small paracrystalline areas (arrows) in a dense matrix. Intracristate granule at *g*. × 45,000.

that are unidentified as to origin or function (Figs. 3, 4). A major component of the hyaline cytoplasm is a fine fibrous material (Figs. 2–5). The fibrils are on the order of 50 A in diameter. In some regions they appear to be oriented in parallel; in others, in a randomly disposed network. Occasionally the fibrils appear in bundles (Figs. 3, 5), and the bundles may penetrate the endoplasm.

Microprojections, only about 0.1 μ in diameter, are also present on the surface of the ameba. They have a core of the same fibrils which make up the cortex of the cell (Fig. 6).

The surface of the fixed amebae rarely appears smooth in outline. There is at least one type of well-defined surface invagination, which is a tubule, roughly 650 A in diameter, that ends in a "coated pit." The tubule may be very short, as in Fig. 9, or it may extend a micron or more into the cell.

Cytoplasmic Membrane Systems

Acanthamoeba contains a number of cytoplasmic membrane systems, not all of which can be clearly characterized at the present time. The major systems, however, can be easily identified; they are the Golgi complex, smooth and rough forms of endoplasmic reticulum, a vacuolar digestive system, and the contractile vacuole or water-expulsion vesicle (50).

The *Golgi complex* comprises two or more polarized stacks of 3–5 cisternae. The cisternae are separated by a space of about 200 A (Figs. 7, 8). The space between the membranes of a cisterna is also approximately 200 A, except at the margins where the sacs are slightly dilated. Many small

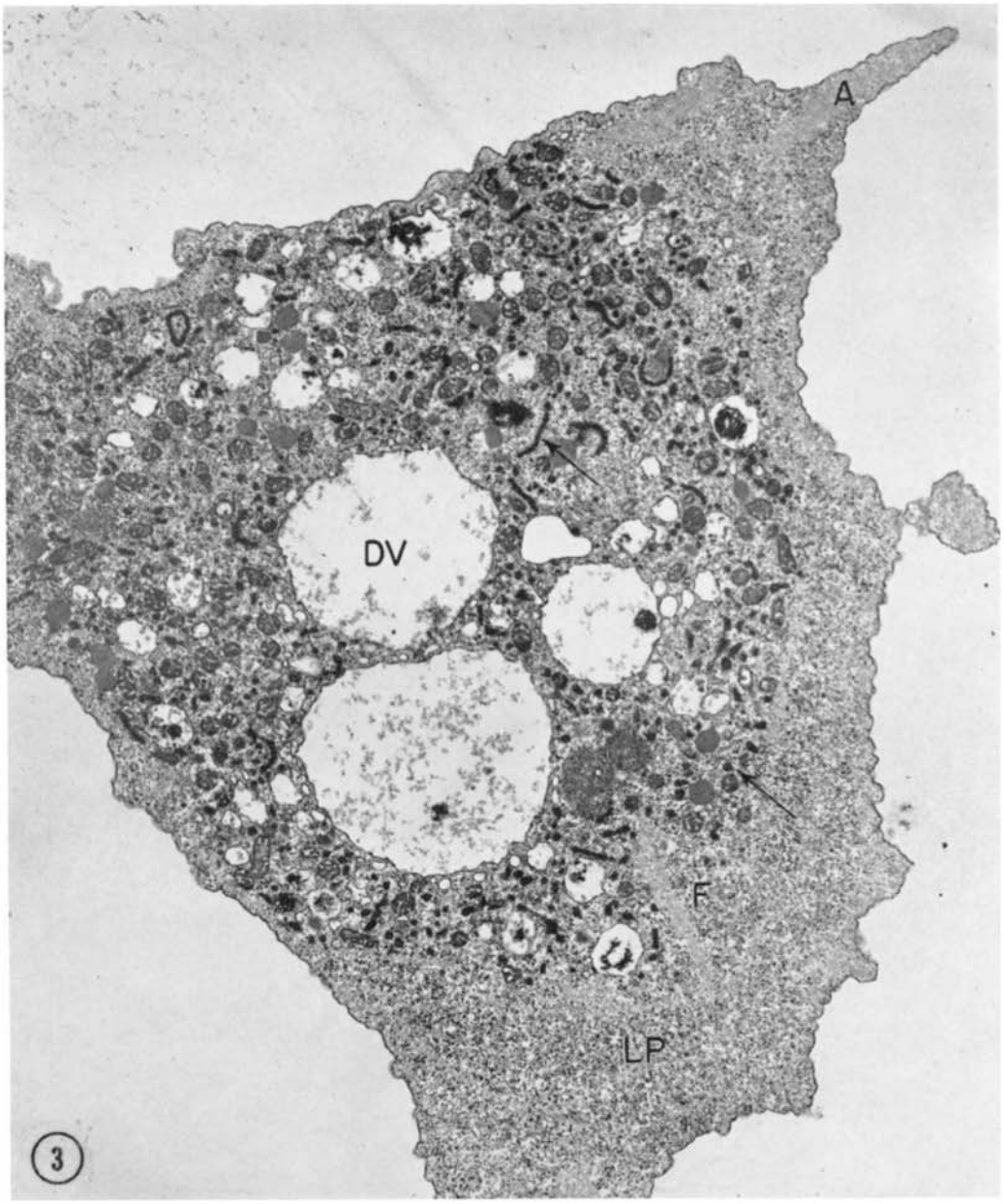

FIGURE 3 Cell from an agitated culture, 8 days after subculture. The section includes a broad locomotor pseudopodium (*LP*) and the base of an acanthopodium (*A*). Large numbers of glycogen granules (small black dots) are present in the pseudopodium. Arrows indicate examples of cross- and longitudinal sections of tubular ER that is filled with densely staining material. Digestive vacuole, *DV*; fibrous bundle, *F*. × 8180.

vesicles and randomly oriented tubules are concentrated on the concave face of the stacked cisternae. The vesicles are about 300 A in diameter and appear to derive from both the periphery of the stacked cisternae and the tubules. Some of the vesicles appear to be of the "coated" variety. The vesicles and associated tubules appear somewhat indistinct, for two reasons. First,

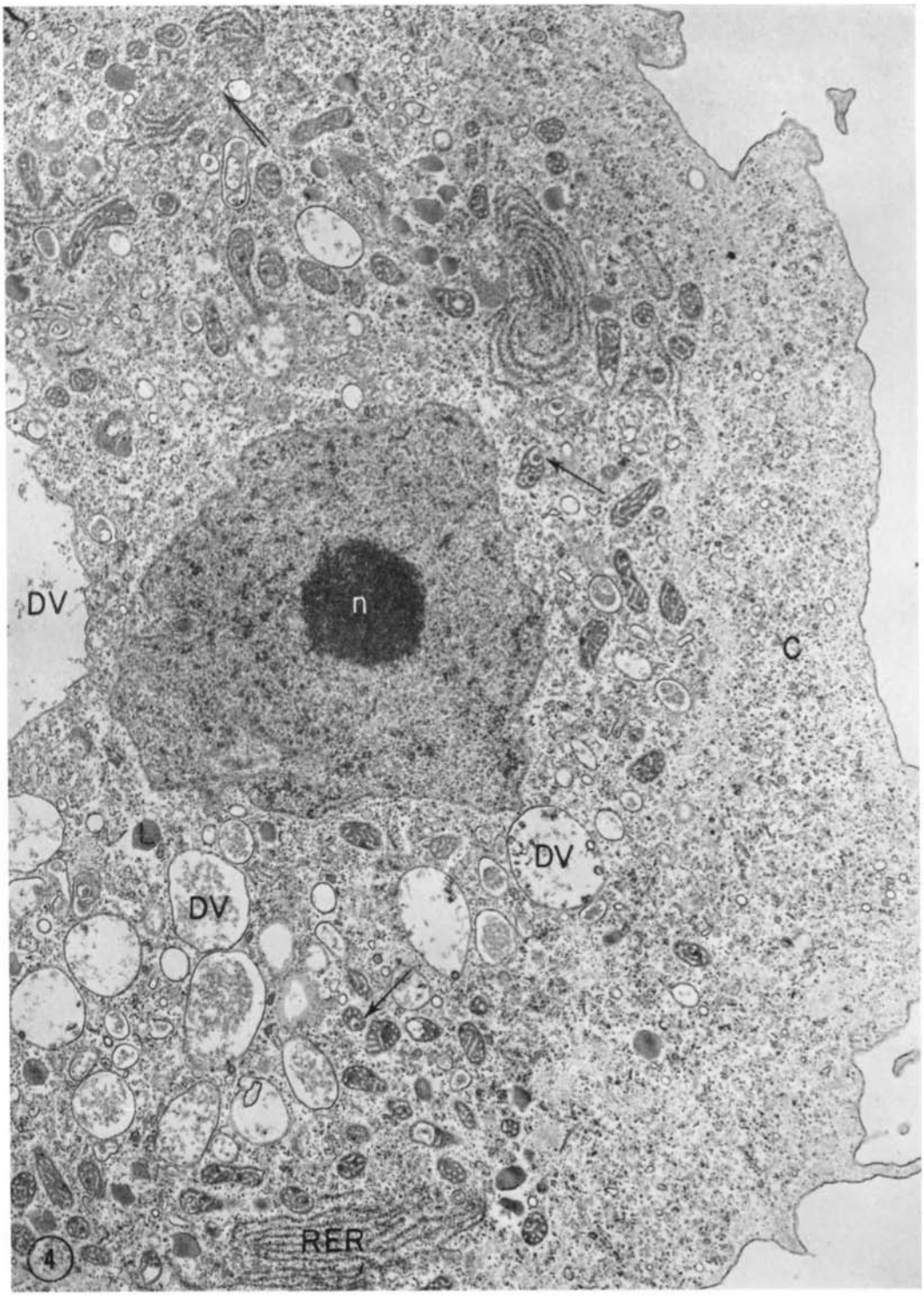

FIGURE 4 Portion of a cell from a stationary culture, 14 days after subculture. The rough endoplasmic reticulum (RER) is in the cisternal form. A small region of smooth ER is present at double-stemmed arrow. Intracristate granules in the mitochondria are visible at small arrows. A compact nucleolus (*n*) is present in the nucleus. Cortex, *C*; digestive vacuoles, *DV*; lipid, *L*. × 9500.

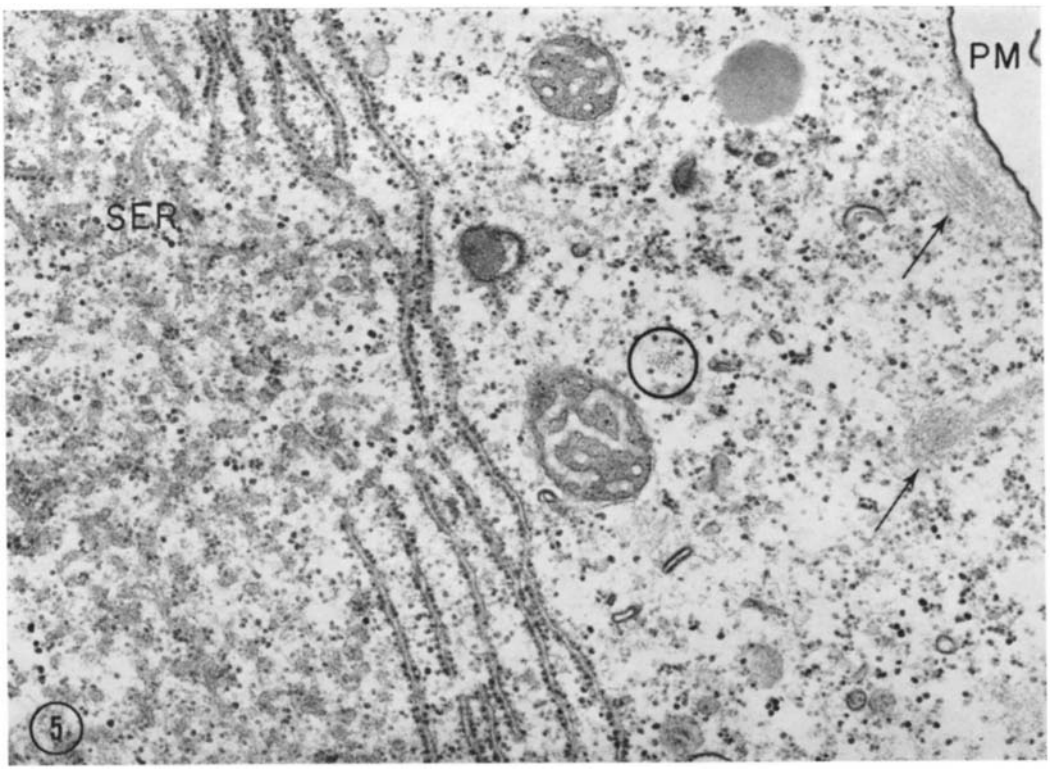

FIGURE 5 A portion of a cell from a stationary culture, 14 days after subculture. Bundles of fibrous material are present in the cell cortex (arrows). One bundle is seen in cross-section in circle. The cisternal form of the rough endoplasmic reticulum borders a region of tubular smooth ER (*SER*). Plasma membrane, *PM*. × 30,500.

the structures are small enough to lie within the section thickness, and second, there is an ill-defined, filamentous material which surrounds and extends between the structures in this region.

The *rough endoplasmic reticulum* (RER) in this ameba is remarkable for its morphological variation. In many cells the profiles consist entirely of circular cross-sections and short longitudinal lengths; this suggests a highly branched tubular system. The tubules are generally filled with a densely staining material (Figs. 1, 3, 9) that at high magnification appears granular in nature. The material is not homogeneously distributed within the cavity of the ER, and in longitudinal section it sometimes gives the appearance of a tubule within a tubule (Fig. 9). In the tubular configuration, the RER appears to ramify fairly uniformly throughout the central region of the cell.

In an alternate configuration, many long profiles (up to 19 μ long) with very few, if any, circular cross-sections, are observed (Figs. 2, 4, 5). In this form one also sees tangential views of cisternae with arrays of ribosomes on their surfaces. In some cases the cisternae are stacked in parallel (Fig. 4); in others they appear randomly oriented. There are instances as well in which the cisternae are seen in a circular configuration, most often enclosing a mitochondrion. The cisternal form of the RER generally lacks the strongly staining content of the tubular form (Figs. 2, 4, 5).

Neither the tubular nor the cisternal form of the RER penetrates the cortex of the cell; indeed, the cisternal elements are often found aligned at the boundary of the cortex.

The stacked cisternal form of RER sometimes encircles patches of *smooth endoplasmic reticulum* (SER) (Fig. 5). The SER is in the form of fine tubules, less than 500 A in diameter. Occasionally we have also been able to identify SER dispersed

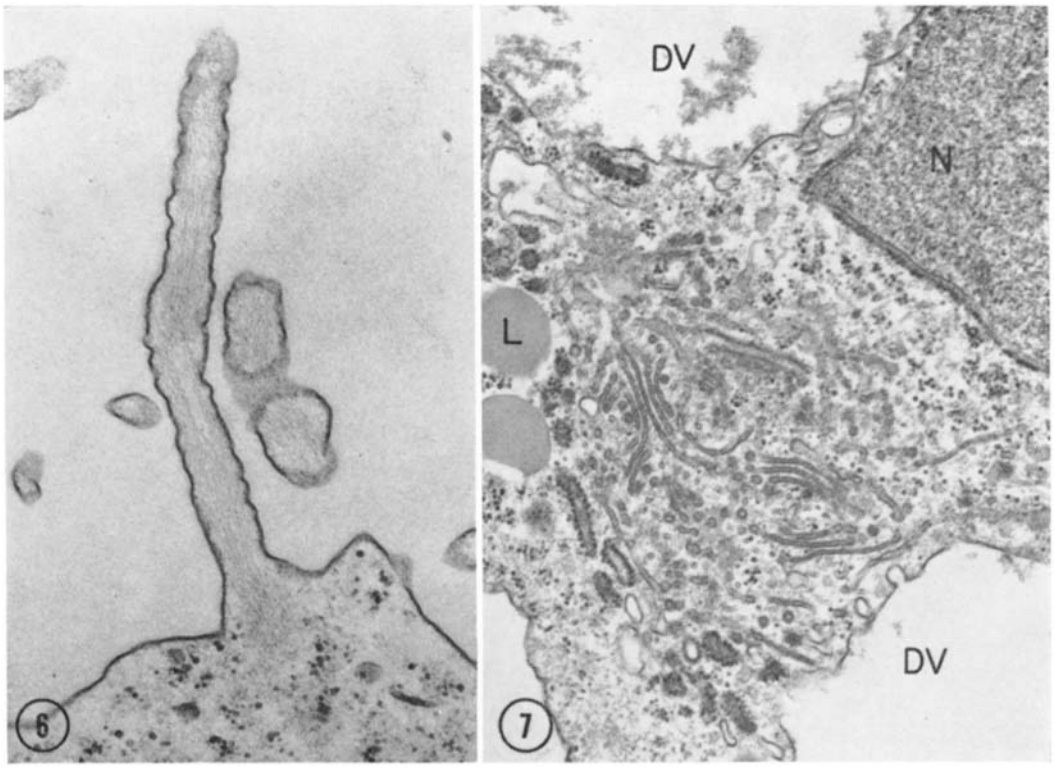

FIGURE 6 A microprojection from the cell surface, showing a supporting core of fibers. × 77,500.

FIGURE 7 A large Golgi complex showing several stacks of cisternae and accumulations of small vesicles and tubules. Digestive vacuole, *DV*; nucleus, *N*; lipid, *L*. × 21,000.

among the other cell constituents. Failure to see SER more often is probably due to the difficulty in distinguishing it among the numerous small vesicular components of the cytoplasm.

Although these striking differences in the morphology of the ER are undoubtedly related to the physiology of the cell, we cannot, at present, discern the relation. It should be noted that both the tubular form with stainable content and the cisternal form without stainable content may occur in the same cell. Furthermore, the full range of forms has been observed in cells of each culture regardless of age or type of culture (agitated or stationary).

Vacuoles are conspicuous elements in the cytoplasm of *Acanthamoeba*, in both the living and fixed cells. They comprise at least two separate systems: the water-expulsion vesicle that is involved in osmotic regulation (19) and the digestive vacuoles which normally function in the breakdown of ingested particulates (18).

With the light microscope the *water-expulsion vesicle* (WEV) in *Acanthamoeba* is seen to discharge periodically, usually in approximately the same position in the cell. The WEV does not always discharge completely, but, whether discharge is complete or not, it appears to fragment into a number of collapsed subsidiary vesicles which then refill, by some means invisible at the light microscope level, and coalesce with one another to form the large, definitive WEV.

In the electron microscope, the WEV can be distinguished from a digestive vacuole by the *absence* of flocculent content (Figs. 1, 9). In addition, the water-expulsion vesicle is usually surrounded by profiles of an accompanying canicular system or spongiome (Fig. 10). The spongiome comprises at least two morphologically distinguish-

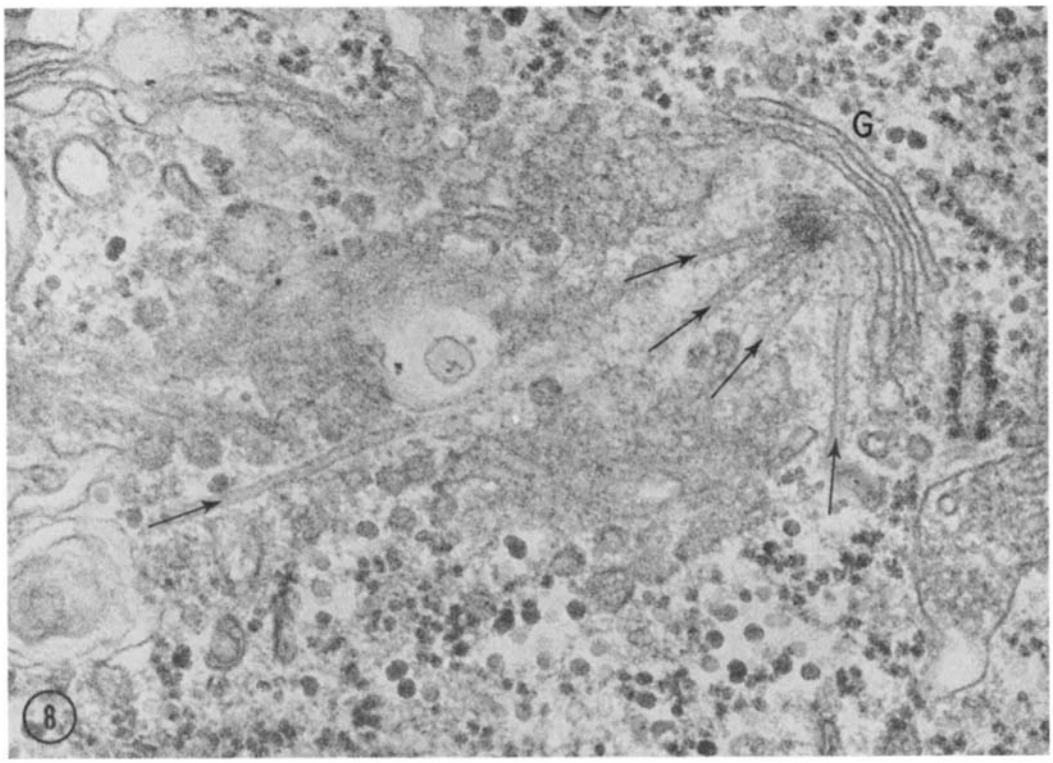

FIGURE 8 Stack of three Golgi cisternae (*G*) with microtubule focus at the concave side. Note dense material, similar to that illustrated in Fig. 13, in which microtubules (arrows) insert. × 65,000.

able components: (*a*) a system of tubules, roughly 600 A in diameter, which are confluent with (*b*) vesicles or ampullae about 1,300 A in diameter. The tubules are lined with a delicate material that extends a uniform distance of about 140 A into the lumen. This material is most easily visualized in cross-sections of the tubules, where it appears as a single dark line concentric with the tubule profile (Fig. 11). That the material is filamentous in nature is suggested by its image in longitudinal sections of the tubule as shown in Fig. 10. The material appears there as fine linear densities crisscrossing the diameter of the tubule. The tubule diameter (600 A) and the section thickness (500–800 A) are such that one would expect to catch one wall of the tubule within the plane of section in most profiles; this accounts for the fact that the filamentous material appears to extend completely across the lumen in longitudinal sections. The vesicular dilatations lack the filamentous lining present in the tubules (Fig. 10).

In many images of the WEV only small vesicular profiles are present around the periphery of the main vesicle (Fig. 9). In these cases we have not so far observed any continuity of the small vesicles with the main vesicle, but if the tubular system is present individual tubules are often seen to open into the main vesicle at a number of points within the spongiome (Figs. 11, 12). We discerned no common ducts (see reference 47). The spongiome is not uniformly ranged around the periphery of the expulsion vesicle, but seems instead to be confined to several randomly distributed regions.

The WEV commonly shows small convolutions in its bounding membrane. These convolutions seem to derive from the opening up of the spongiome tubules into shallow troughs as the tubules join the surface of the vesicle. At low magnification the membrane limiting the WEV appears indistinct relative to the more sharply defined membrane of the digestive vacuoles (Fig. 9). The "fuzziness" of the WEV boundary may be due in part to the high frequency of oblique

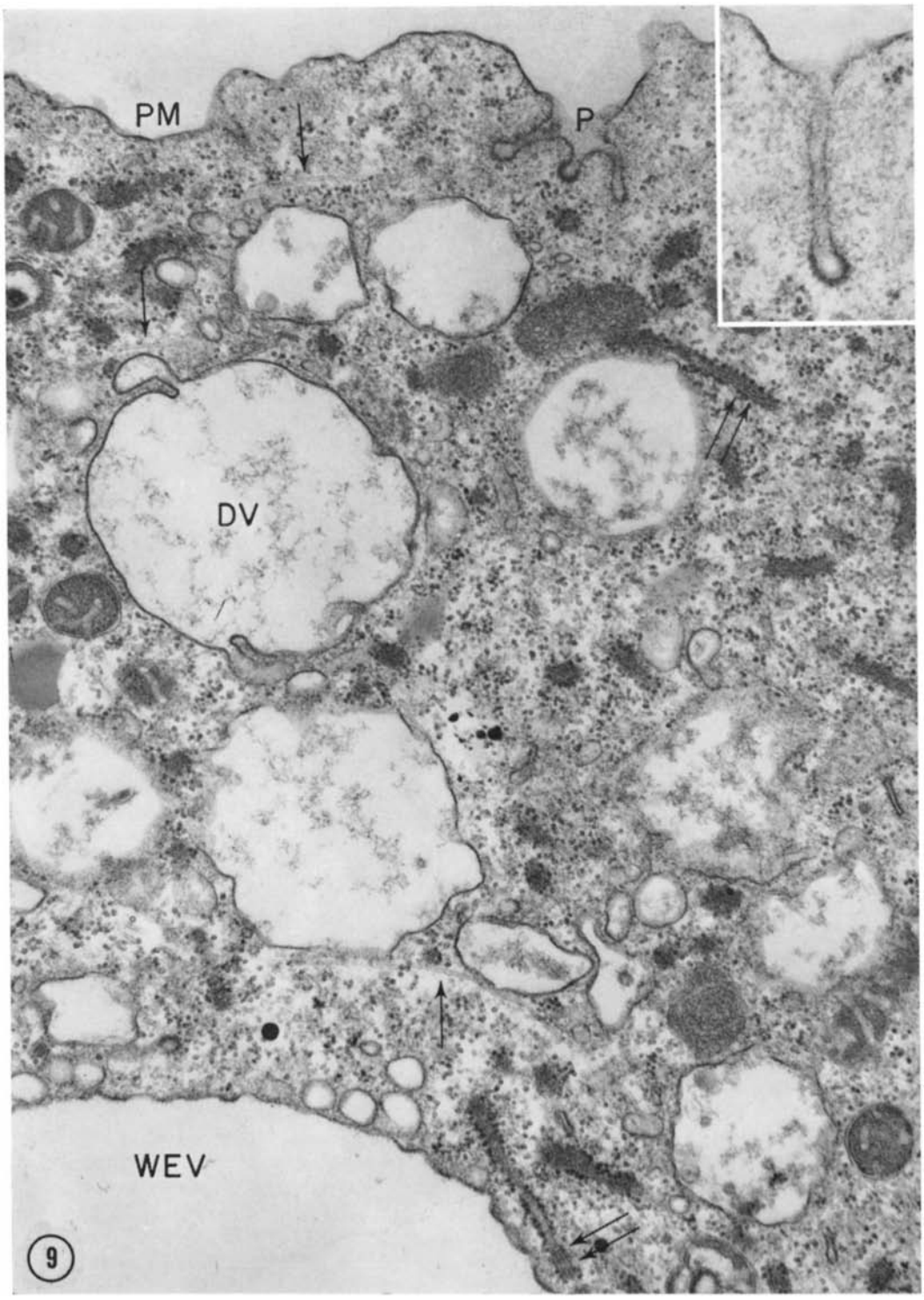

FIGURE 9 Edge of a cell from an agitated culture, 8 days after subculture. The plasma membrane (*PM*) has invaginations which end in coated pits (*P*). A coated pit is shown at higher magnification in inset. The tubular form of the rough ER is present. Its content in longitudinal section shows a linear organization (double arrows). A digestive vacuole (*DV*) displays typical membrane evaginations. Compare the appearance of the membrane of the water expulsion vesicle (*WEV*) with the membrane of the digestive vacuole and the plasma membrane. Microtubules (arrows). × 31,000. Inset; × 42,500.

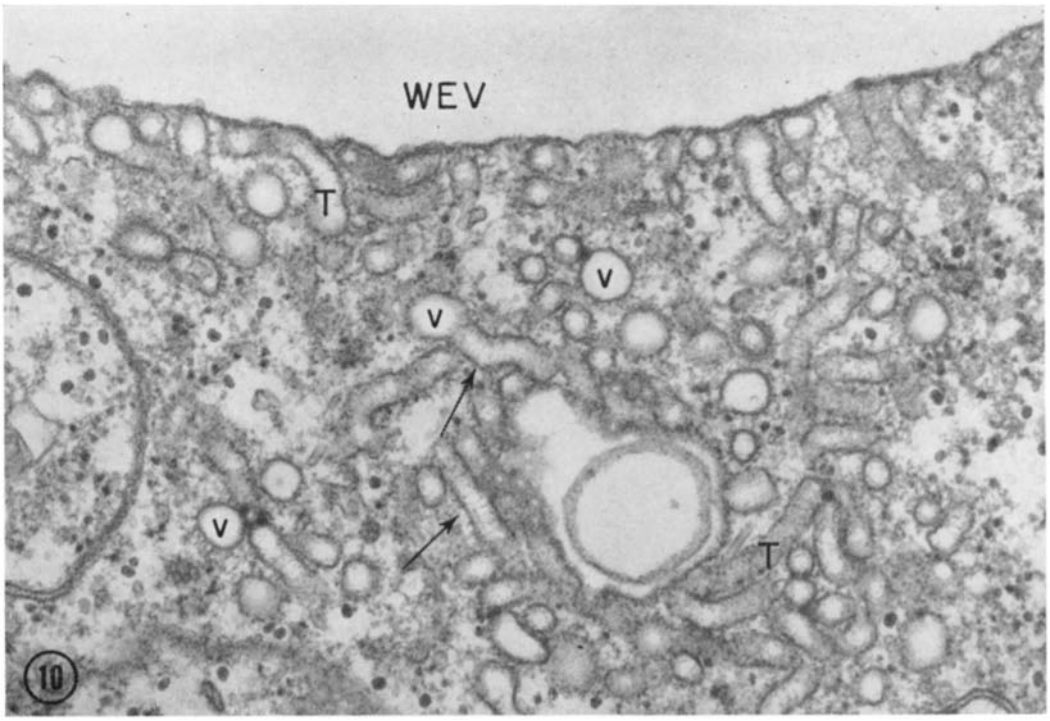

FIGURE 10 Spongiome associated with the water expulsion vesicle (*WEV*). Tubules (*T*) containing a filamentous lining are confluent with vesicles (*V*) lacking the lining. Arrows point to longitudinal sections of tubules in which the filamentous nature of lining material is suggested. Small circular profiles are cross-sections of tubules, and the larger circular profiles are cross-sections of vesicles. The membrane of the *WEV* is trilaminar, but without asymmetry. The cytoplasmic densities associated with the membrane may be real or may be due to oblique sections of the convoluted surface of the membrane. × 59,000.

cuts through the membrane due to the convolutions. When its trilaminar structure can be resolved (Fig. 10), the membrane bounding the WEV does not show the same asymmetry as the plasma membrane and the membrane bounding the digestive vacuole (see below).

Our observations confirm those of other investigators (34, 41): that no system of fibrils or microtubules is associated with the water-expulsion vesicle in amebae.

Large vacuoles which correspond morphologically to digestive vacuoles are numerous in the cytoplasm of these amebae, even though the amebae have been cultured in soluble nutrients (Figs. 1, 3, 4). Vacuoles range from a size of 0.1 μ in diameter to a size larger than the nucleus. They are bounded by a membrane which resembles the plasma membrane in size and staining characteristics (Figs. 2, 9). The membrane image is asymmetric in that the dense line facing the vacuole stains more intensely than the dense line facing the cytoplasm. The vacuoles usually contain a sparse, flocculent precipitate and occasionally dense concentrations of amorphous substances as well (Figs. 1, 3). The limiting membrane of the larger digestive vacuoles is often thrown into folds, and tubular and vesicular connections with the vacuole extend into the surrounding cytoplasm (Figs. 1, 9).

Cytoplasmic Reserve Materials

The trophozoite contains both lipid and polysaccharide reserves. The *lipid* is found as spherical droplets with average diameters of about 0.5 μ (Figs. 1, 4). That the droplets are osmiophilic would be expected because 80% of the fatty acids found in *Acanthamoeba* are unsaturated (22). The droplets are not surrounded by a trilaminar membrane, but sometimes show a thin, dense line at their boundary. Droplets are often con-

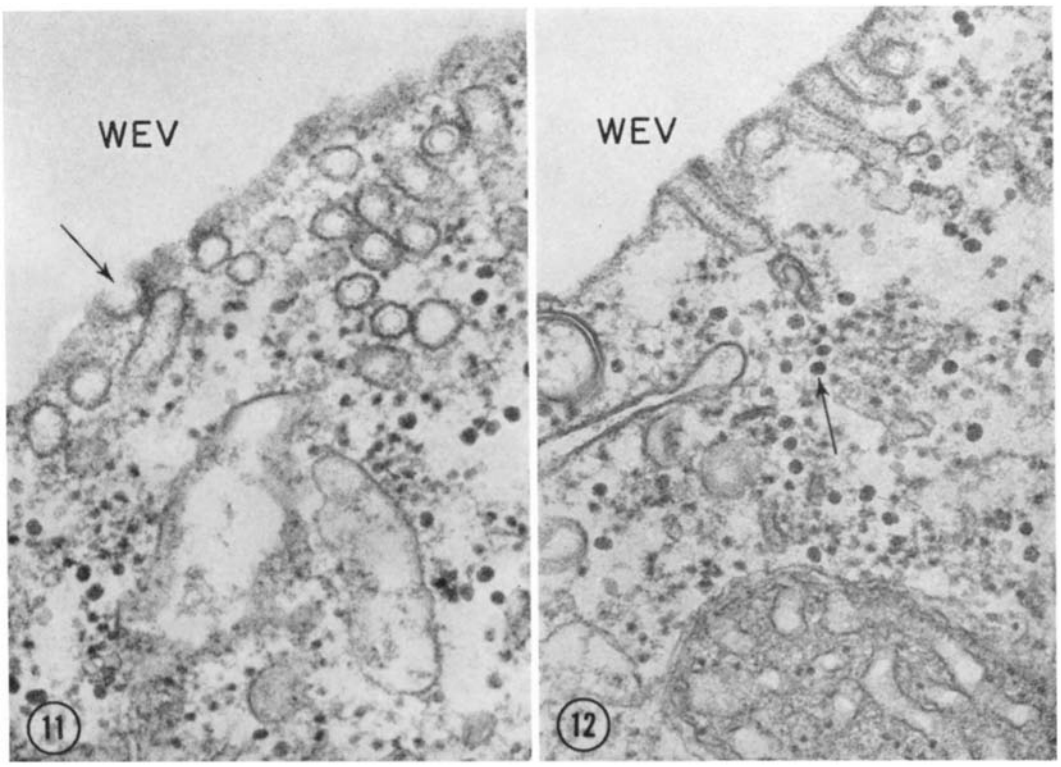

FIGURE 11 Cross-sections of tubules in which the inner layer of material is especially distinct. One tubule is seen opening into the *WEV* at arrow. × 67,500.

FIGURE 12 Spongiome tubules with filamentous lining shown opening into the *WEV*. Glycogen particles, arrow. × 67,500.

tiguous, but only occasionally fuse to form a very large droplet.

The polysaccharide reserve is *glycogen*. It was identified by its characteristic red color with I_2-KI, by its digestion with α-amylase, by the identification of glucose as the sole sugar after hydrolysis in 1 N H_2SO_4 for 1 hr at 100°C, and by the infrared spectrum which was identical with that of authentic glycogen with characteristic absorption bands at 765 cm^{-1} and 937 cm^{-1} for the 1 → 4 linkage and at 855 cm^{-1} for the β-configuration (4). Neff (Personal communication.) has also identified glycogen in *Acanthamoeba*.

Glycogen occurs in the cytoplasm as single granules, or β-particles (13), rather than the more commonly found rosettes. The smaller granules of glycogen are difficult to distinguish from free ribosomes at lower magnifications. The granules can be as large as 400 A in diameter. The glycogen particles are found throughout the ground substance, but when present in large amounts they accumulate in regions from which other formed material is absent (Fig. 2).

In cells from agitated cultures, 7 days after subculture, neutral lipid and glycogen each represents about 10% of the dry weight.

Other Organelles

The *mitochondria* are in either of two forms: elongate and slightly dumbbell-shaped or spherical (Figs. 1–4). Branched or cup-shaped profiles have not been observed (see reference 47). The cristae are formed by branching, anastomosing tubular extensions of the inner mitochondrial membrane. The tubular cristae are about 600 A in diameter; they lie in a dense, heterogeneous matrix that contains filamentous patches, paracrystalline areas, and small granules resembling

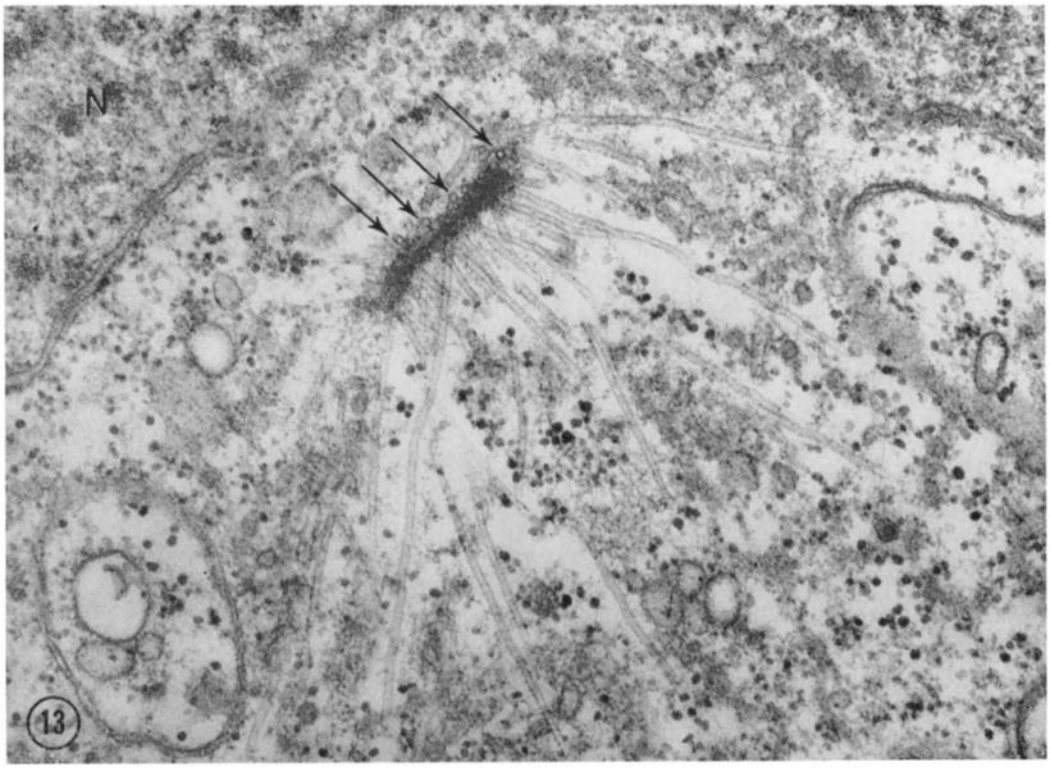

FIGURE 13 An elongate aggregation of material showing insertion of numerous cytoplasmic microtubules. Note cross-sections of microtubules along upper edge of dense material (arrows). Nucleus, *N*. × 31,500.

ribosomes (Figs. 2, 5). The mitochondria commonly contain one or more amorphous granules, about 800 A in diameter, which lie in an enlargement of the tubular space, i.e., in that space which is continuous with the space between the inner and outer mitochondrial membranes (Figs. 1, 4). These granules are variable in density, are often ring-shaped (Fig. 2), and alter their appearance during encystment of amebae (9, 46).

Microtubules are found throughout the cytoplasm. They are about 250 A in diameter and are generally quite straight (Fig. 9). They often radiate from the Golgi complex where they originate from dense material having the same texture as the *centriolar satellites* described in other organisms (5, 6, 12). One of these regions is illustrated in Fig. 8. A less frequently encountered image is illustrated in Fig. 13. In this case the dense material in which the microtubules insert is not associated with the Golgi complex and has either a rod or plaquelike configuration. No structures corresponding to *centrioles* themselves have so far been observed. The microtubules appear to be excluded from the hyaline cytoplasm.

We have observed in these cells nothing which would indicate the presence of symbionts (see reference 47).

Nucleus

The trophozoite of *Acanthamoeba* is typically uninucleate, but multinucleate individuals are numerous in laboratory cultures grown with constant agitation (10). In fixed specimens the nucleus is lobate to varying degrees. It is mildly scalloped in some cases (Fig. 14) and deeply cleft by the cytoplasm in others. As observed in both living and fixed cells, the nucleus is easily deformable, and sometimes it is severely constricted as it passes through a narrow neck of cytoplasm. We have seen no fibrous lamina lining the nuclear envelope, as found in the giant amebae (17, 26, 33). The membranes of the nuclear envelope are

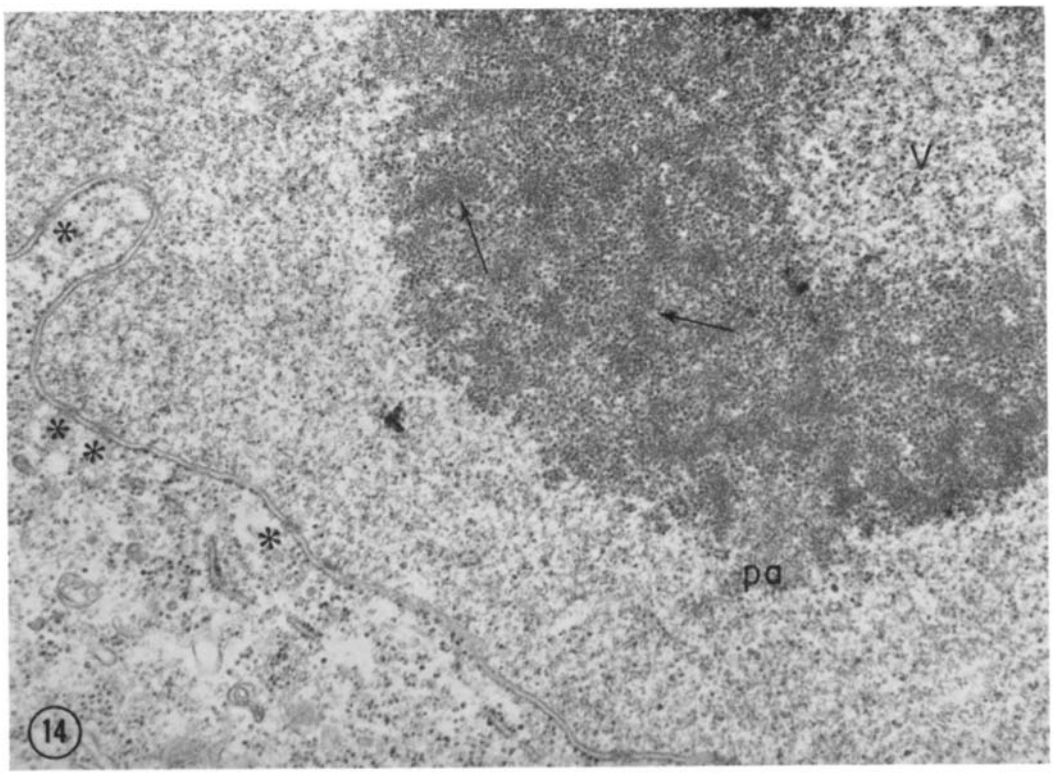

FIGURE 14 Nucleus of a cell from an agitated culture, 8 days after subculture. The nucleolonema (arrows) is surrounded by ribosome-like granules. A segment of the pars amorpha is shown at *pa*. Nucleolar vesicle, *V*; nuclear pores, asterisk. × 26,600.

separated by a distance of about 350 A, and nuclear pores are numerous. The pores are about 1,040 A in diameter and have a central dense granule. Ribosomes are present on the outer membrane of the envelope (Fig. 14).

The *nucleolus* is large, about 2.4 μ in diameter, and occupies approximately one-eighteenth of the nuclear volume. It is variable in morphology; it sometimes appears diffuse (Fig. 1) but is more often compact and spherical (Fig. 4). It is frequently vesiculate, often to the extent of appearing ring-shaped in cross-section. Two nucleoli per nucleus are not uncommon. The substructure of the nucleolus conforms to that of nucleoli seen in most animal cells (Fig. 14). The major component is the nucleolonema, a network of densely staining fibrillar material. The interstices of the nucleolonema are filled with clouds of granules similar in size and appearance to cytoplasmic ribosomes. The pars amorpha is evident as broad fibrous bands which stain less intensely than the nucleolonema. Nuclear regions outside the nucleolus usually appear homogeneous and consist of evenly dispersed, fine fibrillar and granular material (Figs. 1, 14). In some cases, however, the chromatin is differentially stainable (Fig. 4).

DISCUSSION

In main outline *Acanthamoeba* does not differ greatly at the ultrastructural level from a mammalian cell. We can see very little in the nucleus or among the cytoplasmic components which seems to be uniquely protozoan, and we are reassured therefore that this organism represents an appropriate object of study for a "cell biologist." Several of the structures described (Golgi complex, microtubules, fibrils of the hyaloplasm, and smooth ER) have not been consistently found in amebae, probably because of fixation difficulties. In general our observations confirm and extend those of Vickerman (47) on *Acanthamoeba*,

but several new observations in this study deserve brief comment.

Of particular interest in elucidating the transport properties of *Acanthamoeba* is the observation of narrow, tubular invaginations of the cell surface which end in coated pits. Such pits are especially prominent on the surfaces of cells known to be engaged in protein uptake (2, 8, 38) and in several different systems have been demonstrated to take up peroxidase or ferritin (16, 37, 44). It is conceivable that these pits play a similar role in amebae and provide sites for ingestion of macromolecules. We have no estimate of the quantitative importance of the surface pits, but their presence should probably be considered in analyses of uptake properties of these cells.

In a recent study of the genus *Acanthamoeba* with the light microscope, Page (32) did not observe any centrioles; nor have we encountered any with the electron microscope. Negative findings, especially with the electron microscope, are not always convincing, but the fact that *Acanthamoeba* lacks a flagellate phase in its life cycle (32) is at least consistent with a lack of centrioles.

In interphase *Acanthamoeba* cells microtubules are commonly found inserted in a dense material that resembles centriolar satellites (12, 15). These focuses of microtubules are frequently found in the center of a Golgi complex, which is often the location of centrioles in other cell types (3, 6). Occasionally microtubules are found radiating from an elongate aggregation of dense material, as illustrated in Fig. 13, as if from an aggregation of satellites. A similar situation has been reported recently in the yeast cell, *Saccharomyces*, by Robinow and Marak (36) and in the fungus, *Coprinus*, by Lu (24). In both cases spindle microtubules have been observed to converge on dense bodies which lack the associated ring of nine triplet tubules found in centrioles and basal bodies. In *Acanthamoeba* the dense material probably functions as a "centriolar equivalent" for the organization of cytoplasmic and spindle microtubules without the presence of organized structure of the basal body type. This finding perhaps serves to emphasize the importance of the less well-defined components of the centriolar complex in carrying out some of the centriole's presumed cellular functions, such as determination of cell polarity.

It is generally agreed that the major function of the water-expulsion vesicle (WEV) in protozoa is to control the water content of the organism, but almost nothing is known about the way in which this function is carried out (see reference 19 for recent review). Schmidt-Nielsen and Schrauger (39), using a micropuncture technique, have actually measured the osmolality of the vesicle fluid in *A. proteus* and find that the fluid is hypoosmotic to the cytoplasm. For several reasons they believe that the best working hypothesis is that an iso-osmotic fluid is secreted and salt is reabsorbed. Presumably these two events are spatially separated and require at least two "compartments." The spongiome of *Acanthamoeba* contains at least two morphologically distinct components (lined tubules and ampullae or vesicles) which presumably represent functionally different elements. Operations resulting in water secretion may occur exclusively in the spongiome, and the water-expulsion vesicle itself may be a relatively impermeable reservoir. The macromolecular lining of the tubules cannot be interpreted at the present time, but it is interesting that mucopolysaccharide layers have been demonstrated along secretory channels in certain salt-secreting cells, e.g., in reptiles (14).

It seems likely that the tubular spongiome in *Acanthamoeba* is functionally equivalent to the spongiome surrounding the WEV in other protozoa, despite some striking morphological differences. The WEV of the ciliates has been examined in greatest detail (11, 40). These ciliates, unlike the amebae, have a WEV that is fixed in position. They have two distinct kinds of tubules in the spongiome: one type is a smooth-walled, highly convoluted tubule, and the other, which has a larger diameter, is a thicker-walled (40) or coated (11) tubule, groups of which are organized into fasciculi. Ampullae and varicosities are present in the system of convoluted tubules (11). *Acanthamoeba* appears to contain only a system corresponding to the convoluted tubule portion of the ciliate spongiome, and the tubules are 2–3 times larger in diameter than those of the ciliates.

This study has benefited from the excellent technical assistance of Mr. Thomas Olszewski.

Received 5 April 1968, and in revised form 23 May 1968.

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