AN ELECTRON MICROSCOPIC STUDY OF PINOCYTOSIS IN AMEBA

II. The Cytoplasmic Uptake Phase

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ABSTRACT

This report is devoted principally to a consideration of the fate of the pinocytotic vacuole and its content in the ameba *Pelomyxa carolinensis* (*Chaos chaos*). High resolution micrographs of the plasmalemma have shown it to consist of three layers, *i.e.*, an outermost filamentiferous zone, a middle homogeneous zone, and an inner zone which appears to be a unit membrane. The three zones can be identified in the membranes lining the pinoyctotic tunnels and vacuoles of amebas fixed shortly after pinocytosis occurred. The first apparent change in the pinocytotic vacuole is an increase in the surface-to-volume ratio which occurs during the 1st hour of its existence. Within 24 hours the marker substance commonly collects in defecation vacuoles which can be identified by the profiles of bacteria usually found in the lumen. Occasionally, however, thorotrast can be seen in the lumen of the contractile vacuole. The thorotrast appears to enter the two excretory organelles by the coalescence of vesicular fragments of the pinocytotic vacuoles with the limiting membranes of the excretory organelles.

INTRODUCTION

Pinocytosis appears to be a device for coupling the unique properties of the surface membrane to the physiological and biochemical activities of the cytoplasm. Part I (6) of this series of reports was concerned with the demonstration of the site of attachment of pinocytosis-stimulating substances to the plasmalemma of ameba. This subsequent report is devoted principally to a consideration of the fate of the pinocytotic vacuoles consequent to the adsorption of substances onto the plasmalemma as well as to further studies on the nature of the initial adsorption site. Higher resolution of the plasmalemma obtained by studying Araldite-embedded amebas has demonstrated that it is a laminated structure and that only the most superficial layer seems to be involved in the initial adsorption.

A number of hypotheses concerning the fate of pinocytotic vacuoles were tested in part at least by exposing amebas to thorium dioxide suspensions or ferritin solutions for a short interval during which some of the particles were pinocytosed. At a series of intervals after this exposure the cells were fixed and prepared for study in the electron microscope.

Earlier (4, 6) it was suggested that the adsorption to the plasmalemma of molecules in solution, or particles in suspension, evokes pinocytosis in the test cells. Since the adsorption of solute transforms it to another phase, it is noted that ultimately the cell may be responding not to a solution but perhaps to a solid. In this respect there appears to be a close relationship between pinocytosis and phagocytosis. It may be useful, how-

ever, to continue to distinguish between the two until they are more fully understood.

MATERIALS AND METHODS

The culture and fixation techniques employed in this study of pinocytosis, using the ameba *Pelomyxa carolinensis* (*Chaos chaos*), were reported previously (6). In addition to the methacrylate embedding technique employed in the earlier study, Araldite embedding was used according to the technique of Bunge *et al.* (7). The final embedding mixture was 10.0 ml Araldite 502, 10.0 ml of dodecenyl succinic anhydride, 1.0 ml of dibutyl phthalate, and 0.3 ml of tridimethylaminomethylphenol. The mixture was hardened for 1 to 4 weeks in an oven set at 48°C. Sections were cut on an LKB or a Porter-Blum microtome, mounted on Formvar-coated grids and stained for 2 hours with 7.5 per cent aqueous uranyl acetate. The sections were lightly coated with carbon and examined in an RCA EMU 3-F electron microscope.

The earlier report (6) of this series described experiments in which amebas were exposed to ferritin solutions at a pH of 6.8. However, other work (19) suggested that ferritin was more readily and strongly bound to the plasmalemma nearer to its isoelectric point (pH 4). Therefore, in this study, some amebas were briefly exposed to ferritin solution at approximately pH 4.8.

Ferritin (solution A) at pH 6.8 was prepared and used as described previously (6). To prepare solution B, ferritin (purchased from Pen Tex Corp., Kankakee, Illinois, as 1 gram of ferritin in 10 ml of solution) was briefly shaken with Amberlite MB-1, filtered, and brought to pH 4.8 with 1/100 N HCl. For the experiments, one volume of culture solution carrying the amebas was mixed with one volume of ferritin solution. In addition, suspensions of thorium dioxide (0.01 per cent "thorotrast") were used under conditions reported earlier (6). The amebas were exposed to the test solution for 5 to 10 minutes. They were either fixed immediately, or were returned to the culture fluid for various intervals before fixation.

RESULTS

The earlier report (6) described the attachment of ferritin and thorium dioxide to the hair-like or filamentous extensions of the ameba plasmalemma. It was suggested that these extensions composed the extraneous coat of the surface membrane. In order to elucidate the relationship of the filamentous extensions to the plasma membrane and to follow any changes in fine structure of the membrane complex which may occur after its incorporation into vacuoles, high resolution micrographs of Araldite-embedded amebas were obtained.

Fig. 1 is an electron micrograph of a portion of an Araldite-embedded ameba which was exposed to ferritin (solution B) for 5 to 6 minutes before fixation. The filaments of the plasmalemma, which appear as soft, irregular strands coated with ferritin molecules, seem to arise from an underlying amorphous layer 200 A thick. Immediately below the amorphous layer are two dense 20 A thick lines separated by a 40 to 50 A space.

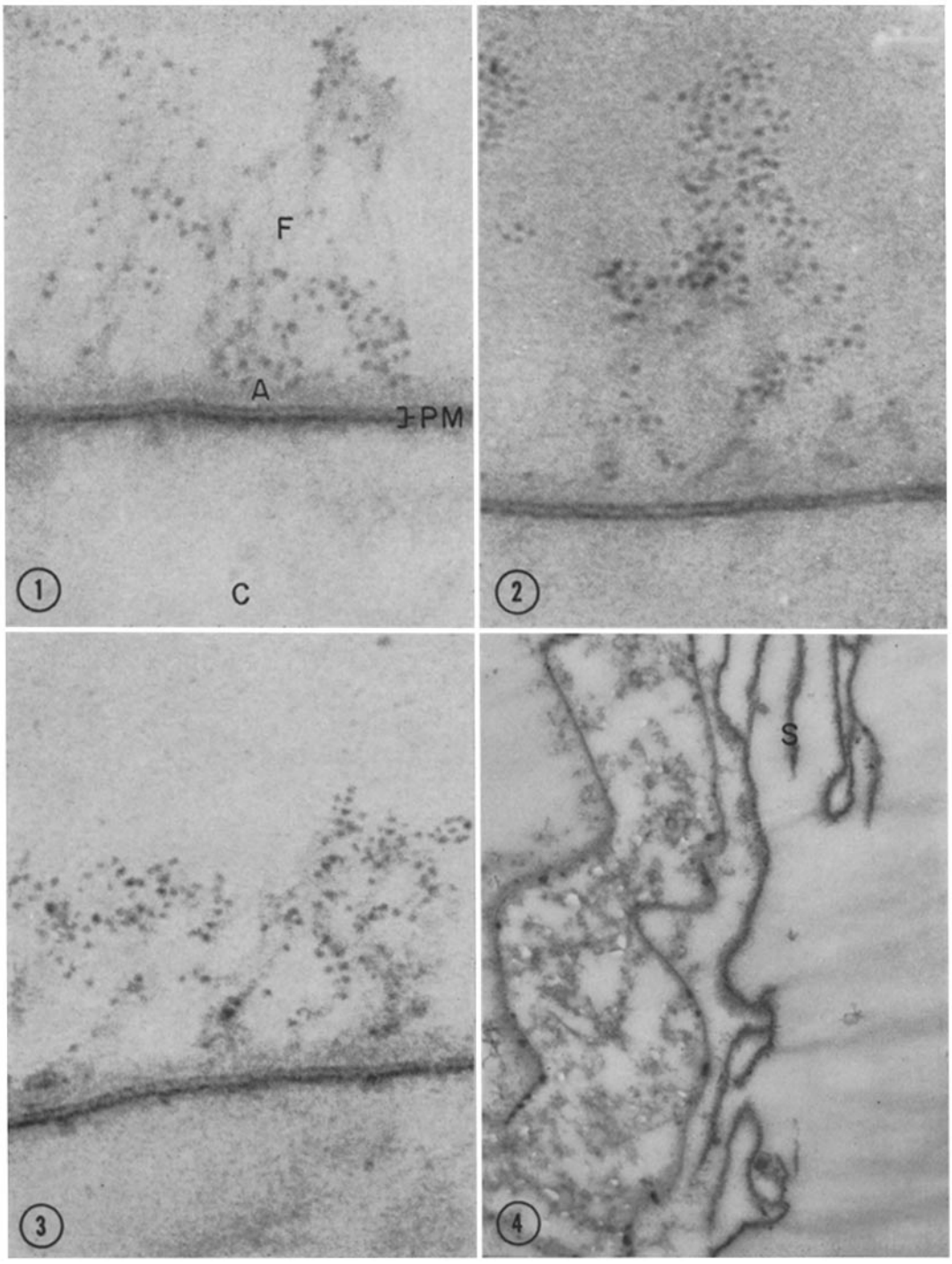
The structure of the complex surface zone is summarized in Fig. 5. Its structure may be compared with the structure of the membrane which limits a pinocytotic vacuole or tunnel. Fig. 2 is a portion of the sheath of a tunnel taken from a cell which was fixed during exposure to the test solution. The structure of the membrane forming the wall of a pinocytotic vacuole is shown in Fig. 3.

FIGURES 1 TO 3

Electron micrographs of the plasmalemma, pinocytotic tunnel membrane, and pinocytotic vacuole membrane, respectively. The filamentiferous zone (*F*), amorphous zone (*A*), plasma membrane (*PM*), and cytoplasm (*C*), are similarly located in all three. Numerous ferritin molecules are attached to the filaments and to the outer surface of the amorphous zone, although no ferritin penetrates below the surface of this homogeneous layer. The amorphous coat therefore represents the initial permeability barrier to ferritin. These figures were taken from cells exposed to ferritin solution approximately 10 minutes. Araldite embedding. × 240,000.

FIGURE 4

Demonstrates dense thick ribbons of ferritin (*S*) adjacent to the plasmalemma. It is probable that these ribbons of ferritin were initially attached to the filamentous extensions of the plasmalemma. Due to the inelasticity that such concentrations imparted to the filaments, a splitting resulted in the filamentiferous zone during cellular movement. Araldite embedding. × 21,000.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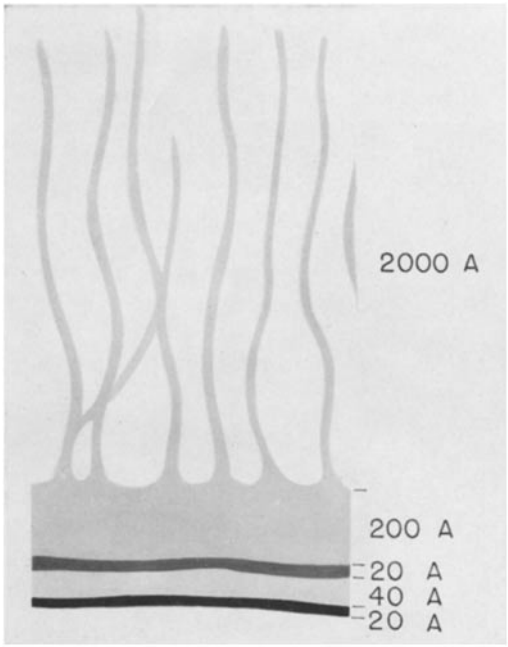


FIGURE 5

This is a diagrammatic representation of the structure of the plasmalemma of *Pelomyxa carolinensis* (*Chaos chaos*). The 2000 A thick layer is termed the filamentiferous zone, and it appears to be a continuation of an amorphous zone 200 A thick beneath it. A zone composed of two 20 A dense lines separated by a 40 A space may represent the plasma membrane. It is evidently a unit membrane similar to that described by Robertson (28).

In this latter figure the hair-like extensions appear to be less defined than the filaments on the free surface (Fig. 1). However the homogeneous zone lying above the plasma membrane unit is readily visible. It is apparent from Figs. 1 to 3 that the ferritin molecule does not penetrate the "homogeneous" zone of the surface membrane, even after the membrane is incorporated into a pinocytotic vacuole for 10 minutes as in Fig. 3.

The amount of the ferritin attached to the filamentous extensions in Fig. 1 is somewhat greater than that reported previously (6) when ferritin was adsorbed from a solution at pH 7.0. Indeed, in these experiments which utilized ferritin at a pH of 4.8, instead of a pH 7.0, splitting of the filamentiferous zone accompanied the heavy accumulations of adsorbed ferritin (Fig. 4). The aggregation of ferritin into a dense layer which splits from the cell surface paralleled the splitting which occurred when cells were exposed to high concentrations of thorium dioxide (6) and gamma globulin (4). The plane of splitting when ferritin was densely adsorbed appears to be close to the free tips of the filaments, whereas the plane of split reported for experiments using thorium dioxide suspensions was near the base of the hairs (6).

Figures 6 to 8 demonstrate several different forms of pinocytotic tunnels and droplets which may represent the various stages of formation of pinocytotic vacuoles. In Fig. 6 a pinocytotic tunnel (*T*) is seen coiling from the cell surface into the cytoplasm. It appears to bifurcate shortly below its continuity with the cell surface. The filamentous coating of the plasmalemma both on the cell surface and in the tunnels is heavily encrusted with particles of thorium dioxide. In the cytoplasm a dense aggregation of thorium dioxide particles (*PV*) can be seen which is the result of an exposure to the test solution 4 hours earlier. The amebas were fixed immediately after the second 15-minute exposure to the test solution.

A portion of a tunnel evoked by an exposure to ferritin solution B can be seen in Fig. 7. Examination shows clumps of ferritin molecules attached to the filamentous extensions of the plasmalemma. In Fig. 8 a small tunnel, a pinocytotic vacuole with thorium attached to the walls, and part of one with the thorium confined to the center are found. The cell was fixed during exposure to the test solution.

Figures 9 to 12 demonstrate some of the changes which occur in the vacuoles in the first four hours after formation. In Fig. 9 the vacuoles PV_1 and PV_2 have irregular outlines, their contents are dispersed, and they are surrounded by numerous small vesicles containing particles of thorium dioxide. The small vesicles which contain particles may be fragments or extensions of the large vacuoles. In Fig. 10 the surface area of the pinocytotic vacuole is markedly extended by numerous projections of the wall into the lumen of the vacuole. In Fig. 11 a somewhat irregularly shaped vacuole densely packed with ferritin is seen, while in Fig. 12 the ferritin is much more dispersed and the surface-to-volume ratio of the vacuoles is much increased.

Contrary to the tendency of the pinocytotic vacuoles to have increased surface-to-volume ratios with time is the occasional appearance of contracted vacuoles containing thorium dioxide particles (Fig. 13). Since these are encountered in

amebas which were exposed to the test solution 24 hours prior to fixation, they may represent a type of defecation vacuole containing the marker.

Defecation vacuoles in amebas normally appear to contain many bacteria. However, in amebas treated with thorium dioxide the defecation vacuoles frequently contain in addition a layer of the tracer particles (Fig. 14). Membranous projections of the defecation vacuole suggest that a process of cytoplasmic exchange mediated by membranous sacs was occurring at fixation. Particles can be seen in small and large vacuoles in the near-by cytoplasm. It is evident that, as an indigestible inclusion, thorium dioxide is eventually segregated into these excretory organelles. However, fluid recovery from the defecation vacuole mediated by membrane-bounded vacuoles may recycle the marker into the cytoplasm.

Occasionally, thorium dioxide is found in the contractile vacuole (Fig. 15). This is a rather infrequent finding, and it may not represent a major mode of excretion of these particles, yet it is of interest since the particles mark a pathway of fluid transfer from the pinocytotic vacuole to the contractile vacuole. Such a concept is suggested by the appearance of small, particle-containing vesicles frequently continuous with large vacuoles containing thorotrast particles (Figs. 16, 17, at

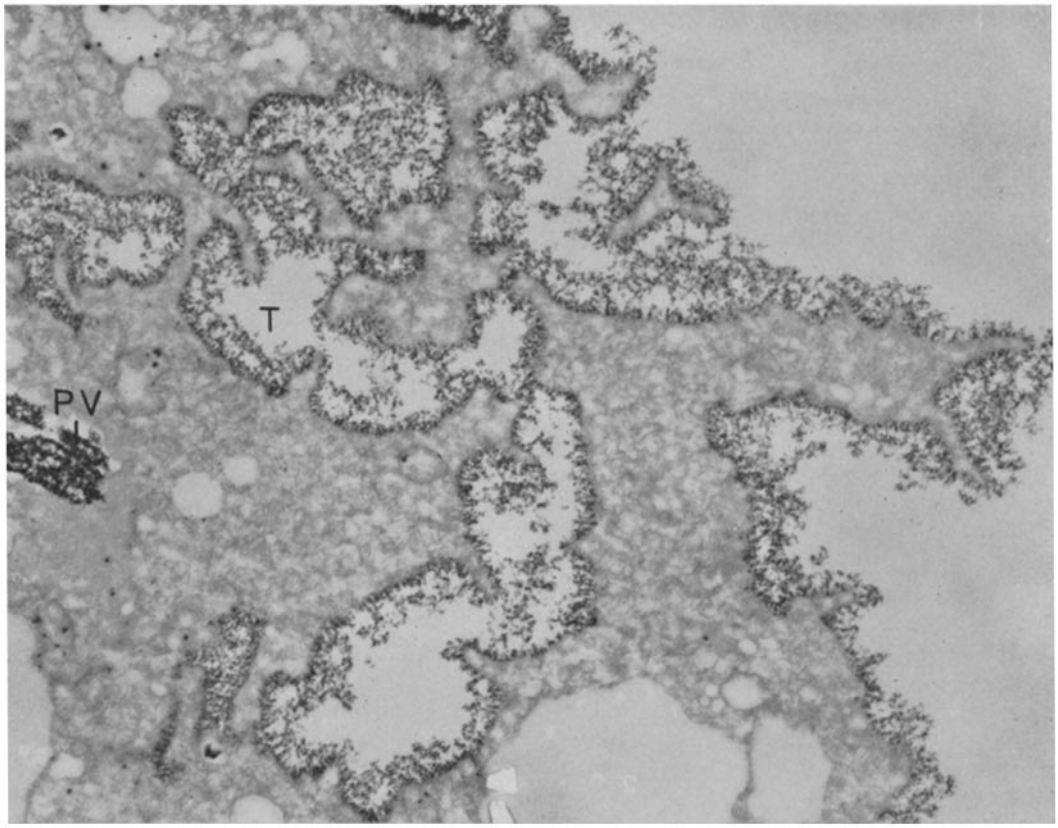


FIGURE 6

An electron micrograph of a pinocytotic tunnel (*T*) which coils from the cell surface into the cytoplasm. It appears to bifurcate shortly below its attachment to the cell surface. The filamentous extensions of the plasmalemma on the cell surface and in the tunnels are heavily encrusted with particles of thorium dioxide. In the cytoplasm a dense aggregation of membrane-limited particles (*PV*) is found which is the result of an exposure 4 hours earlier to the test solution. The cell was fixed immediately after the second 15-minute exposure to the test solution. Methacrylate embedding. × 7,650.

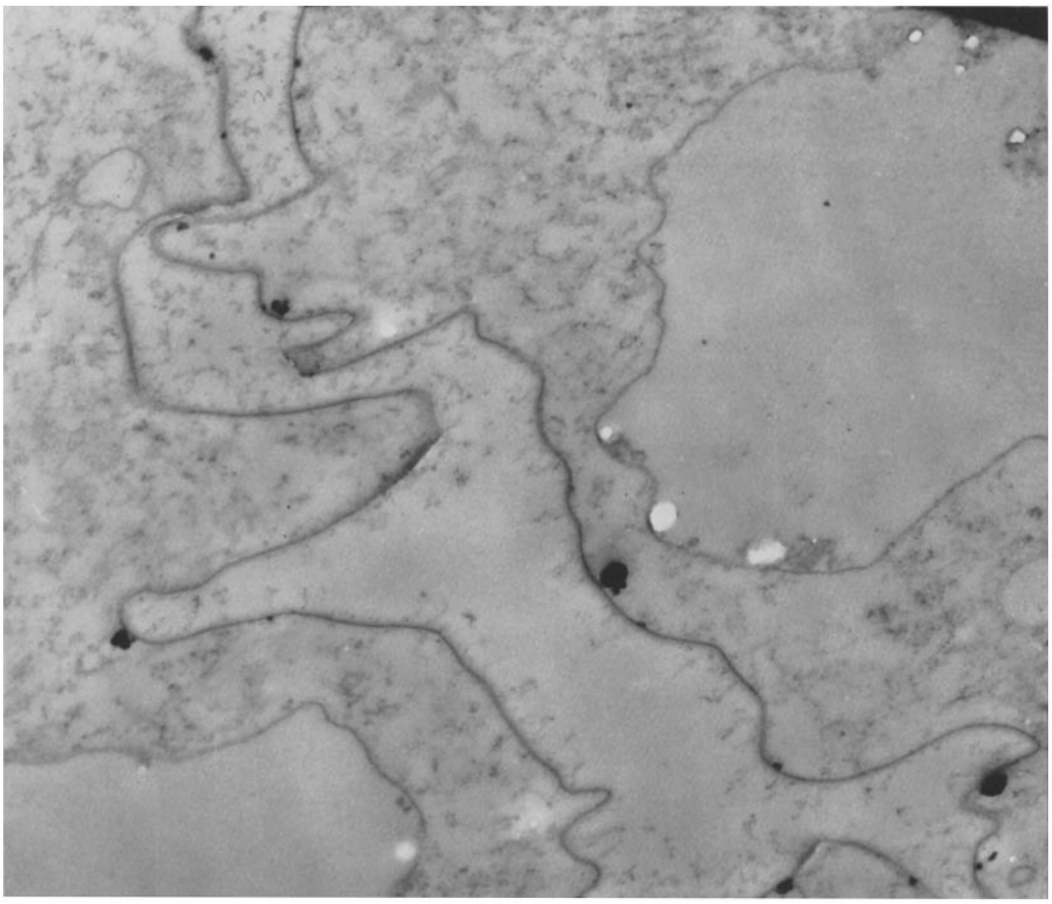


FIGURE 7

A portion of a pinocytotic tunnel evoked by the exposure of the cell to ferritin solution B. Clumps of ferritin molecules appear to be attached to the filamentous extensions of the plasmalemma. See Fig. 2 for a higher power of the tunnel. Araldite embedding. × 22,000.

arrows). The small thorotrast-marked vesicles resemble the numerous vesicles surrounding the contractile vacuole. Some of the small vesicles in the zone of the contractile vacuole contain particles (double arrows, Figs. 16, 17). It appears that the small vesicles surrounding the contractile vacuole add their content to it during diastole (26).

DISCUSSION

The first stage in pinocytosis is the change in phase of a component of the environment. This component becomes attached to the filamentous extensions of the ameba plasmalemma by a process which presumably requires a redistribution of energy in the system. It was suggested in part I (6) of this series and earlier (4) that the plasmalemma–medium interface tension is lowered when the pinocytosis-stimulating agent is adsorbed onto the extraneous layer of the plasmalemma.

In Fig. 1 the filamentous extensions of the plasmalemma are seen to arise from a homogeneous layer of medium electron transparency. Immediately beneath the homogeneous zone are two opaque lines separated by a relatively transparent space. Although the two dense lines measure 20 A each, which is substantially in agreement with Robertson's (28) data for other cell types, the transparent space separating them is 40 to 50 A or somewhat wider. It is not evident whether the wider spacing of the plasmalemmal

membrane components (Fig. 1) represents a normal structural feature of *P. carolinensis*, or is due to the preparation techniques used, since a closer spacing of the two dense lines has been reported for *Amoeba proteus* (17). The wide spacing of the two 20 A lines is not found in the membranes of the cell organelles, however, for the dense lines are spaced only 20 to 30 A apart and are similar in spacing to Robertson's (28) unit membrane.

It can be seen that the newly formed pinocytotic vacuole (Fig. 3) has a spacing of the unit membrane similar to that of the plasmalemma. Since the spacing of this unit membrane differs from that of the other membranes of the cyto-

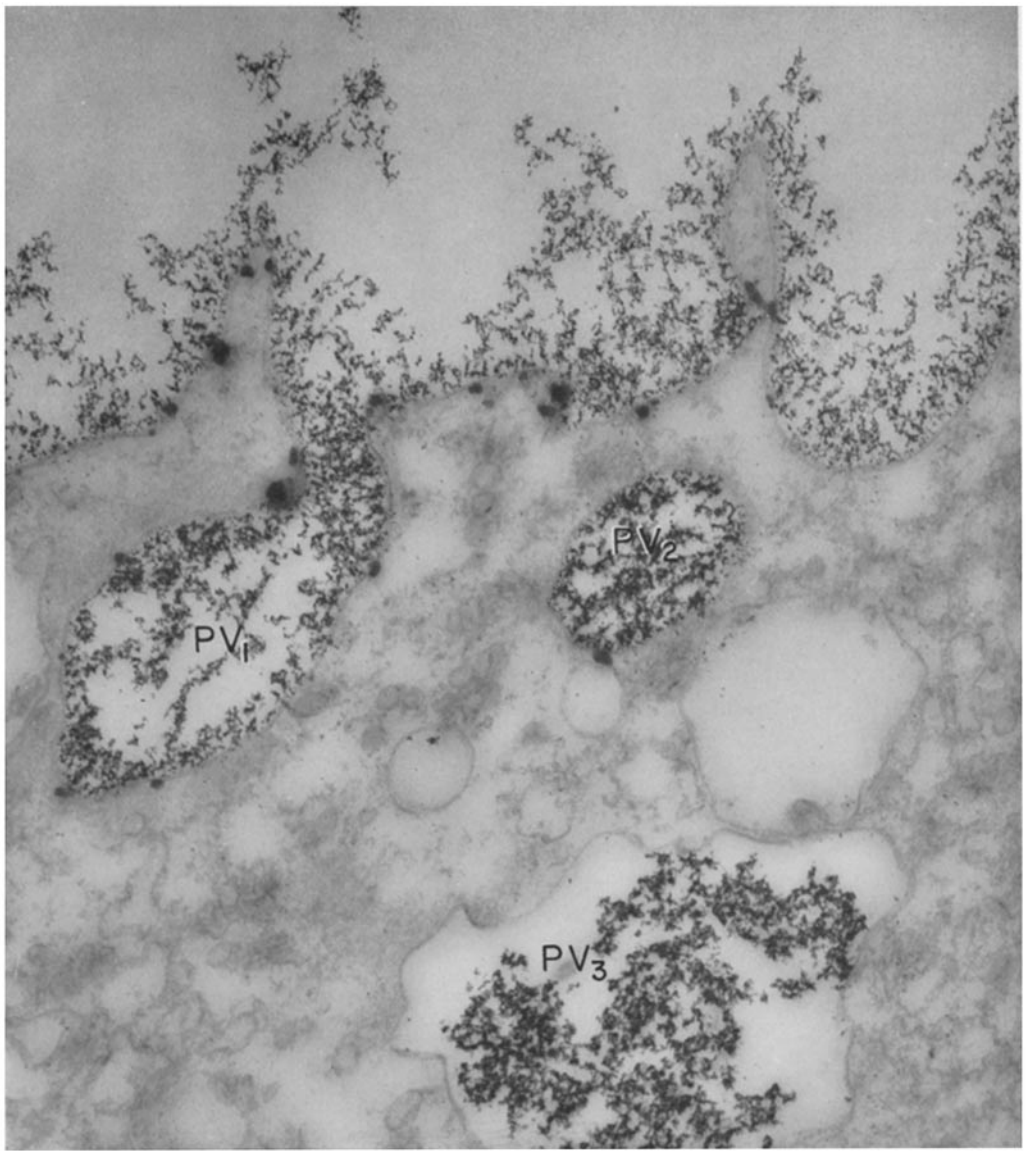


FIGURE 8

Demonstrates what appears to be a small pinocytotic vacuole (PV_1) in the process of being internalized. The vacuole PV_2 appears completely detached from its surface origin while the contents of the vacuole PV_3 are no longer attached to the limiting membrane. Methacrylate embedding. × 25,000.

plasm, further studies must be made to determine whether or not the membrane of the pinocytotic vacuole alters with time to conform to other cytoplasmic membranes.

It would appear reasonable to designate the zone consisting of two opaque 20 A lines separated by a 40 A space as the plasma membrane, *i.e.* a unit membrane forming the major permeability barrier to water (6), and the 200 A thick amorphous zone and 2000 A thick filamentiferous zone as an "extraneous" coat (8). The first stage in pinocytosis therefore is predominantly a reaction between the fluids bathing the cell and the extraneous coat of the plasmalemma.

It is evident (Figs. 1 to 3) that the homogeneous zone of the extraneous coat is impermeable to ferritin molecules, therefore it too must be considered as a factor in the over-all permeability of the cell to solute. Such extraneous coats may operate as an initial filter and prevent or retard the diffusion of large molecules into the plasma membrane. It is evident that ferritin at pH 4.8 (Fig. 1) penetrates the filamentiferous zone more deeply than ferritin at pH 7.0 (6). This evidence supports the suggestion that molecular charge is an important factor in determining not only the adsorption properties but also the permeability properties of the extraneous coat.

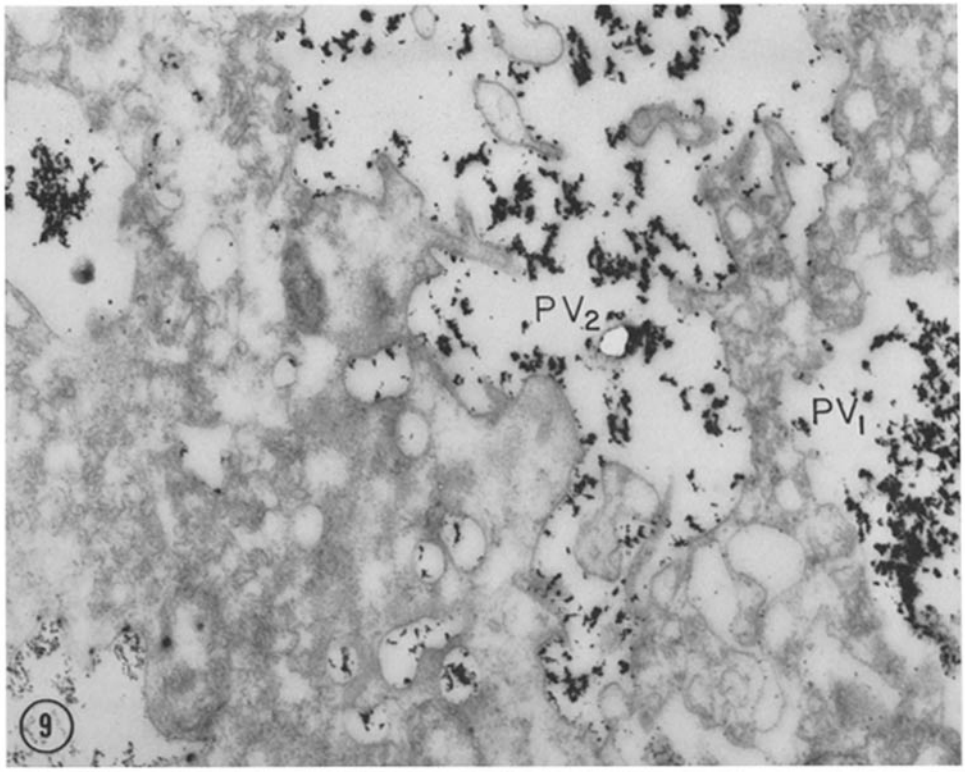
The second phase of pinocytosis involves the incorporation of the plasmalemma and the adsorbed material into pinocytotic inclusions or vacuoles. In what fashion and by what mechanism the presence of solute or particles adsorbed to the plasmalemma stimulates the cell to ingest its membrane is not known. It is conceivable that the redistribution of interfacial energy brought about by the adsorpton of solute produces a change in the plasmalemma which may be the "trigger" initiating the second phase of pinocytosis (4).

The invagination of the cell membrane to form the limiting membranes of pinocytotic tunnels and vacuoles has been demonstrated by a number of techniques (4, 6, 10, 13). It was demonstrated previously with the fluorescence microscope that the tunnels and vacuoles are lined with a concentrated coat of the agent which stimulates pinocytosis (4). Chapman-Andresen and Nilsson (10) published electron micrographs of pinocytotic channels; however, the techniques used by them did not resolve the extraneous coats nor was the pinocytosis-stimulating substance visualized. In Figs. 2, 3, 6 to 8, various stages of the incorporation of the cell membrane into pinocytotic vacuoles can be seen. These findings confirm the earlier light microscopic evidence (4) which suggested that pinocytotic tunnels and vacuoles are lined with a coating of the pinocytosis-evoking agent.

Evidence presented by Chapman-Andresen and Holter (9) suggests that the permeability of the pinocytotic vacuolar membrane changes within 45 minutes after the membrane transfers from the cell surface to an inclusion vacuole. Fig. 2 and 3 show the fine structure of the membrane lining a pinocytotic tunnel and newly formed vacuole. The morphology of the plasma membrane unit does not appear to have changed from that of the plasmalemma (Fig. 1), although the filamentiferous zone of the extraneous coat is less well defined in the vacuole. However, the membranes pictured in Figs. 2 and 3 were intracytoplasmic 10 minutes or less, whereas the membranes of the pinocytotic vessels observed by Chapman-Andresen and Holter (9) were studied 45 minutes after incorporation. It may be that changes occur in the fine structure of the membrane within 45 minutes. All of the figures of pinocytotic vacuoles which were intracytoplasmic more than 10 minutes were of cells embedded in methacrylate. Similarly treated cells embedded in Araldite must be studied to determine the exact structure of the

FIGURES 9 AND 10

The varieties of pinocytotic vacuoles which are found in the cytoplasm 20 minutes to several hours after the exposure to the test solutions. In Fig. 9, PV_1 has a fairly regular contour whereas PV_2 has become quite irregular in outline. The limiting membrane of the large vacuole in Fig. 10 is extensively folded; extensions of the vacuole project into the cytoplasm, and microvillous extensions from the cytoplasm project into the vacuole. It appears that there is an increase in the membrane volume ratio of the pinocytotic vacuole after the first 20 minutes of intracytoplasmic existence. It is suggested that this is a progressive change which is coincident with the onset of the digestion phase. Methacrylate embedding. × 23,000.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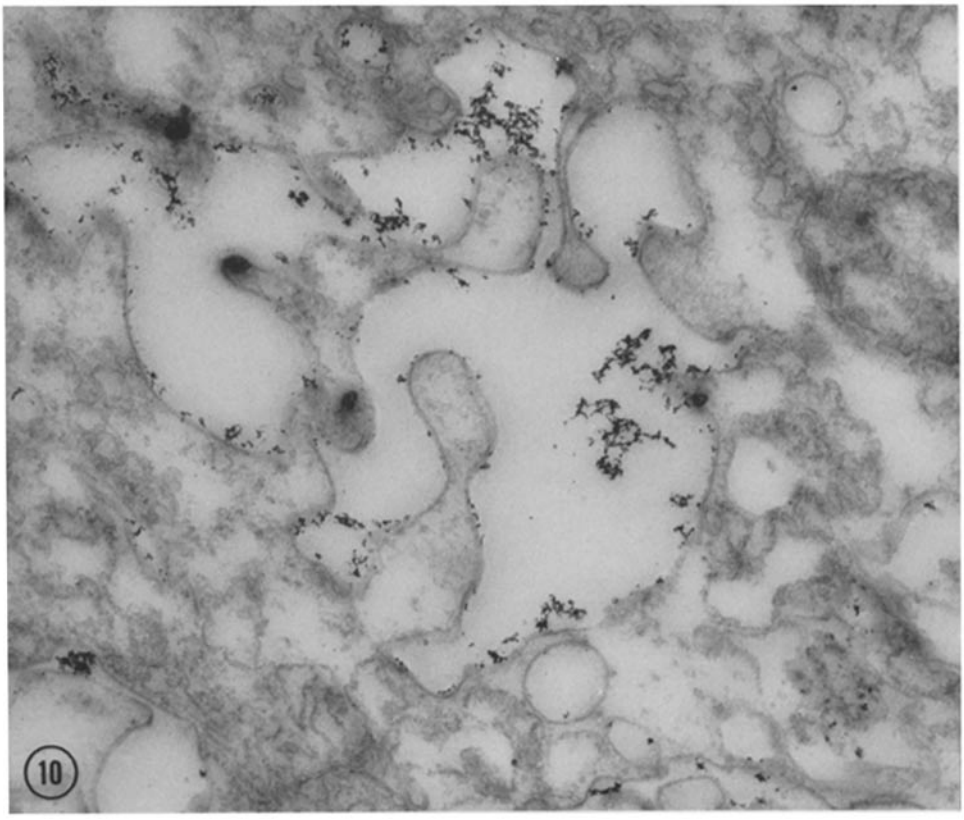
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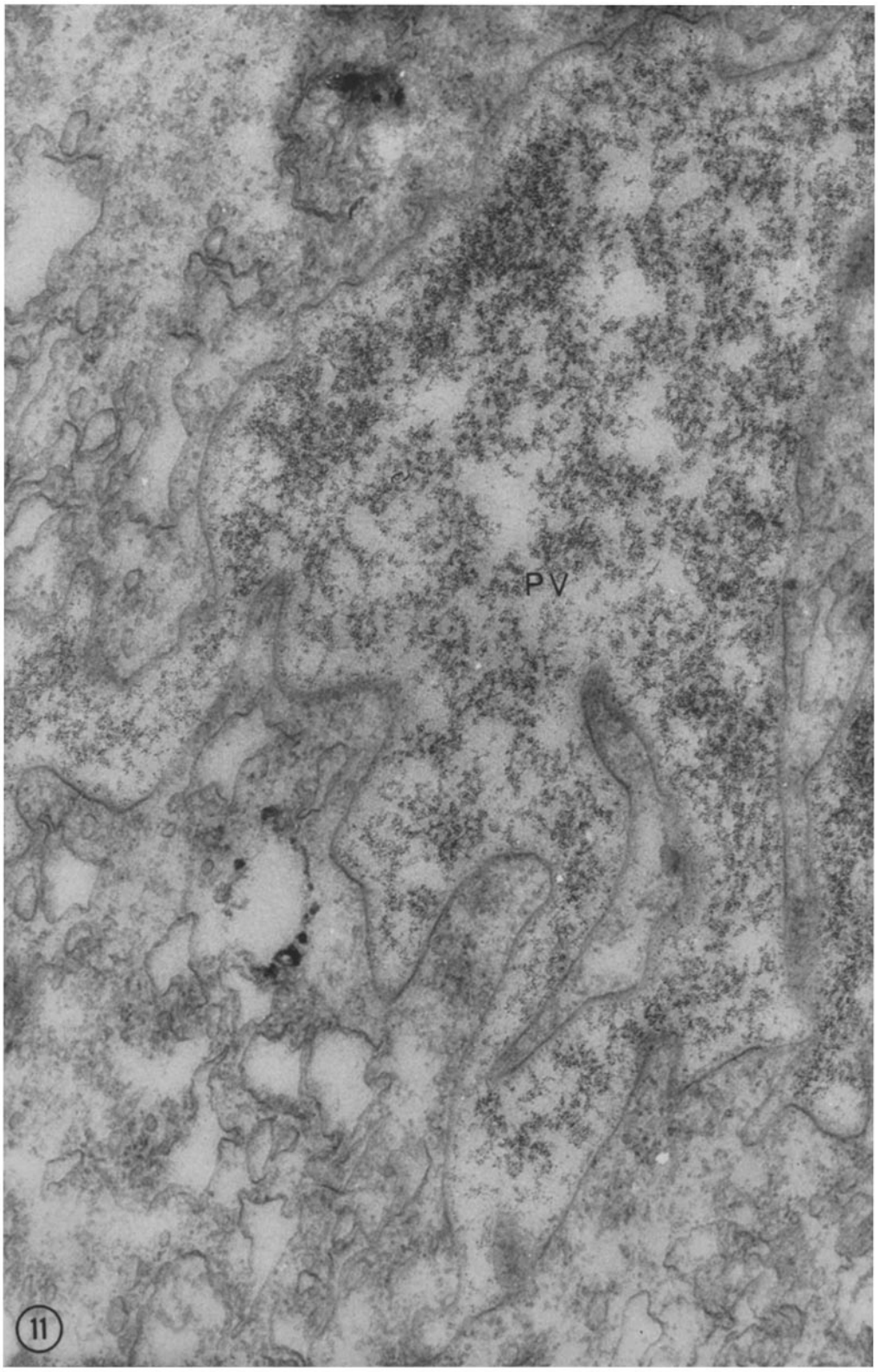
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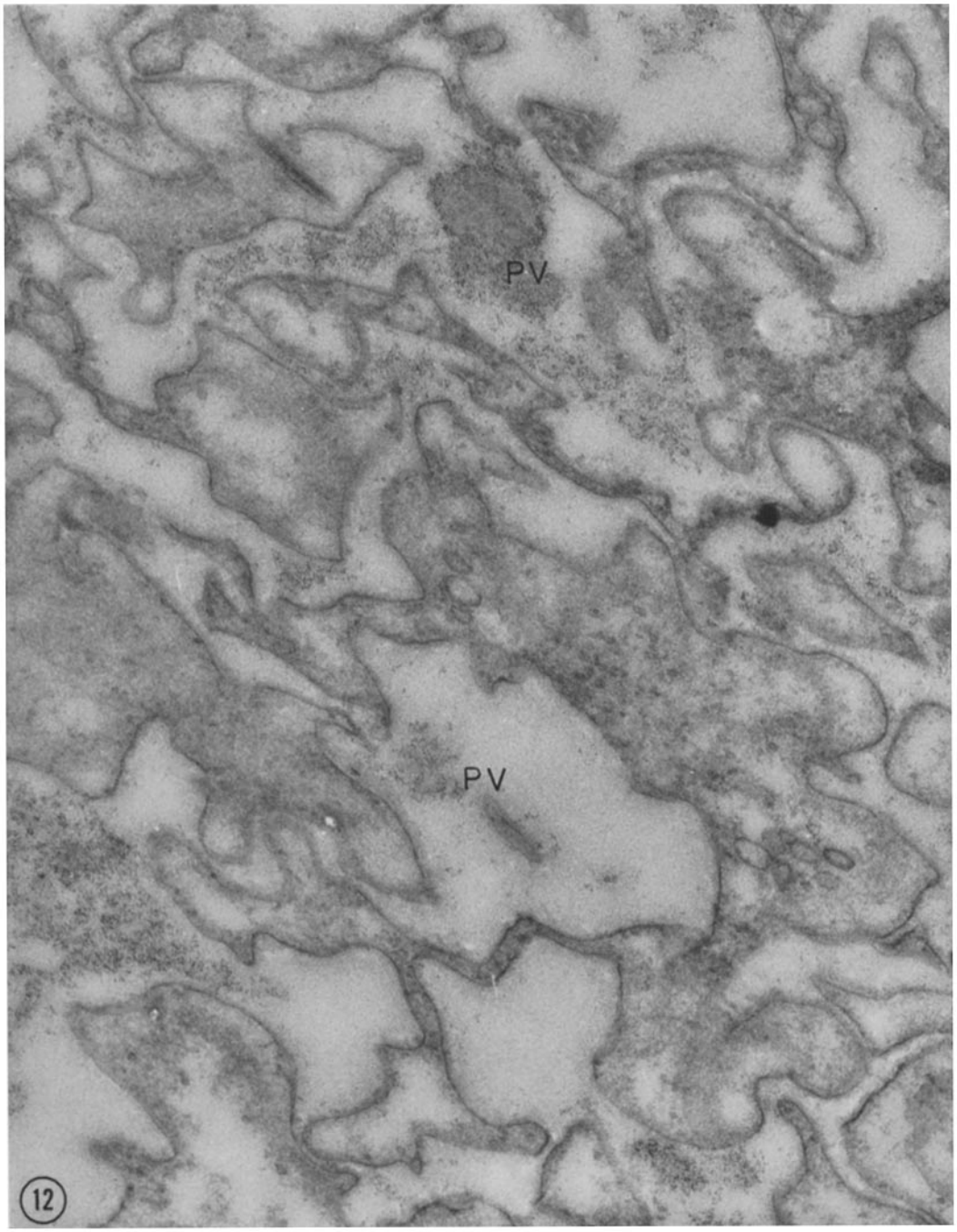
FIGURES 11 AND 12

The changes which occur in pinocytotic vacuoles (*PV*) between 10 minutes (Fig. 11) and about 1 hour (Fig. 12) when the test substance is ferritin. There are several changes which can be correlated with time. The image of the ferritin becomes increasingly "fuzzy" and it tends to be found in the lumen and not associated with the limiting membrane. The surface-to-volume ratio of the pinocytotic vacuole appears to increase enormously between 10 minutes and 1 hour. It is suggested that the change in ratio is coincident with digestion. Fig. 11, × 40,000; Fig. 12, × 52,500.



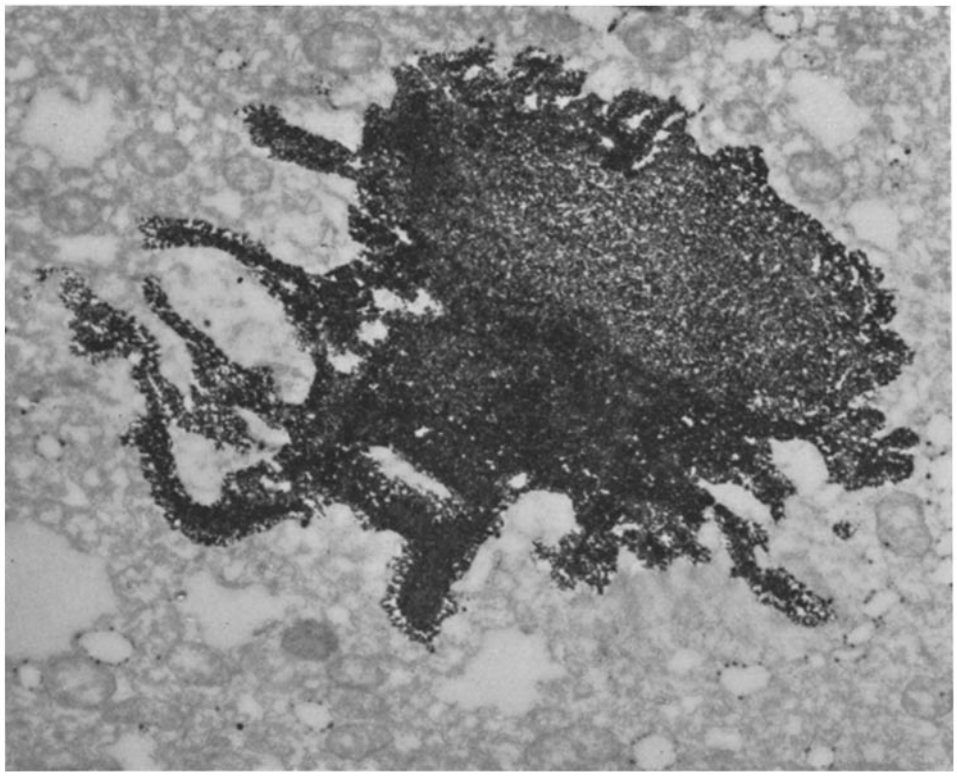


FIGURE 13

An electron micrograph of a vacuole compactly filled with thorium dioxide particles. The contents of the vacuole were accumulated in the ameba by pinocytosis 24 hours previous to fixation. It is probable that this indigestible material has been segregated from the highly diluted material found in pinocytotic vacuoles which were intracytoplasmic about an hour. It is suggested that this vacuole is a defecation vacuole without a complement of bacteria. Methacrylate embedding. × 16,000.

vacuole membrane since the unit membrane is not easily resolved in methacrylate-treated material.

The Fate of the Pinocytotic Vacuole and Its Contents

The pathway that pinocytosed material can take through the cells was demonstrated in part by following the course of thorium dioxide particles. It was possible to trace these particles from the pinocytotic vacuoles through various vacuolar systems in the cytoplasm, and into the excretory organelles of the amebas. At no time was pinocytosed material found free in the cytoplasm; it was always enclosed by membranes. The most apparent change in the pinocytotic vacuole is the rapid and large increase in the membrane-to-volume ratio (Figs. 10 and 12). By the mechanism of numerous infoldings and outpocketings of the vacuole membrane the surface-to-volume ratio is increased, but this ratio may be increased also by subdivision of the vacuole into very small vesicles (Figs. 9, 10, 16, and 17). It is suggested that this change in surface-to-volume ratios is coincidental with digestion. A summary of the sequential events by which particles attach to the surface membrane, cycle through the cytoplasm, and reach the defecation vacuole is diagrammed in Fig. 18.

It is interesting to speculate on the relationship, suggested by Bennett (3), between pinocytotic vacuoles and lysosomes (1, 11, 20). If pinocytotic vacuoles are a type of "food" vacuole, as suggested above, they would eventually contain the lysing class of enzymes. It would be necessary to combine the techniques of marking pinocytotic vacuoles with the techniques of histochemistry and of

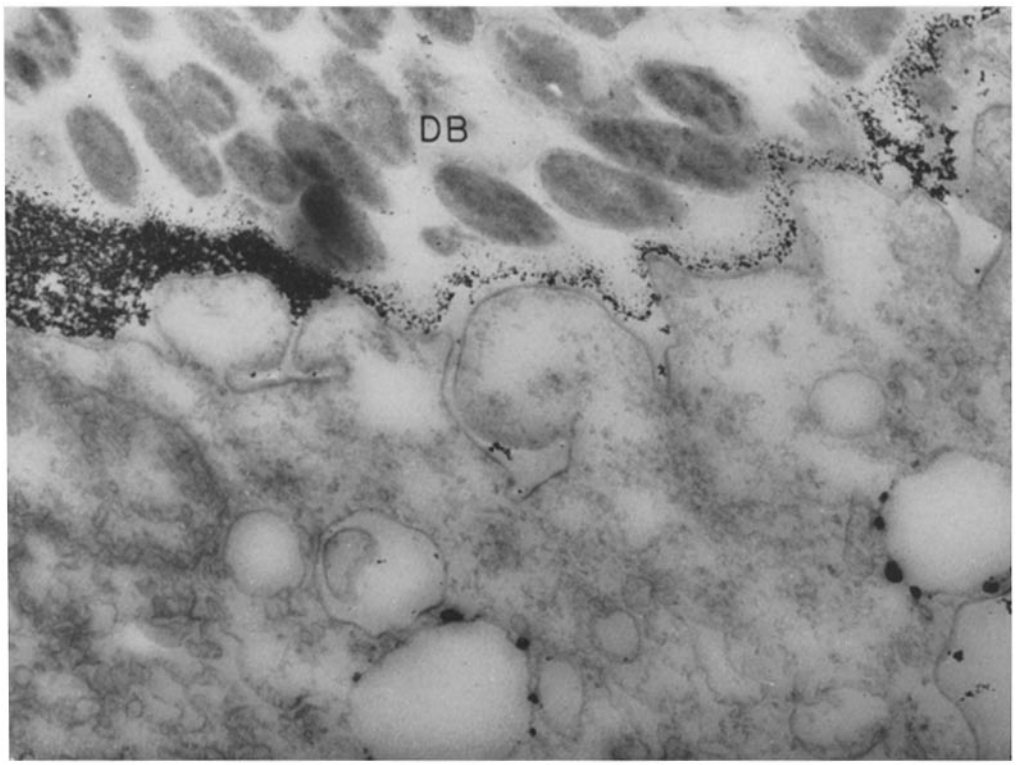


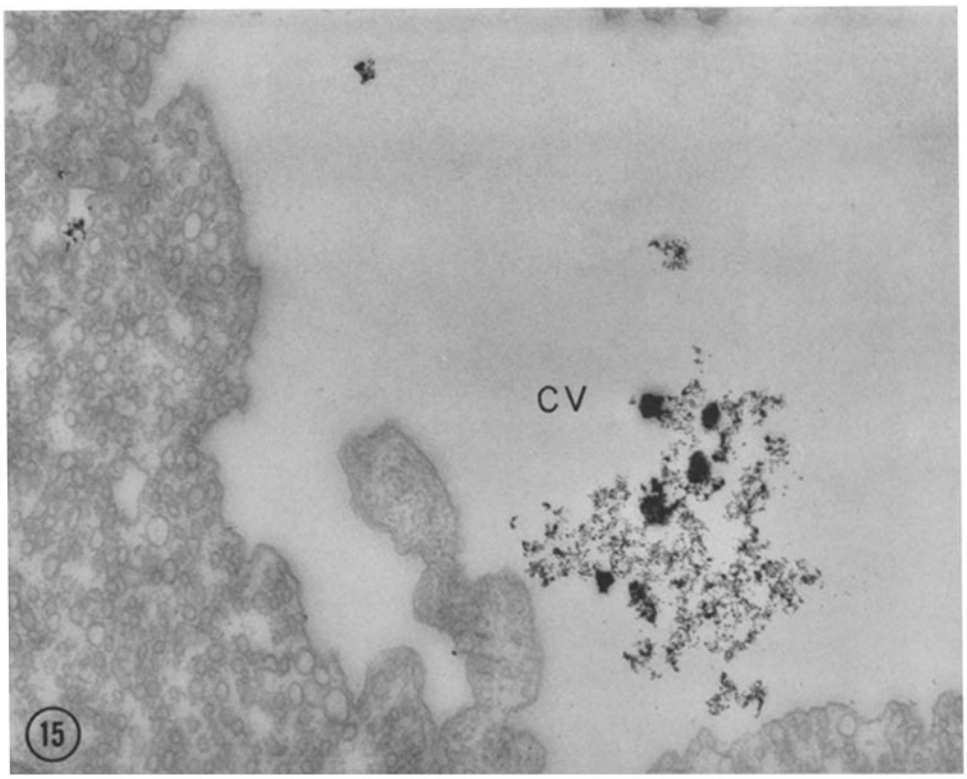
FIGURE 14

An electron micrograph of a defecation ball (*DB*) which can be identified by the profiles of bacteria found in the lumen. In this experiment the ameba was exposed to a suspension of thorium dioxide particles for 10 minutes 24 hours prior to fixation. Numerous particles can be seen in the cortex of the defecation ball, in small projections of the waste vacuole, and in vacuoles of the cytoplasm. It is apparent that in general the particles are being concentrated in the waste vacuole; however, some mechanism of fluid recovery from the vacuole may tend to recycle the marker back to the cytoplasm. Whether the projections of the waste vacuole represent transport of particles to the vacuole or recovery of fluid from the vacuole cannot therefore be determined by these static experiments. Methacrylate embedding. × 26,000.

differential centrifugation of cell homogenates in order to explore this possibility.

It was possible to test several other suggestions concerning the fate of the pinocytotic vacuoles under these experimental conditions. Gey (12) suggested from observations on living cells in tissue culture that pinocytotic vacuoles become mitochondria. Similarities between mitochondria and pinocytotic vacuoles in amebas have been reported in the literature (4, 13, 14); however, all these observations were limited by the resolution of the light microscope. It is more accurate to identify mitochondria by their internal structure as revealed by the higher resolution of the electron microscope (21, 23). In this electron microscopic study, no pinocytotic vacuoles marked with thorium dioxide, or any other marker, were found which had the internal structure of ameba mitochondria (27). Although these experiments do not necessarily constitute a final test of Gey's hypothesis, they and other work (5, 13) suggest that there is no transition from pinocytotic vacuoles to mitochondria.

Bennett proposed (2) a hypothesis of "membrane flow or vesiculation" and linked it to pinocytosis. He envisaged a mechanism whereby material trapped on the cell membrane would be internalized by pinocytosis. Following this, the membranes of the vacuole would be dissolved and trapped material released into the cytoplasm.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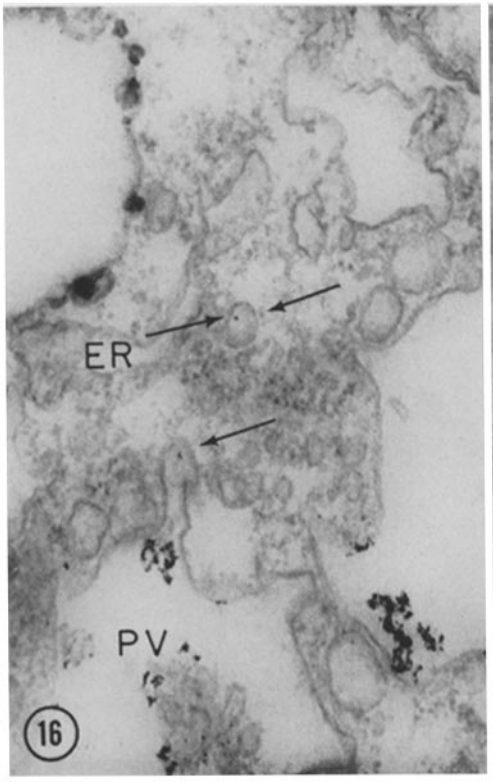
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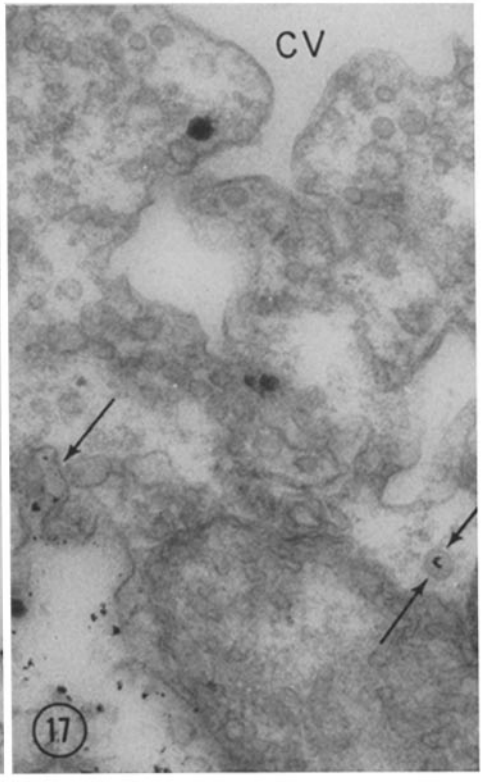
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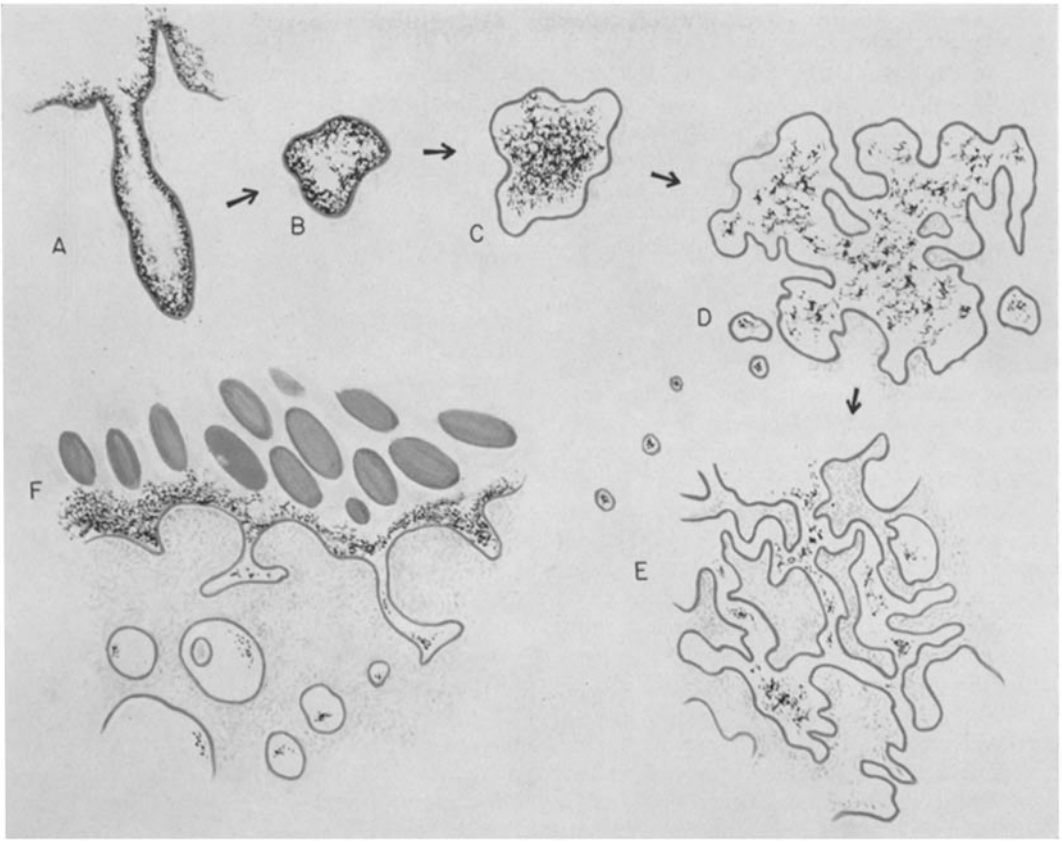

FIGURE 18

The pathway is diagrammed by which particles taken up into pinocytotic vacuoles (steps *A* and *B*) are eventually transferred to the defecation vacuole in step *F*. The steps *C*, *D*, and *E* represent the increased surface-to-volume ratio which occurs in the first several hours of the intracytoplasmic existence of the vacuole. Small vesicular fragments (at *E*) of the larger pinocytotic vacuole may directly contribute their contents to the contractile vacuole during diastole.

Some of the data presented in this report substantiate Bennett's hypothesis. Material adsorbed on the cell membrane of the amebas was internalized in a pinocytotic vacuole. Eventually, the pinocytotic vacuolar walls lost some of their resemblance to the plasmalemma by the disappearance of the filamentous extensions. However, the vacuole membrane did not "dissolve," as Bennett's

FIGURES 15 TO 17

Electron micrographs of portions of a contractile vacuole (*CV*) or cytoplasm in its immediate vicinity. In Fig. 15, thorium dioxide particles can be seen in the lumen of the contractile vacuole which can be identified by the numerous small vesicles surrounding it. In Figs. 16 and 17, small vesicles (double arrows) containing marker particles can be seen which resemble the evaginations (single arrows) of the pinocytotic vacuole (*PV*). It is suggested that fluid may be transported directly from the pinocytotic vesicle to the contractile vacuole in small vesicles. Elements of the endoplasmic reticulum and its attached rosets of granules (*ER*) can be seen in Fig. 16. Methacrylate embedding. Fig. 15, × 23,000; Fig. 16, × 34,000; Fig. 17, × 46,000.

hypothesis suggests. Instead, the pinocytotic vacuolar walls appeared increasingly similar to the alveolar structures in the cytoplasm of the amebas (Figs. 11 and 16). Some of these alveolar structures have been identified as elements of the endoplasmic reticular component of the cytoplasm of the amebas (25). No conclusion can be drawn about the relationship of the pinocytotic vacuoles to the granular endoplasmic reticulum, although they may be related to the agranular reticulum.

In rare instances, thorium dioxide could be traced into the lumen of the contractile vacuole. It is unusual that this organelle which appears to operate as a pump to remove excess water from the cytoplasm (15, 16) should contain particulate material. There are several possible pathways by which water and occasional particles could be extracted to effect the concentration of the pinocytotic vacuole noted in Fig. 13 and to enter the contractile vacuole. Water may be withdrawn from the pinocytotic vacuole by the osmotic pressure of the cytoplasm, or it may be actively transported across the membrane of the pinocytotic vacuole. In either of these two cases, it would enter the contractile vacuole from the ground substance of the cytoplasm. An alternative method can be suggested, based on the evidence presented in Figs. 16 and 17. The fluid and an occasional marker particle could be carried in membrane-bounded vesicles directly from the pinocytotic vacuole to the contractile vacuole. In this postulated mechanism, water and dissolved substances are mechanically sieved out of the pinocytotic vacuole into small membrane-bounded vesicles or packets, but large insoluble particles are usually left in the vacuole. Solute may be extracted from the packets by active transport across the walls of the packets (26) while the fluid remaining in the packets eventually empties into the lumen of the contractile vacuole. The substances which are not removed from the packets would eventually be extruded from the cell without ever entering the ground substance of the cytoplasm.

This proposed mechanism drawn from Figs. 16 and 17 amounts to micropinocytosis involving the membrane of the pinocytotic vacuole. It is conceivable that this micropinocytosis is more analogous to the pinocytosis observed by Palade (22, 24) and Moore and Ruska (18) in mammalian capillary endothelium than to the initial and larger plasmalemmal response of the amebas.

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