AN ELECTRON MICROSCOPIC STUDY OF PINOCYTOSIS IN AMEBA

I. The Surface Attachment Phase

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ABSTRACT

The attachment to the surface of the ameba (*Chaos chaos* L. (*Pelomyxa carolinensis*, Wilson)) of two proteins, ribonuclease and ferritin, and two colloidal suspensions, thorium dioxide and gold, was studied in the electron microscope. The initial step in the pinocytosis of ferritin and thorium dioxide particles by amebas is shown to be the attachment of these substances to the "hairlike" extensions of the plasmalemma. Ribonuclease caused alterations in the structure of the plasmalemma, but on account of its relative lack of density, it could not be definitely localized. Colloidal gold did not appear to be active with respect to pinocytosis in amebas. Since molecules in solution and particles in suspension are taken up by the same mechanism, the first step of which is their attachment to the cell surface, it is suggested that a single mechanism underlies phagocytosis, pinocytosis, ropheocytosis, cytopempsis, and potocytosis.

INTRODUCTION

Pinocytosis or "cell drinking" was described first by Lewis (26) from observations on living mammalian cells in tissue culture. Lewis suggested that pinocytosis was a phenomenon distinct from phagocytosis, for he reasoned that this process is apparently a mechanism by which cells internalize fluid droplets rather than particulate material.

In 1934, Mast and Doyle (30) reported that pinocytosis occurred in the free-living fresh water ameba, *Amoeba proteus*. Using this cell they began to explore some of the parameters of pinocytosis. From their data, it is apparent that pinocytosis is not directed by simple osmotic forces; in general, salts and proteins in the culture medium stimulate the amebas to exhibit pinocytosis, whereas carbohydrates do not. These results have been confirmed and extended by the work of Chapman-Andresen (10–12), Holter and Marshall (24), Brandt (5), and Schumaker (49) using a variety of techniques on free-living amebas.

Lewis (27) followed by others (3, 9, 15, 17, 18, 23) continued to describe pinocytosis from observations on mammalian cells made by using various light microscopic techniques. More recently, on the basis of electron microscopic studies, it has been suggested that pinocytosis is a normal activity of mammalian cells *in situ* (6, 14, 33, 36–38). The morphological criterion by which cells were adjudged to be active with respect to pinocytosis was the presence of small vesicles in the cytoplasm. These vesicles found near, and sometimes continuous with, the cell membrane are approximately 500 A in diameter, and about 100 times smaller than the pinocytosis "vacuoles"

This investigation was supported, in part, by research grants (B-1202 and B-2314) from the National Institute of Neurological Disease and Blindness of the National Institutes of Health, United States Public Health Service.
Received for publication, April 19, 1960.

seen in living cells with the light microscope. If the criterion used in the electron microscopic observations is correct, it can be concluded that pinocytotic activity is a normal and almost universal attribute of animal cells. It may therefore be an important factor in the transfer of substances into the cell.

Studies on free-living amebas by Brandt (5) and Schumaker (49) have demonstrated that the earliest phase in pinocytosis is the attachment of the pinocytosis-stimulating agent to the cell surface. Brandt (5), using a fluorescent antibody technique, demonstrated that proteins that stimulate pinocytosis are initially attached to the cell surface and apparently concentrated to some extent by the attachment. The cell membrane, with its attached "solute," is then incorporated within the cytoplasm, forming the wall of the pinocytosis vacuole. Schumaker (49) studied the rate of uptake of isotope-tagged proteins, under various conditions of protein concentration and temperature. He concluded, from the reversibility of the early phase of uptake, that protein is first bound to the cell surface and secondarily incorporated within the ameba. This second phase of uptake is not reversible. Schumaker demonstrated that the initial, reversible, binding phase resulted in the concentration of the protein by as much as fifty times. Pinocytosis, for amebas at least, is therefore a mechanism for the transfer of solute into the cell, and also a mechanism for concentrating the solute.

Although an important step in pinocytosis is the attachment of solute to the ameba plasmalemma (5, 49), the limited resolution of the earlier light microscopic studies prevented the precise site of the attachment from being visualized. Work by Odor (36) suggested that a similar surface attachment can be observed in mammalian cells with the aid of the electron microscope. She was able to follow the movement of dense "submicroscopic" particles from the peritoneal cavity into the cells which line it.

The work reported here is concerned with demonstrating the precise locus at which pinocytosis-stimulating agents attach to the ameba plasmalemma. To demonstrate these loci, amebas were exposed for various times before fixation, to one of several solutes or colloidal suspensions, some of which are relatively opaque to an electron beam. After fixation the cells were processed and then examined in the electron microscope.

MATERIALS AND METHODS

Methods for the Culture of Chaos chaos L. (*Pelomyxa carolinensis* Wilson): The giant, multinucleated ameba *Chaos chaos*, used in this study was grown in a culture solution which can be described as an artificial spring water. This culture solution was considerably higher in salt concentration than those usually employed (45) because the paramecia used to feed the amebas were grown in a comparatively salty medium. The final culture medium for the amebas represented a compromise between a highly dilute spring water and the culture medium of the paramecia. The ameba culture solution was composed of 6 μM sodium chloride, 0.75 μM potassium nitrate, 0.85 μM magnesium sulfate, 0.165 μM monobasic calcium phosphate, and 0.2 per cent spring water as a source of trace ions. It was made up in ion-free water produced by passage of distilled water through a column of Amberlite MB-1, and it was adjusted to pH 6.5 with 1.0 N sodium hydroxide solution.

Mass cultures of amebas were grown in 8-inch finger bowls. The culture fluid was changed each day, and living *Paramecium aurelia* was added as food. The paramecia were grown in 5-gallon carboys according to the technique of Preer (44). The paramecia were harvested from large volumes of their culture fluid with a mechanical cream separator, which collected the paramecia in the dirt trap. The paramecia were washed, by passing a liter of ameba culture fluid through the separator, and then were transferred to a beaker. The thickly concentrated paramecia obtained in this manner could be kept in good condition for several days. Before being fed to the amebas, the paramecia were lightly packed in a centrifuge, the supernatant was discarded, and the cells were resuspended in ameba culture fluid.

Methods for Fixation and Embedding Amebas: One to twenty amebas were pipetted directly from the experimental solution, or from a wash solution consisting of culture fluid, into the fixative, which consisted of 1 per cent osmium tetroxide buffered to pH 8.6 by 1/140 M veronal acetate solution. This concentration of buffer appeared to cause the least osmotic damage during the process of fixation. The amebas were fixed for 5 minutes at 0°C. They were kept in each step of the graded alcohol series for 5 minutes at a temperature of -10°C. After dehydration, several amebas were placed in each gelatin capsule (size 00) which was then filled with the methacrylate mixture consisting of 10 per cent methyl and 88 per cent butyl methacrylate, 2 per cent Lupercol (50 per cent 2,4-dichlorobenzoyl peroxide with dibutyl phthalate, Wallace Tiernan Inc., Buffalo, N. Y.), and 0.1 per cent uranyl nitrate (51). The block was polymerized overnight in an oven set at 43°C. Thin sections (40 to 80 mμ) were

cut on a Porter-Blum microtome and examined in an RCA EMU 3C electron microscope.

Methods for the Preparation of the Experimental Solutions: Ribonuclease solution was prepared by dissolving 0.1 mg./ml. of ribonuclease (Worthington Biochemical Corp.) in ameba culture solution. The resultant solution was used immediately as the test solution.

Thorium dioxide suspensions were obtained by dialyzing Thorotrast (Testagar and Co.) against ion-free water. The dialyzate was changed until all impurities were diluted at least 10^6 times. The resultant solution (pH 6.5 to 7.0) contained 25 per cent thorium dioxide stabilized with 25 per cent dextrin. This solution was diluted from 50 to 500 times with ion-free water at pH 6.5 before the amebas were exposed to it.

Colloidal gold suspensions were prepared by dialyzing Long Colloidal Gold Solution (Magar Chemicals, Inc., Cornwall Landing, N. Y.) against ion-free water of pH 6.5.

Ferritin was isolated from horse spleen according to the method of Laufberger (28) as modified by Wissig (52). The final preparation was dialyzed against 0.01 M Krebs phosphate buffer, pH 7.4, until the final dilution of dialyzable impurities had been reduced 10^6-fold. The solution was dialyzed further against ion-free water, pH 7.0, until the phosphate buffer solution had been diluted at least 10^6 times. This solution was used directly as a test solution for the amebas or diluted up to 10 times with ion-free water.

RESULTS

The Attachment of Pinocytosis-Stimulating Agents to the Ameba Plasmalemma

It has been established that proteins introduced into the culture medium become attached to the ameba membrane prior to being taken up into pinocytosis vacuoles (5, 22, 49). Pappas (40), who first described the "hair-like" extensions of the

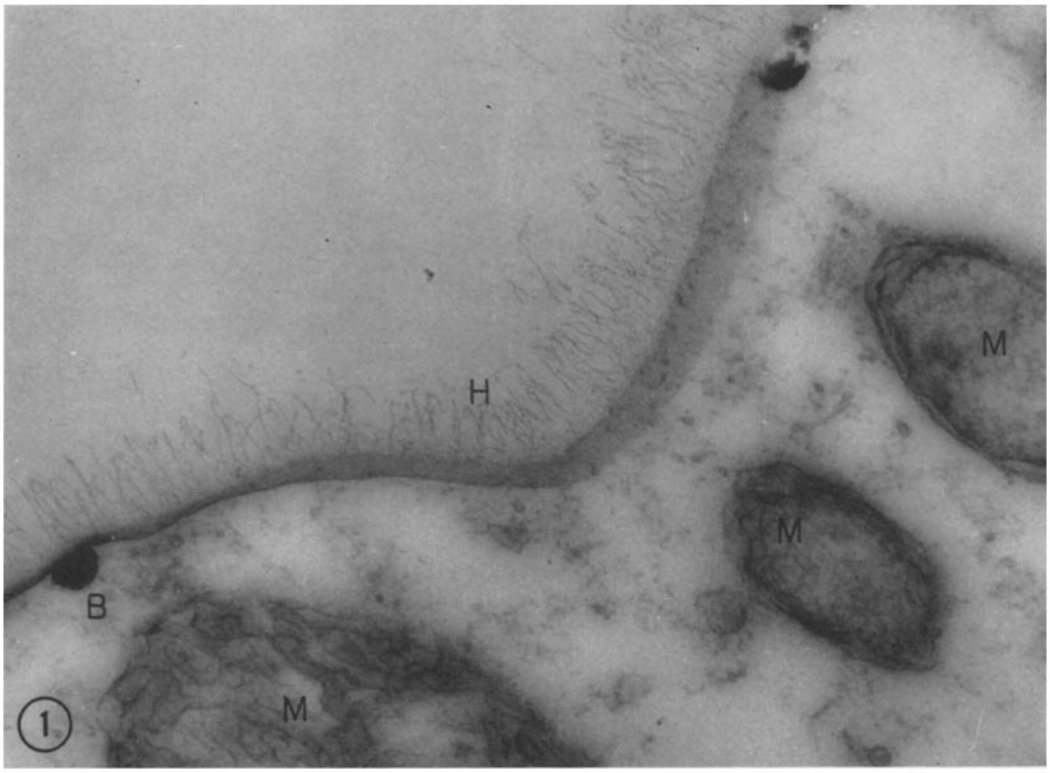


FIGURE 1

An electron micrograph showing the hairlike (*H*) extensions of the plasmalemma of the ameba *Chaos chaos*. The extensions are 50 to 80 A in diameter, and vary between 1000 and 2000 A in length. Profiles of of parts mitochondria (*M*) can be seen in the cytoplasm. The dense bodies (*B*) are frequently found associated with membranes, and are believed to be artifacts of fixation. × 55,000.

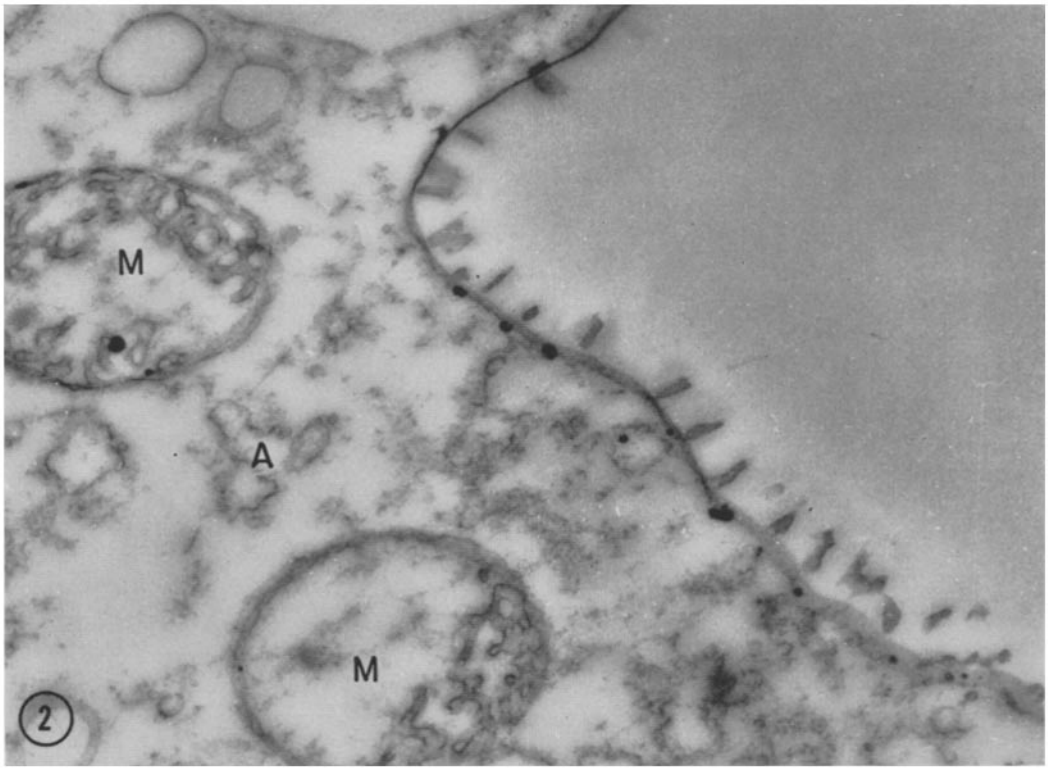


FIGURE 2

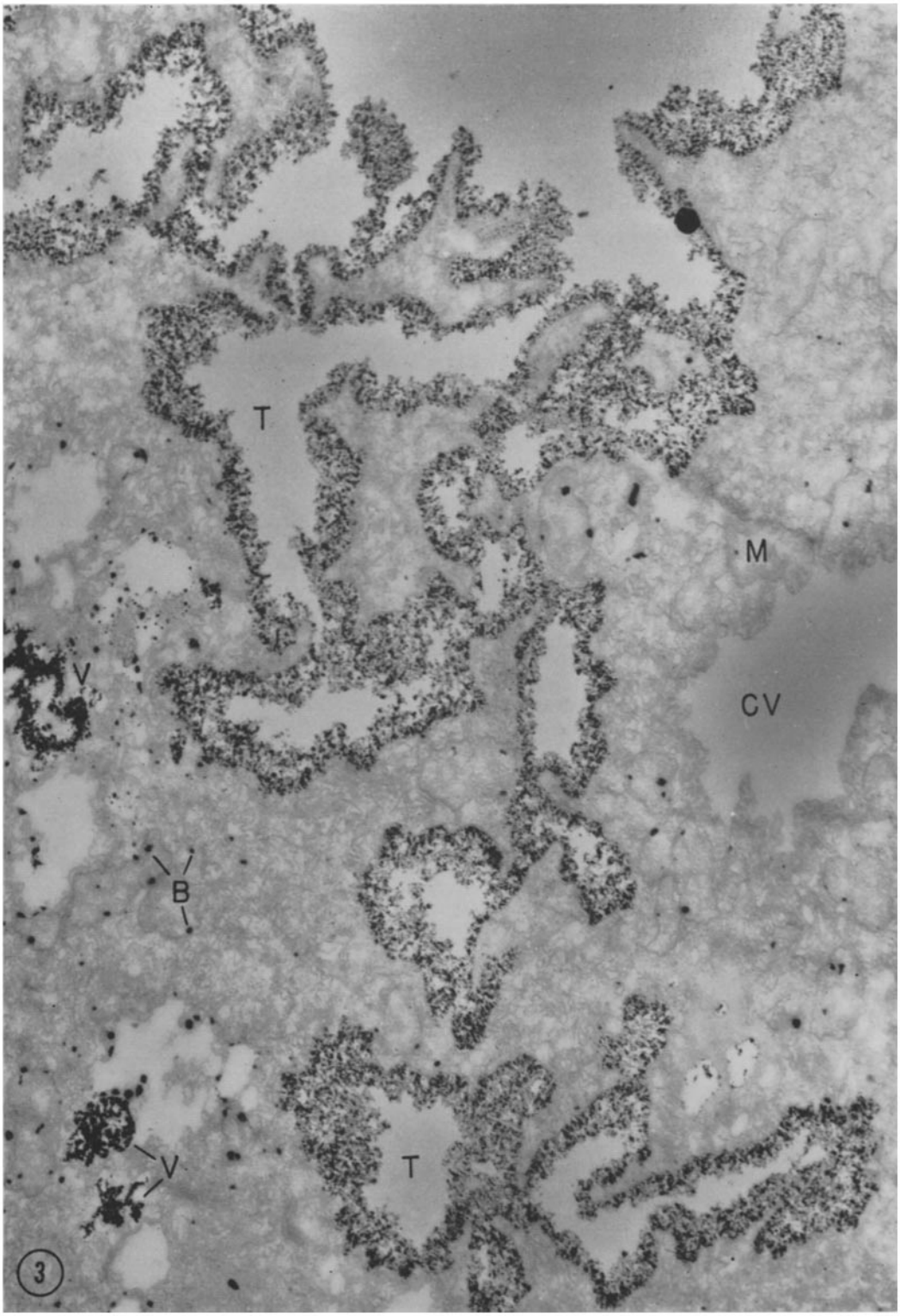
An electron micrograph which shows a portion of an ameba treated *in vivo* with ribonuclease. The number of hairlike extensions of the plasmalemma is greatly reduced. The remaining plasmalemma extensions are considerably thicker (300 to 450 A in diameter) than usual, although their length is unchanged. The mitochondria (*M*) are apparently swollen. The cristae are farther apart, and they tend to be localized more in one end. The alveolar structures (*A*) in the cytoplasm are widely separated and somewhat "ragged" in appearance. × 21,000.

plasmalemma, suggested that these structures may be the site of the attachment of pinocytosis-stimulating agents. Fig. 1 is an electron micrograph of the plasmalemma of *Chaos chaos* showing the extensions of the membrane. The "hairs" are approximately 50 to 80 A in diameter, and vary between 1000 to 2000 A in length.

Amebas were placed in one of four test solutions in order to determine which reagents stimulated pinocytosis, and also which reagents became

FIGURE 3

An electron micrograph of a section through the convolutions of a pinocytosis tunnel (*T*) formed by the invagination of the cell membrane (at top of figure). The invaginated plasmalemma is lined with attached thorium dioxide particles. The tunnel is 2 μ or less in diameter and of indeterminate length. The pinocytosis vacuoles (*V*) in the cytoplasm are more densely packed with thorium dioxide particles than the tunnel, and are the result of another earlier exposure to the test suspension. A contractile vacuole (*CV*) can be seen at right. It is typically surrounded by mitochondria (*M*). The numerous dense bodies (*B*) frequently associated with the mitochondria, or other membranes, are not thorium dioxide particles and are believed to be artifacts of fixation. × 13,000.


T
M
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3

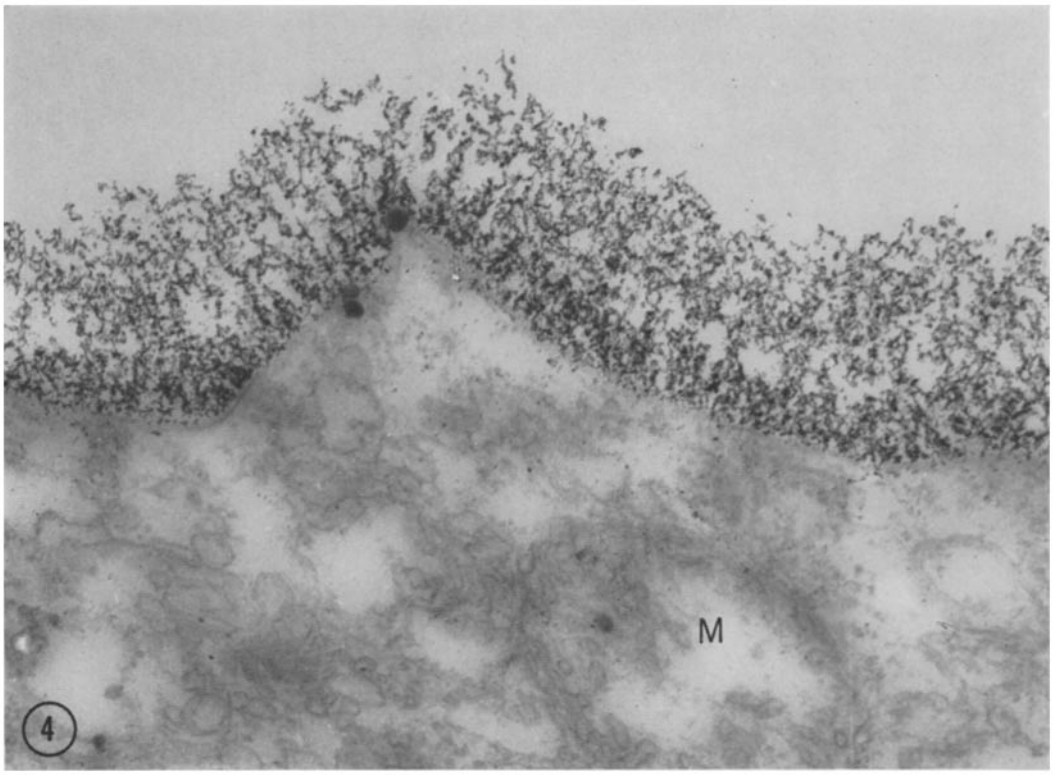


FIGURE 4

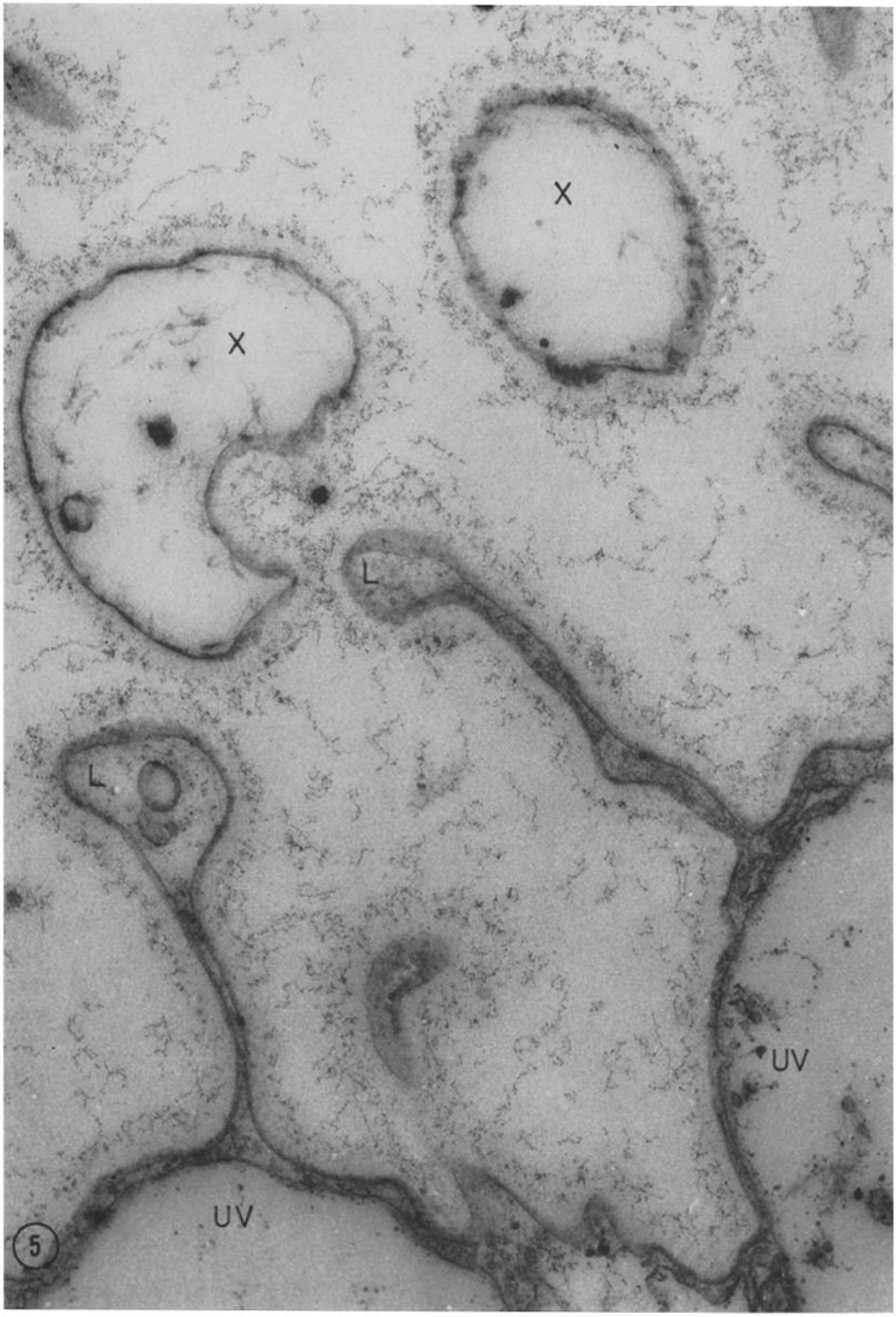
A high magnification of the plasmalemma with attached thorium dioxide particles. The inner third of the attached layer of particles (2000 A) is more concentrated than the outer two-thirds. The orientation of the particles in rows normal to the plane of the membranes implicates the hairlike extensions as the underlying structures to which the particles are attached. The hairs are not visible, but are obscured by the attached particles. It is suggested that adsorption forces at the medium-membrane interface are responsible for the attachment. A mitochondrion (*M*) can be seen in the cytoplasm. The random scattering of a few thorium dioxide particles in the cytoplasm is believed to be artifact. They were probably displaced from the plasmalemma by the passage of the knife through the block. × 38,000.

attached to the cell surface. Ribonuclease had been used by Schumaker (49) to study quantitatively protein uptake in amebas during pinocytosis. Even though this protein is not particularly opaque, it was used in this study because information on its site of attachment could be correlated with the quantitative data on its uptake.

Amebas were fixed after a 40-minute exposure

FIGURE 5

An electron micrograph of a portion of the ameba plasmalemma which demonstrates the attachment of ferritin molecules to the hairlike extensions. The extensions of the plasmalemma are of normal length (1500 A). The ferritin appears to be fixed at the ends of the hairs; under the experimental conditions used, it rarely seems to penetrate deeply into this layer. The section is through a very irregularly shaped portion of the ameba. Cross sections (*X*) as well as longitudinal sections (*L*) of pseudopods can be seen. The visible "cell body" consists mostly of two unidentifiable vacuoles (*UV*). × 35,000.


X
X
L
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UV
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5

to a 0.1 mg./ml. solution of ribonuclease. During the exposure approximately 10 per cent of the amebas dissolved or cytolyzed. Fig. 2, which is an electron micrograph of the plasmalemma of a ribonuclease-treated ameba, shows a considerable decrease in the number of the hairlike components. Although the remaining plasmalemma extensions are of normal length (1000 to 2000 A), they are much thicker (300 to 450 A). This thickening of the remaining hairs could be due to the accretion of protein or to clumping.

By using relatively opaque molecules or colloidal particles, the site of attachment to the plasmalemma of some of the pinocytosis-stimulating agents was demonstrated. Amebas were immersed for 20 minutes in various concentrations of ferritin, thorium dioxide, and colloidal gold. Subsequently the cells were washed very briefly and then fixed. Essentially similar results were obtained with solutions of ferritin and suspensions of thorium dioxide. Ameba fixed 20 minutes or longer after exposure were found to contain numerous vacuoles (V) filled with the test solution (Fig. 3). Therefore, both were judged active with respect to pinocytosis. However, when amebas were treated with colloidal gold suspensions, gold particles did not become attached or incorporated.

The attachment of thorium dioxide particles to the plasmalemma can be seen in Fig. 3. The depth of the attached layer (5000 A) is almost three times the length of the normal hairs of the ameba membrane. The cytoplasm and mitochondria appear to be normal, particularly when compared with the swollen mitochondria and ragged cytoplasm of the ribonuclease-treated amebas (Fig. 2). Fig. 4 is a higher magnification of the plasmalemma and attached thorium dioxide particles. The inner one-third of the attached layer (2000 A) is more concentrated than the outer two-thirds. The orientation of thorium dioxide particles in rows normal to the plane of the plasmalemma indicates that the hairs are the

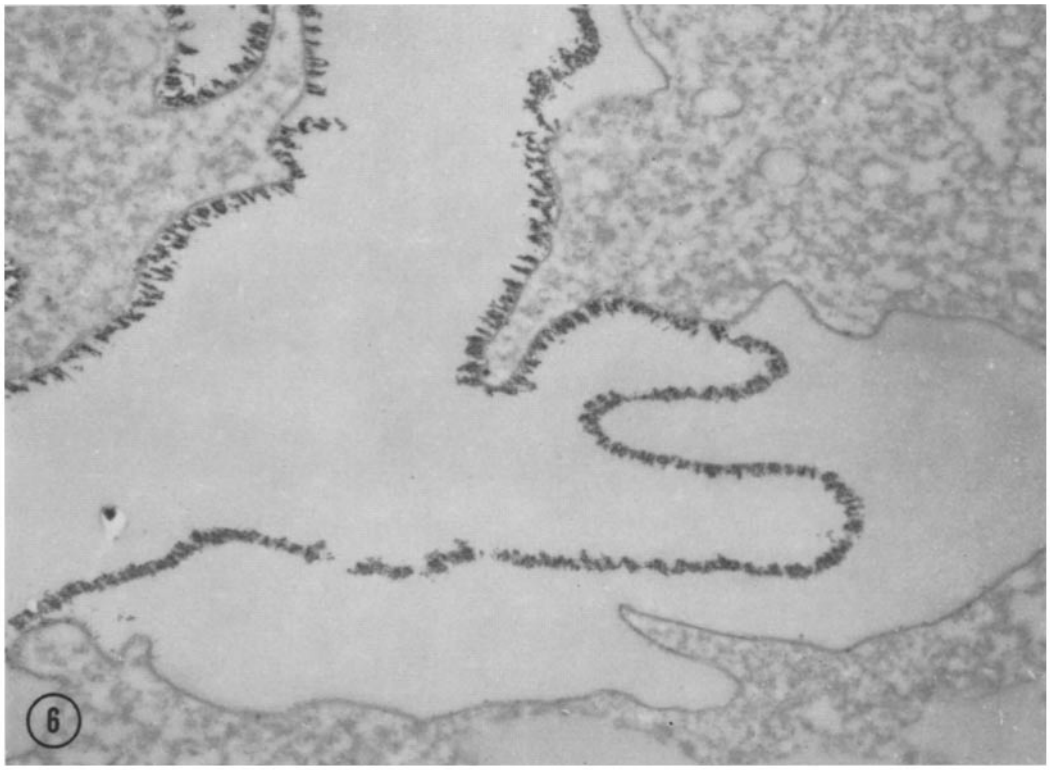

FIGURE 6

An electron micrograph showing the splitting off of the "hair" component of the plasmalemma after the heavy accumulating of thorium dioxide particles. (× 8000)

underlying structures to which the particles are attached. The hairs which are not visible are probably obscured by the attached particles.

The ferritin experiments differ from the thorium dioxide experiments in that the former are based on true solutions whereas the latter are based on colloidal suspensions. Ferritin molecules appear to attach less strongly to the plasmalemma than do the thorium dioxide particles. Fig. 5 is an electron micrograph of the plasmalemma demonstrating the attachment of ferritin. The ferritin molecules may be recognized by their diameter (60 A) and density. Ferritin seldom penetrates deeply into the hair layer in contradistinction to the thorium dioxide particles, but remains fixed near the ends of the hairs. Fig. 5 represents the plasmalemma of a very irregular part of the cell surface. It was not possible to demonstrate the attachment of ferritin to the smoother, more exposed portions of the plasmalemma. This, as well as its lesser penetration into the hair layer, suggests that ferritin is more loosely attached to the plasmalemma than the thorium dioxide particles. It is possible, therefore, that ferritin attaches to the more exposed portions of the plasmalemma and is subsequently removed during the fixation and dehydration.

Figs. 3, 4, and 5 show the attachment of ferritin and thorium dioxide particles when optimum concentrations of these substances are used. The optimum concentration of ferritin was between 1.0 per cent and 2.0 per cent. Higher concentrations were not tested and concentrations of 0.1 per cent resulted in considerably less attached material. The optimum concentration of thorium dioxide was found to be 0.1 per cent; Figs. 3 and 4 were taken from experiments using this concentration. Other concentrations of thorium dioxide suspensions led to quite different results. Considerably lower concentrations (0.01 per cent) resulted in the attachment of fewer particles to the plasmalemma. The lower concentrations were also less effective in causing the amebas to pinocytose the particles. While concentrations of 0.1 per cent resulted in a large number of membrane-attached particles, concentrations of 0.25 to 5.0 per cent often showed no membrane-attached particles. Fig. 6 demonstrates that the higher concentrations of thorium dioxide resulted in such massive accumulations of particles on the hairs that these structures were split off the membrane and left it bare in places. Such a splitting off of a membrane component in the presence of attached proteins has been reported previously (5).

DISCUSSION

The Relationship of the Cell Surface to Pinocytosis-Stimulating Agents

Morphological evidence is presented in this report supporting the concept that solute attaches and concentrates on the cell membrane from the surrounding medium. Figs. 3 to 5 show the attachment of ferritin and thorium dioxide particles to the surface of the amebas. It appears that the thorium dioxide attached to the plasmalemma is more concentrated than the 0.1 per cent test suspension. The attachment (5) and concentration (49) of proteins on the plasmalemma of amebas has been demonstrated previously by light microscopy and autoradiographic techniques. Odor (36) reported that thorium dioxide particles attach to the surface of mesothelial cells of the abdominal cavity after an injection of the suspension into the cavity. Hampton (20) demonstrated a similar attachment of particles to the surface of liver cells when thorium dioxide suspensions were injected into the venous system. Pappas *et al.* (41) showed the attachment of thorium dioxide particles to the surface of the ciliary epithelium after the injection of this substance into the posterior chamber of the eye. Tennyson (50) demonstrated that thorium dioxide particles attach to the surface of the choroid plexus epithelium when it is introduced into the lateral ventricle of the telencephalon. Ferritin attaches to the surface of the erythroblast prior to being taken up into minute cytoplasmic vesicles. In this latter case, the authors (2, 43) described the process as being related to pinocytosis, but called it "ropheocytosis." In all these studies the subsequent uptake of the particles into small cytoplasmic vesicles was reported.

An important role in the binding of particles to the cell surface may be played by the "extraneous" coats of the cell membrane. Chambers (7, 8) and Kopac (25) demonstrated that the sea urchin egg possesses several superficial coats which can be removed without causing the egg to cytolyze. They termed these removable coats "extraneous" for, although they are components of the cell membrane, they do not represent the diffusion barrier or plasma membrane. These findings (7, 8, 25) and other evidence (46–48), suggest that the plasma membrane is about 70 A

thick and lies adjacent to the cytoplasm beneath the extraneous coats.

Certainly the plasmalemma hairs do not represent the diffusion barrier of the ameba cell membrane, and therefore they may be considered extraneous. In fact, this PAS positive material (4, 39) can be almost completely removed from the membrane by treating the amebas with versene-lysozyme solutions (4, 29), or by splitting it off with excess thorium dioxide. "Extraneous" coats may operate as more than just a mechanical buffer protecting the delicate plasma membrane beneath, as suggested by Chambers (8). They also may act as an "adsorbing" surface in certain instances.

The suggestion that proteins are attached to the surface of the plasmalemma by nonspecific forces of adsorption (5) is consistent with certain thermodynamic considerations. Adsorption is a phenomenon which occurs at interfaces such as must exist between the aqueous environment and the cell membrane. The cell membrane may be considered a separate nonaqueous phase (13) of a plastic nature, for it separates two aqueous phases, and it can be separated by mechanical means from the other cell components (1, 32, 35, 42).

Adsorption as a step in the process of active transport is not a new concept. Höber (21) suggests such an interpretation for the active transport of such dyestuffs as phenol red through the kidney tubule epithelium. He states (p. 619) concerning a curve depicting phenol red excretion plotted against plasma concentration: "The curve resembles an adsorption isotherm with its increments decreasing with increasing concentration, until an adsorption maximum has been attained, possibly indicating a saturation of the surface of the adsorbent." Schumaker's data (49) on protein uptake by amebas, when plotted as concentration against uptake, also resemble an adsorption isotherm. In an earlier section of the same chapter Höber (21) reviews the concept that the actual transport of these dyestuffs through the cytoplasm may be in "carriers" such as submicroscopic vesicles. The only link missing in his conceptualization was the evidence from electron microscopic studies relating the adsorption of particles or solute on the cell membrane to the formation of submicroscopic pinocytosis vesicles. Historically it is interesting to note that Meltzer (31), writing in 1904, postulated that the transport of fluids across the capillary endothelium may occur in submicroscopic vacuoles. He related this postulated mechanism to phagocytosis, but termed it "potocytosis." Palade (38) recently published evidence in support of Meltzer's concept.

The interface in a complex biological system is probably not homogeneous, and several types of adsorption sites may be available. Chemisorption is usually not reversible; therefore, Schumaker's demonstration (49) of the reversibility of the initial protein uptake suggests that proteins are adsorbed on the cell membrane of amebas by van der Waals forces.

It is less satisfactory to explain the pinocytosis-stimulating effect of solutes such as sodium, potassium, and lithium chlorides (5, 10, 12) on the basis of physical adsorption. These solutes as a class are generally negatively adsorbed, *i.e.*, they leave the vicinity of an aqueous-solid interface. However, this effect can be reversed by strong electrostatic forces (19).

The cell surface may have electrostatic force fields, due to structural proteins, as well as fields directed by van der Waals forces. A heterogeneity of forces could be most significant if adsorption is a part of the active transport mechanism of cells. Many different types of molecules are actively transported by cells, and not a few are the products of chemists (21). Indeed a "universal" hypothesis of active transport, based on the initial adsorption of the substrate, may explain the enigma surrounding the variety of biological and nonbiological substances which are actively transported by cells.

An important corollary of adsorption is the concomitant decrease in the surface tensions forming the interface. When amebas are exposed to ribonuclease for short periods of time, some of the plasmalemma hairs appear to be removed. Longer exposures seem to dissolve the cell membrane, although there is no reason to suspect that ribonucleic acid is a structural component of the plasmalemma. This action of ribonuclease may not be a specific enzymatic function, but brought about by its strong adsorption causing the dispersion of the plasmalemma. In the respect the action of ribonuclease would be as nonspecific as the action of a detergent.

When amebas are exposed to thorium dioxide suspensions, the depth of the attached layer of particles is up to three times the length of the normal hairlike extensions of the plasmalemma. In Fig. 4 the depth of the inner, more concentrated layer (2000 A) roughly corresponds to the normal hair length, and the outer, less concentrated layer

to the additional length. The increased length of the hairs accompanying the adsorption of particles may be correlated with a decrease in their surface tension. Schumaker (49) reported a "break" in the plot of protein uptake against concentration, which he suggested was caused by the appearance of new binding sites on the plasmalemma. The break in the curve may be correlated with an increase in the surface area of the plasmalemmal extensions following the initial adsorption of material.

The Relationship of Pinocytosis to Phagocytosis

Pinocytosis was distinguished from phagocytosis by Lewis (26) because cells were observed to take up globules of fluid when no known particles, which could have been a stimulus for phagocytosis, were present. However, the two phenomena have much in common—perhaps too much to maintain the distinction (12, 22).

Both pinocytosis and phagocytosis result in the internalization of solute or particles surrounded by a derivative of the cell membrane; both appear to be greatly dependent on surface factors. Evidence for the dependence of pinocytosis on surface factors has already been discussed. It has long been recognized that the sticking of particles to the cell surface may be an important step in phagocytosis (16, 34). The effect of opsonins and surface tension factors on phagocytosis suggested to Mudd *et al.* (34) that surface reactions are of paramount importance in this phenomenon. Protein opsonins increase the rate at which bacteria are phagocytosed, perhaps primarily due to the ability of the cell surface to adsorb the protein-coated bacteria more readily than the polysaccharide capsule or cell wall.

The definition given pinocytosis by Lewis (26) seems to depend, in the light of present knowledge, more on the condition of the substrate than on the reaction of the cell. It is true that cells will take up globules of fluid. However, this may be only the most obvious component of pinocytosis and not the most significant in terms of the quantity of materials carried into the cell or in terms of the stimulus to the cell to react to the external fluid. The solute concentrated by adsorption on the membrane may not only stimulate the cell to pinocytose but also may result in the transport of considerably more solute than that trapped as fluid in the vacuole (5, 49).

It is suggested that the mode by which cells take up large particles or solutes into vacuoles is a continuum. At one extreme, large insoluble particles are said to be "phagocytosed," and at the other extreme, solute molecules are said to be "pinocytosed." Since between these two extremes are dispersion classes which shade into the extremes, a definition of uptake cannot depend on the condition of the substrate. Until some distinction in the mechanism initiating pinocytosis and phagocytosis can be made, the two classifications of uptake must be considered as a single phenomenon.

It is apparent that this single category also includes "ropheocytosis" (2, 43), "potocytosis" (31), and "cytopempsis" (33). Ropheocytosis was differentiated from pinocytosis by Policard and Bessis (2, 43) because they observed the attachment of ferritin to the cell membrane prior to the uptake of this substance. Since this attachment often accompanies pinocytosis, it is not necessary to consider ropheocytosis a special class of uptake.

Moore and Ruska (33) observed that pinocytosis vesicles lined both the luminal surface and the basal surface of the capillary endothelial cells. They suggested that fluid was transferred across the endothelium in these vesicles, and they differentiated such a transcellular process from pinocytosis because it could not "benefit" the cell as pinocytosis might. They termed the process "cytopempsis" although it is identical with the "potocytosis" mechanism suggested by Meltzer (31). Such a hypothetical mechanism can only be maintained as a special class of transport if the following criteria can be satisfied: (*a*) It can be shown that intact vesicles cross the endothelium; (*b*) the process does not, as they suggest (33), benefit the cell; (*c*) it can be shown that the stimulus to the cell to form the vesicles differs from that of pinocytosis. Since these criteria have not been satisfactorily demonstrated, cytopempsis and potocytosis may be considered only as hypothetical transport mechanisms based on pinocytosis. Palade, who first described pinocytosis in endothelial cells, also suggests fluid may be transported across the endothelial cells in vesicles. However, he does not distinguish the formation of these vesicles from pinocytosis (37).

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