PINOCYTOSIS IN Acanthamoeba Castellanii

Kinetics and Morphology

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ABSTRACT

The uptake of radioactively labeled albumin, inulin, leucine, and glucose by Acanthamoeba castellanii (Neff strain) was measured. The uptake is linear with time and appears to be continuous under the conditions of these experiments. Uptake is abolished at 0°C. No evidence for saturation of the uptake mechanism was obtained with either albumin or leucine. Each of the four tracer molecules enters the ameba at a similar rate when the uptake is calculated as volume of fluid ingested per unit time. The data suggest that each of these molecules enters the cell by pinocytosis. The highest rate of uptake was obtained with cells in their usual culture medium containing proteose peptone, glucose, and salts but pinocytosis also continued at a reduced rate in a simple salt solution. The calculated volume of fluid taken in during pinocytosis in culture medium was about 2 μl/hr per 10^6 cells. The route of uptake was examined in the electron microscope using horseradish peroxidase (HRP) as a tracer. HRP activity was found exclusively within membrane profiles within the cytoplasm, confirming the pinocytotic mode of uptake. An estimate of the rate of surface membrane turnover due to pinocytosis was made using the biochemical and morphological data obtained. This estimate suggests that the plasma membrane turnover of one cell is on the order of several times an hour.

INTRODUCTION

Weisman and Korn (29), while studying phagocytosis by the small soil ameba, Acanthamoeba castellanii, found evidence for a low rate of uptake of soluble molecules from the incubation medium. The uptake occurred in the presence or absence of particle uptake. We have now examined this slow uptake of solutes more closely to determine its mechanism. We present here biochemical and morphological evidence that the uptake of five quite different soluble molecules occurs by pinocytosis in Acanthamoeba. Pinocytosis goes on continuously and appears to be the feeding mechanism in a culture medium containing no particles. The results further show that although the total volume of medium ingested by pinocytosis is not large, the surface to volume ratio of most pinosomes is high. As a consequence, pinocytosis results in a high rate of surface internalization in Acanthamoeba.

MATERIALS AND METHODS

Cell Incubations

Acanthamoeba castellanii (Neff strain) was cultured axenically in a medium containing 1.5% proteose peptone and 1.5% glucose as described by Korn (12). After 5-7 days of growth the cells were harvested by low speed centrifugation and either re-suspended in...
cells/ml, or washed twice and resuspended in replacement medium at a concentration of 1.2 × 10^6 cells/ml. Cell concentrations were determined by light scattering (28). 10 ml of the cell suspension, in a total volume of 12 ml, were used in each experimental flask, giving a final cell concentration of 10^6 cells/ml. Cell protein content as determined by the Lowry method (13), using bovine serum albumin as a standard, was 0.53 mg/10^6 cells. Osmolalities of suspending media were determined by freezing-point depression with an Advanced Instruments Osmometer (Advanced Instruments Inc., Newton Highlands, Mass.).

The amebas were equilibrated for 15 min on a reciprocal shaker (80 strokes/min) at 30°C, then the radioactive tracer was added and the incubation continued for the required length of time. Duplicate 2 ml samples from each flask were pipetted into 10 ml of ice-cold wash solution (0.1 M NaH_2PO_4, Na_2HPO_4 buffer, pH 6.8) to terminate the incubation. For the albumin uptake studies, the wash solution contained 1 mg/ml nonradioactive human serum albumin (Sigma Chemical Co., St. Louis, Mo.). The presence or absence of nonradioactive inulin or leucine in the wash solutions had no effect on the measurement of the uptake of these two molecules.

The cells were washed three times with 5 ml of wash solution in a refrigerated centrifuge and pelleted. The wash solution was removed and the pellet was suspended in 0.5 ml of ice-cold wash solution (0.1 M NaH_2PO_4, Na_2HPO_4 buffer, pH 6.8) to terminate the incubation. For the albumin uptake studies, the wash solution contained 1 mg/ml nonradioactive human serum albumin (Sigma Chemical Co., St. Louis, Mo.). The presence of nonradioactive albumin or leucine in the wash solutions had no effect on the measurement of the uptake of these two molecules.

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Tracer Molecules

Tracer molecules were human serum albumin-[^131]I (Abbott Laboratories, North Chicago, Ill.), inulin-[^3H], uniformly labeled d-glucose-[^14C], uniformly labeled l-leucine-[^14C], and l-leucine-[^4,5-3H] (all from New England Nuclear Corp., Boston, Mass.). The albumin was filtered on a Sephadex G-25 column (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) before use to remove unincorporated[^131]I. About 95% of the counts were recovered in the void volume, and this fraction was used for the experiments. The concentration of albumin in the column fraction was determined from a standard curve by reading the absorption at 280 nm. Inulin counts chromatographed in a single broad peak on Sephadex G-50 and were all in the void volume after filtration on Sephadex G-15; therefore, the inulin was used without further purification. Glucose and leucine were also used without further purification.

Electron Microscopy

For morphological studies on the uptake of horseradish peroxidase (HRP), amebas were incubated in medium containing 1 mg/ml of HRP (type HPOD, Worthington Biochemical Corp., Freehold, N. J.). After suitable intervals, the cells were fixed for 1 hr by the addition of 2 vol of 3% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8. For the demonstration of peroxidase activity the fixed cells were rinsed and incubated for 15 min in the medium described by Graham and Karnovsky (10), postfixed in 1% OsO_4, dehydrated in ethanol, and embedded in Epon 812 (14). As controls, cells exposed to exogenous HRP were incubated in the Graham-Karnovsky medium containing 0.01 M NaCN, and cells not exposed to exogenous HRP were incubated in complete medium. In neither case was activity observed.

RESULTS

Albumin Uptake

Pinocytosis was initially tested by using isotopically labeled human serum albumin as a marker for fluid uptake. Cells at a concentration of 10^6/ml were incubated in culture medium containing about 0.02 mg/ml of albumin[^3H] plus nonradioactive albumin to make a total concentration of 1 mg/ml. The uptake was examined as a function of time and temperature (Fig 1). The uptake at 30°C is linear for about 15 min and then begins to plateau after 30 min. There is no uptake at 0°C, indicating that the uptake process is not simply a physical binding of albumin to the surface.

The plateauing of the uptake curve after 30 min might be due to cessation of uptake or to loss of label due to excretion of[^3H] after metabolism of the ingested protein. To distinguish between these alternatives a second experiment was performed with the results shown in Table I. In Experiment A, cells were preincubated with 4 mg/ml of nonradioactive albumin for 15, 30, or 45 min and then a trace amount of albumin[^3H]
Table 1

| Albumin<sup>125I</sup> Uptake by Acanthamoeba | Uptake per 10<sup>6</sup> cells per 15 min | cpm | µg protein |
|---------------------------------------------|-------------------------------------------|-----|-----------|
| Experiment A<sup>a</sup>                   |                                            |     |           |
| None                                        | 432                                        | 0.04|           |
| 15                                          | 461                                        | 0.04|           |
| 30                                          | 439                                        | 0.04|           |
| 45                                          | 476                                        | 0.05|           |
| Experiment B<sup>b</sup>                    |                                            |     |           |
| None                                        | 242                                        | 0.57|           |
| 15                                          | 258                                        | 0.60|           |
| 30                                          | 263                                        | 0.61|           |
| 45                                          | 281                                        | 0.66|           |

* 0.17 mg/ml albumin<sup>125I</sup> (1.8 × 10<sup>4</sup> cpm/ml) was added after preincubation in culture medium for the indicated times and the uptake was determined after 15 min.

† Identical to Experiment A, except that albumin<sup>125I</sup> was added after preincubation in culture medium containing 4 mg/ml of nonradioactive albumin. Both Experiments A and B were performed with the same cell batch.

was added and the uptake measured over an interval of 15 min. In a parallel experiment, Experiment A, the cells were preincubated in culture medium without the addition of nonradioactive albumin. In both these experiments, the uptake measured over 15-min intervals is constant for at least 60 min. The flattening of the curve in Fig 1 can then be attributed to loss of <sup>125I</sup>I from the cells rather than to a diminution of albumin uptake with time. This experiment also shows that our incubation conditions do not result in any decrease in cell activity over a period of an hour.

The loss of isotope from cells was investigated directly after preloading with albumin<sup>125I</sup> or inulin<sup>3H</sup> for 15 min. The cells were washed free of external isotope in the cold and reincubated in culture medium at 30°C. The results of this experiment, shown in Fig. 2, support the idea that the protein may be rapidly degraded after uptake, with the consequent loss of <sup>125I</sup>I from the cells. Over 60% of the counts were lost from albumin-loaded cells after 30 min of reincubation, as compared with only about 15% from inulin-loaded cells.

The uptake of albumin is nearly linear with concentration, at least to concentrations as high as 10 mg/ml (Fig. 3). We interpret the break in the curve at about 4 mg/ml to signify a general inhibition of cellular activity by high concentrations of albumin rather than any saturation of albumin uptake. This inhibition of uptake in the presence of albumin is also shown in Table 1. Experiments A and B were performed with the same batch of cells and the medium contained identical amounts of radioactivity, but in this experiment, in the presence of 4 mg/ml of albumin total, uptake is depressed to about half that with trace amounts of albumin. Decrease in uptake of other solute molecules in the presence of albumin has also been observed, so this cannot be interpreted as overload of some transport mechanism specific for albumin, but is related to an unidentified effect of albumin on cellular activity.

Active transport processes, which are mediated by enzymes, characteristically show a limiting rate, or saturation, of transport with increasing concentrations of substrate. The inability to obtain saturation of the uptake mechanism in this instance is therefore consistent with a mechanism of bulk transport for this molecule.

Inulin Uptake

Inulin, a polysaccharide of intermediate molecular weight (5000–5500), is in general not enzymatically degraded or actively transported by cells. It therefore might be expected to provide a more convenient marker than albumin for measuring uptake processes in Acanthamoeba.

Experiments similar to those with albumin were performed using inulin<sup>3H</sup> as a tracer molecule. Fig. 4 shows that inulin, like albumin, is removed from the culture medium at a constant rate. We were not able to do a meaningful test of uptake as a function of concentration with inulin because of its limited solubility.

Uptake measurements were routinely carried out with amebas in their culture medium. The culture medium contains 1.5% proteose-peptone and 1.5% glucose as major components. The effect of proteins and amino acids on stimulating pinocytosis in the large amebas has been well documented (5), and there is evidence in another protozoan, Tetrahymena, that proteose-peptone solution stimulates vacuole formation and fluid uptake (24). In order to rule out that the proteose-peptone solution might be inducing pinocytosis in Acanthamoeba, inulin uptake was measured...
Figure 1 Uptake of albumin as a function of incubation time and temperature. Cells were incubated as described under Methods with human serum albumin-131I (1.9 X 10^5 cpm/ml). Nonradioactive albumin was added to obtain a concentration of 1 mg/ml.

Figure 2 Loss of label from cells preloaded with albumin-131I (circles) or inulin-3H (triangles). Cells were prelabeled for 15 min, washed free of external isotope, and re-incubated in culture medium. Each time point is percent of initial cellular radioactivity. For albumin-loaded cells, initial radioactivity was 1400 cpm/10^6 cells, and for inulin-loaded cells, 975 cpm/10^6 cells.

after washing the cells free from culture medium with 0.02 M NaCl containing 0.1 M glucose, or 0.1 M glucose plus 0.001 M MgCl_2, or with 0.02 M NaCl alone (Fig 4). In each case uptake continued. The rate of uptake found in the glucose-salt solution was within the range of values we obtained with cells in culture medium, so that, for short-term experiments at least, the rate is not significantly altered from that found in culture medium. In the salt solution alone, the rate was about one-half that found with the same batch of cells in the salt solution plus glucose. The presence or absence of small amounts of Mg^{++} in the incubating solution had no effect. The reasons for the reduction in rate in the 0.02 M NaCl solution have not been further explored, but two obvious variables are the marked change in osmolality, from 200 mosmols for the culture medium to 40 mosmols for the salt solution, and the lack of an energy source. In any event, these results show the
Figure 3. Uptake of human serum albumin–¹³¹I as a function of concentration in the medium. Results from three experiments are plotted. Uptake was measured for 15 min with total concentrations of albumin of 0.08, 0.16, 0.32, 0.50, 1, 3, 4, 6, and 10 mg/ml. A constant amount of radioactive albumin was used (about 18 μg/ml), and the final concentration achieved with nonradioactive human serum albumin. In the experiments described by the open symbols the medium contained 1.9 × 10⁵ cpm/ml. In the experiment described by the closed triangles, the medium contained 2.4 × 10⁵ cpm/ml. Protein taken up was calculated from the specific activity of the protein and the cpm found in the cells.

Figure 4. Uptake of inulin–³H. Three experiments are plotted: (a) the uptake of inulin by cells in culture medium (circles); (b) uptake in 0.02 M NaCl, 0.1 M glucose, 0.001 M MgCl₂ (open triangles); and (c) a comparison with the same cell batch of uptake in 0.02 M NaCl, 0.1 M glucose (closed triangles) with uptake in 0.02 M NaCl (squares). The concentration of inulin in these experiments was 0.9 × 10⁻⁶ M; 1 × 10⁵ cpm/ml.

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uptake process to be a continuous function that proceeds readily in a simple salt solution with an energy source. The experiment shown in Fig. 2 shows that the rate of loss of counts from cells which were preloaded with inulin-3H is only about 3.4 of the rate of accumulation. This result contrasts with that found for albumin-14C and is consistent with the presumed inability of amebas to metabolize inulin. The experiment also indicates that uptake as measured in the experiment in Fig. 4 is in fact a summation of the uptake and loss of label from the amebas. In the case of inulin, the rate of loss is too low to affect the constancy of uptake over a period of 1 hr.

Leucine and Glucose Uptake

By analogy with other cells, small molecules such as amino acids and sugars might be expected to be taken up by active-transport mechanisms. We therefore examined the uptake of L-leucine-14C and D-glucose-14C by Acanthamoeba.

The kinetics of leucine uptake are shown in Fig. 5 to be the same as for the uptake of albumin and inulin. Leucine is removed from the medium in a temperature-dependent process that is constant for at least an hour. Counts from leucine-14C were rapidly incorporated into triethylacetic acid-precipitable material and thus loss of counts through metabolism was not observed during these short experiments. The rate of leucine ingestion is proportional to its concentration in the medium up to a concentration of 0.01 M (Fig. 6).

At 0.1 M leucine, uptake is slightly depressed. This is presumably due to the high osmolality of the solution (300 mosmols), rather than to any saturation of an uptake mechanism. Osmolarities in this range have been shown to inhibit particle uptake in Acanthamoeba (18, 29), and it is likely that they would affect the uptake of soluble molecules also. The absence of saturation kinetics with leucine, as with albumin, is evidence for bulk transport rather than active transport of this amino acid.

The data for uptake of albumin, inulin, and leucine have been given in terms of the amount of radioactivity taken in per 10⁶ cells per unit time. These tracer molecules were of different specific activities and were present in the medium in different concentrations. If it is assumed that each of them goes into the cell by a mechanism of bulk transport, then their uptake rates may be more directly compared by calculating the rate of uptake as the volume of medium ingested per 10⁶ cells per unit time. Values calculated in this way are identical for replicate flasks within a given experiment. For example, four replicate flasks in an albumin-14C experiment gave 0.42 ± 0.01 μCi/10⁶ cells per 15 min. There was, of course, considerably greater variation between uptakes with different batches of cells. The average uptake expressed in μCi/10⁶ cells per 15 min for four different experiments with albumin-14C was 0.29 ± 0.08; for seven experiments with inulin-3H was 0.59 ± 0.15; and for two experiments with leucine-14C was 0.65 (0.55 and 0.75). In a single experiment with glucose-14C, uptake for 15 min was equivalent to a rate of 0.47 μCi/10⁶ cells per 15 min. Radioactive CO₂ collected during the incubation accounted for an additional 10% uptake. This value of 0.5 μCi/10⁶ cells per 15 min is virtually identical with those found for the other molecules tested and indicates that glucose is ingested in the same manner.

Thus, the rate of uptake, when expressed as volume of medium ingested per unit time, is the same regardless of the tracer used or its concentration in the medium. This finding points to a mechanism of uptake that does not involve selective binding of the substance transported to the cell surface. In other words, the uptake mechanism appears to be nonconcentrative, and a true, nonspecific "gulping" of the medium.

Morphology of Pinocytosis

In order to visualize the uptake process of Acanthamoeba in the electron microscope, we have used horseradish peroxidase (HRP) as the tracer molecule. Incubations were performed exactly as for the biochemical experiments, except that HRP was added instead of a radioactive solute molecule and the incubations were terminated by the addition of fixative. We used a very short incubation time (20 sec) to attempt to visualize the primary events of transport, but have also used a variety of longer incubation times up to 1 hr. As expected, all HRP found within the cells was enclosed within membrane profiles, confirming the pinocytotic nature of the uptake process.

Figs. 7 and 8 illustrate some of the variety of labeled profiles observed in cells after a very brief exposure to tracer.3 After longer incubation

1 The HRP activity of the pinosomes most often is localized along the membrane of the vesicles rather than being uniformly distributed within them. Although this might appear contradictory to the conclusion reached from the biochemical data that up-
periods the images are similar but more numerous, and there is a relative increase in the number of intermediate size (0.3-0.7 μm diameter) profiles. In incubation periods extending for several minutes it is clear that small pinosomes fuse with larger, unlabeled vesicles, causing some ambiguity as to which are the initial uptake vesicles. For this reason we have looked most carefully at cells exposed to tracer for only 20 sec, the minimum time for a reasonable amount of label to go into the cells. In order to illustrate the range of profile sizes observed and their frequency,
Figure 7  Section of *Acanthamoeba* cytoplasm showing small HRP-containing vesicles (arrows). The cell was exposed to HRP for 20 sec before fixation. Section is stained with lead citrate and uranyl acetate. *PM*, plasma membrane; *DV*, digestive vacuole. X 31,500.
Figure 8 a-c  Shows the range of HRP-labeled profiles found in cells exposed to HRP for 20 sec. Sections are unstained. PM, plasma membrane; DV, digestive vacuole. X 29,000.
we measured all the labeled profiles in a number of micrographs from three different 20-sec uptake experiments. The results are plotted as a histogram in Fig. 9. This is not a statistically valid, random sample because the number of labeled vesicles within any thin section was so small that it was not feasible to obtain a true random sample. The micrographs were obtained by scanning the sections and photographing the labeled profiles observed. Vesicles of large size might be overrepresented, if anything, because of their greater visibility as compared with vesicles of 120 nm diameter.

The histogram indicates that the most frequently observed profile is around 120 nm in diameter, with a whole spectrum of sizes extending up to about 2.5 μm in diameter occurring much less frequently. The smaller vesicles were usually spherical or cucumber-shaped, while the larger profiles were often more irregular in shape. Some of them appear to be profiles from finger-like invaginations of the surface (Fig. 8 c), others from flattened spheroids (Fig. 8 b). Most of the labeled profiles were shapes of high surface to volume ratio, that is, quite small, or, if large, flattened, or spindle-shaped.

Labeled vesicles often occurred in clusters (Fig. 7), and single vesicles or clusters were frequently seen at one or two widely separated sites around the periphery of a single cell. This appearance suggests that one or more random sites on the surface are probably engaged in pinocytosis within any given time interval.

The image seen in Fig. 8 c has some special characteristics. It is found close to the edge of the cell and has the appearance of a narrow, highly convoluted invagination, often with cytoplasmic protrusions in the center. We have included these images in the frequency chart, but feel that they probably do not contribute significantly to uptake. In unpublished observations of cells incubated at 0°C and exposed to HRP for 15 min, we found a number of similar channels labeled with HRP, but in similar experiments we find no measurable uptake of radioactive molecules at 0°C. The volumes of such invaginations may be very small or they may represent incompletely closed-off channels in the surface from which trapped molecules can be removed by extensive washing (as in the experiments with radioactive tracers).

DISCUSSION

We have examined the uptake of four quite different kinds of molecules by Acanthamoeba. Serum albumin is a large protein with a molecular weight of 65,000 and a net negative charge at the pH of these experiments. Inulin is a neutral sugar, intermediate in molecular weight (5000-5500). Glucose (mol wt 180) and leucine (mol wt 131) are small molecules that enter readily
investigations on pinocytosis have been carried out
with two large amebas, Chaos chaos and Amoeba proteus (11). Those amebas have in common a prominent mucoid surface coating (15, 17). They can be induced to pinocytose intensively for short periods of time by certain cations and other positively charged molecules (5) which appear to initiate pinocytosis by interacting with the mucoid coat (4, 15, 16). The cells are also capable of concentrative uptake of proteins and other materials that bind to the mucoid coat (5, 15, 23). In induced pinocytosis the amebas characteristically cease locomotion and a number of protruberances are formed on the surface. A channel-like invagination forms in each protruberance and pinches off vesicles at its terminus that are large enough to see with the light microscope (5). Induced pinocytosis in these amebas appears to be a highly artificial state (23). Although some spontaneous or "permanent" pinocytosis may occur in the culture medium (30) the amebas cannot be cultured axenically. Thus, the rate or extent of this permanent pinocytosis is presumably insufficient to support satisfactory growth. In Acanthamoeba, on the other hand, we have not been able to demonstrate a surface coat in thin-sectioned material (2), nor have we any evidence that surface binding of molecules plays a major role in their ingestion. Furthermore, pinocytosis appears to be a continuous process in agitated cultures and is not enhanced by molecules (e.g., serum albumin) that induce pinocytosis in Chaos chaos or Amoeba proteus. Finally, the morphology of uptake appears somewhat different in Acanthamoeba than in the large amebas. There are obvious limitations to the interpretation of static images, but our electron microscope images suggest that the most common mode of ingestion is by vesiculations that are too small to be seen with the light microscope.

Although there is a plethora of morphological observations on pinocytosis in a variety of cell types, there are relatively little kinetic data. Most of these data come from mammalian cells, and some of the data that are directly comparable to values we have obtained with Acanthamoeba are tabulated in Table II. In each instance listed in the table, the uptake is at least an order of magnitude lower than that found for Acanthamoeba. This result is perhaps not surprising since pinocytosis seems to perform no significant nutritional function in mammalian cells (9, 21, 27),
Table II
Rates of Pinocytosis in Acanthamoeba and Other Cells

| Cell type                          | Tracer (conc.) | Molecules/cell/hr | nl/10^6 cells/hr | Reference         |
|-----------------------------------|----------------|-------------------|------------------|------------------|
| Acanthamoeba                      | Albumin-^{131}I (0.4%) | 6 × 10^4         | 1600-2400       | Ryser, 1963 (19) |
| Ehrlich ascites                   | Albumin-^{131}I (0.5%) | 8 × 10^4         |                  | Ryser, 1963 (19) |
| Sarcoma 180                       | Albumin-^{131}I (0.4%) | 6 × 10^4         |                  | Ryser, 1963 (19) |
| Chinese hamster cell line (A12 VIII) | Chondroitin Sulfate-^{35}S | 48-114         |                  | Saito and Uzman, 1971 (22) |
| Guinea pig PMN                    | Inulin-^{14}C | 21-31             |                  | Berger and Karnovsky, 1966 (1) |

Abbreviation: PMN, polymorphonuclear leucocytes.
* Value from Fig. 3.
† Range observed with albumin and inulin.

and indeed its function is not well understood (20).

The electron microscope images show that a major pathway in pinocytotic uptake is by quite small vesiculations. Some images suggest that these vesiculations occur directly at the surface (Fig. 8 a), others, that they may form from the breakup of surface channels or larger vesiculations (Fig. 8 b). Perhaps both occur. In any event the images suggest a high surface to volume ratio in the pinocytotic uptake event. The largest vesicular profiles with pinocytotic content were found infrequently and we are uncertain as to their significance in the uptake process. Even though they are not numerous in the small sample obtained with the electron microscope, they could account for the largest part of the volume of uptake. In spite of these uncertainties, it is possible to make a reasonable estimate of the rate of internalization of surface membrane due to pinocytosis in Acanthamoeba. Amebas were incubated under conditions in which they took up two to six times their surface in about 30 min by phagocytosis, and the rate of incorporation of radioactive precursors into cell phospholipids was compared with the rate in nonphagocytosing cells. Whether measured as incorporation into whole cell lipids (26) or into lipids of isolated plasma membranes (A. G. Ulsamer and E. D. Korn, personal communication), the rate was not appreciably affected by phagocytosis. This would be the expected result if pinocytosis in nonphagocytosing cells results in such a high surface turnover rate that it would not be appreciably different in cells forced to phagocytose. Studies on the effect of phagocytosis on pinocytosis (Bowers, unpublished) show a proportional decrease in pinocytosis with increasing amounts of phagocytosis, so that the two events are not additive with respect to surface turnover.
The rapid deletion of the cell surface suggests that it is not all replaced by de novo synthesis, but instead points to some recirculation of surface membrane components. The nature of the postulated recirculating unit is entirely unknown although Chlapowski and Band (7) have suggested, mainly on morphological grounds, that "collapsed vesicles" commonly seen in the closely related ameba they studied may be vehicles for membrane transfer. In Acanthamoeba, we note that similar collapsed vesicles (Fig. 8 a) appear to be related to pinocytotic uptake but we have no evidence on the possibility of reinsertion into the membrane as suggested by Chlapowski and Band.

The estimated rate of ingestion of surface membrane by Acanthamoeba of several times an hour is high in comparison with estimates from some other cells that exhibit pinocytosis. For example, Cohn (8) has calculated that the macrophage may internalize 50% of its surface in 2-5 hr during active pinocytosis. Chapman-Anderson (6) estimates that up to 30% of the surface of Amoeba proteus may be ingested during one pinocytotic cycle (15-30 min), but there is a required rest period before a new cycle can be initiated. Thus surface turnover in Acanthamoeba is remarkable not only for its high rate, but also for the fact that it appears to be continuous under our culture conditions.

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