Morphometric Analysis of Volumes and Surface Areas in Membrane Compartments during Endocytosis in Acanthamoeba

BLAIR BOWERS, T. E. OLSZEWSKI, and JOHN HYDE
Laboratory of Cell Biology and Biometrics Research Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

ABSTRACT Stereologic analysis was made of cell surface membrane (PM) and two interrelated cytoplasmic membrane systems, the vacuole membranes (VM) and small vesicle membranes (SVM). Volumes and surface areas of the three membrane compartments were measured during steady-state pinocytosis, when membrane recycling is rapid, and during phagocytosis, when a shift to a lower rate of membrane uptake by endocytosis occurs (B. Bowers, 1977, Exp. Cell Res. 110:409). Total membrane area in the three compartments was 3.2 µm²/µm³ of protoplasmic volume and was constant throughout the experiments. In pinocytosing cells, 32% of the membrane was in the PM, 25% in the VM, and 43% in the SVM. The vacuole compartment occupies ~20% of the total cell volume, and the small vesicle, ~3%. As the endocytic uptake of membrane from the surface decreased, there was an increase in PM area and a marked decrease in SVM area. The VM area remained constant even though "empty" vacuoles were almost completely replaced by newly formed phagosomes within 45 min. This demonstrates directly a rapid flux of membrane through this compartment. A model, taking into consideration these and other data on Acanthamoeba, is proposed to account for the observed membrane shifts. The data suggest that the vacuolar (digestive) system of Acanthamoeba is central to cellular control of endocytosis and membrane recycling.

Evidence from several different kinds of studies suggests that the bulk of cell surface membrane in free-living endocytic cells is in a reversible equilibrium with cytoplasmic vacuolar system (4, 6, 12, 15–18). The rate of cell surface turnover in these cells is very high, on the order of minutes (4, 17, 18). The dynamics of the membrane flow and the cytoplasmic membrane relationships are complex so that a complete definition of the pathway of membrane flow in a single system is not yet available. Even fewer clues exist as to how the cell modulates the process. We are using the small amoeba, Acanthamoeba castellanii as a model system for exploring these questions. Acanthamoeba is especially useful because it is easy to culture, it pinocytoses continuously and at a high rate, and the membrane composition appears to be simpler than that of mammalian cells (8).

The present study seeks to establish basic information about the volume and membrane area in three interrelated membrane compartments that appear to comprise the bulk of the recirculating membrane in Acanthamoeba. The compartments examined were: plasma membrane, vacuolar membrane (the amoeba digestive system), and an associated membrane system of small vesicles. We have made a morphometric analysis of electron micrographs of pinocytosing cells to establish a baseline. We then determined shifts in the distribution of membrane in the three compartments when phagocytosis at a maximum rate was superimposed on pinocytosis. Phagocytosis pre-empts the pinocytic mechanism so that the rate of fluid uptake decreases (1). The total volume of uptake remains virtually constant regardless of whether the cell is taking in large (solid) or small (liquid) quanta. The rate of surface uptake, on the other hand, is decreased in phagocytic cells because the average volume of the phagosomes is large compared to that of the pinosomes; hence the surface to volume ratio is smaller. These experiments, in addition to establishing the volumes and surface areas of those compartments directly involved in the endocytic process, give information about the flow of membrane through the cell by measuring the shift of membrane when the surface turnover is altered by this nonperturbing method.

A brief report of part of this work has been given elsewhere (5).
MATERIALS AND METHODS

Materials

Routine cell counts were performed on a cell counter (Particle Data Inc., Elmhurst, Ill.). Cell volumes were determined with a cell counter equipped with a multichannel analyzer and plotter (Coulter Electronics, Inc., Hialeah, Fla.). Polystyrene beads (0.93 μm diameter) were obtained from Dow Chemical Co., (Indianapolis, Ind.). *Acanthamoeba castellanii* (Neff strain) was cultured as described by Korn (7).

Uptake Experiment

20 ml of a cell suspension (10⁶ cells/ml) in culture medium were equilibrated for 15 min at 30°C on a reciprocating shaker. 1 ml of a concentrated suspension of “light” yeast in 0.1 M sodium phosphate buffer was added. The light yeast fraction was prepared as described by Oates and Touster (13) and facilitated removal of uningested yeast after saturation of the cells. The uptake of the yeast particles was monitored by light microscopy. A control flask contained no added yeast, but was otherwise treated identically with the experimental flasks. After 45 min, the cells were washed free of uningested yeast by two low-speed centrifugations and resuspended in fresh culture medium. The cells were re-equilibrated for 15 min and ~500 polystyrene beads/cell were added. Cell samples taken at 15-min intervals were assayed for yeast content and bead uptake. The bead content was determined spectrophotometrically as described previously (21).

Morphometry Experiment

Cells in culture medium (10⁶/ml) were incubated on a shaking water bath at 30°C. They were sampled at 0 time (represents value for pinocytosing cells) and 15, 30, and 45 min after the cells were presented with saturating loads of particles (lipid-extracted yeast). The cells were fixed for 1 h at room temperature in 3 or 5% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8, enrobed in agar, postfixed in 1% OsO₄ in the same buffer, and embedded in Epon 812 (9). Three separate experiments (A, B, and C) with identical experimental protocol were performed and analyzed. Particle uptake was monitored with the light microscope.

Morphometry Methods

Initial requirements for random sampling of the cell population are easily met with *Acanthamoeba*. There is no cell polarity and a random sample of the population is included in each block. For microscopy pale silver to gray sections were picked up on 200-mesh grids. All cell profiles in which the entire perimeter could be photographed were recorded at x 2,100 from a single section per block. 45-85 profiles per time point were analyzed with a coherent multipurpose test system (19) on micrographs enlarged to a final magnification of 10,500. These micrographs were used to determine cell surface, vacuole surface, and volume relationships. Vesicles (defined as membrane profiles <0.5 μm in their longest dimension) were too small to be seen at this magnification. Vesicle surface and volume were measured in a second stage of analysis in which micrographs were taken at 1,500 and enlarged to a final magnification of 46,500. A grid with higher density of test lines was used for the second-stage analysis. Micrographs were taken sequentially across all cell profiles in a single section per block, taking care not to overlap images. 35-45 micrographs were analyzed for each time point in the second stage of analysis. For both stages of analysis the reference volume was the protoplasm. Surface densities (Sv) and volume fractions (Vv) were computed by summing the individual counts for each profile and taking the ratio of the sums (10) using the formulas (19):  

\[ S_v = \frac{4 \cdot I_v}{2 \cdot P_v} \]

where \( z \) = test line length in μm, \( I_v \) = intersections of test line with membrane, and \( P_v \) = points in protoplasm; and

\[ V_v = \frac{P_v}{P_v} \]

where \( P_v = \) points in compartment and \( P_v = \) points in protoplasm.

For the individual experiments, the jackknife technique (11) was used to reduce the bias in the estimate of the ratios and to compute the standard errors. The method is as follows:

(a) Compute ratio using all micrographs; get value \( r \).

(b) For each micrograph, compute ratio with that micrograph removed. Obtain value \( r \) for each micrograph.

(c) For each micrograph define \( P_v = n \cdot (n - 1) \cdot r \), where \( n \) is the total number of micrographs. \( P_i \) is called a pseudovalue.

(d) Using values \( P_i \), compute the mean and the standard error in the usual way:

\[ \text{Mean} = \frac{\sum P_i}{n} \]

\[ \text{Standard error} = \sqrt{\frac{\sum (P_i - \text{mean})^2}{n(n-1)}} \]

RESULTS

Definition of Membrane Compartments

The three membrane-bounded compartments measured in this study are illustrated in Figs 1 and 2. The first, the surface membrane and the included cell volume, is unambiguous. At the outset we could not predict whether the cell volume would be constant during the period of the experiment. It was possible, for example, that the ingestion of large numbers of particles would result in an increase in total cell volume. We therefore choose to refer surface areas to the “protoplasmic” volume which would be expected to change very little during the 45-min period of the experiment. 45 min is ~5% of the doubling time of the cells (12-14 h). Protoplasmic volume included the nucleus, and the cytoplasmic ground substance with organelles, but excluded the space of the vacuolar and vesicular systems described below.

The second compartment, a large vacuole system, is always present in the cells under our culture conditions. As seen in the light microscope, axenically cultured amoebas normally contain 15-20 vacuoles up to 3 μm in diameter. Acid phosphatase activity has been demonstrated in the vacuoles (14) and it is clear that they represent the digestive system of the amoeba. For purposes of these experiments any membrane profile diameter >0.5 μm was considered part of the vacuolar system (Fig. 1).

A third compartment, comprised of small vesicles, included all membrane profiles with a diameter <0.5 μm (Fig. 2). These were almost all considerably <0.5 μm in diameter so that there were few profiles whose assignment was ambiguous. The vesicle compartment is certainly of multiple origins, although it appears to be composed mainly of small pinosomes and vesicles that fuse with or bud off vacuoles (2). The vesicles occur in a variety of three-dimensional shapes ranging from tubular to spherical.

Other membrane systems within the cytoplasm, namely the endoplasmic reticulum, the contractile vacuole, and Golgi apparatus are morphologically distinct (3) and were included as part of the protoplasmic volume.

Phagocytic Uptake

Amoebas, like multicellular organisms, behave as individuals. For any given experiment the percentage of cells that take up particles may vary. Because the sample for morphometric analysis is small, we determined the pattern of uptake in each experiment to be sure that the cell response was reasonably uniform. The pattern of uptake at 45 min is shown for the three experiments in Fig. 3. The graph shows that 96-99% of the cells in these experiments took in many particles. The variation in number of yeast per cell is in part caused by variation in size of individual amoebas rather than failure of a maximum rate of uptake. A different plot of the data from each of the time points is shown in Fig. 4. This graph shows that the cell population was essentially saturated with particles between 30 and 45 min after the initiation of uptake. The 15-min time point shows the most variation in both sets of data. Exp A is
more typical of uptake curves of *Acanthamoeba*. The cells in exp B were slow to being uptake and the cells in exp C, faster, but at 45 min the total uptake was very similar (Fig. 3).

Interpretation of the morphometric data relies on earlier data that showed that pinocytic uptake, and, as a consequence, surface turnover, is reduced in phagocytosing cells (1). Furthermore, the assumption has been that “saturated” cells have ceased taking in particles. It was possible, however that the appearance of saturation resulted from uptake and release of particles at the same rate so that, in fact, the membrane was still turning over at an appreciable rate because of a phagocytic component. To rule out this possibility, cells were first saturated with yeast, then washed free of uningested particles and exposed to latex beads to test further particle uptake. The results...
Figure 3: Internalized yeast were counted in 100-200 cells in the light microscope for each of the time points. Histograms of the 45-min time points are shown. The data show that >95% of the cells in each experiment were loaded with particles by 45 min so that they represent a relatively homogeneous cell population for analysis.

Figure 4: A plot of the average number of yeast per cell, obtained from light microscope counts as in Fig. 3, shows the overall uptake pattern in the three experiments. The cells reach the same level of saturation after 45 min, although the early kinetics of uptake is different in the three experiments. Note that the curves exactly parallel the morphometric data on the fractional volume of yeast-filled vacuoles, obtained with a much smaller sample in the electron microscope analysis (see Fig. 6).

Figure 5: The number of yeast taken up per cell was counted with the light microscope and the average for 100 cells computed (A). After 45-min exposure to yeast, the cells were washed free of uningested yeast and reincubated with 0.9-µm polystyrene beads (B) for an additional 45 min. The uptake of polystyrene beads was measured spectrophotometrically after extraction with dioxane (20). As a control, cells were carried through the same incubation and wash procedures in the absence of yeast particles, and the uptake of beads in these cells was measured after 45 min (C).

Figure 6: The volume fractions \( V_c \) plotted are the ratios of the compartment volume to the "protoplasmic" volume, rather than to total cell volume, in order to have a constant denominator. The protoplasm is defined as the nucleus plus the cytoplasmic ground substance and its included organelles. Three experiments (A, B, and C) are shown and standard error bars are included in the plots. Values plotted in the "combined" panel are the average of the jackknifed estimates of the values from the three experiments. The circled points are statistically significantly different from the 0 time value \( P \leq 0.05 \).

Morphometry

Volumes of the measured compartments, as volume fraction of protoplasmic volume, are shown in Fig. 6. The percentages given below have been recalculated as percent of total cell volume rather than protoplasmic volume and are based on the average values for the three experiments. The space of the vacuolar compartment accounts for 20% of the total cell volume in pinocytosing (0 time) cells, whereas the vesicle compartment volume is only 3% of the cell volume. As the cell fills with particles, "empty" vacuoles disappear in a reciprocal fashion. The loss of empty vacuoles is slower than the rate of phagosome acquisition, however, so that an increase in total vacuolar volume of 9% is observed during the experiment. The increase in vacuolar volume parallels, and presumably accounts for, the increase in total cell volume. Only the volume of the vesicle compartment decreased, from 3% of the total cell volume in
pinocytosing cells to 1% in saturated phagocytic cells.

Data on surface densities are given in Fig. 7. The largest fraction of membrane in pinocytosing cells is found in the small vesicle compartment. It accounts for 43% of the total membrane of the three compartments. The plasma membrane is 32% of the total and vacuolar membrane, 25%.

An unexpected finding was that the plasma membrane area increased by ~24% in phagocytosing cells. The increase was seen in each experiment and is statistically significant. This result indicates a temporary imbalance between surface replacement and surface internalization caused by endocytosis, and suggests that the two events are not tightly coupled. Because both the cell surface and the cell volume increased during the experiment, we examined the cell surface to total cell volume ratios to determine if a change in this relationship occurred (Table I). With the exception of one point in exp A, (at 30 min) the ratio is the same, within limits of the analysis, for a given batch of cells. The average values for the three experiments show no statistically significant differences between time points. However, each of the three experiments shows the same slight increase in surface to volume ratio that returns toward the value in pinocytic cells after saturation, suggesting that the shift may be real.

The membrane area of the vacuolar system remains essentially constant during the experiment and the same approximately reciprocal relationship is seen between particle-filled and empty vacuoles as was seen in the volume measurements. About 80% of the membrane in the vacuolar system surrounds particles after 45 min of phagocytosis. Because most of this represents membrane that has entered the cytoplasm from the cell surface, the implication is that the vacuolar membrane is not a permanent intracellular membrane, but turns over with the surface membrane. The disappearance of empty vacuoles cannot be accounted for by their fusion with phagosomes, as the majority of phagosomes remain tightly wrapped by membrane during the course of the experiment (Table I). The morphometric data indicate that the vacuoles fragment. The surface/volume ratio of individual empty vacuoles on the average increased during the experiment. For the 0-, 15-, 30-, and 45-min samples, the ratios averaged for the three experiments were 3.2, 3.7, 5.1 and 3.9, respectively. In contrast the S/V ratios for the yeast-containing vacuoles were constant at 1.8 for each of the samples.

The surface of the small vesicle compartment declines sharply during phagocytosis (Fig. 7). After 45 min of phagocytosis, the membrane area in vesicles decreased by almost 40%. The membrane in this compartment undergoes much larger fluctuations than membrane in the other two compartments, and thus appears not to be so closely regulated by the cell. This finding is also consistent with the idea that vesicles provide a pool of membrane that is replacing surface membrane.

The total membrane surface area, i.e., the sum of the three compartments, is the same for each time point. There is one exceptional value: the total membrane at 45 min in exp A is significantly less than that found in the preceding three time points, a finding for which we have no explanation. The constancy of total membrane found within the experiments serves as a check on the validity of the method, and also indicates that there is no appreciable loss of membrane with intensive phagocytosis. The total membrane surface area for the three compartments was 3.2 ± 0.3 μm²/μm³ of protoplasmic volume (average of the four time points for three experiments).

The volumes of glutaraldehyde-fixed *Acanthamoeba* cells from cultures grown to a density of 1 x 10⁶ cells/ml were determined with a cell counter calibrated for volume measurements. The average volume was 2,540 ± 0.9 μm³, corresponding to a cell diameter of 17 μm. The surface area of a smooth sphere 17 μm in diameter is 908 μm². The surface area determined by morphology in these experiments is 2,388 μm², using S/V of 0.94 and the measured average cell volume. Thus the actual cell surface area is ~2.6 greater than that calculated for a sphere of the same volume.

![Figure 7](https://example.com)  
**Figure 7** The surface densities (S₂) are the ratio of the compartment surface area to the protoplasmic volume (see Fig. 6 legend). In some cases the individual values for each of the three experiments (e.g., empty vacuole membrane) were significantly different from the 0 time value, but there was enough variability between experiments that the averaged value for all three experiments did not show a P value <0.05.

| Table I |
|---------|
| **Cell Surface to Total Cell Volume Ratios** |
| **Time, min** | **0** | **15** | **30** | **45** |
| **exp A** | 0.67 ± 0.05 | 0.77 ± 0.07 | 0.82 ± 0.05 | 0.65 ± 0.04 |
| **exp B** | 0.94 ± 0.06 | 1.01 ± 0.08 | 0.97 ± 0.10 | 0.92 ± 0.06 |
| **exp C** | 0.94 ± 0.07 | 0.96 ± 0.10 | 1.04 ± 0.08 | 0.99 ± 0.07 |
| **avg** | 0.85 ± 0.09 | 0.91 ± 0.07 | 0.94 ± 0.06 | 0.86 ± 0.10 |

Values are ± standard error of the mean. There are no statistically significant differences between values within any experiment, except the 30-min point for exp A has a P value of 0.036 when compared to 0 time, and 0.008 when compared to 45 min. The average values are not significantly different from one another.

Bowers ET AL. Morphometry of Membranes in Acanthamoeba
The vesicle compartment represents membrane in transit both to and from the plasma membrane. Flow from the surface via small vesicles has previously been documented in *Acanthamoeba* using horseradish peroxidase (HRP) as a tracer (2, 4). The return of membrane to the surface by way of small vesicles is not shown directly in these experiments, but is the most reasonable interpretation of the morphometric data. The morphometric experiment shows that at least 95% (exp C) of the vacuolar membrane can be replaced in 45 min. This value represents a minimum rate of turnover and is direct evidence for a rapid flux of membrane through the cell. There are several possible routes by which membrane might leave the vacuolar compartment.

(a) The empty vacuoles do not appear to fuse with the phagosomes. Table II shows that 54–85% of the phagosomes are tightly wrapped with membrane after 45 min of phagocytosis, and there is no trend toward loosening the membrane with time. The percentage of phagosomes that do show some loosening of the membrane coincides with the proportion (about one-third) found in cytochemical experiments to show acid phosphatase activity after 1 h of yeast uptake (14). We have also observed (B. Bowers, unpublished results) that when HRP was given simultaneously with yeast and the cells were washed free of HRP and reincubated for 2 h, cytochemically demonstrable HRP almost disappeared after 2 h. The small amount of reaction product observed was confined to tight phagosomes and none was found in loose phagosomes. Thus, loosening of the membrane probably reflects the addition of membrane-enclosed hydrolytic enzymes.

(b) The empty vacuoles do not fragment into units that are not recognizable as membrane in electron micrographs, nor does the membrane appear to be degraded. During the period of greatest loss of empty vacuole membrane, the total morphologically identifiable membrane in the three compartments is constant. If the membrane is degraded, then it must be replaced by a steady synthesis of new membrane. If that were the case, the sudden decrease in membrane turnover as the cell becomes saturated with particles should result in membrane accumulation, which was not observed.

(c) It is unlikely, although difficult to rule out, that an empty vacuole of equivalent surface area adds to the plasma membrane in exchange for each phagosome taken in. Such a mechanism does not account for the small vesicle compartment nor is the plasma membrane balance as precise as this mechanism would imply.

(d) An alternative possibility, that vacuoles fragment into smaller vesicles as they proceed through their life cycle, best fits the data. Morphological studies have shown large numbers of vesicles attached to and associated with vacuoles, as well as the appearance of vacuolar fragmentation (2, 6). Surface to volume ratios of vacuoles increase at a time when few new vacuoles are being formed, indicating that the average vacuole is becoming smaller. Finally, the pattern of decrease in the size of the vesicle compartment is consistent with a major component being contributed by membrane flowing to the plasma membrane, as the decrease continued at a time when the pinocytic contribution should be stable at a lower rate or increasing.

Fig. 8 summarizes a model of membrane dynamics that fits
the currently available data. The continued decline in the small vesicle compartment is interpreted to be the result of the decrease in rate of pinocytosis that occurs when phagocytosis is superimposed on the cell plus the decreased rate of processing of digestive vacuoles. Fig. 5 shows that the cells retain yeast particles for a period of hours, whereas "empty" digestive vacuoles are displaced (turned over) within 45 min (Figs. 6 and 7). This suggests that the vacuoles are processed more rapidly than phagosomes containing digestible particles, which seems reasonable as their content is largely water and the low molecular weight solutes of the culture medium. An important corollary of this interpretation is that modulation of the flow of membrane appears to be related to the digestive process and not the endocytic process.

The molecular significance of the constancy of vacuolar dimensions in the cell cannot be meaningfully interpreted at the present time, but the observations reported here serve to focus attention on some important properties of the internal membrane systems of endocytic cells.

Received for publication 18 August 1980, and in revised form 17 November 1980.

REFERENCES

1. Bowers, B. 1977. Comparison of pinocytosis and phagocytosis in Acanthamoeba castellanii. Exp. Cell Res. 110:409-417.
2. Bowers, B. 1980. A morphological study of the plasma and phagosome membranes during endocytosis in Acanthamoeba. J. Cell Biol. 84:246-260.
3. Bowers, B., and E. D. Korn. 1968. The fine structure of Acanthamoeba castellanii. I. The trophosome. J. Cell Biol. 39:95-111.
4. Bowers, B., and T. E. Olserwski. 1972. Pinocytosis in Acanthamoeba castellanii. Kinetics and morphology. J. Cell Biol. 53:681-694.
5. Bowers, B., and T. E. Olserwski. 1978. Stereological measurements on phagocytosing Acanthamoeba. J. Cell Biol. 79:2, Pt. 2:237a (Abstr.).
6. Chlapowski, F. J., and R. N. Band. 1971. Assembly of lipids into membranes in Acanthamoeba palestinensis. II. The origin and fate of glycerol-3H-labeled phospholipids of cellular membranes. J. Cell Biol. 56:634-651.
7. Korn, E. D. 1974. The isolation of the amoeba plasma membrane and the use of latex beads for the isolation of phagocytic vacuole (phagosome) membranes from amoeba including the culture techniques for amoeba. Methods Enzymol. 31:686-698.
8. Korn, E. D., and P. L. Wright. 1973. Macromolecular composition of an amoeba plasma membrane. J. Biol. Chem. 248:459-447.
9. Luft, John H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409-414.
10. Mayhew, T. M., and L. M. Cruz Orive. 1974. Caveat on the use of the Delesse principle of areal analysis for estimating component volume densities. J. Microsc. (Oxf.). 102:195-207.
11. Miller, R. G. 1974. The jackknife—a review. Biometrika. 61:1-15.
12. Muller, W. A., R. M. Steinman, and Z. A. Cohn. 1980. The membrane proteins of the vacuolar system. II. Bidirectional flow between secondary lysosomes and the plasma membrane. J. Cell Biol. 86:304-314.
13. Oates, P. J., and O. Touster. 1976. In vitro fusion of Acanthamoeba phagolysosomes. I. Demonstration and quantitation of vacuole fusion in Acanthamoeba homogenates. J. Cell Biol. 68:319-334.
14. Ryter, A., and B. Bowers. 1976. Localization of acid phosphatase in Acanthamoeba castellanii with light and electron microscopy during growth and after phagocytosis. J. Cell Biol. 70:309-321.
15. Ryter, A., and C. de Chastellier. 1977. Morphometric and cytochemical studies of Dictyostelium discoideum in vegetative phase. Digestive system and membrane turnover. J. Cell Biol. 75:200-217.
16. Schneider, Y. J., P. Tulkens, C. de Duve, and A. Trouet. 1979. Fate of plasma membrane during endocytosis. II. Evidence for recycling (shuttle) of plasma membrane constituents. J. Cell Biol. 82:666-674.
17. Steinman, R. M., S. E. Brodie, and Z. A. Cohn. 1976. Membrane flow during pinocytosis. A stereological analysis. J. Cell Biol. 78:665-677.
18. Thilo, L., and G. Vogel. 1980. Kinetics of membrane internalization and recycling during pinocytosis in Dictyostelium discoideum. Proc. Nat. Acad. Sci. U. S. A. 77:1015-1019.
19. Weibel, E. R., and R. P. Bolender. 1973. Stereological techniques for electron microscopic morphometry. In Principles and Techniques of Electron Microscopy. M. A. Hayat, Editor. Van Nostrand Reinhold Co., New York. 3:237-296.
20. Weibel, E. R., and D. Paumgarner. 1977. Integrated stereological and biochemical studies on hepatoplastic membranes. II. Correction of section thickness effect on volume and surface density estimates. J. Cell Biol. 87:584-597.
21. Wiegman, R. A., and E. D. Korn. 1967. Phagocytosis of latex beads by Acanthamoeba. I. Biochemical properties. Biochemistry. 6:483-497.
22. Williams, M. A. 1977. Stereological Techniques. In Practical Methods in Electron Microscopy. A. M. Glauert, Editor. North Holland Publishing Co., Amsterdam. 6:1-94.