Disappearance of Macrophage Surface Folds after Antibody-dependent Phagocytosis

HOWARD R. PETTY, DEAN G. HAFEMAN, and HARDEN M. McCONNELL
Stauffer Laboratory for Physical Chemistry, Stanford University, Stanford, California 94305

ABSTRACT We have employed the method of Burwen and Satir (*J. Cell Biol.*, 1977, 74:690) to measure the disappearance of surface folds from resident guinea pig peritoneal macrophages after antibody-dependent phagocytosis. Unilamellar phospholipid vesicles containing dimyristoylphosphatidylcholine and 1 mol % dinitrophenyl-ϵ-aminocaproyl-phosphatidylethanolamine, a lipid that possesses a hapten headgroup, were prepared by an ether injection technique. These vesicles were taken up by macrophages in a time- and temperature-dependent fashion. Vesicles that contained ferritin trapped in the internal aqueous volume were identified within macrophages by transmission electron microscopy. Scanning electron microscopy has shown that macrophage surface folds decrease dramatically after phagocytosis. The surface fold length (μm) per unit smooth sphere surface area (μm^2) decreases from $1.3 \pm 0.3\ \mu m^{-1}$ to $0.53 \pm 0.25\ \mu m^{-1}$ when cells are incubated in the presence of specific anti-DNP antibody and vesicles at 37°C. No significant effect was observed in the presence of antibody only or vesicles only. Our studies show that phagocytosis is associated with a loss of cell surface folds and a loss of cell surface area, which is consonant with current views of the endocytic process. On the basis of our uptake data, we estimate that ~400 μm^2 of vesicle surface membrane is internalized. The guinea pig macrophage plasma membrane has a total area of ~400 μm^2 in control studies, whereas the cells have roughly 300 μm^2 after phagocytosis. These estimates of surface areas include membrane ruffles and changes directly related to changes in cell volume. We suggest that during antibody-dependent phagocytosis a membrane reservoir is made available to the cell surface.

The macrophage plasma membrane possesses receptors for the Fc domain of the immunoglobulin molecule that can mediate immunologic recognition leading to phagocytosis or cytolysis of a target (for reviews see references 21–23). A variety of targets has been employed as models of antibody-dependent and antibody-independent phagocytosis (e.g., sheep erythrocytes and latex beads, respectively). Recent research in this laboratory (12, 16) and others (11) has examined the antibody-dependent phagocytosis of lipid-hapten-containing phospholipid vesicles. This system has several distinct advantages over the more conventional methods because: (*a*) it is chemically well characterized, (*b*) the physical characteristics ("fluidity") of the membrane can be controlled, (*c*) membrane hapten density is known and can be varied, (*d*) surface charge density can be altered, (*e*) the cholesterol-phospholipid ratio can be changed, and (*f*) various materials can be delivered to the cells by entrapment in the internal aqueous volume of the vesicle. It is clear that such vesicles have many potential advantages for studies of molecular events involved in immunologic recognition, triggering, and subsequent effector functions.

To understand the role of cell surface components during antibody-dependent phagocytosis it is helpful to have an understanding of variations in plasma membrane surface area as well as turnover. This description requires knowledge of four interrelated parameters: (*a*) cell surface area before phagocytosis, (*b*) cell surface area after phagocytosis, (*c*) area of cell surface membrane internalized, and (*d*) the area of membrane shuttled to the plasmalemma during this process. In the present study we have measured parameters *a*, *b*, and *c* to infer the magnitude of *d*. We have used resident guinea pig peritoneal macrophages in a study of the antibody-dependent phagocytosis of single-shell lipid vesicles containing lipid haptens, and radiolabeled lipid, or ferritin (FT) trapped in the internal aqueous volume.

© The Rockefeller University Press · 0021-9525/81/05/0223/07$1.00

MATERIALS AND METHODS

Materials

Cell buffer was composed of 2.0 mM $CaCl_2$, 1.5 mM $MgCl_2$, 5.4 mM KCl, 1 mM Na_2HPO_4, 5.6 mM glucose, 120 mM NaCl, 0.2% bovine serum albumin (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.; fatty acid poor), and 25 mM HEPES at pH 7.4. Horse spleen FT was obtained from Sigma Chemical Co. (St. Louis, Mo.). Glutaraldehyde, collidine buffer, and OsO_4 were obtained from Polysciences, Inc. (Warrington, Pa.). Scanning electron microscopy (SEM) specimen stubs were obtained from Pelco, Inc. (Tustin, Calif.).

Phospholipid Vesicles

FT-containing, ~1-μm unilamellar vesicles were prepared by a modification of the ether injection method of Deamer and Bangham (5) as described previously (12). Vesicle lipid composition was 99 mol % dimyristoylphosphatidylcholine (DMPC) and 1 mol % dinitrophenyl-ε-aminocaproyl-phosphatidylethanolamine (DNP-cap-PE). FT was trapped within the vesicles by inclusion of 18–22 mg/ml FT in the phosphate-buffered saline (PBS; 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4) injection buffer. To remove unentrapped FT the FT vesicles were washed three times by centrifugation on a 3-ml cushion of 15% Ficoll in PBS (12,000 *g* for 30 min).

For quantitative studies of vesicle-cell binding, 0.25 mol % radiolabeled phospholipid L-[1-^{14}C]α-dipalmitoylphosphatidylethanolamine (55 μCi/mol, Applied Science Labs, Inc., State College, Pa.) and 1 mol % of the fluorescent phospholipid *N*-4-nitrobenzo-2-oxa-1,3-diazole phosphatidylethanolamine (NBD-PE), prepared from egg lecithin (Avanti Biochemicals, Inc., Birmingham, Ala.) were included in the lipid mixture with a concomitant decrease in the amount of DMPC. FT was omitted from the injection buffer to facilitate later separation of unbound vesicles and cells by density gradient centrifugation.

Cell Preparation

Resident peritoneal cells were collected from Hartley guinea pigs (EZH Caviary, Williams, Calif.). The anesthetized animals were sacrificed by cardiac puncture followed by cervical dislocation. The cells were collected in 100 ml of cold Ca^{++}, Mg^{++}-free Hanks' balanced salt solution (HBSS) (Grand Island Biological Co., Santa Clara, Calif.). The cells were then washed with HBSS. The peritoneal cells were almost exclusively macrophages, eosinophils, and erythrocytes as previously determined (6). The macrophages were purified by step-density gradient centrifugation on lymphocyte separation medium (Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, Md.) as described by Boyum (1). The cell preparation (~5×10^6/animal) was made up of ≥95% phagocytes as indicated by latex bead uptake and confirmed by fluorescence microscopy and transmission electron microscopy (TEM). Differential counts were made with Wright's stain and the viable percentage (≥95%) of cells was determined by trypan blue exclusion.

Antihapten Antibody

Rabbit anti-DNP antiserum was a gift from Dr. Alfred Esser (Scripps Clinic and Research Foundation, La Jolla, Calif.). The anti-DNP IgG was fractionated from serum by affinity chromatography using protein A coupled to cyanogen bromide-actived Sepharose (8). In all experiments the anti-DNP was centrifuged at 110,000 *g* for 15 min in a Beckman airfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) to remove aggregates.

Phagocytosis of Vesicles

To 1.2 × 5.0 cm polypropylene vials (E & K Scientific Products, Inc., Saratoga, Calif.), 100 μl of vesicles (0.05 μmol total lipid, suspended in PBS), and antibody diluted into 100 μl of PBS were added and thoroughly mixed. 1×10^6 macrophages were added in 300 μl of cell buffer. The mixtures were incubated at 37°C in a gyratory water-bath shaker (model G76, New Brunswick Scientific Co., Inc., Edison, N. J.) at 170 cycles/min. After various periods of time the vials were chilled in ice water.

Measurement of Cell-associated Lipid Vesicles

To measure vesicle uptake (binding followed by phagocytosis) by macrophages, we employed ^{14}C-labeled lipids (see above for preparation). In all determinations of vesicle uptake, control experiments were performed in the presence of radiolabeled vesicles and the absence of anti-DNP antibody. After incubation with vesicles, an aliquot of 0.5 ml was then layered with a polypropylene pipette tip onto 0.5 ml of a 3:2 mixture of 10% Ficoll (Pharmacia, Uppsala, Sweden) in cell buffer and Ficoll-Hypaque (density = 1.077) in a 1.0-ml polystyrene centrifuge tube (Fisher Scientific Co., Pittsburgh, Pa.). The tube was capped and spun at 500 *g* for 20 min, frozen, and the lower tip was removed with a hacksaw. The contents of the tip were digested overnight with 0.5 ml of NCS solubilizer (Amersham Corp., Arlington Heights, Ill.) and the radioactivity was determined by adding 10 ml of a toluene-based scintillation fluor (Preblend 2a70; RPI Corp., Elk Grove Village, Ill.) and counting in a liquid scintillation counter. The channel discriminators were set to exclude background light caused by chemiluminescence-phosphorescence, and the relative counting efficiency was determined later by adding an internal ^{14}C standard.

Cell Volume

The intracellular volume accessible to water was determined using methods similar to those previously described (4). [^{14}C]polyethylene glycol 4000 (21.7 mCi/g) and ^3H_2O (5 Ci/ml) were obtained from Amersham Corp. Incubation of macrophages with or without vesicles was carried out as described under Phagocytosis of Vesicles, except that all volumes and reagents were increased by a factor of 2.5. After a 30-min incubation with vesicles, 5×10^6 macrophages were pelleted by centrifuging at 800 *g* for 10 min and resuspended to 140 μl in HBSS. 15 μl of a mixture of [^{14}C]polyethylene glycol (50 μCi/ml) and ^3H_2O (50 μCi/ml) was added and the suspension was incubated at 25°C for 30 min. The suspended cells were then layered over a 250-μl cushion of dibutyl phthalate and paraffin oil in a 400-μl polypropylene centrifuge tube and centrifuged at 1,000 *g* for 2 min. The lower portion of the centrifuge tubes containing the cells was cut off. The cell pellet was resuspended in 0.1 ml of H_2O and 0.5 ml of NCS solubilizer and digested overnight. ^{14}C and ^3H were determined as described under Phagocytosis of Vesicles in a dual-channel Beckman LS-230 counter.

Transmission Electron Microscopy

Macrophages were fixed with 1% glutaraldehyde in PBS for 1 h at room temperature. The macrophages were postfixed with 1% OsO_4 in 0.1 M collidine buffer (pH 7.0) at room temperature for 1 h. The cells were dehydrated in acetone and embedded in Spurr's resin (18). The embedded cells were thin-sectioned on an AMR ultramicrotome (AMR Corp., Bedford, Mass.) equipped with a diamond knife. The thin sections were examined in a Hitachi HU-11E electron microscope.

Scanning Electron Microscopy

Macrophages were fixed in suspension with 1% glutaraldehyde in PBS for 1 h at room temperature. The cells were then washed in 0.1 M collidine buffer and postfixed for 1 h in 1% OsO_4 in collidine buffer at room temperature. The cells were dehydrated in an increasing series of acetone in water with three changes in 100% acetone. The cells were placed in modified BEEM capsules (Better Equipment for Electron Microscopy, Inc., Bronx, N. Y.) with a Millipore filter (Millipore Corp., Bedford, Mass.; pore size 1.0 μm) affixed to one end (15). The macrophages were critical-point dried in CO_2. Cells were dispersed on a stub coated with double-sticky tape before sputter coating with gold. Specimens were examined in the ISI-40 scanning electron microscope. Micrographs were taken with Polaroid type 55 P/N film.

Measurement of Surface Fold Length

We have employed the method developed by Burwen and Satir (2, 3) to measure macrophage surface fold length per unit surface area before and after phagocytosis. Briefly, cells were randomly selected at low magnification. Micrographs taken at a magnification of ~7,000 were enlarged to a nominal magnification of 20,000. The cell radius (neglecting surface projections) was determined as described (3) by using a series of concentric circles on plexiglass. Folds were traced with a map reader to 80% of the cell radius. The fold length measured is compared to the surface area that a smooth sphere of the cell's radius would occupy in a two-dimensional projection at 0.8 × radius (see reference 3).

RESULTS

Uptake of Vesicles

Macrophages bearing Fc receptors bind specific antibody liganded phospholipid vesicles in a time-dependent fashion. The rate of binding is dependent on the surface density of bound antibody molecules and on the physical properties of the vesicle lipid bilayer (12). The time-course of binding a large excess of DMPC vesicles containing 1 mol % DNP-cap-PE at 37°C to guinea pig macrophages is shown in Fig. 1. After 30

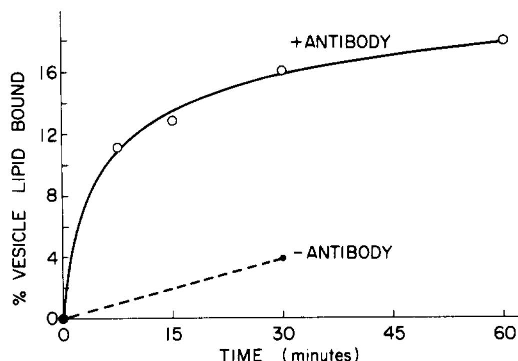

FIGURE 1 Rate of vesicle uptake by macrophages. DMPC vesicles contained 0.25 mol % radiolabeled phospholipid, 1 mol % fluorescent NBD-PE, and 1 mol % DNP-cap-PE hapten (○, with anti-DNP rabbit IgG; ●, without). Points show the mean of duplicate determinations; standard deviations are ±10% of the measured values.

min, 16% of the vesicle lipid was bound in the presence of anti-DNP antibody vs. 4% in its absence. Incubation on ice for 15 min resulted in insignificant cell-associated radioactivity. The antibody-dependent binding and subsequent phagocytosis of the fluorescent vesicles was confirmed by direct observation using epifluorescence microscopy.

TEM of FT Vesicles

The uptake of FT vesicles was examined by thin-section TEM. A representative cell is shown in Fig. 2. FT-containing vesicular structures may be seen throughout the cytoplasm. Such structures have not been observed in untreated macrophages (data not shown). It is also known that FT is not present in peritoneal macrophages under normal conditions (9). We suggest that these structures are the internalized FT vesicles. The phagocytic vesicles may have fused with lysosomes.

Scanning Electron Microscopy

The surfaces of untreated resident guinea pig peritoneal macrophages possess many folds and ridges. A representative cell that was incubated at 37°C for 30 min is shown in Fig. 3 *a*. A few microvilli can be seen on occasional cells. Nearly all cells observed are spherical in shape. The folds were similar in width, although fold height appears to vary more in macrophages than in mast cells (3). Macrophages are heterogeneous with regard to both cell size (Table I) and fold length. To provide a significant amount of quantitative data in convenient form, we have employed the method of Burwen and Satir (3) to assess the ratio of surface fold length to surface area. In Fig. 4*A* we show a histogram of this ratio for untreated cells. These data represent a composite of three separate experiments. The mean fold length:area ratio of 1.1 μm^{-1} is similar to that of 1.3 μm^{-1} reported for rat peritoneal mast cells (3). In additional control experiments, we have shown that macrophages treated with antibody alone (Fig. 3*b*) or FT vesicles alone (Fig. 3*c*) are very similar to the untreated cells. Macrophages were exposed to either reagent for 30 min at 37°C.

A substantial reduction in the number of surface folds occurs when macrophages are incubated with FT vesicles in the presence of specific antihapten antibodies. We show a typical cell in Fig. 3*d*. These cells were treated with antibodies and FT vesicles for 30 min at 37°C as described above. Identical results were obtained on four separate occasions, each consisting of pooled cells from several animals in each. In Fig. 4*B* we show fold length:area ratio for this cell population, which is significantly less than that of control cells (Fig. 4*A*). A break in the histogram of Fig. 4*B* can be observed. However, the reason for this discontinuity is uncertain. It may, for example, represent subpopulations of macrophages that differ in surface properties after phagocytosis. Additional experiments (data not shown) have indicated that the change in surface folds is time-dependent.

In addition to data on surface folds, this technique also provides information with regard to cell size. The mean cell radii, neglecting surface projections, in control (absence of both antibody and vesicles) and phagocytosis experiments are 3.3 ± 0.06 and 3.8 ± 0.06 μm, respectively (see also Table I). The cell radius, as measured by SEM, increased ~15%.

Cell Volume

Because cell radius is an indirect measure of cell volume, we considered it advisable to determine cell volume by a more direct means. We have employed ^3H_2O as a probe for total pellet volume and [^{14}C]polyethylene glycol as a probe of extracellular volume to measure total cell volume. Macrophage volume was found to be 160 ± 50 μm^3/cell and 250 ± 70 μm^3/cell before and after phagocytosis, respectively (see Table I). Extracellular volume was somewhat greater after phagocytosis, suggesting a different packing behavior of the cells. These values are similar to those calculated from cell radii with the assumption that the cells are smooth spheres (see Table I).

Calculation of Macrophage and Vesicle Surface Areas

From the data obtained, one may directly calculate the influence of membrane folds upon macrophage surface area. The fold length:surface area ratio is first adjusted by a small, but significant, geometrical factor (1.16) that corrects for image foreshortening (up to 0.8 r) in the SEM micrographs. The only assumption is that the cells are randomly placed on the SEM stub. The area of the fold is then calculated as described (3) with fold height estimated as 0.6 μm, fold width is 0.1 μm, and the area of a smooth sphere of the cell radius is 140 μm^2. Fold width was estimated with TEM and SEM. Fold height was estimated from thin-section TEM micrographs by measuring the distance from cell body to end of the fold. This underestimates fold height and decreases slightly the magnitude of the differences we have observed. The surface area of a cell, including surface fold length and our estimates of height and width, is calculated to be ~400 μm^2 (values are summarized in Table I). Surface area after phagocytosis (fold height ~0.2 μm), including the increase in volume, is ~300 μm^2.

The surface area internalized may be calculated from the data given in Fig. 1. With the assumption of random vesicle uptake, we calculate an internalized surface area of ~400 μm^2/cell. We have employed the value of 50 Å^2/lipid molecule and assumed 0.3 mol of exposed lipid/mole lipid in this calculation, as previously determined for similar vesicles (17). In experiments utilizing the fluorescent phospholipid NBD-PE and fluorescence microscopy as described (12), we estimate that at least one-half of the vesicles are internalized under the conditions employed for guinea pig macrophages. Previous workers have established that the surface area of internalized particles is very nearly equal to the surface area of plasmalemma

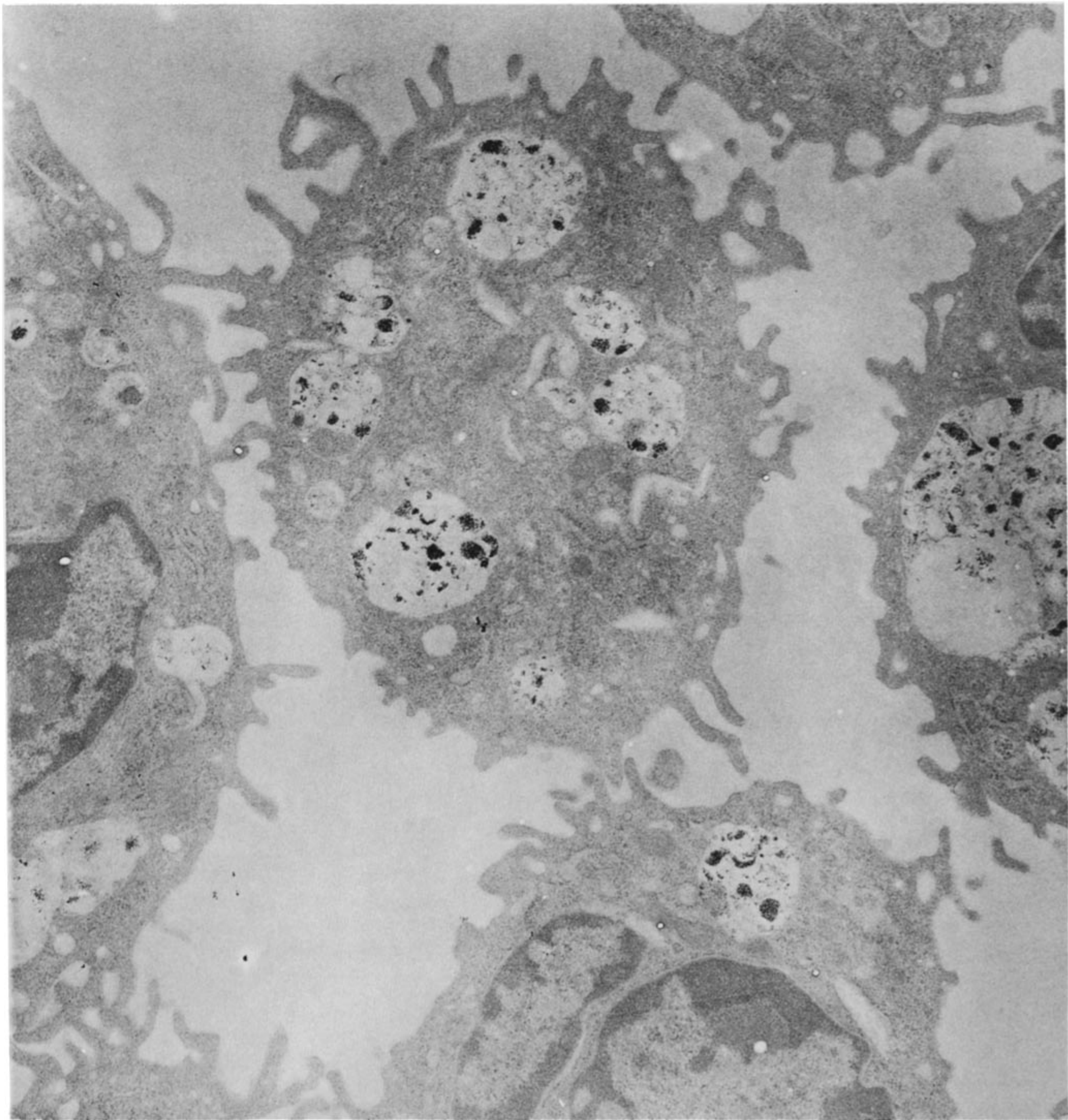

FIGURE 2 TEM of an FT vesicle and specific antibody-treated resident guinea pig peritoneal macrophage. Macrophages were exposed to FT vesicles and antihapten antibody for 30 min at 37°C with agitation as described in Materials and Methods. A number of vesicular cytoplasmic structures that contain the FT label may be discerned. The vesicle membranes are not visualized because the saturated fatty acids employed in this study are not osmiophilic. This thin section has not been counterstained. × 17,000.

internalized. This has also been demonstrated (by M. McCloskey in this laboratory) for the antibody-dependent uptake of lipid vesicles with freeze-fracture techniques.

The above calculations show that the amount of surface area internalized is approximately equal to that originally present at the cell surface. Although the cell surface area decreases after phagocytosis (while the volume increases), this does not account for the amount of plasmalemma internalized.

DISCUSSION

Our studies have shown that there is a dramatic reduction of macrophage surface folds after antibody-dependent phagocytosis of lipid-hapten and ferritin-containing phospholipid vesicles. The disappearance of surface folds requires the presence of both specific antibody and FT vesicles because antibody or FT vesicles alone have no effect. The reduction in fold length

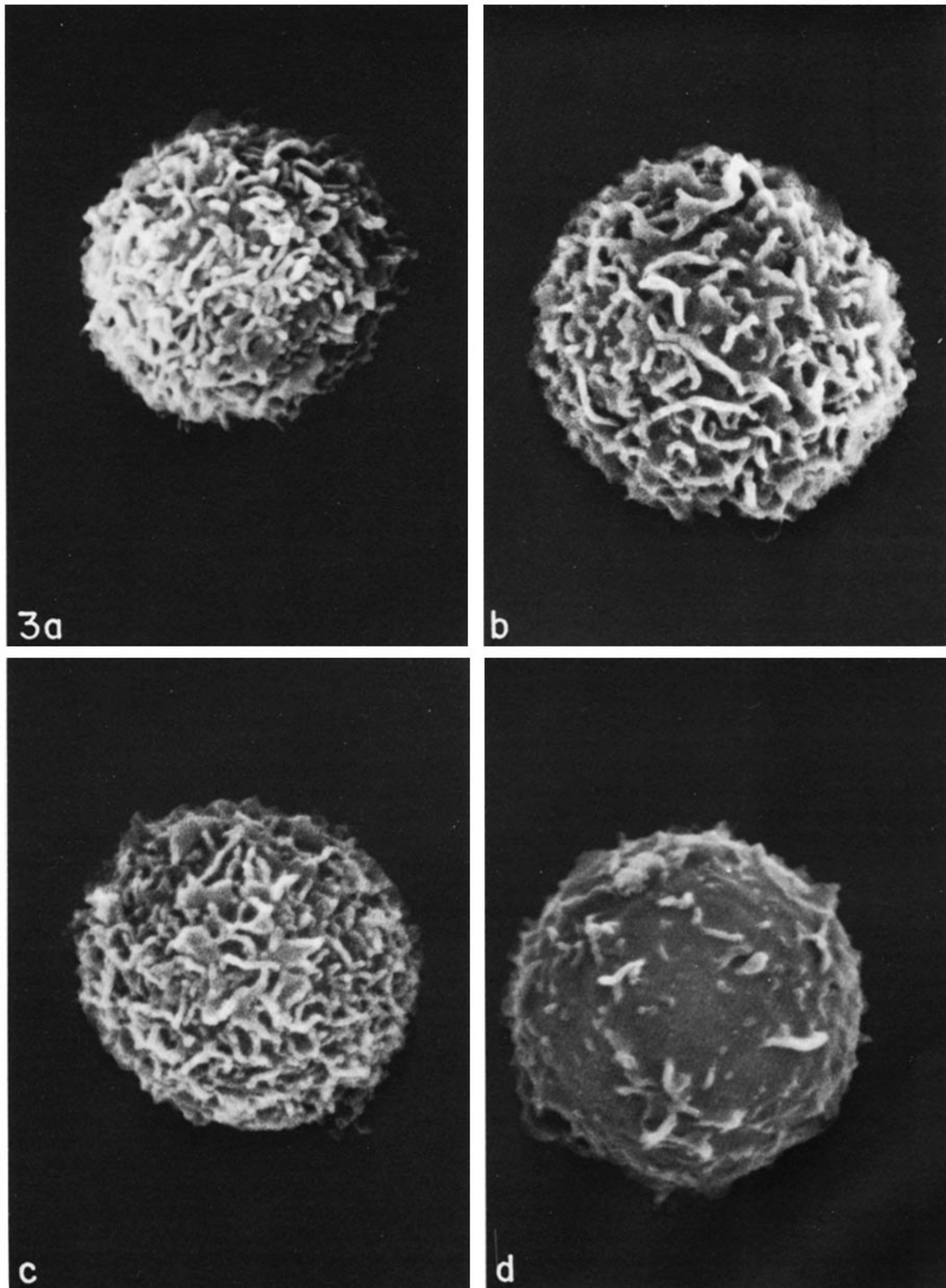

FIGURE 3 (*a*) SEM of a resident guinea pig peritoneal macrophage incubated at 37°C for 30 min. These cells possess many surface folds. (*b*) A typical SEM of a macrophage treated with anti-DNP antibody at 37°C for 30 min. (*c*) SEM of a macrophage exposed to FT vesicles at 37°C for 30 min. This cell population and that of *b* are indistinguishable from the cells of *a*. (*d*) In this SEM, macrophages were treated with both specific antibody and FT vesicles under identical conditions at 37°C for 30 min. A substantial reduction in the number of surface folds has taken place. All micrographs are × 7,200.

TABLE I
Properties of Macrophages

Quantities	Control	Phagocytosis
Measured		
Cell radius (μm)*	3.3 ± 0.06	3.8 ± 0.06
Surface fold length/surface area (μm^{-1})*	1.1 ± 0.03	0.53 ± 0.04
Cell volume (μm^3/cell)‡	160 ± 50	250 ± 70
Calculated		
Cell volume*(μm^3/cell)	150	230
Surface area of smooth sphere (μm^2)*	140	180
Surface area including folds (μm^2)*	410	300
Approximate surface area of internal vesicles (μm^2)§	—	~400

* Based on SEM data (mean ± SEM); n = 41 for each entry.
‡ Based on ^3H_2O tracer volume data (mean ± SD of duplicate determinations).
§ Based on binding data. See text for details.

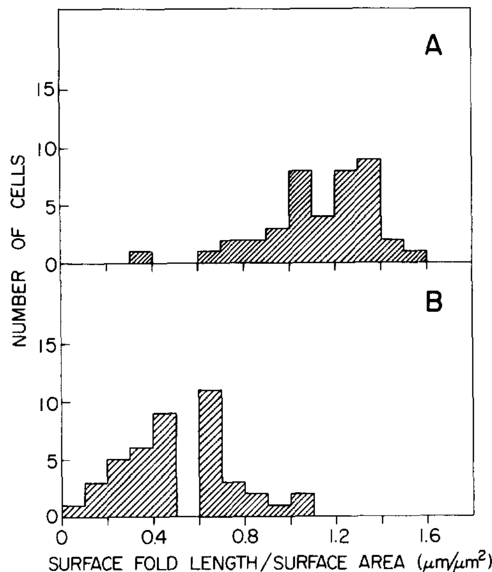

FIGURE 4 A histogram of the surface fold length (μm) per unit smooth sphere surface area (μm^2) of macrophages. (*A*) Control macrophages (both antibody and vesicles are absent) incubated at 37°C for 30 min. (*B*) Macrophages were treated with both antibody and FT vesicles. Both cell populations were examined during four separate experiments with pooled cells from several animals in each. The data given in the histogram are from randomly selected cells in three experiments (n = 41 for each panel; see also Table I).

was concomitant with the appearance of FT vesicles in the cytoplasm and the binding of ^{14}C-labeled phospholipid vesicles to macrophages. The data indicate that the internalization of plasma membrane during phagocytosis results in a decrease in surface folds.

In previous studies (14, 15) one of us has shown that the adsorptive pinocytosis of concanavalin A results in a global decrease in macrophage surface folds. Various workers (for a review see reference 22) have suggested that localized decreases in surface folds occur near the location of phagocytosis. However, the latter qualitative experiments are complicated by the facts that it is difficult to know a posteriori where the phagocytic event took place and that the responses are generally not high (for example, because of antibody-independent conditions). We have, therefore, employed the method of Burwen and Satir to measure the decrease of surface folds in a highly responsive antibody-dependent system. Our data (Table I) show that a significant area of the cell surface membrane is removed.

TEM studies of the uptake of FT vesicles have shown clearly the presence of FT-containing vesicular structures within macrophages. These structures probably represent the internalized FT vesicles. The presence of these structures indicates that vesicle morphology is not altered dramatically by phagocytosis. The vesicle membrane was not visualized in the thin sections because the saturated fatty acid employed in these preparations is not osmiophilic. This method of visualizing vesicle phagocytosis might be useful in examining vesicle clearance in drug delivery systems.

From our binding and SEM experiments, we suggest that a membrane reservoir is made available to the plasmalemma during phagocytosis. This is based upon the following data: (*a*) cell surface area before phagocytosis is ≃400 μm^2, (*b*) this value after phagocytosis is ≃300 μm^2, and (*c*) the binding data indicate that ≃400 μm^2 of vesicle surface area is internalized. (This value is analogous to results in experiments utilizing RAW264 macrophages where these cells took up an area equivalent to 100% of the cell surface area during phagocytosis [12].) Roughly 300 μm^2 of surface area in addition to the ≃100 μm^2 contributed by the decrease in membrane folds would need to be made available to the cell surface. We have not yet examined the possible sources of this area; however, the Golgi apparatus and the plasmalemma-like membrane which is recycled during pinocytosis (20) are likely candidates.

Previous studies of macrophage membrane flow have focused upon (*a*) surface area internalized during bulk phase pinocytosis in resident (20) and activated (7) macrophages; (*b*) the recyling of trypsin-α macroglobulin receptors after adsorptive pinocytosis (10, 19); and (*c*) the recycling of membrane components after antibody-independent phagocytosis of latex beads (13).

Antibody-dependent lipid vesicle phagocytosis has been examined in our laboratory (12, 16) and in others (11). In the presence of specific antibody, these vesicles activate the CN^--insensitive respiratory burst of RAW264 macrophages (12) and guinea pig macrophages (unpublished observations). The dependence of this respiratory burst on vesicle lipid composition ("fluid" or "solid") is similar for both types of macrophages. The binding of vesicles to cells has been examined by fluorescence microscopy (12) and a fluorescence-activated cell sorter (11). A quantitative theory of Fc receptor motion and depletion after antibody-dependent phagocytosis of vesicles has been presented (12) and this theory has been supported by rosetting experiments (16). The experiments described herein provide additional important ultrastructural data relevant to the mechanism of Fc receptor depletion and membrane turnover.

We thank Dr. R. Beuerman and Ms. S. Lauber of the Department of Opthamology, Stanford University Medical School for making the scanning electron microscope available. We are grateful to the Department of Biological Sciences, Stanford University for the use of their transmission electron microscope. We thank Ms. F. Thomas for her expert technical assistance.

This work was supported by grant 2R01 AI13587 from the National Institutes of Health. H. R. Petty is a fellow of the Damon Runyon-Walter Winchell Cancer Fund (DRG-396F).

Received for publication 29 September 1980, and in revised form 30 December 1980.

REFERENCES

1. Boyum, A. 1976. Isolation of lymphocytes, granulocytes and macrophages. *Scand. J. Immunol. Suppl.* 5:9–15.
2. Burwen, S. J. 1977. Measurement of microfold length per unit area in two-dimensional projection of mast cells. Scanning Electron Microscopy/1977, Vol. II. IIT Research Institute. Chicago, Ill. 21–28.
3. Burwen, S. J., and B. H. Satir. 1977. Plasma membrane folds on the mast cell surface and their relationship to secretory activity. *J. Cell Biol.* 74:690–697.
4. Castranova, V., L. Bowman, and P. R. Miles. 1979. Transmembrane potential and ionic content of rat alveolar macrophages. *J. Cell. Physiol.* 101:471–480.
5. Deamer, D., and A. D. Bangham. 1976. Large volume liposomes by an ether vaporization method. *Biochim. Biophys. Acta.* 443:629–634.
6. Daems, W. Th., and P. Brederoo. 1973. Electron microscopical studies on the structure, phagocytic properties, and peroxidatic activity of resident and exudate peritoneal macrophages in the guinea pig. *Z. Zellforsch. Mikrosk. Arat.* 144:247–297.
7. Edelson, P. J., R. Zwiebel, and Z. A. Cohn. 1975. The pinocytic rate of activated macrophages. *J. Exp. Med.* 142:1150–1164.
8. Esser, A. F., R. M. Bartholomew, J. W. Parce, and H. M. McConnell. 1979. The physical state of membrane lipids modulates the activation of the first component of complement. *J. Biol. Chem.* 254:1768–1770.
9. Fedorko, M. E., N. L. Cross, and J. G. Hirsch. 1973. Appearance and distribution of ferritin in mouse peritoneal macrophages *in vitro* after uptake of heterologous erythrocytes. *J. Cell Biol.* 57:289–305.
10. Kaplan, J. 1980. Evidence for revitalization of surface receptors for α-macroglobulin protease complexes in rabbit alveolar macrophages. *Cell.* 19:197–205.
11. Lesserman, L. D., J. N. Weinstein, R. Blumenthal, and W. D. Terry. 1980. Receptor-mediated endocytosis of antibody-opsonized liposomes by tumor cells. *Proc. Natl. Acad. Sci. U. S. A.* 77:4089–4093.
12. Lewis, J. T., D. G. Hafeman, and H. M. McConnell. 1980. Kinetics of antibody-dependent binding of haptenated phospholipid vesicles to a macrophage-related cell line. *Biochemistry.* 19:5376–5386.
13. 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 plasma membrane. *J. Cell Biol.* 86:304–314.
14. Petty, H. R. 1979. The inflammatory macrophage-concanavalin A interaction. *In* Proceedings of the Electron Microscopy Society of America. G. W. Bailey, editor. Claitor Publishing, Baton Rouge, La. 154–155.
15. Petty, H. R. 1980. Response of the resident macrophage to concanavalin A: Alterations of surface morphology and anionic site distribution. *Exp. Cell Res.* 128:439–454.
16. Petty, H. R., D. G. Hafeman, and H. M. McConnell. 1980. Specific antibody-dependent phagocytosis of lipid vesicles by RAW264 macrophages results in the loss of cell surface Fc but not C3b receptor activity. *J. Immunol.* 125:2391–2396.
17. Schwartz, M. A., and H. M. McConnell. 1978. Surface areas of liposomes and lipid vesicles. *Biochemistry.* 17:837–840.
18. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium of electron microscopy. *J. Ultrastruct. Res.* 26:31–43.
19. Stahl, P., P. H. Schlessinger, E. Sigardson, J. S. Rodman, and Y. C. Lee. 1980. Receptor-mediated pinocytosis of mannose glyconconjugates by macrophages: characterization and evidence for receptor recycling. Cell. 19:207–215.
20. Steinman, R. M., S. E. Brodie, and Z. A. Cohn. 1976. Membrane flow during pinocytosis. A sterologic analysis. *J. Cell Biol.* 68:665–687.
21. Stossel, T. P. 1977. Endocytosis. *In* Receptors and Recognition, Series A, Vol. 4 P. Cuatrecasas and M. F. Greaves, editors. Chapman and Hall, London. 103–141.
22. Walters, M. N.-I., and J. M. Papadimitriou. 1978. Phagocytosis: A review. *CRC Crit. Rev. Toxicol.* 377–421.
23. Zuckerman, S. H., and S. D. Douglas. 1979. The characterization and functional significance of plasma membrane Fc receptors. *CRC Crit. Rev. Microbiol. Immunol.* 1–26.