

Polarization of Endocytosis and Receptor Topography on Cultured Macrophages

ROBERT J. WALTER, RICHARD D. BERLIN, JANET R. PFEIFFER, and JANET M. OLIVER
Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032

ABSTRACT In the J774.2 macrophage cell line, microtubule disassembly by colchicine causes the polarization of membrane functions and structure. Colchicine-treated cells develop a bulge or protuberance that is bordered by microvillous membrane. The protuberance is the site of concanavalin A cap formation. The fluid pinocytosis of horseradish peroxidase and of fluorescein- and rhodamine-conjugated high molecular-weight dextrans, the adsorptive pinocytosis of concanavalin A, and the concentration and phagocytosis at 37°C of a range of phagocytic particles (IgG- and complement-opsonized erythrocytes, complement-opsonized zymosan, latex spheres, albumin-stabilized oil droplets) are all similarly restricted to the protuberance. A reduction in the rate of dextran pinocytosis, determined by fluorimetry, and reductions in phagocytic rates for oil emulsion and IgG-opsonized erythrocytes accompany the polarization of endocytic activity in colchicine-treated J774.2 macrophages.

Membrane receptors for phagocytic particles are not confined to the protuberance but rather may display their own unique topographical asymmetry. The inherent topography of receptors was inferred from particle distribution under conditions that limit particle-receptor redistribution (after labeling at 4°C or a very brief incubation at 37°C). Under these restrictive conditions, latex binding sites were detected over the whole membrane whereas receptors for IgG-opsonized erythrocytes, aggregated IgG, complement-opsonized erythrocytes, and complement-opsonized zymosan were excluded from the protuberance. Thus, functional (endocytosis) and structural (inherent receptor distribution) analyses of membrane topography define different patterns of asymmetry in protuberant cells.

The asymmetry induced in J774.2 macrophages by colchicine is highly analogous to the functional and structural polarity of epithelial cells. Exploration of this analogy may provide insight into the development of polarized epithelia and, more generally, into mechanisms by which specialized areas of membrane are established.

In a variety of leukocytes and macrophages, microtubule disassembly by colchicine leads to development of a ruffled protuberance underlain by microfilaments at one pole of the cell. We and others have shown that the protuberance provides a focus for the movement at 37°C of concanavalin A (Con A)-receptor complexes from their inherently dispersed distribution into a surface cap (1). In the course of this earlier work, we also noted the similar shapes and ultrastructure of colchicine-treated leukocytes and polarized epithelial cells. In particular, the microfilamentous meshwork that underlies the protuberance of microtubule-depleted leukocytes is reminiscent of the terminal web and brush border of intestinal epithelium (23). It is well known that many surface functions and enzymatic activities are polarized to the microvillous border of epithelial

cells (22, 37, 47). Therefore, by analogy, we predicted that polarization of surface functions and of surface molecular topography might extend to colchicine-treated cells of non-epithelial origin.

We report here the induction by colchicine of asymmetry in the ultrastructure, function, and molecular topography of J774.2 mouse macrophages.

MATERIALS AND METHODS

Cells

J774.2 macrophages were kindly provided by Dr. O. Rosen and Dr. B. Bloom, Albert Einstein School of Medicine, New York. They were selected from a line originally developed by Dr. P. Ralph, Sloan-Kettering Institute, New York (32). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supple-

mented with 20% horse serum as described before (8). For studies in cell suspension, J774.2 macrophages were cultured on sterile plastic Petri plates. As shown by Muschel and co-workers (24), macrophages are readily collected from these plates by pipetting. Cell monolayers used for fluorescence microscopy were grown on 13-mm diameter glass coverslips in 35-mm diameter Falcon dishes (Falcon Plastics, Oxnard, Calif.). Monolayers for electron microscopy were grown directly on Falcon dishes. J774.2 cells adhere tightly and assume a fibroblastic cell shape on these latter surfaces.

Con A Labeling

Monolayers of J774.2 cells were labeled with fluorescein- or rhodamine isothiocyanate-conjugated Con A (both 10–25 $\mu\text{g}/\text{ml}$) for fluorescence microscopy or with a complex of biotinyl-Con A (Vector Laboratories Inc., Burlingame, Calif.; 100 $\mu\text{g}/\text{ml}$) and avidin-peroxidase (Vector Laboratories Inc.; 25 $\mu\text{g}/\text{ml}$) for electron microscopy. In general, fluorescent Con A labeling was done for 5 min in Dulbecco's phosphate-buffered saline with 5 mM glucose (PBS) at either 4° or 37°C followed by fixation in 2% paraformaldehyde in PBS as described before (8). For electron microscopy, cells were usually incubated with biotinyl-Con A in PBS for 5 min at 4°C, rinsed thoroughly in PBS at 4°C, incubated for 5 min at 4°C with avidin-peroxidase, rinsed again, and then warmed at 37°C for 5 min in DMEM. Control monolayers received avidin-peroxidase only or biotinyl-Con A only. Fixation and development of the peroxidase reaction product with diaminobenzidine and H_2O_2 was carried out as described before for light microscopy of peroxidase uptake (8). However, for electron microscopy, the cell pellets were postfixed in 2% osmium tetroxide, embedded in Epon, thin-sectioned with a diamond knife, and examined without staining in a Philips 300 or JEOL 100 CX/ASID electron microscope. Fluorescence microscopy was used to optimize the experimental conditions before electron microscopy: this was possible by substitution of avidin-fluorescein or avidin-rhodamine (also from Vector Laboratories Inc.) for avidin-peroxidase.

Before use, avidin-peroxidase was treated with sodium periodate to destroy sugar residues with potential affinity for surface receptors (39), followed by overnight dialysis and ultracentrifugation to remove particulates. After this treatment, no avidin-peroxidase was bound at 4°C to macrophages in the absence of biotinyl-Con A. At 37°C no surface binding occurred but some avidin-peroxidase could be internalized by fluid pinocytosis. The protocol described above, in which cells were warmed to 37°C only after treatment at 4°C with both Con A and peroxidase and careful removal of unbound ligands by washing, was developed to eliminate any contribution of fluid pinocytosis to the observed distribution of peroxidase.

Horseradish Peroxidase Uptake

For ultrastructural studies of fluid pinocytosis, monolayers of macrophages were incubated for various times with 200 $\mu\text{g}/\text{ml}$ horseradish peroxidase (HRP) in complete medium. The cells were subsequently fixed and processed for electron microscopy as described above. The HRP was passed through a 0.22- μm filter before use. Preliminary experiments showed that yeast mannan (500 $\mu\text{g}/\text{ml}$), which inhibits a receptor-mediated component of HRP uptake in alveolar macrophages (39), does not affect HRP uptake in J774.2 cells.

Dextran Uptake

A more dynamic measure of fluid pinocytosis was obtained from the uptake of fluorescent, high molecular-weight dextrans. J774.2 cells were incubated in complete medium supplemented with various concentrations of either fluorescein-dextran (Sigma FD-70, average mol wt 70,000 Sigma Chemical Co., St. Louis, Mo.) or rhodamine-dextran (average mol wt 84,000, Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N.J.; prepared by incubation of dextran overnight with rhodamine-isothiocyanate followed by either three cycles of ethanol precipitation and resolubilization in water or exhaustive dialysis against PBS). After extensive washing, usually at 4°C, the cells were either fixed with 2% paraformaldehyde in PBS or extracted by the addition of 0.1% Triton-X-100 in PBS (1.0 ml) and vortexing. The fixed cells were examined and photographed on Kodak Tri-X-Pan film with a Zeiss Photomicroscope III. 0.75-ml portions of the cell extracts were diluted with 0.7 ml PBS and fluorescence intensity caused by dextran was measured in a Hitachi Perkin-Elmer MP4 spectrofluorimeter (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.) using excitation and emission wavelengths of 485 and 515 nm, respectively.

Phagocytic Particles

J774.2 cells were incubated with two particles whose binding and uptake are independent of opsonization: 0.77- μm diameter latex particles (Polysciences, Inc., Warrington, Pa.) and bovine serum albumin-stabilized paraffin oil emulsion that

was prepared according to Stossel and co-workers (43) using oil containing the fluorochrome, perylene (6).

Two particles whose binding and uptake is mediated by the macrophage Fc receptor were also studied. Sheep erythrocytes (Cordis Laboratories Inc., Miami, Fla.) were opsonized with rabbit anti-sheep erythrocyte IgG (N. L. Cappel Laboratories Inc., Cochranville, Pa., or Cordis Laboratories Inc.) according to Griffin et al. (17). Aggregated IgG was prepared by heating rhodamine-conjugated rabbit anti-goat IgG (15 mg/ml) at 63°C for 30 min followed by low-speed centrifugation to remove large clumps (3, 13).

Finally, two different complement-opsonized particles were used. Zymosan particles (Sigma Chemical Co.) were opsonized as before (10) by incubation of 20 mg of zymosan at 37°C for 15–30 min with 1 ml of fresh human serum followed by washing and resuspension in 4 ml of complete medium. Sheep erythrocytes were opsonized by sequential incubation with rabbit anti-sheep erythrocyte IgM (N. L. Cappel Laboratories Inc.) and fresh mouse serum according to the method of Griffin and co-workers (17).

Phagocytosis: Functional Topography and Quantitation

Latex particles suspended in complete medium were incubated for various times with either monolayers or suspensions of J774.2 cells. The cells were rinsed in PBS, fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, washed in cacodylate buffer, postfixed in 1% osmium tetroxide, and then dehydrated through a graded series of ethanols as described before (7). In some experiments, cell monolayers were then prepared for scanning electron microscopy by critical-point drying using liquid CO_2 and coating with gold using a Denton vacuum evaporator equipped with a cold sputter module (Denton Vacuum Inc., Cherry Hill, N.J.). The cell surface distribution of particles was observed at 20 kV in a JEOL 100 CX/ASID electron microscope. Alternatively, the cells were further dehydrated with acetone (propylene oxide was avoided to prevent solubilization of the beads), then embedded in Epon, thin-sectioned, and stained with uranyl acetate and lead citrate. For quantitative studies the number of beads per cell was counted in at least 20 random micrographs from each cell preparation. The cross-sectional areas of the same cells (minus nuclei) were determined by use of a sonic digitizer (graf/pen, Science Accessories Corp., Southport, Conn.), and the data were finally expressed as number of ingested beads per unit area of cytoplasm (in square micrometers).

Bovine serum albumin-stabilized paraffin oil was diluted in complete medium and incubated for various times with macrophage suspensions followed by careful washing to remove extracellular oil droplets. For simple observation of the distribution of ingested particles, cells were then fixed with 2% paraformaldehyde and examined in the fluorescence microscope. For quantitative studies of oil uptake, the cell pellets were extracted with *n*-butanol and the fluorescence intensities of the extracts were measured in a Hitachi-Perkin-Elmer MP4 spectrofluorimeter (Perkin-Elmer Corp.). Details of this sensitive fluorimetric assay for phagocytosis have been reported before (6).

IgG-opsonized erythrocytes suspended in complete medium were added to cell monolayers and incubated for 5 to 10 min at 37°C. Incubation was terminated by gentle rinsing through four changes of PBS. Particle uptake was quantified as before (8) by counting intracellular erythrocytes per cell in the light microscope after hypotonic lysis of adherent erythrocytes and glutaraldehyde fixation of the macrophages. Before each experiment, a preliminary titration of opsonin concentration and hematocrit was performed to obtain an average of six to nine erythrocytes bound or ingested per macrophage after 5–10 min of incubation at 37°C. Nonopsonized erythrocytes were included as controls in each experiment. These particles showed no binding activity under any conditions.

Complement-opsonized erythrocytes and zymosan were incubated with macrophages as described for IgG-opsonized erythrocytes. Nonopsonized zymosan did not bind to J774.2 cells.

Phagocytosis: Receptor Topography

The topography of latex binding sites and of Fc and complement receptors was inferred from the distribution of the same particles described above but with the incubation conditions adjusted to minimize the movement of particle-receptor complexes. In most cases, particles (diluted as above) or aggregated IgG (1–30 dilution in complete medium) were bound to surface receptors during incubation with macrophages at 0–4°C for 15–30 min. However, certain receptors failed to bind particles during short incubation at 4°C. Consequently, an alternative procedure was also followed: macrophages were exposed to a short pulse (5–60 s) of more concentrated ligand suspensions at 37°C. Under the latter conditions, both IgG-opsonized erythrocytes and complement-opsonized erythrocytes were used at a 5% hematocrit. Complement-opsonized zymosan was used at 20 mg/ml .

After particle-receptor binding by either protocol, macrophages were rinsed

briefly (15–20 s) in Hanks' buffer at the same temperature used for binding and either fixed immediately in 2% paraformaldehyde or 2% glutaraldehyde or incubated further at 37°C for 5–10 min and then fixed. The distribution of rhodamine-IgG was observed by epifluorescence microscopy and photographed with a $\times 63$ or $\times 100$ neofluor objective. Latex particles bound to macrophages were observed by scanning electron microscopy (SEM), and the distribution of opsonized erythrocytes and zymosan on macrophages was observed with Nomarski optics in a Zeiss Photomicroscope III.

RESULTS

The Effect of Colchicine on Cell Shape

More than 90% of untreated J774.2 cells attached to glass coverslips maintain a rounded or spread morphology (Fig. 1 a). In contrast, after exposure in complete medium to the inhibitors of microtubule assembly, 2 μ M colchicine (30 min) or 1 μ M nocodazole (30 min), ~ 90 –95% of these cells develop a distinct protuberance (Fig. 1 b). The protuberance is separated from the cell body by a constricted region that resembles a cleavage furrow. Granules and vesicles are excluded from large portions of the periphery of the protuberance, consistent with the presence of a microfilamentous gel that is more typically highly organized in primary leukocytes and has been described in detail previously (1, 7, 26–28). The time-course of this change in cell shape is given in Fig. 2 (solid circles).

The Effect of Colchicine on the Distribution of Con-A Receptor Complexes

J774.2 macrophages incubated at 4°C with Con A, in the form of either fluorescein-Con A or the biotin Con A-avidin

peroxidase complex, display a uniform distribution of surface ligand on both control (Fig. 3 a) and colchicine-treated cells (Fig. 3 b). When Con A-labeled J774.2 macrophages are warmed at 37°C, the bound lectin is rapidly redistributed. In cells incubated with medium alone, Con A-receptor complexes are internalized rapidly and uniformly by the process of adsorptive pinocytosis (Fig. 4 a). In cells exposed to colchicine, Con A moves rapidly to the protuberance forming a cap that is in turn internalized through the protuberance (Fig. 4 b).

The Topography and Kinetics of Pinocytosis after Colchicine

In mouse peritoneal macrophages the uptake of the soluble protein, HRP occurs by fluid pinocytosis: that is, HRP does not bind to surface receptors but enters the cells during their normal uptake of medium (40, 41). The uptake of HRP in J774.2 cells follows these same kinetics (data not shown). Furthermore, in J774.2 cells fluorescein- and rhodamine-conjugated dextrans similarly follow the accepted kinetics of fluid pinocytosis: their rates of uptake are directly proportional to medium concentration (Fig. 5 a) and are not saturated over long periods of incubation (Fig. 5 b).

In normal, rounded J774.2 macrophages, HRP uptake occurs over the entire cell. This is illustrated in Fig. 6 a: a control cell exposed to HRP for 10 min shows a membrane free of peroxidase and shows a large number of pinocytic vesicles distributed uniformly in the peripheral cytoplasm. By contrast, after colchicine treatment pinocytosis is confined to the protuberance

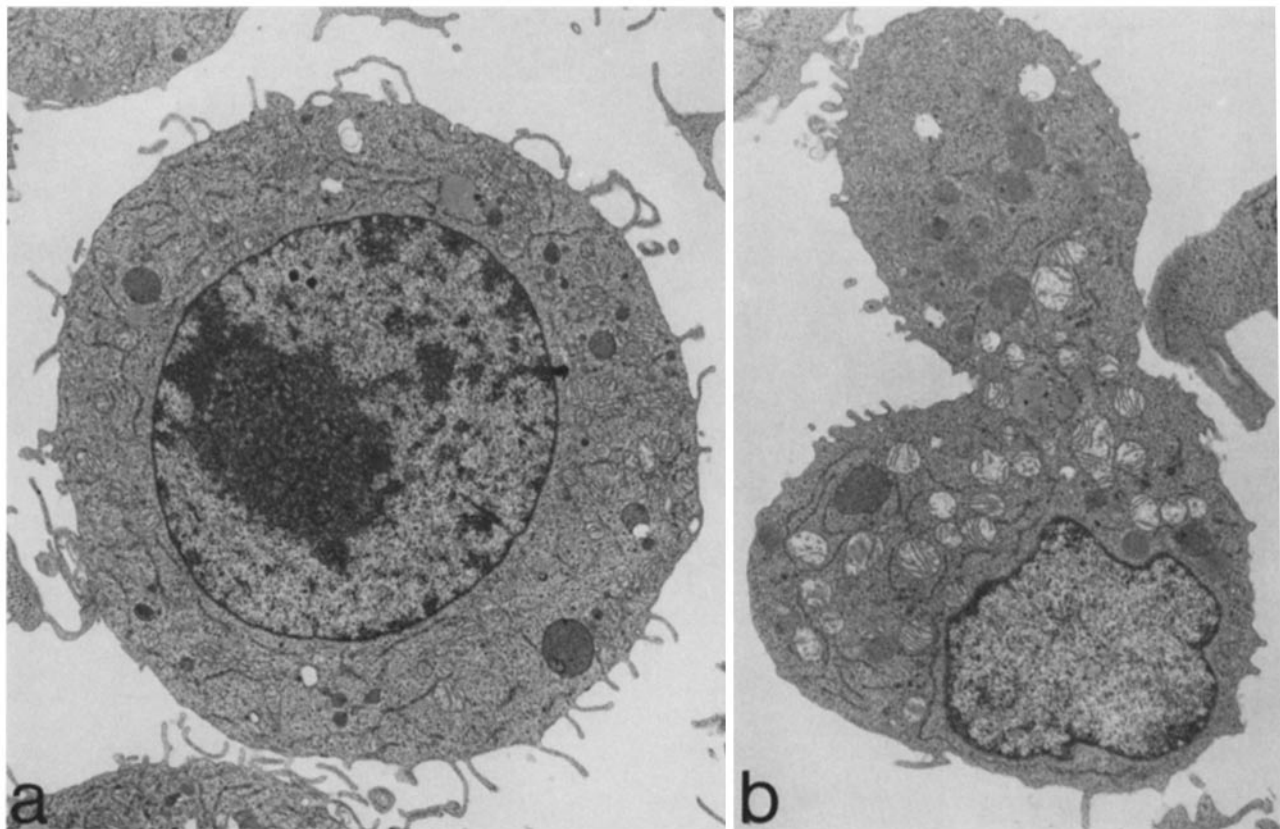


FIGURE 1 Morphology of interphase J774.2 macrophages. A thin section through a typical interphase macrophage (a) reveals a rather uniformly ruffled cell with prominent endoplasmic reticulum, mitochondria, vacuoles, and lysosomal granules. Colchicine treatment causes the appearance of a large, ruffled protuberance connected to the cell body by a constricted neck region. Cytoplasmic organelles are restricted to the core of the protuberance (b). (a) $\times 5,775$. (b) $\times 6,600$.

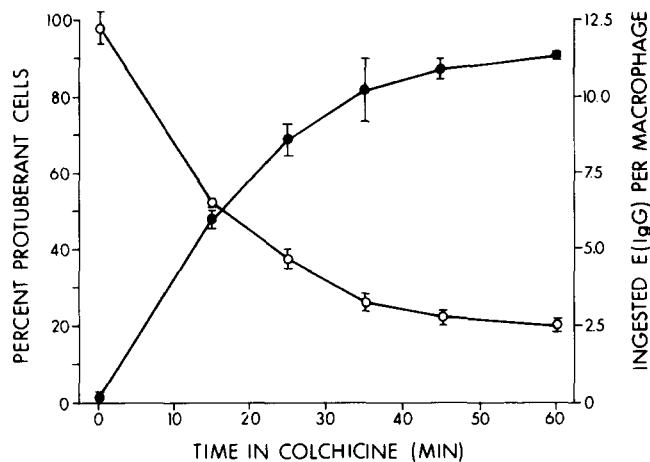


FIGURE 2 The effect of colchicine on cell shape and phagocytosis. J774.2 monolayers were incubated with $2 \mu\text{M}$ colchicine for varying times at 37°C . IgG-opsonized erythrocytes were present during the last 10 min of incubation. The monolayers were then rinsed in hypotonic medium to lyse extracellular erythrocytes, fixed in 2% glutaraldehyde, and examined in a Zeiss Photomicroscope III. At least 200 cells were scored at each time to determine the percent protuberant cells (○) and the average number of ingested erythrocytes per cell (●).

and little uptake of HRP occurs from other regions of the cell (Fig. 7b).

By studying the distribution of pinocytized material with time, it was possible to show that pinocytotic vesicles form in the protuberance and move unidirectionally through it towards the cell body. Macrophages were incubated sequentially with fluorescein- and rhodamine-labeled dextrans, and the direction of movement of vesicles entering by fluid pinocytosis was determined from the resulting distributions of fluorescence. Under these conditions in nonprotuberant cells, as shown in Fig. 7 a-c, fluorescence caused by fluorescein-dextran that had been present during the initial 20 min of incubation and rhodamine-dextran that had been present in the final 5 min of incubation is present in clearly separate vesicles that are distributed diffusely throughout the cytoplasm. By contrast, the protuberant cells shown in Fig. 7 d-f were incubated with colchicine before dextran uptake. The first marker, fluorescein-dextran, is accumulated in the cell body and it shows a very limited distribution in the protuberance. However, the rhodamine-dextran that is in process of internalization is located primarily in the protuberance. Thus, pinocytotic vesicles may move down through the protuberance into the cytoplasm where presumably they fuse with lysosomes. However, the resulting secondary lysosomes, like mitochondria and other organelles, show a very limited capacity to move back into the protuberance.

In the course of these experiments it became clear that colchicine not only polarizes the uptake of HRP and dextran but also causes a variable reduction in the extent of their uptake. This inhibition can be demonstrated with either marker. It is illustrated here (Fig. 8) for the uptake of dextran in cell suspensions. The fluorimetric data show that both control and colchicine-treated cell suspensions maintain an essentially linear rate of uptake. However, the linear rate is much lower after colchicine.

The Topography and Kinetics of Phagocytosis after Colchicine

Colchicine had a similar effect on the topography of phago-

cytosis at 37°C . In control J774.2 cells, binding of all particles and their phagocytosis occurred over the whole cell surface. By contrast, in colchicine-treated cells all types of particles studied after 5–10-min incubation at 37°C were concentrated over the protuberance and were internalized through the protuberance. Complement-opsonized particles, though bound, showed little or no uptake by J774.2 cells. Oil emulsion observed by fluorescence microscopy was distributed only within and not on the protuberance, because of the ready removal of adsorbed emulsion by washing. For all particles the redistribution into the protuberance developed very rapidly (within 5 min at 37°C) and occurred on the great majority (at least 90%) of cells.

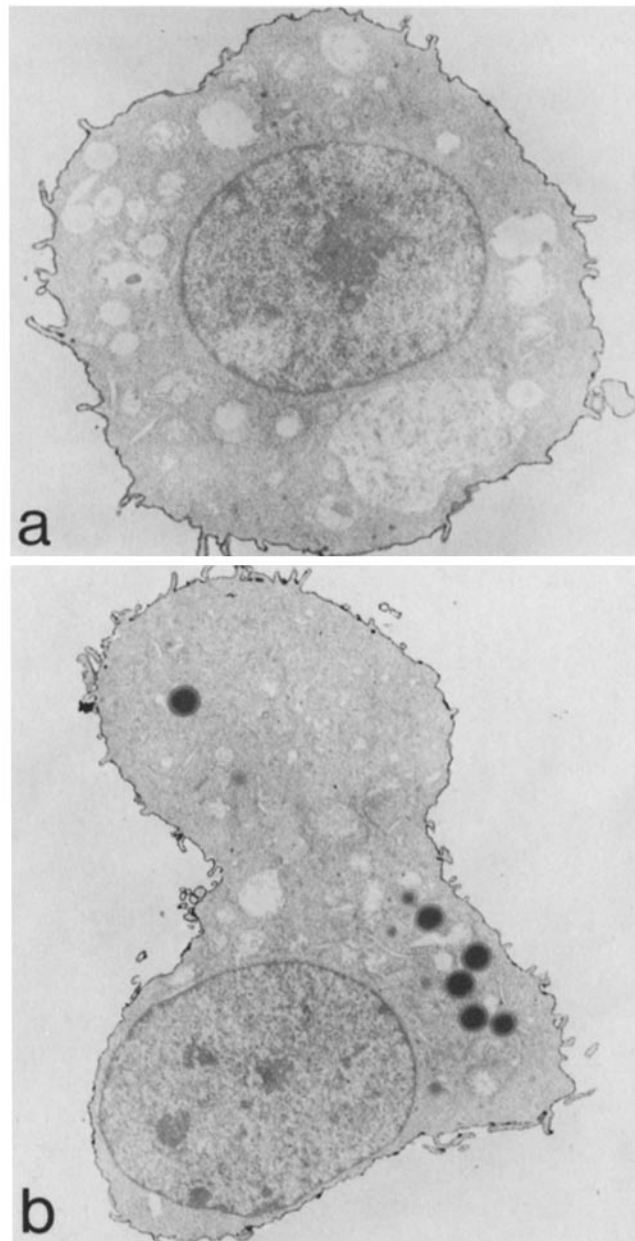


FIGURE 3 The distribution of Con A at 4°C on J774.2 macrophages. Monolayers of J774.2 cells were incubated with either medium alone (a) or medium that contained $2 \mu\text{M}$ colchicine (b) for 30 min before labeling at 4°C with biotinyl-Con A and avidin-peroxidase and processing as described in Materials and Methods. The distribution of Con A at 4°C is uniform whether or not a protuberance has developed. Unstained sections. (a) $\times 6,600$. (b) $\times 5,800$.

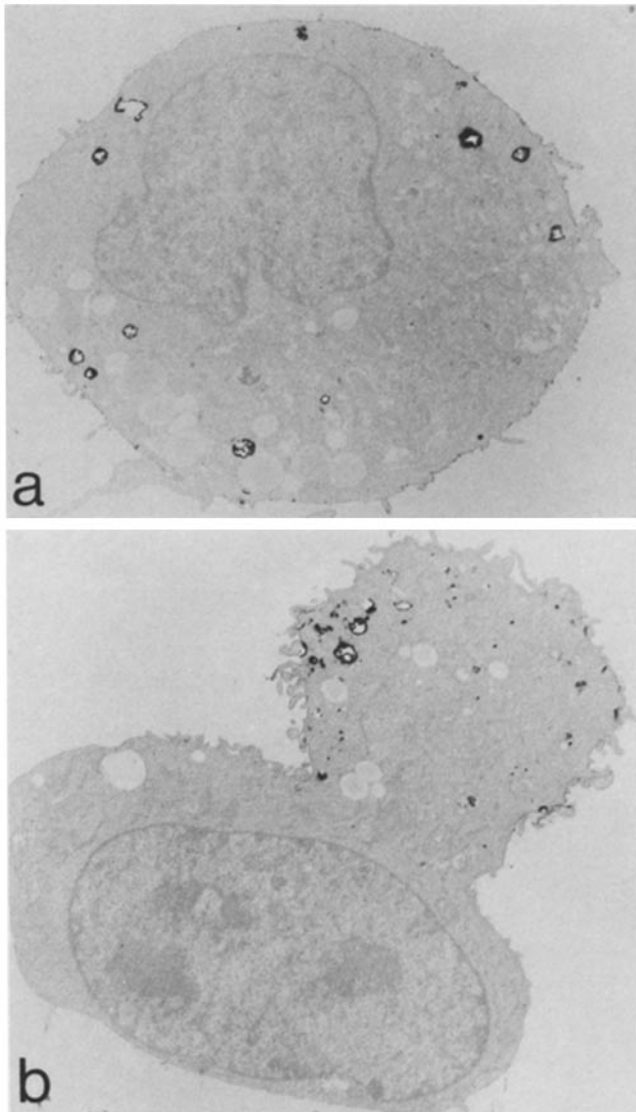


FIGURE 4 The distribution of Con A at 37°C on J774.2 macrophages. Cells were incubated as described in Fig. 3, except that 4°C surface labeling with biotinyl-Con A and avidin-peroxidase was followed by a 5-min incubation in DMEM at 37°C. In the control cell (a) Con A is internalized rapidly and uniformly over the whole membrane. In contrast, Con A has capped on the protuberant cell (b) and is in process of being internalized through the protuberance. Unstained sections. $\times 7,000$.

Like fluid pinocytosis, the polarization of phagocytosis was associated with a reduction in phagocytic rate in colchicine-treated cells. For latex, this reduction was determined from data obtained from micrographs of thin sections through variously treated cells. In random thin sections of control cells incubated for 10 min at 37°C with latex, 0.32 intracellular particles were observed per square micrometer of cytoplasmic area, ($n = 34$ cells) whereas colchicine reduced this number to 0.05 intracellular particles per square micrometer ($n = 52$ cells). Direct measurement of the uptake of perylene-containing oil emulsion, made possible by the ready removal of extracellular oil by washing, confirmed the reduction in phagocytic rate for nonopsonized particles after colchicine in J774.2 macrophages. As shown in Fig. 9, uptake is a saturable process whose rate is depressed at least fourfold by colchicine. Colchicine also caused a significant impairment of the ingestion of IgG-opsonized

erythrocytes in J774.2 cells. As described above, colchicine causes a time-dependent change in J774.2 shape from round or spread to the typically protuberant cell. This shape change is almost maximal by a 30-min exposure to 2 μM colchicine. The data in Fig. 2 (solid circles) demonstrate that the average number of ingested erythrocytes is inversely proportional to the percent of protuberant cells (Fig. 2, open circles). That is, the reduction in phagocytosis of IgG-opsonized erythrocytes is highly correlated with protuberance formation.

The Topography of Membrane Receptors for Phagocytic Particles

It seemed possible that the topographical restriction of phagocytosis to the protuberance reflected simply a corresponding asymmetry of receptors for phagocytic particles. To explore this, inherent receptor distributions were inferred from the distribution of particles under conditions that limit ligand-receptor mobility and were then compared with the topography of particle-receptor complexes under conditions that enabled their redistribution in the membrane. Several different methods were employed to achieve immobility for the diverse particle-receptor complexes studied. Although chemical fixation has been used successfully to establish the inherent distribution of Con A receptors (1, 11) and other membrane markers (33), we found in preliminary experiments that the opsonized particles used here do not bind to aldehyde-fixed macrophages. Thus, as one alternative, particle-receptor binding was studied at 4°C where mobility is greatly reduced. This approach proved suitable to observe the distribution of IgG-opsonized erythrocytes and aggregated IgG bound to macrophage Fc receptors. However, it was found that complement-opsonized particles do not bind at 4°C to J774.2 cells. Hence a third approach was developed in which complement-opsonized particles were added to macrophage monolayers during very brief (5–60 s) periods at 37°C. In both control and colchicine-treated cells the binding patterns of latex particles, IgG-opsonized erythrocytes, and aggregated IgG obtained after a brief 37°C pulse

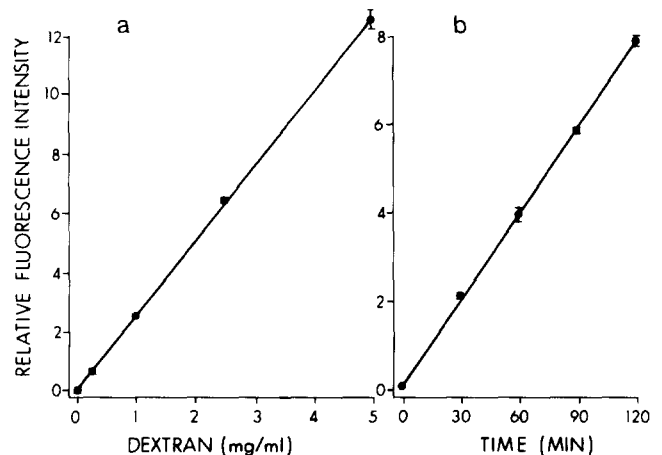


FIGURE 5 The kinetics of uptake of fluorescent dextran by J774.2 macrophages. Cell suspensions (2×10^6 macrophages per ml) in complete medium were incubated either for 30 min at 37°C with a range of concentrations of fluorescein-dextran (a) or for various times with 1 mg/ml fluorescein-dextran (b). 1-ml cell suspensions were subsequently washed three times by centrifugation with cold PBS, extracted in PBS that contained 0.1% Triton X-100, and dextran uptake was measured fluorimetrically. The data are the average \pm SD of triplicate determinations.

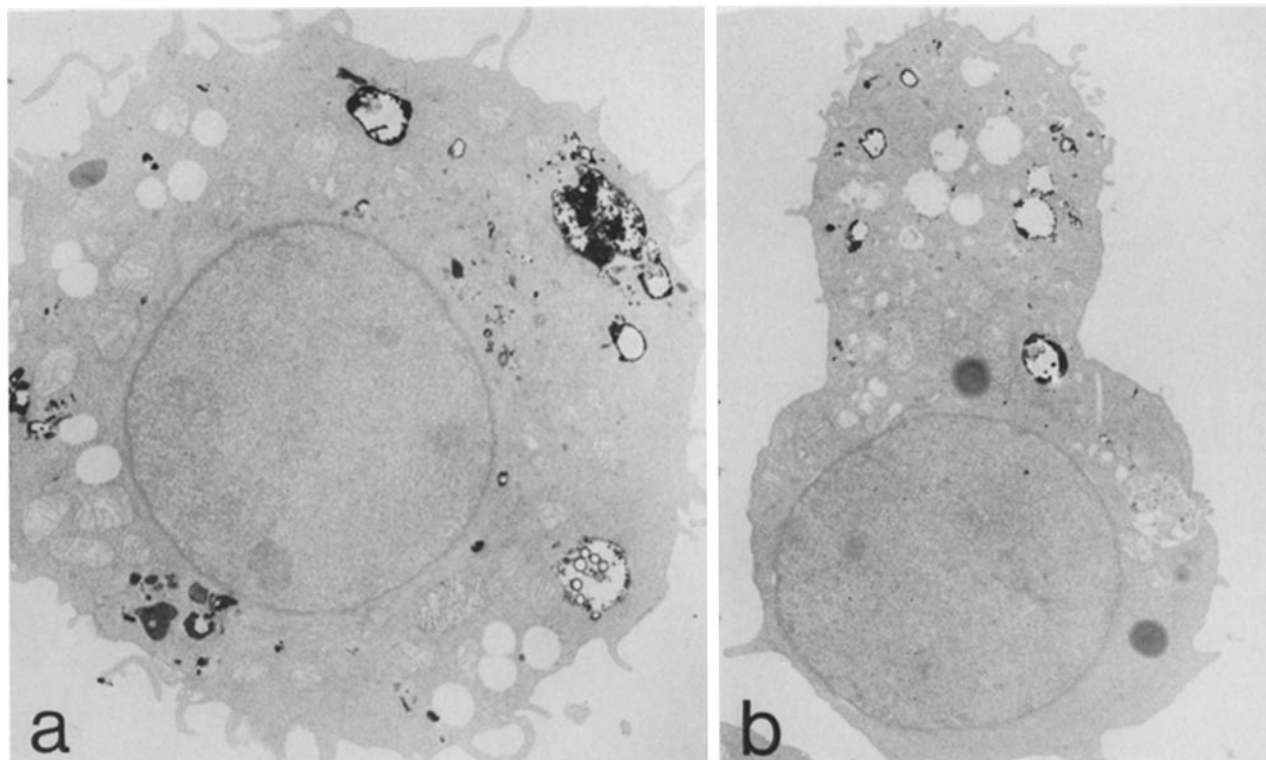


FIGURE 6 The uptake of HRP by J774.2 cells. Cell monolayers were incubated for 45 min in medium alone or medium plus $2 \mu\text{M}$ colchicine; followed by 10 min at 37°C with $200 \mu\text{g}/\text{ml}$ HRP. The control cell (a) shows a uniform uptake of HRP, whereas the colchicine-treated cell (b) shows uptake that is confined to the protuberance. Unstained sections. (a) $\times 7,100$. (b) $\times 6,400$.

were identical to those obtained during 4°C incubations (see below). We therefore consider that the distribution of markers obtained by either of our binding assays (i.e., extended 4°C incubation or brief 37°C pulse) provides a semiquantitative localization of the corresponding membrane receptors.

Latex

Latex particles were bound over the entire surface of colchicine-treated cells after 4°C incubation or a 37°C pulse. Some preference for association of latex with the protuberance was suggested but many particles were bound over the opposite or nuclear end of all cells observed in SEM samples (Fig. 10a). Thus, the binding sites for latex appear to exhibit a rather uniform inherent distribution on J774.2 cells. If these uniformly labeled cells were warmed for 5 min at 37°C before fixation, the latex particles were present almost exclusively over the protuberance (Fig. 10b).

IgG-Opsonized Erythrocytes

In contrast to latex particles, IgG-opsonized erythrocytes were excluded from the protuberance during incubation at 4°C or after a short pulse at 37°C . For example, the cell in Fig. 11a was fixed after a 5-s incubation with IgG-opsonized erythrocytes at 37°C . Numerous particles are bound over the nuclear region of the cell and none are associated with the protuberance. However, a similar cell that was rinsed after a 5-s incubation with particles and the incubation of which was continued at 37°C for 5 min before fixation shows erythrocytes located exclusively over the protuberance (Fig. 11b). For comparison, untreated, nonprotuberant cells are shown that bound erythrocytes uniformly during both brief (Fig. 11c) and extended (Fig. 11d) incubations at 37°C .

The asymmetric distributions of these particle-receptor complexes were quantified by counting the numbers of erythrocytes bound to the cell body and protuberance after 4°C incubation, a short pulse at 37°C , or a short pulse at 37°C followed by rinsing and further incubation for 5 min at 37°C . By varying the opsonin concentration and hematocrit, conditions were established that enabled binding of between six and nine erythrocytes per macrophage. For each condition, at least 500 cells were then assigned to one of five categories ranging from cells that had all particles attached to the cell body to cells that had all particles concentrated over the protuberance. These categories are represented schematically in Fig. 12. It may be seen that the distribution of particles is closely similar between cells incubated for 15 min at 4°C and those incubated for 5–10 s at 37°C : in both cases, the great majority of cells show particles bound almost exclusively over the nuclear end of the cells. A clear and dramatic shift of these particles to the protuberance is apparent during a subsequent 5-min incubation at 37°C . These data indicate that the inherent distribution of Fc receptors is away from the protuberance. The accumulation of erythrocytes over this structure after some minutes at 37°C thus appears to involve binding of erythrocytes to receptors and subsequent translocation of complexes into the region that supports their ingestion.

We considered that mechanical or steric interference might prevent the large erythrocytes from binding to the highly irregular surface of the protuberance at 4°C or during the pulse procedure at 37°C . We therefore extended our investigation of Fc receptor distribution by using a much smaller Fc probe, aggregated IgG. As shown in Fig. 13, the majority of aggregated IgG, like IgG-opsonized erythrocytes, binds away from the protuberance at 4°C (Fig. 13a) and accumulates rapidly on and within the protuberance at 37°C (Fig. 13b).

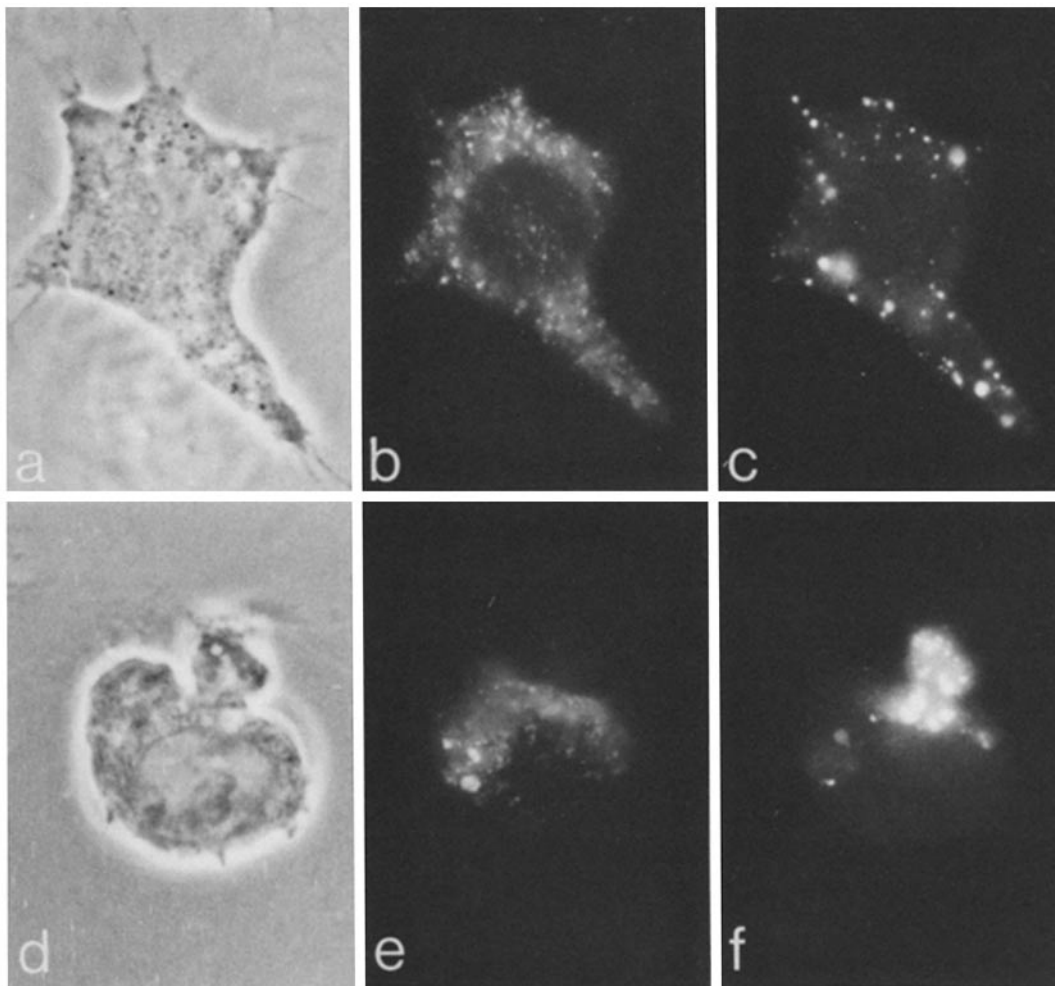


FIGURE 7 The polarization of fluid pinocytosis by colchicine. Cell monolayers were incubated in medium alone or in medium containing $2 \mu\text{M}$ colchicine for 30 min. The cells were subsequently incubated for 20 min at 37°C with 5 mg/ml fluorescein-dextran, rinsed thoroughly in DMEM, incubated 10 min in medium alone, and finally exposed for 5 min at 37°C to 10 mg/ml rhodamine-dextran. The control cells are well spread (a) and maintain a similar, uniform distribution in separate vesicles of fluorescein fluorescence (b) and rhodamine fluorescence (c). The colchicine-treated cells, on the other hand, maintain an almost complete topographical separation of the two markers. The protuberance (d) excludes the majority of fluorescein-dextran fluorescence (e) and includes the bulk of rhodamine fluorescence (f). $\times 1,260$.

Complement-Opsonized Erythrocytes

Complement-opsonized particles do not bind at 4°C to macrophages. However, after a brief (45 s) incubation at 37°C , both complement-opsonized erythrocytes and complement-opsonized zymosan were bound primarily over the nuclear region of the cell. This typical distribution is illustrated for both particles in Fig. 14a and c. The population analysis summarized in Fig. 15 emphasizes that the great majority of cells localize complement-opsonized erythrocytes away from the protuberance during a 45-s binding period. Nevertheless, after washing away unbound erythrocytes or zymosan and a further 5-min incubation at 37°C , most of the bound particles moved toward the protuberance (Figs. 14b, d, and 15).

Although the inherent distributions of Fc and complement receptors thus seem similar, three differences were noted between the behavior of these particles. First, to obtain binding of complement-opsonized particles comparable to that of IgG-opsonized particles, it was necessary to increase the labeling period from ~ 5 to ~ 45 s. Second, upon rinsing and a further 5-min incubation at 37°C the IgG-opsonized particles moved over and into the entire protuberance, whereas the comple-

ment-opsonized particles were preferentially accumulated at the constricted region between the protuberance and the cell body (compare the distributions given in Figs. 14 and 15 with those in Figs. 11 and 12). Third, the complement-opsonized particles remained bound to the protuberance but were not phagocytized even after extended incubation.

We emphasize that these nonrandom ligand distributions occur only on protuberant cells. The binding not only of IgG-opsonized erythrocytes (Fig. 14c and d) but also of latex particles, aggregated IgG, and complement-opsonized erythrocytes and zymosan in untreated, nonprotuberant cells was essentially uniform after a 4°C incubation or 37°C pulse. On the other hand, spontaneously protuberant cells, comprising $\sim 2\%$ of the control cell population, displayed the same nonrandom distributions evident on colchicine- or nocodazole-treated macrophages.

The topographical data presented above are summarized in Fig. 16.

DISCUSSION

The high levels of pinocytosis and phagocytosis characteristic

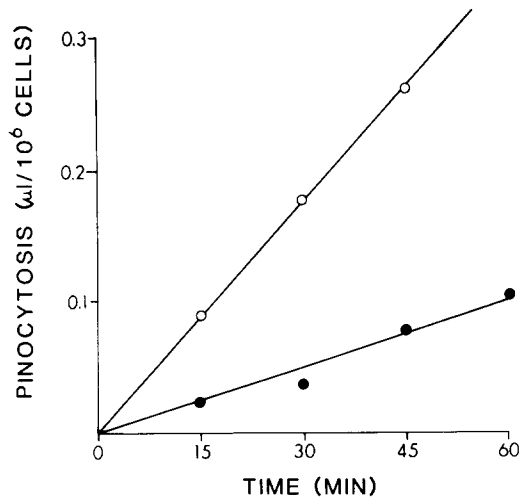


FIGURE 8 The inhibition of pinocytosis by colchicine. Cell suspensions were incubated in triplicate with fluorescein-dextran as described in Fig. 7, except that some cells were exposed to $2 \mu\text{M}$ colchicine for 30 min before the dextran was added. Fluorescence intensity was subsequently measured in extracts of the washed cells and in samples of the medium (1 mg dextran/ml). (○) Control cells. (●) Colchicine-treated cells.

of macrophages have allowed detailed examination of the spatial distribution of these functions over the cell surface. We show that whereas the capacity for endocytosis can be expressed over the entire surface of symmetric cells, all endocytic functions (fluid and adsorptive pinocytosis and phagocytosis of both opsonized and nonopsonized particles) are localized to the protuberance that develops after colchicine treatment in J774.2 macrophages. The protuberance, which has been described in detail before in other cells (1, 7, 26–28), consists of a region of ruffled membrane whose underlying cytoplasm is enriched for microfilaments. Although large aggregates of microfilaments are not so apparent in protuberant J774.2 cells as in neutrophils (7) or monocytes (1), large cytoplasmic granules and vesicles tend to be excluded and there is frequently a narrow neck between protuberance and cell body, suggesting a contractile ring. Spontaneously protuberant cells that com-

prise a small proportion (around 5%) of macrophages show the same functional polarization. Polarization is thus a physiological process and not dependent on colchicine or other drugs which simply increase the proportion of cells expressing this dramatic polarization.

In the course of these studies, a wide range of techniques were employed to observe pinocytosis and phagocytosis. We emphasize the significant potential of one new technique, the use of fluorescent high molecular-weight dextrans to measure pinocytosis. The uptake of these compounds is easily and sensitively measured by fluorimetry. In J774.2 cells, this uptake follows the kinetics of fluid pinocytosis as defined from work with HRP by Steinman and others (40, 41): that is, dextran uptake is linear with time and with dextran concentration. Furthermore, use of fluorescent dextrans avoids several com-

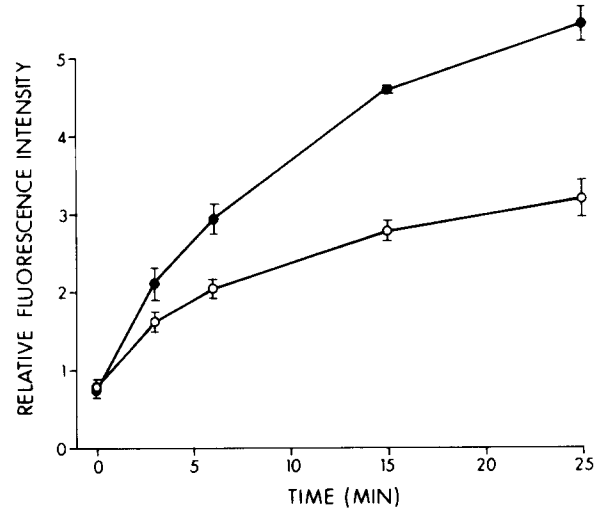


FIGURE 9 The inhibition of phagocytosis by colchicine in J774.2 macrophages. Cells were incubated in medium with or without $2 \mu\text{M}$ colchicine for 30 min before albumin-stabilized oil emulsion containing the fluorochrome perylene was added. Phagocytosis was terminated by washing, the cell pellets were extracted with *n*-butanol, and oil uptake was estimated from the fluorescence intensity due to perylene in the supernatant extracts. (●) Control cells. (○) Colchicine-treated cells.

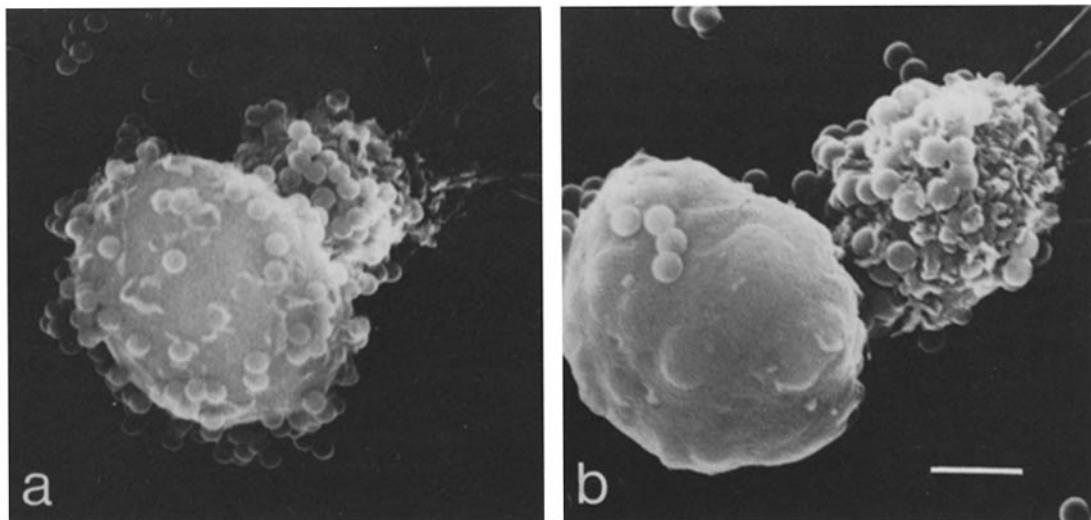


FIGURE 10 The distribution of latex particles on colchicine-treated macrophages. At 4°C , latex particles bind over the entire cell (a). In contrast, after the cells are rinsed to remove unbound particles, followed by a 5-min incubation at 37°C , particles are concentrated over the protuberance (b). Bar, $5 \mu\text{m}$.

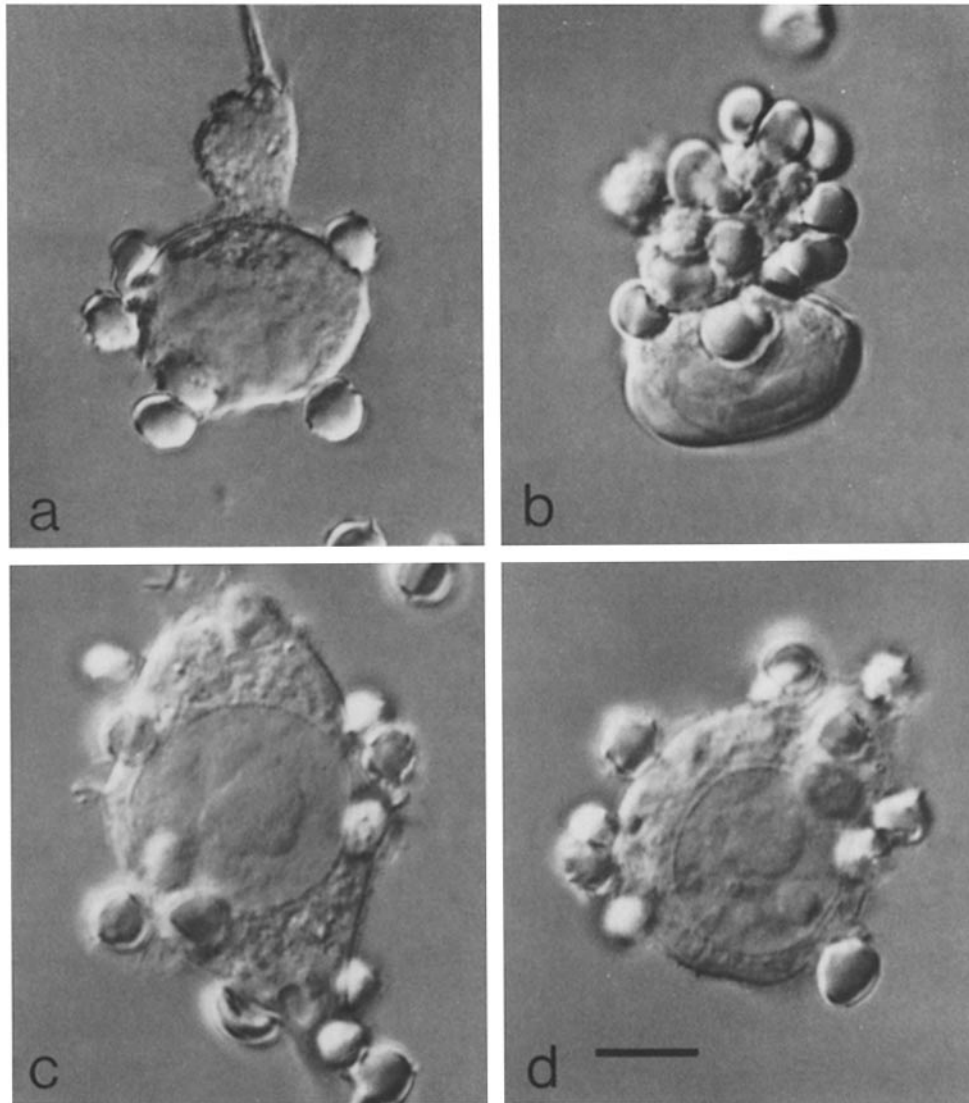


FIGURE 11 The distribution of IgG-opsonized erythrocytes on J774.2 macrophages. In colchicine-treated cells, erythrocytes are concentrated over the cell body after incubation for 5 s at 37°C (a) but move into the protuberance when incubation is continued for 5 min at 37°C (b). Untreated cells maintain a uniform distribution of bound erythrocytes whether incubation is for 5 s (c) or 5 min (d). Bar, 10 μ m.

plications of the HRP assay, particularly the requirements for repeated washes to remove extracellular marker and for kinetic assay of enzymatic activity in cell extracts. In addition, the distribution of the fluorescent dextrans is readily observed by fluorescence microscopy, again without the necessity for development of an enzyme reaction; the application of dextrans to mark fluid pinocytosis in the electron microscope has already been established (38); and the fluorescent conjugates are completely stable over several days of incubation with macrophages (unpublished results) whereas the half-life of HRP is variably reported as being between 5 and 10 h. In a recent publication, Quintart et al. (31) suggested that the uptake of 125 I-dextran by hepatoma cells may occur in part by adsorptive pinocytosis and that the intracellular processing of dextran and HRP may occur by different pathways. However, no evidence in support of either of these proposals was obtained with fluorescent dextrans in J774.2 cells. We suggest that these dextrans may thus be the most versatile, simple and reliable tools now available to study the topography and kinetics of fluid pinocytosis.

Our data establish that the polarization of phagocytosis to the protuberance does not simply reflect the prior concentration of receptors for phagocytic particles in this region. Under conditions that restrict the mobility of particle-receptor complexes, Fc receptors and complement receptors are actually excluded from the protuberance and so accumulate over the nuclear region of the cells, while binding sites for latex are distributed over the whole cell surface. An analogous tendency for certain receptors to concentrate away from the spontaneously formed uropod (protuberance) of T lymphocytes has been reported by DePetris (11). However, other surface markers are distributed differently. Con A-receptor complexes show a uniform (1, 35) or incompletely antiprotuberant distribution (11) whereas surface immunoglobulin is associated preferentially with the uropod (35). The overall picture of the surface that emerges is one of inherently highly polarized membrane receptors and functions. It seems likely that such topographical specialization develops on a smaller scale with the formation of processes and other shape changes. In this view the cell is continually rearranging membrane elements and produces

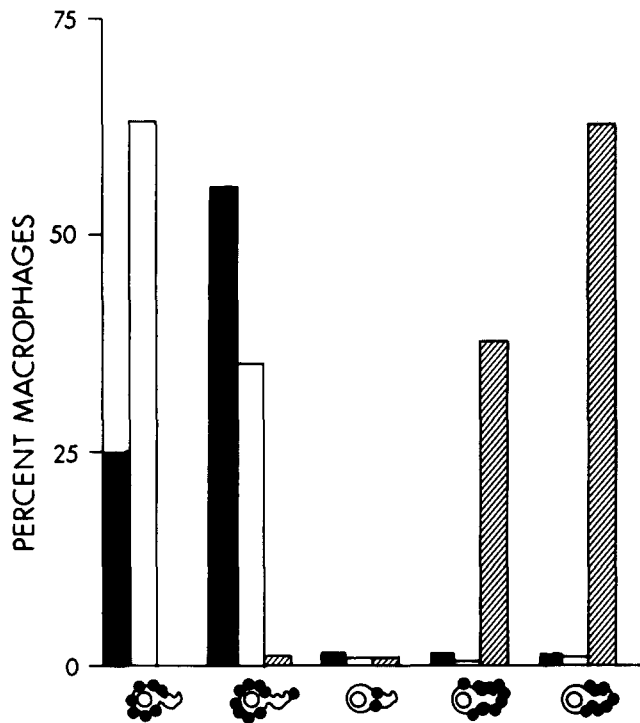


FIGURE 12 The distribution of IgG-opsonized erythrocytes on colchicine-treated macrophages. After a 15-min incubation with erythrocytes at 4°C (solid bars) or a 5-s pulse of erythrocytes at 37°C (open bars), most erythrocytes are bound over the nuclear end of each macrophage. When cells are rinsed after a 5-s pulse and are further incubated for 5 min at 37°C (crosshatched bars), the majority of erythrocytes are concentrated over the protuberance. Numbers are the average of two experiments in which at least 500 cells were observed under each incubation condition.

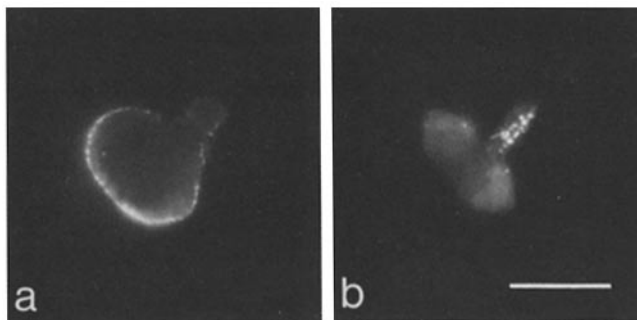


FIGURE 13 The distribution of aggregated rhodamine-IgG on colchicine-treated macrophages. After binding for 15 min at 4°C, IgG is concentrated over the nuclear end of the cell (a). When cells are rinsed and incubated for 5 min more at 37°C, the aggregates are clustered and internalized in the protuberance (b). Bar, 20 μ m.

functional change coordinately. Pinocytosis at the leading edges of lamellipodia, filopodia, or growth cones may be examples.

It seems unlikely that this topographical differentiation can result from direct or indirect connections of membrane constituents with microfilaments as proposed by others (14, 25, 36). Separate subsets of microfilaments (for which there is little structural or biochemical evidence) would be required to explain the different and specific distributions of receptors, for example latex binding sites and Con A and immunoglobulin receptors.

We propose that the asymmetric distribution of receptors is

directly dependent on local membrane properties and only indirectly on properties of the underlying cytoskeleton. Protuberance formation may occur by the segregation of specific proteins and lipids to form a unique region of membrane. Other proteins and lipids that are excluded from this cooperative process would become enriched outside the protuberance. Some receptors, for example Fc and complement receptors, might be thus "frozen out" of the protuberance while others (35) (as suggested from the work on immunoglobulin distribution on lymphocytes) are "frozen in". Still other receptors, for example latex binding sites and Con A receptors, might be equally distributed depending on their affinity for the protein-lipid matrix. The microfilaments in the protuberance may serve to nucleate or to stabilize this segregation. A similar hypothesis was suggested before to explain the specific retention of several transport systems (44) as well as certain membrane lipids (5) in the plasma membrane of polymorphonuclear leukocytes that had undergone extensive phagocytosis. Their concentration in surface membrane may reflect a "freezing out" from the pseudopods by a process that is analogous to the exclusion of Fc and complement receptors from the protuberance of colchicine-treated macrophages.

Whatever their inherent distribution, all particle-receptor complexes move into the protuberance after ligand binding and subsequent incubation at 37°C. The final distribution of ligand-receptor complexes into protuberance membrane follows a common theme previously emphasized and demonstrated for the movement of Con A-receptor complexes into caps (1, 28), pseudopods (7), and into the cleavage furrow of dividing cells (8). Existing models cannot satisfactorily explain

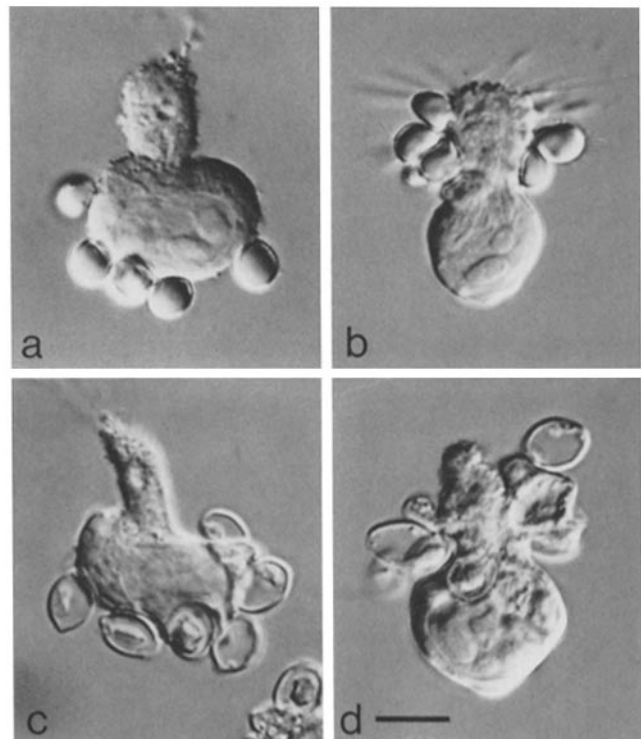


FIGURE 14 The distribution of complement-opsonized erythrocytes and complement-opsonized zymosan on colchicine-treated macrophages. After 45 s at 37°C, erythrocytes (a) and zymosan (c) are bound away from the protuberance. After incubation for 5 min more at 37°C, erythrocytes (b) and zymosan (d) are concentrated on the protuberance and in the constricted region between the cell body and the protuberance. Bar, 10 μ m.

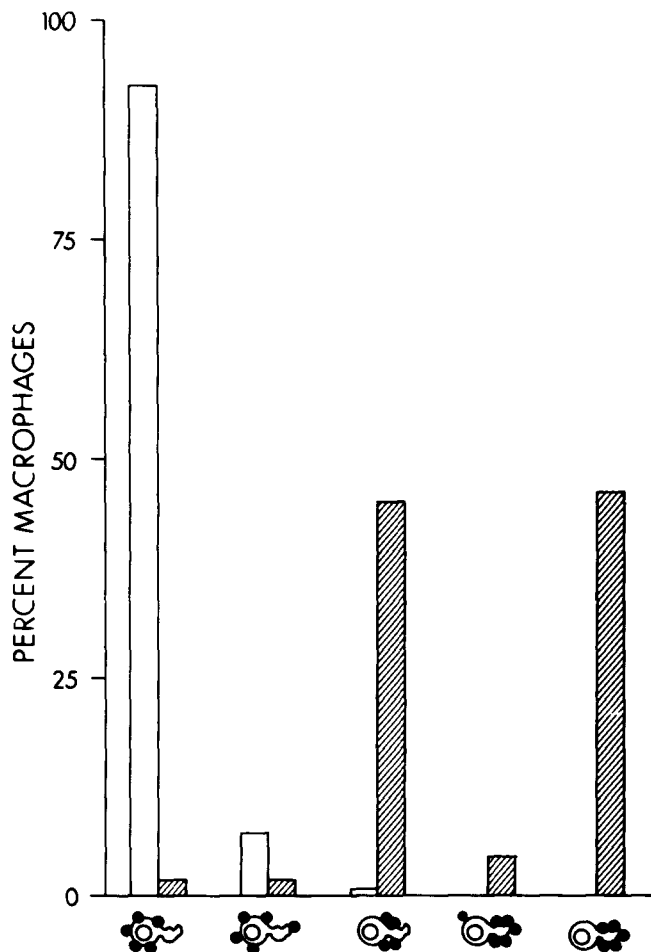


FIGURE 15 The distribution of complement-opsonized erythrocytes on colchicine-treated macrophages. After a 45-s pulse at 37°C (open bars), erythrocytes are bound mainly to the cell body of each macrophage. When such cultures are further incubated for 5 min at 37°C (crosshatched bars), erythrocytes are redistributed toward the protuberance. The numbers are averages from two experiments. At least 500 cells were observed for each condition.

this movement as well as the inherent distribution of some of the same receptors away from the protuberance reported here. For example, Stern and Bretscher (42) have developed the hypothesis that a continuous, directed membrane flow carries cross-linked receptors to a "cap". The apparent restriction of certain receptors to membrane regions opposite to that into which they will accumulate after particle binding (i.e. cross-linking) is difficult to reconcile with a simple membrane flow concept. Another model proposed by DePetris and Raff (12) suggests that a countercurrent movement of "free" membrane molecules occurs against the movement of other cross-linked components. This could provide a gradient of unoccupied receptors away from the protuberance but only when other ligand-receptor complexes are in the process of capping.

The model we proposed above to explain receptor asymmetry could be extended to suggest that particle-receptor complexes (as opposed to receptors) are modified so as to be frozen into the membrane overlying the protuberance. However, although such a model could explain the trapping of complexes, it does not explain their movement. Simple diffusion of complexes seems unlikely because of the largeness of the particle-receptor complex and the rapidity (1-5 min) of redistribution, although this remains to be examined experimentally. Alter-

natively, complex formation may alter local surface tension and thereby promote membrane flow or recruit new specific proteins or lipids, effectively displacing or pushing complexes into the protuberance. According to Greenspan (16), changes in surface tension occurring in cleavage furrow or lamellipodium membrane could also provide the main force for cytokinesis and chemotaxis. The ultimate role of microfilaments may thus be in terms of stabilizing changes in surface tension. These and other possible mechanisms of particle-receptor complex movement remain to be investigated.

The redistribution of IgG-opsonized erythrocytes bound to Fc receptors from an "antiprotuberant" distribution into the protuberance before their ingestion by colchicine-treated J774.2 cells has an additional implication related to the mechanism of phagocytosis. According to Griffin and colleagues (17, 18), particle binding engages receptors which in turn activate and/or recruit microfilaments to the segment of plasma membrane immediately adjacent to the particle. We show here that on protuberant cells the initial site of particle binding is at a distance from the eventual region of endocytosis. That is, ingestion at the immediate site of particle binding is not critical to the phagocytic process. To rationalize this discrepancy, one might hypothesize that a special population of microfilaments or other elements of the contractile mechanism essential for ingestion is localized in the protuberance and that ingestion cannot occur until the particle-receptor complex reaches them. However, movement into the protuberance is a necessary but insufficient condition for internalization. Complement-opsonized particles move from a predominant distribution over the cell body into the protuberance when incubated with particles at 37°C, but they are not subsequently internalized in J774.2 macrophages. Particle binding, lateral movement of receptor complexes and internalization may be thus spatially as well as temporally separable.

Why is endocytosis restricted to the protuberance? Phagocytosis and (less completely) fluid pinocytosis are processes that appear, from pharmacological and ultrastructural studies, to depend on microfilament function (2, 7, 19, 30, 46). Thus, the increased density of microfilaments in the protuberance may in part explain the restriction. However, as noted above, it seems clear from the work of Griffin et al. in macrophages (17) and in the rapid development of pseudopods in neutrophils (7) that, in nonprotuberant cells, microfilaments are rapidly recruited to the site of particle binding. It appears that it is this capacity for recruitment that is lost from non-protuberant membrane. Components that mediate the aggregation or cross-linking of actin filaments by gelation factors or the polymerization of soluble actin on a continuous basis (for pinocytosis) or after surface binding events (for phagocytosis) may become restricted to the protuberance.

The localization of the machinery for internalization to a relatively small segment of the surface may explain the inhibition of endocytosis by colchicine. For example, large phagocytized particles would be sterically hindered from moving into the down a narrow protuberance. This problem would also be encountered in pinocytosis after multiple fusions of endocytic vesicles. In addition, Steinman and others (40) have emphasized the necessity in macrophages of very rapid membrane recycling to support their usual high pinocytotic rates. This membrane recycling could be delayed because of the exclusion of lysosomes from the protuberance. In any event, it should be noted that some cells (rabbit and human neutrophils, mouse peritoneal macrophages: 9, 21, 29, 45) continue to phagocytize





















| PARTICLE | CONTROL | | COLCHICINE | |
|-----------------------------------|---|--|---|---|
| | Fixed | Mobile | Fixed | Mobile |
| Latex |  |  |  |  |
| IgG-Opsonized Erythrocytes |  |  |  |  |
| Aggregated IgG |  |  |  |  |
| Complement-Opsonized Erythrocytes |  |  |  |  |
| Complement-Opsonized Zymosan |  |  |  |  |

FIGURE 16 Summary view of receptor distributions on untreated and colchicine-treated macrophages before and after particle-induced rearrangements. The "fixed" distributions are those observed after incubating macrophages with ligands at 4°C or after a brief pulse at 37°C. Upon being rinsed and further incubated for 5 min in buffer at 37°C, the "mobile" distributions of particle-receptor complexes are observed. Complement-opsonized particles fail to bind at 4°C to interphase cells, so the fixed distributions of these particles were obtained after pulse-labeling macrophages at 37°C. For all other particles, the same fixed distributions are obtained whether binding is at 4°C or during very brief incubations at 37°C.

and/or pinocytize at normal rates after colchicine, whereas others, for example chick chondrocytes and guinea pig neutrophils, show impairment of endocytosis consistent with that observed here (30, 43).

Finally, while the extent of polarization of cell shape and surface topography in colchicine-treated J774.2 cells may appear remarkable, a clear precedent exists in the organization of epithelial cells. We have previously noted the ultrastructural similarities between the microvillous protuberance and the brush border-terminal web region of epithelia. Endocytosis is localized to the brush border as it is to the protuberance. For example, proteins are reabsorbed from the renal tubular epithelium via the brush border (22); and thyroglobulin via the brush border of cells lining the thyroid follicle (37, 47). Molecular topography is also polarized in epithelia. In transport epithelia, the intestine for example, some membrane enzymes such as sucrase are localized to the brush border whereas others such as the Na⁺, K⁺-dependent ATPase are confined to the basolateral borders (15, 34). By analogy with the segregation of macrophage receptors about the protuberance, this segregation may develop partly by migration of proteins into or out of the developing brush border. The major difference between epithelia and colchicine-treated leukocytes may thus lie in the stability of structure: in the case of epithelia, the polarity of structure and function appears to be stabilized by tight junctions, whereas in colchicine-treated J774.2 macrophages and leukocytes the polarization is dynamic. Further explanation of the protuberance-polarized epithelium analogy may give insight into the molecular development of polarized epithelia

from nonpolarized precursors and also guide future exploration of membrane structural and functional asymmetry in nonepithelial cells.

This work was supported in part by National Institutes of Health grants CA-18564, ES-01106, CA-15544, GM-22621, and HL-23192 and by grant BC179 from the American Cancer Society. Dr. Walter is a postdoctoral fellow of the American Cancer Society. Dr. Oliver holds an American Cancer Society Faculty Research Award.

Received for publication 22 October 1979, and in revised form 19 February 1980.

REFERENCES

- Albertini, D. F., R. D. Berlin, and J. M. Oliver. 1977. The mechanism of Concanavalin A cap formation in leukocytes. *J. Cell Sci.* 26:57-75.
- Allison, A. C., and P. Davies. 1974. Mechanisms of endocytosis and exocytosis. *Symp. Soc. Exp. Biol.* 28:419-444.
- Anderson, C. L., and H. M. Grey. 1974. Receptors for aggregated IgG on mouse lymphocytes. Their presence on thymocytes, thymus-derived and bone marrow-derived lymphocytes. *J. Exp. Med.* 139:1175-1188.
- Ash, J. F., D. Louvard, and S. J. Singer. 1977. Antibody-induced linkage of plasma membrane proteins to intracellular actomyosin-containing filaments in cultured cells. *Proc. Natl. Acad. Sci. U.S.A.* 74:5584-5588.
- Berlin, R. D., and J. P. Fera. 1977. Changes in membrane microviscosity associated with phagocytosis. Effect of colchicine. *Proc. Natl. Acad. Sci. U.S.A.* 74:1072-1076.
- Berlin, R. D., J. P. Fera, and J. R. Pfeiffer. 1978. Reversible phagocytosis in rabbit polymorphonuclear leukocytes. *J. Clin. Invest.* 63:1137-1144.
- Berlin, R. D., and J. M. Oliver. 1978. Analogous ultrastructure and surface properties during capping and phagocytosis in leukocytes. *J. Cell Biol.* 77:789-804.
- Berlin, R. D., J. M. Oliver, and R. J. Walter. 1978. Surface functions during mitosis. I. Phagocytosis, pinocytosis and mobility of surface-bound Con A. *Cell.* 15:327-341.
- Bhisey, A. N., and J. J. Freed. 1971. Altered movement of endosomes in colchicine-treated cultured macrophages. *Exp. Cell Res.* 64:430-438.
- Burchill, B. R., J. M. Oliver, C. B. Pearson, E. D. Leinback, and R. D. Berlin. 1978. Microtubule dynamics and glutathione metabolism in phagocytizing human polymorphonuclear leukocytes. *J. Cell Biol.* 76:439-447.

11. DePetris, S. 1978. Nonuniform distribution of Concanavalin A receptors and surface antigens on uropod-forming thymocytes. *J. Cell Biol.* 79:235-251.
12. DePetris, S., and M. C. Raff. 1973. Fluidity of the plasma membrane and its implications for cell movement. Locomotion of tissue cells. *CIBA Found. Symp.* 14:27-41.
13. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ -globulin with B lymphocytes. *J. Exp. Med.* 136:191-196.
14. Edelman, G. M., J. L. Wang, and I. Yahara. 1976. Surface modulating assemblies in mammalian cells. In *Cell Motility*. R. Goldman, T. Pollard, and J. Rosenbaum, editors. 305-321.
15. Fujita, M., H. Ohta, K. Kawai, H. Matsui, and M. Nakao. 1972. Differential isolation of microvillous and basolateral plasma membranes from intestinal mucosa: mutually exclusive distribution of digestive enzymes and ouabain-inhibitable ATPase. *Biochim. Biophys. Acta.* 274:336-347.
16. Greenspan, H. P. 1978. On fluid-mechanical simulations of cell division and movement. *J. Theor. Biol.* 70:125-134.
17. Griffin, F. M., Jr., J. A. Griffin, J. E. Lieber, and S. C. Silverstein. 1975. Studies on the mechanism of phagocytosis. I. Requirements for circumferential attachment of particle-bound ligands to specific receptors on the macrophage plasma membrane. *J. Exp. Med.* 142:1263-1282.
18. Griffin, F. M. Jr., J. A. Griffin, and S. C. Silverstein. 1976. Studies on the mechanism of phagocytosis. II. The interaction of macrophages with antiimmunoglobulin IgG-coated bone marrow-derived lymphocytes. *J. Exp. Med.* 144:788-809.
19. Hartwig, J. H., W. A. Davies, and T. P. Stossel. 1977. Evidence for contractile protein translocations in macrophages spreading, phagocytosis and phagolysosome formation. *J. Cell Biol.* 75:956-967.
20. Klaus, G. G. 1973. Cytochalasin dissociation of pinocytosis and phagocytosis by peritoneal macrophages. *Exp. Cell Res.* 69:73-78.
21. Malawista, S. G. 1975. Microtubules and the mobilization of lysosomes in phagocytizing human leukocytes. *Ann. N. Y. Acad. Sci.* 253:738-749.
22. Maunsbach, A. B. 1973. Ultrastructure of the proximal tubule. *Handb. Physiol. Renal Physiology.* 8:31-79.
23. Mooseker, M. S., and L. G. Tilney. 1975. Organization of an actin filament-membrane complex. Filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. *J. Cell Biol.* 67:725-743.
24. Muschel, R. L., N. Rosen, and B. R. Bloom. 1977. Isolation of variants in phagocytosis of a macrophage-like continuous cell line. *J. Exp. Med.* 145:175-186.
25. Nicolson, G. L. 1976. Transmembrane control of the receptors on normal and tumor cells. I. Cytoplasmic influence over cell surface components. *Biochim. Biophys. Acta.* 458:1-72.
26. Oliver, J. M. 1976. Concanavalin A cap formation on human polymorphonuclear leukocytes induced by R17934, a new antitumor drug that interferes with microtubule assembly. *J. Reticuloendothel. Soc.* 19:389-395.
27. Oliver, J. M., D. F. Albertini, and R. D. Berlin. 1976. Effects of glutathione-utilizing agents on microtubule assembly and microtubule-dependent surface properties of human neutrophils. *J. Cell Biol.* 71:921-932.
28. Oliver, J. M., R. Lalchandani, and E. L. Becker. 1976. Actin redistribution during Concanavalin A cap formation in rabbit neutrophils. *J. Reticuloendothel. Soc.* 21:359-364.
29. Oliver, J. M., T. E. Ukena, and R. D. Berlin. 1974. Effect of phagocytosis and colchicine on the distribution of lectin binding sites on cell surfaces. *Proc. Natl. Acad. Sci. U.S.A.* 71:294-398.
30. Piasek, A., and J. Thyberg. 1979. Effects of colchicine on endocytosis and cellular inactivation of horseradish peroxidase in cultured chondrocytes. *J. Cell Biol.* 81:426-437.
31. Quintart, J., M. A. Leroy-Houyet, A. Trouet, and P. Baudhuin. 1979. Endocytosis and chloroquine accumulation during the cell cycle of hepatoma cells in culture. *J. Cell Biol.* 82:644-653.
32. Ralph, P., and I. Nakoinz. 1975. Phagocytosis and cytolysis by a macrophage tumor and its cloned cell line. *Nature (Lond.)* 257:393-394.
33. Sabatini, D. D., K. Bensch, and R. J. Barnett. 1963. Cytochemistry and electron microscopy: the preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* 17:19-58.
34. Sassier, P., and M. Gergeron. 1978. Cellular changes in the small intestine epithelium in the course of cell proliferation and maturation. *Subcell. Biochem.* 5:129-185.
35. Schreiner, G. F., J. Braun, and E. R. Unanue. 1976. Spontaneous redistribution of surface immunoglobulin in the motile B lymphocyte. *J. Exp. Med.* 144:1683-1688.
36. Schreiner, G. F., and E. R. Unanue. 1976. Membrane and cytoplasmic changes in B lymphocytes induced by ligand-surface immunoglobulin interaction. *Adv. Immunol.* 24:38-165.
37. Seljelid, R., and K. F. Nakken. 1968. Endocytosis of thyroglobulin and release of thyroid hormone. *Scand. J. Clin. Lab. Invest.* 22 (Suppl. 106):125-143.
38. Simionescu, N., M. Simionescu, and G. E. Palade. 1972. Permeability of intestinal capillaries. Pathway followed by dextrans and glycogens. *J. Cell Biol.* 53:365-392.
39. Stahl, P. D., J. S. Rodman, M. J. Miller, and P. H. Schlesinger. 1978. Evidence for receptor-mediated binding of glycoproteins, glycoconjugates and lysosomal glycosidases by alveolar macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 75:1399-1403.
40. Steinman, R. M., E. S. Brodie, and Z. A. Cohn. 1976. Membrane flow during pinocytosis. A stereological analysis. *J. Cell Biol.* 68:665-687.
41. Steinman, R. M., J. M. Silver, and Z. A. Cohn. 1974. Pinocytosis in fibroblasts. Quantitative studies in vitro. *J. Cell Biol.* 63:949-969.
42. Stern, P. L., and M. S. Bretscher. 1979. Capping of exogenous Forssman glycolipid on cells. *J. Cell Biol.* 82:829-833.
43. Stossel, T. P., R. J. Mason, J. Hartwig, and M. Vaughan. 1972. Quantitative studies of phagocytosis by polymorphonuclear leukocytes: use of emulsions to measure the initial rate of phagocytosis. *J. Clin. Invest.* 51:615-624.
44. Tsan, M. F., and R. D. Berlin. 1971. Effect of phagocytosis on membrane transport of non-electrolytes. *J. Exp. Med.* 134:1016-1035.
45. Ukena, T. E., and R. D. Berlin. 1972. Effect of colchicine and vinblastine on the topographical separation of membrane functions. *J. Exp. Med.* 136:1-7.
46. Wagner, R., M. Rosenberg, and R. Estensen. 1971. Endocytosis in Chang liver cells. Quantitation by sucrose-³H uptake and inhibition by cytochalasin B. *J. Cell Biol.* 50:804-817.
47. Wetzel, B. K., S. S. Spicer, and S. H. Wollman. 1965. Changes in fine structure and acid phosphatase localization in rat thyroid cells following thyrotropin administration. *J. Cell Biol.* 25:593-618.