

SUBPLASMALEMAL MICROFILAMENTS AND MICROTUBULES IN RESTING AND PHAGOCYTIZING CULTIVATED MACROPHAGES

EVE P. REAVEN and STANTON G. AXLINE

From the Department of Medicine, Stanford University School of Medicine, and Stanford Medical Service, Veterans Administration Hospital, Palo Alto, California 94305

ABSTRACT

The subplasmalemmal organization of the free and glass-attached surfaces of resting and phagocytizing cultivated macrophages were examined in an attempt to define specific membrane-associated structures related to phagocytosis. From analysis of serial thin sections of oriented cells it was found that the subplasmalemmal region of the attached cell surface has a complex microfilament and microtubule organization relative to the subplasmalemmal area of the free surface. A filamentous network composed of 40–50-Å microfilaments extended for a depth of 400–600 Å from the attached plasma membrane. Immediately subjacent to the filamentous network was a zone of oriented bundles of 40–50-Å microfilaments and a zone of microtubules. Additional microtubules were found to extend from the plasma membrane to the interior of the cell in close association with electron-dense, channellike structures. In contrast, the free aspect of the cultivated macrophage contained only the subplasmalemmal filamentous network. However, after a phagocytic pulse with polystyrene particles (14 μm diam) microtubules and oriented filaments similar to those found on the attached surface were observed surrounding the ingested particles. The observations reported in this paper provide support for the hypothesis that microfilaments and/or microtubules play a role in the translocation of plasma membrane required for the functionally similar processes of phagocytosis and cell attachment to glass.

INTRODUCTION

Mononuclear phagocytic cells exhibit a special ability to remove particulate material and soluble macromolecular constituents from their environment by endocytosis. Formation of the endocytic vacuole requires extensive translocation of plasma membrane to accommodate the size and shape of the particle being ingested. However, the physical events associated with the initial phases of this process are still unknown. North (1) has suggested that small particles may be ingested by a process involving nothing more than plasma membrane invagination and closure, whereas the ingestion of

large particles may require extensive evagination and spreading of the cytoplasm of the cell around the particle, a process which is a form of cell movement requiring metabolic energy (2) and perhaps involving contractile elements within the cell (3–11). The spreading of the cell cytoplasm on a glass substratum during cultivation may also be considered to be an evagination of the cell cytoplasm on a flat surface and may well be viewed as an attempt by these specialized cells to phagocytize infinitely large particles (1).

In this study we have examined the sub-

plasmalemmal organization of the free and glass-attached surfaces of resting¹ and phagocytizing macrophages in culture in an attempt to define specific membrane-associated structures related to the ingestion phase of the phagocytic process. Special attention has been given to microfilamentous and microtubular components of the subplasmalemmal region as these structures are thought to be involved in cellular events leading to alteration in shape and movement (12-20).

MATERIALS AND METHODS

Animals

Male mice (25-30 g) of the NCS/PA strain were used in all experiments. The newly designated strain was derived from the NCS (pathogen-free) mice of The Rockefeller University, New York, and has been maintained since 1970 as an outbred colony at the Veterans Administration Hospital, Palo Alto, California.

Cultivation of Mononuclear Phagocytes

The cells from the peritoneal cavity of unstimulated mice were harvested by techniques previously described (21). Monolayers of macrophages free of lymphocytes were prepared and maintained for 48 h in Medium no. 199 containing 20% heat-inactivated newborn calf serum (Grand Island Biological Co., Berkeley, Calif.) before use.

For experiments involving phagocytosis, polystyrene particles of 14.0- μ m diam (Particle Information Service, Los Altos, Calif.) were added to Leighton tube cultures at a final concentration of 4.0×10^7 /ml and phagocytosis was permitted to proceed at 37°C for 60 min.

Techniques for Electron Microscopy

All steps in the processing of macrophages grown in the above manner were carried out while the cells were still affixed to the glass cover slips. The cells were washed briefly with unbuffered saline at 37°C and immediately fixed with 1% glutaraldehyde pH 7.2 in 0.1 M phosphate buffer (mosM = 330) for 3 h at 22°C. The cells were postfixed at room temperature in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h followed by 0.5% uranyl acetate in veronal buffer pH 5.8 for an additional hour. Occasionally cells were fixed in a mixture of osmium tetroxide and glutaraldehyde as described by Hirsch and Fedorko (22). The cells were dehydrated in a routine manner,

¹ In the context of this paper, the word "resting macrophage" refers to cells not in the process of ingesting particles.

infiltrated and embedded in Epon, and removed from the glass cover slips by the method of Branson (23). The final product was a flat disk of hardened plastic, coated on one surface with a monolayer of macrophages. Segments of the Epon disk were trimmed and remounted on precut plastic cylinders and serial sectioned either parallel to the plane on which the cells had originally grown or at cross sections to this plane. In the first instance, sections of cells were obtained as nearly flat to the plane of the block surface as possible, the first sections representing the surface of the cells which had adhered to the glass substrate, the following sections going deeper into the interior of the cells, and the final sections representing the free surface of the cells.

Serial thin sections were mounted on Formvar coated-slot grids (Belden Mfg. Co., Chicago, Ill.) and were sequentially stained with a saturated solution of uranyl acetate for 20 min and Reynolds' lead citrate solution for 40 min. The grids were finally coated with a thin film of carbon and examined with an Hitachi HS-8 electron microscope.

An estimation of the cell depth obtained in sectioning and the thickness of individual subplasmalemmal layers was based on serial sections of approximately 400-600 Å in thickness (24) in which the initial section which transected the plasma membrane was used as a marker.

RESULTS

Freshly explanted peritoneal monocytes resemble circulating blood monocytes. Under the culture conditions employed, cells maintained in vitro as monolayers for 48 h have differentiated into macrophages and exhibit the general morphologic features previously described (25). The cells are well spread and possess numerous pseudopodia extending radially from the free surface of the cell body. For purposes of orientation a low power longitudinal view of a typical mouse peritoneal macrophage is shown in Fig. 1 *a* and *b*. The flattened surface of attachment to the glass substratum is easily distinguished from the free, gently curving, convex surface from which a few microvilli-like projections protrude.

The subplasmalemmal microfilamentous and microtubular structures of the free and attached surface of these macrophages were examined in serial sections cut parallel to the plane of glass attachment with the following results.

Filamentous Network

A filamentous zone estimated to be 400-600 Å in thickness is present directly subjacent and interior to the plasmalemma on both the free and

attached surfaces of all cells and all pseudopodia examined. The zone is composed of randomly oriented segments of 40–50-Å microfilaments which resemble a reticulum or network (Figs. 2, 4 *a*, 5, 8, and 10). The network is not uniform in appearance and in some regions of the cell the subplasmalemmal reticulum has a loose, delicate appearance, whereas in other regions the network appears to be condensed (compare areas 1 and 2 in Fig. 2). Strands of rough endoplasmic reticulum, mitochondria, lysosomes, and other cytoplasmic organelles are located immediately interior to the filamentous network on the free aspect of resting macrophages. However, on the glass-attached surface of these cells or on the free surface participating in phagocytosis the following specialized arrangements of microfilaments and microtubules are observed.

Microfilament Bundles

Bundles of oriented 40–50-Å microfilaments can routinely be found in a 400–600-Å thick zone subjacent to the filamentous network on the attached surface of the cultivated macrophage (Fig. 3). The detailed arrangement of these filaments can be seen in serial sections cut parallel to the surface of attachment of a cell to the substratum; in Figs. 4 *a–d* serial sections of a group of microfilaments from the cell in Fig. 3 have been obtained (see encircled area). Minor undulations of the cell surface as well as slight deviations in the angle of sectioning through the plane of cell attachment usually result in some variation in depth of the cell represented in a single section. Thus, the lower right corner of the series of micrographs of Fig. 4 depicts a slightly deeper slice into the cell than the remaining portions of these serial micrographs. Despite this limitation the morphology of the subplasmalemmal region can be reconstructed by analysis of serial thin sections. The major portion

of the aspect of the cell seen in Fig. 4 *a* is occupied by the filamentous network. In the adjacent section (Fig. 4 *b*) oriented bundles of microfilaments are prominently displayed. Figs. 4 *c* and 4 *d* representing progressively deeper cuts into the cell have passed through the microfilament zones and demonstrate the abrupt transition between the more superficial microfilament-containing zones and the deeper regions containing ribosomes, rough endoplasmic reticulum, and other cytoplasmic organelles.

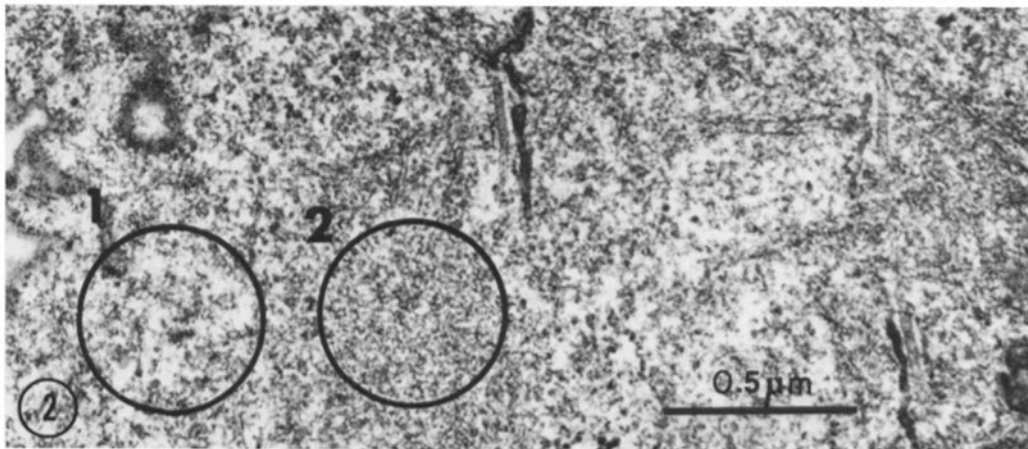
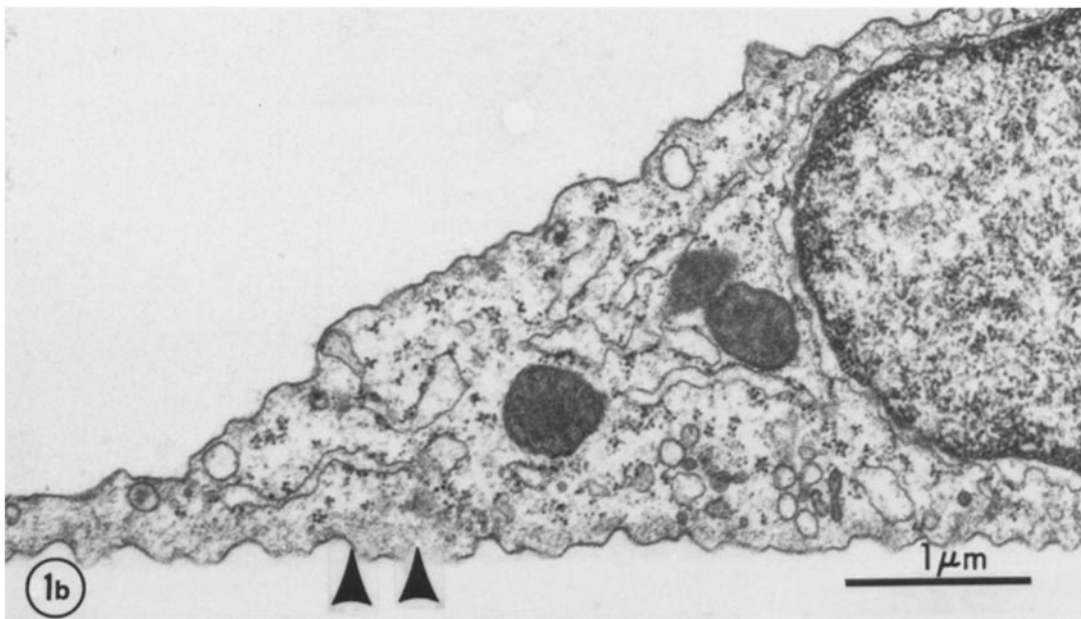
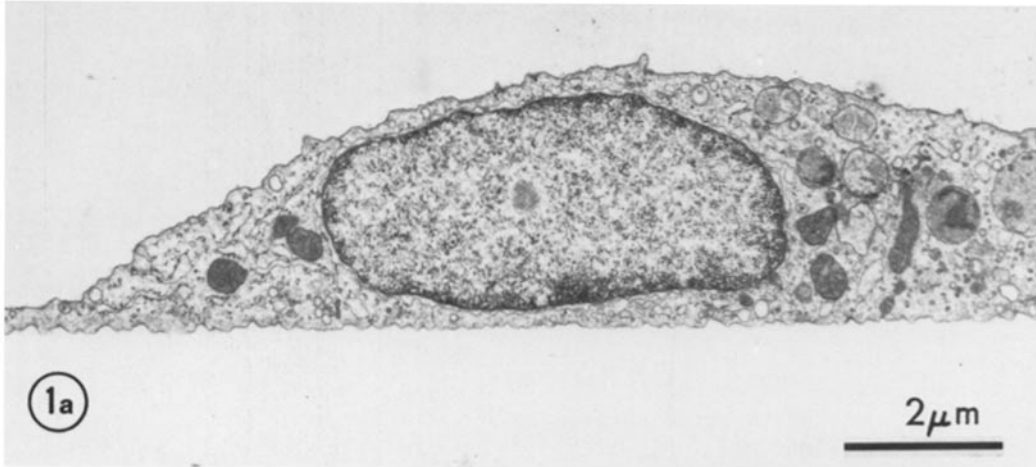
Inspection of a large area of the subplasmalemmal zone (Fig. 5) shows that microfilaments are arranged in oriented bundles forming a plane parallel to the surface of cell attachment to its substratum. Within that plane, microfilaments are arrayed in multiple directions around an axis perpendicular to the plane of glass attachment. The failure to consistently observe oriented microfilament bundles in sections cut perpendicular to the plane of cell attachment to its substratum is most likely a reflection of the variable spacing between, and orientation of, the microfilament bundles just described. As one can see, in Fig. 5 these nonfilament spaces represent a substantial portion of the cytoplasm and in randomly obtained vertical sections through cells it is unusual to find uniformly long stretches of oriented microfilaments. Figure 1 *b* shows a typical vertical cut through a cell with oriented microfilaments visible only in a short stretch of the attached surface. However, in oblique sections the general features of the two filamentous zones of the attached surface, as well as the sharp transition between these zones and the more interior regions of the cell, can be particularly well visualized (Figs. 8 and 10).

Although the orientation of microfilament bundles in the “body” of the cell bears no discernible relationship to cell shape, the orientation of microfilaments in attached regions of pseudopodia is

FIGURE 1 *a* Vertical section through resting cultivated macrophage. Note the slight undulations of the flattened surface of attachment to the glass substratum. Glutaraldehyde fixation. $\times 10,000$.

FIGURE 1 *b* Photographic enlargement of portion of cell seen in 1 *a*. Note specifically the variation in apparent thickness of the subplasmalemmal region along the substrate-attached surface (arrowheads). $\times 25,000$.

FIGURE 2 Illustration of loose delicate nature of filamentous network (circle 1) and more condensed type of filamentous network (circle 2) in adjacent locations of same subplasmalemmal site. Glutaraldehyde fixation. $\times 55,000$.



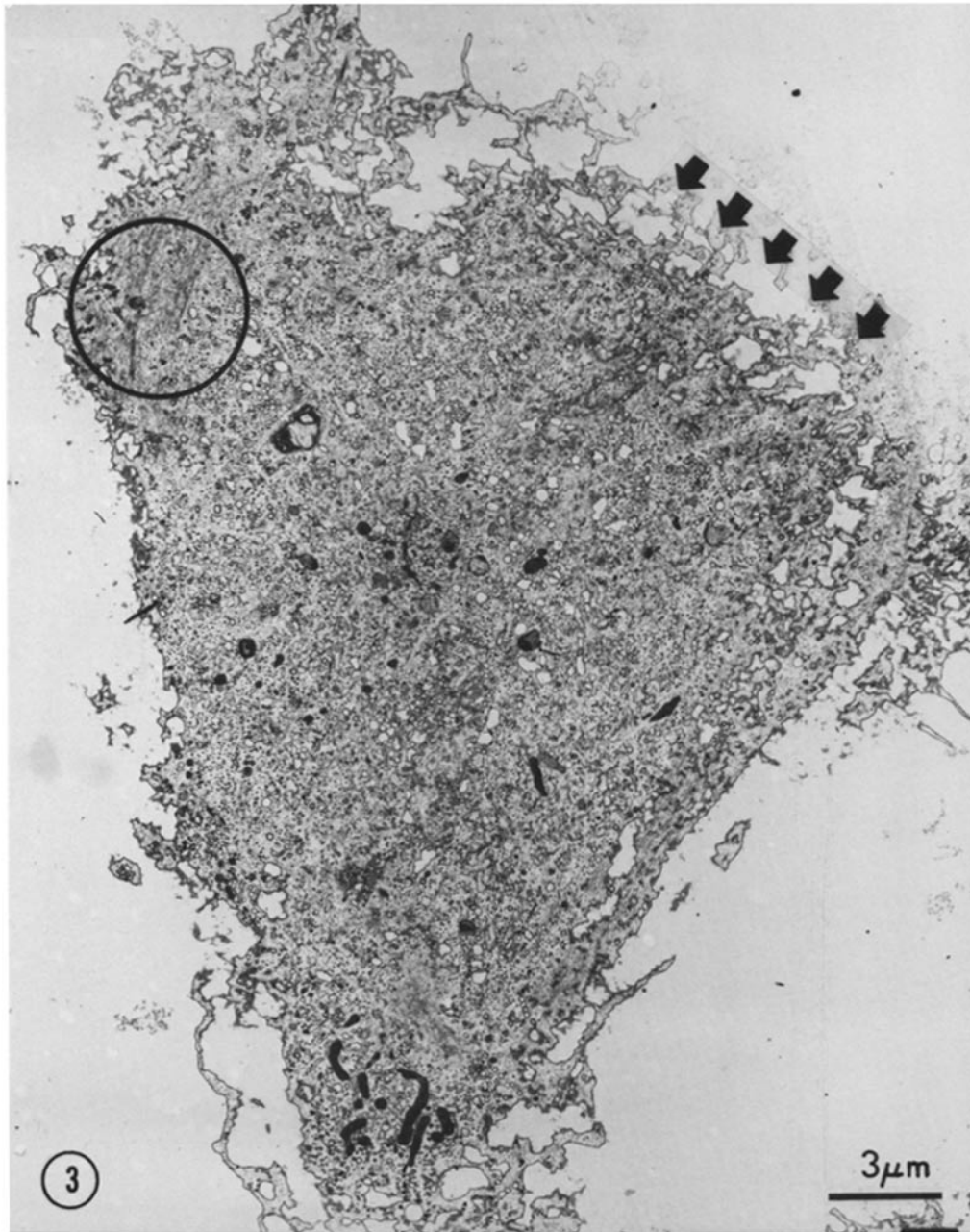


FIGURE 3 Subplasmalemmal region of the attached surface of a resting macrophage. Section was cut parallel to the surface of adhesion of the cell to its substratum. The ragged and incomplete upper right edge of the cell (arrows) represents the region directly beneath the plasma membrane. The other areas of the slice are a few hundred angstroms deeper into the subplasmalemmal zone but the precise depth of the slice varies slightly from region to region because of the undulations of the attached surface. The structural details of the encircled area are shown in Fig. 4. Fixed with a mixture of glutaraldehyde and osmium. $\times 5,200$.

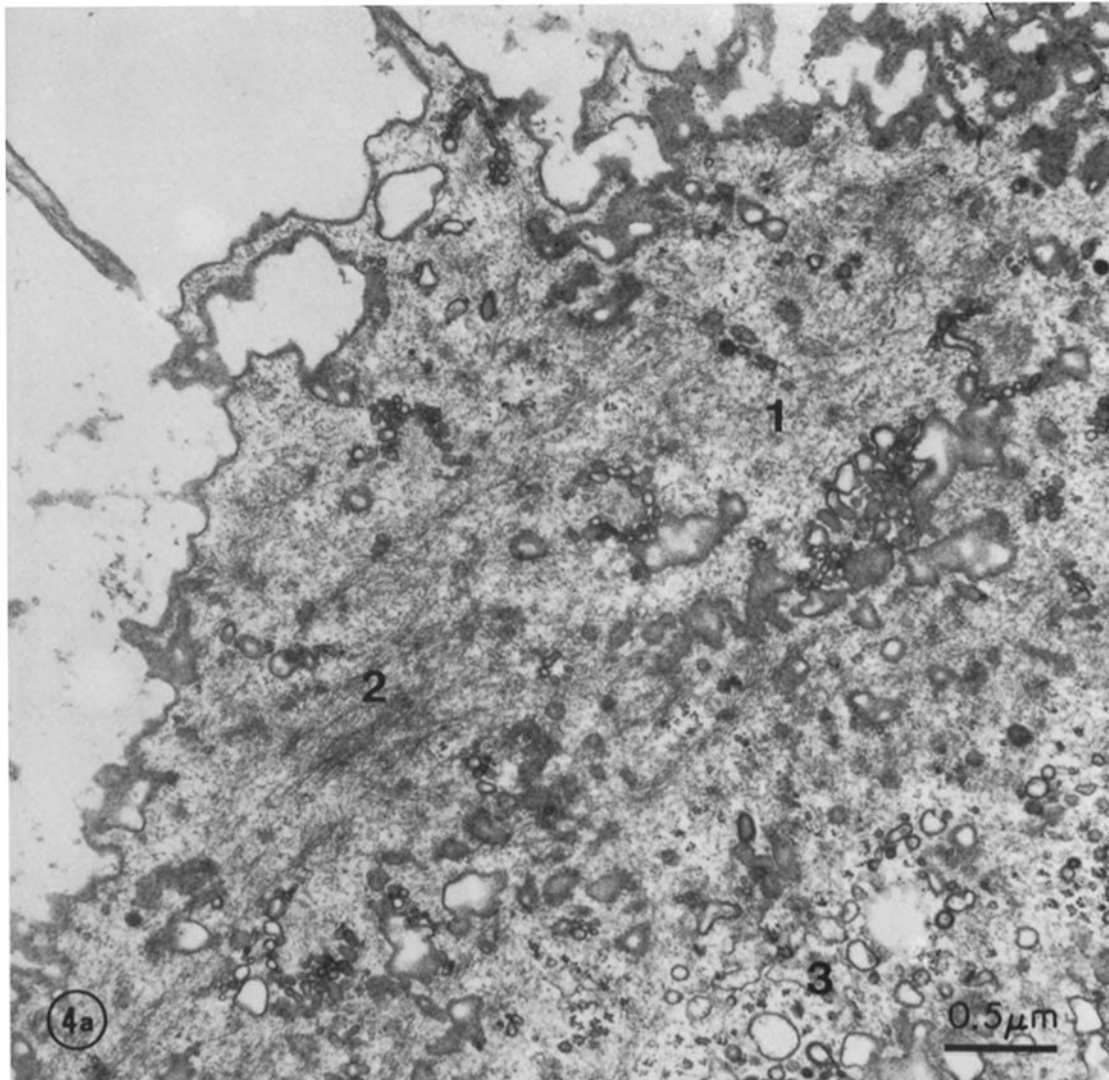


FIGURE 4 *a-d* Serial sections through subplasmalemmal microfilament zone encircled in resting macrophage of Fig. 3. Each successive section represents a progressively deeper (400–600 Å) slice into the cell. Within each section one can also visualize regional differences in depth of slice into the cell. Thus, in each micrograph region 1 represents the filamentous network, region 2 represents the oriented bundles of 40–50-Å microfilaments, and region 3 represents the interior of the cell subjacent to the subplasmalemmal microfilament zone. Fixed with a mixture of glutaraldehyde and osmium. $\times 30,000$.

FIGURE 4 *a* Most superficial section obtained of the attached surface of this cell. This section is estimated to be approximately 500-Å interior to the plasma membrane of the attached surface. The filamentous network, region 1, is especially prominent although some bundles of oriented microfilaments, region 2, can be seen.

frequently found to be parallel to the long axis of the cytoplasmic extensions.

It should be noted that organized bundles of 40–50-Å microfilaments were never observed on

the free surface of resting macrophages despite the fact that large numbers of oblique and vertically oriented serial sections of this surface were examined. We have accepted these findings as highly

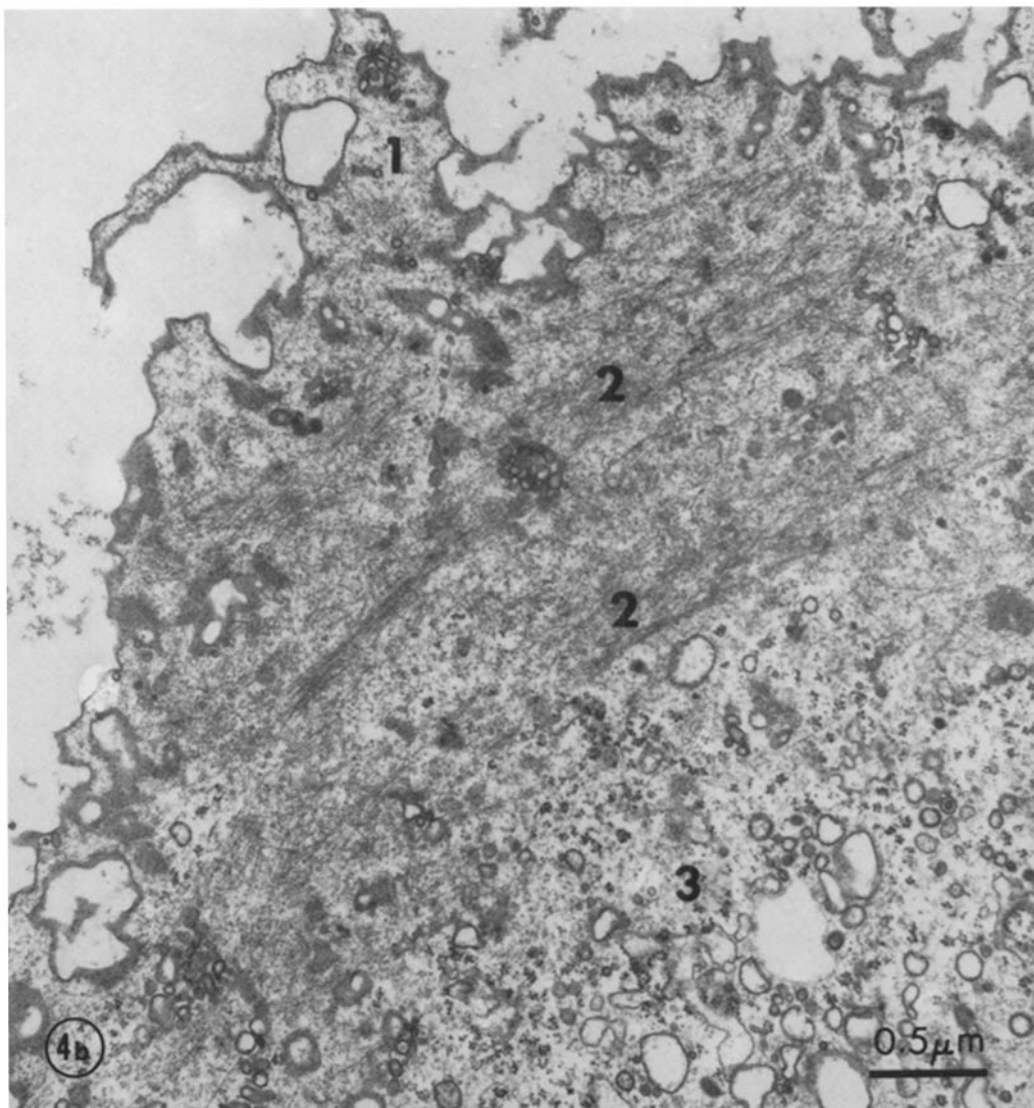


FIGURE 4 *b* Section subjacent to 4 *a* approximately 1,000-Å interior to the plasma membrane. Oriented bundles of microfilaments, region 2, are prominent.

suggestive evidence that oriented microfilaments are not normal constituents of the subplasmalemmal region of the free surface of resting macrophages. It is clear, however, that these negative observations do not provide absolute proof on this point.

Phagocytosis and Microfilaments

The microfilamentous structures of actively phagocytizing macrophages were next examined. The early stages of phagocytic vacuole formation

involving invagination and/or evagination of plasma membrane are of particular interest for the purposes of the present study. Large polystyrene spheres (14-μm diam) which could be only partially interiorized were employed. Use of such large particles permitted investigation of long stretches of membrane participating in endocytosis.

As previously noted, bundles of oriented 40–50-Å microfilaments are not normally associated with the free aspect of macrophages. However, following

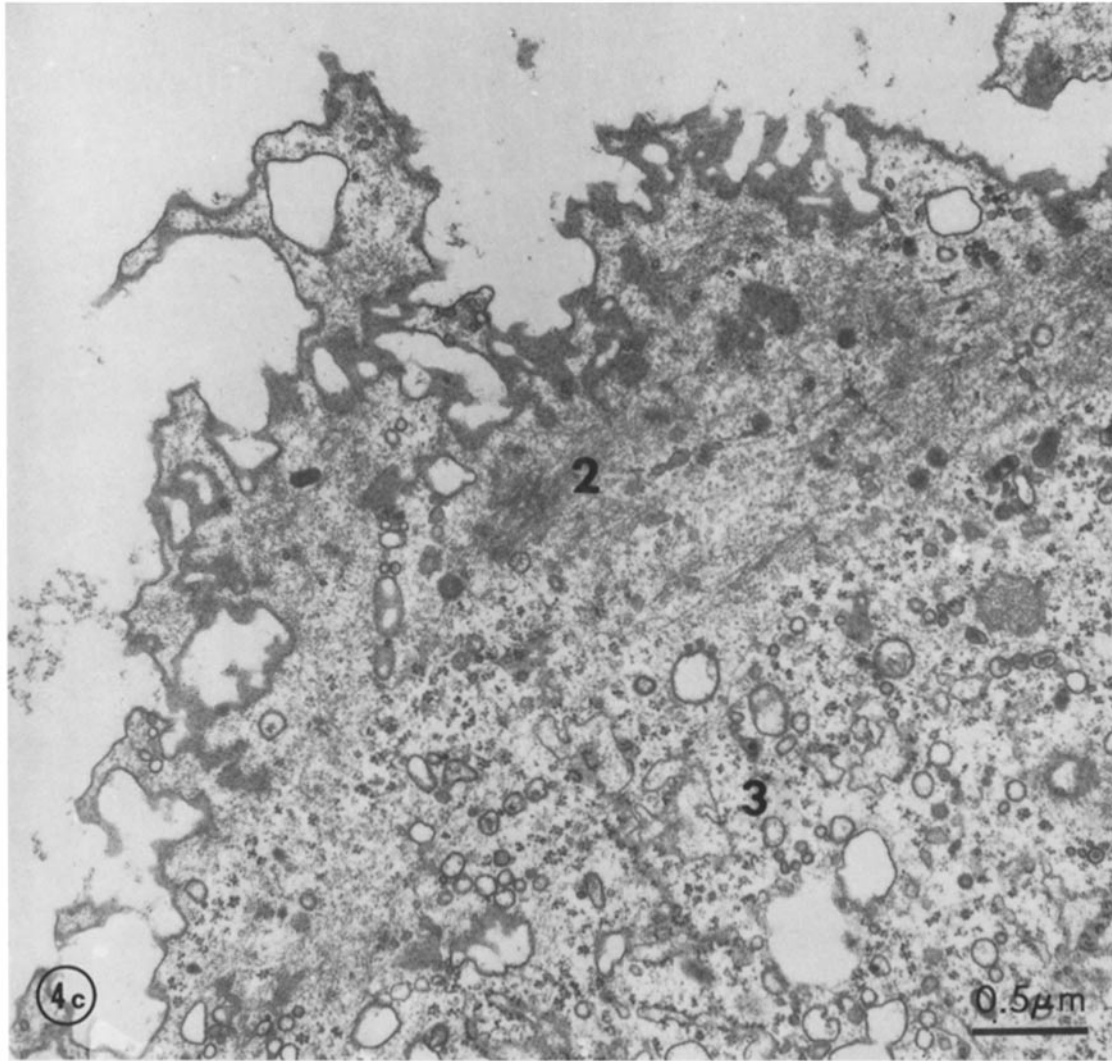


FIGURE 4 *c* Section subjacent to 4 *b* approximately 1,500-Å interior to the plasma membrane of the attached surface. Few bundles of microfilaments, region 2, are seen. Organelles of the interior of the cell, region 3, are prominent.

a phagocytic pulse with polystyrene particles, oriented bundles of microfilaments similar to those described on the glass-attached cell surface are regularly observed surrounding the ingested particles (Figs. 6 and 7). Such microfilaments are not always seen in randomly obtained sections, although, in serial cuts parallel to the plane of the substrate and approaching the base of the phagocytized particles, bundles of oriented microfilaments are always observed going in and out of the plane of sectioning. In addition to the organized bundles of microfilaments just described, larger

100-Å filaments are seen to associate with the 40-50-Å microfilaments, producing a basket-weave effect at the base of the phagocytized particle (Fig. 7).

Microtubules

As shown in Fig. 5, microtubules are found in great abundance in subplasmalemmal regions of the glass-attached surface of the cell. Two distinct patterns of microtubules are present.

Microtubules of one type extend from the

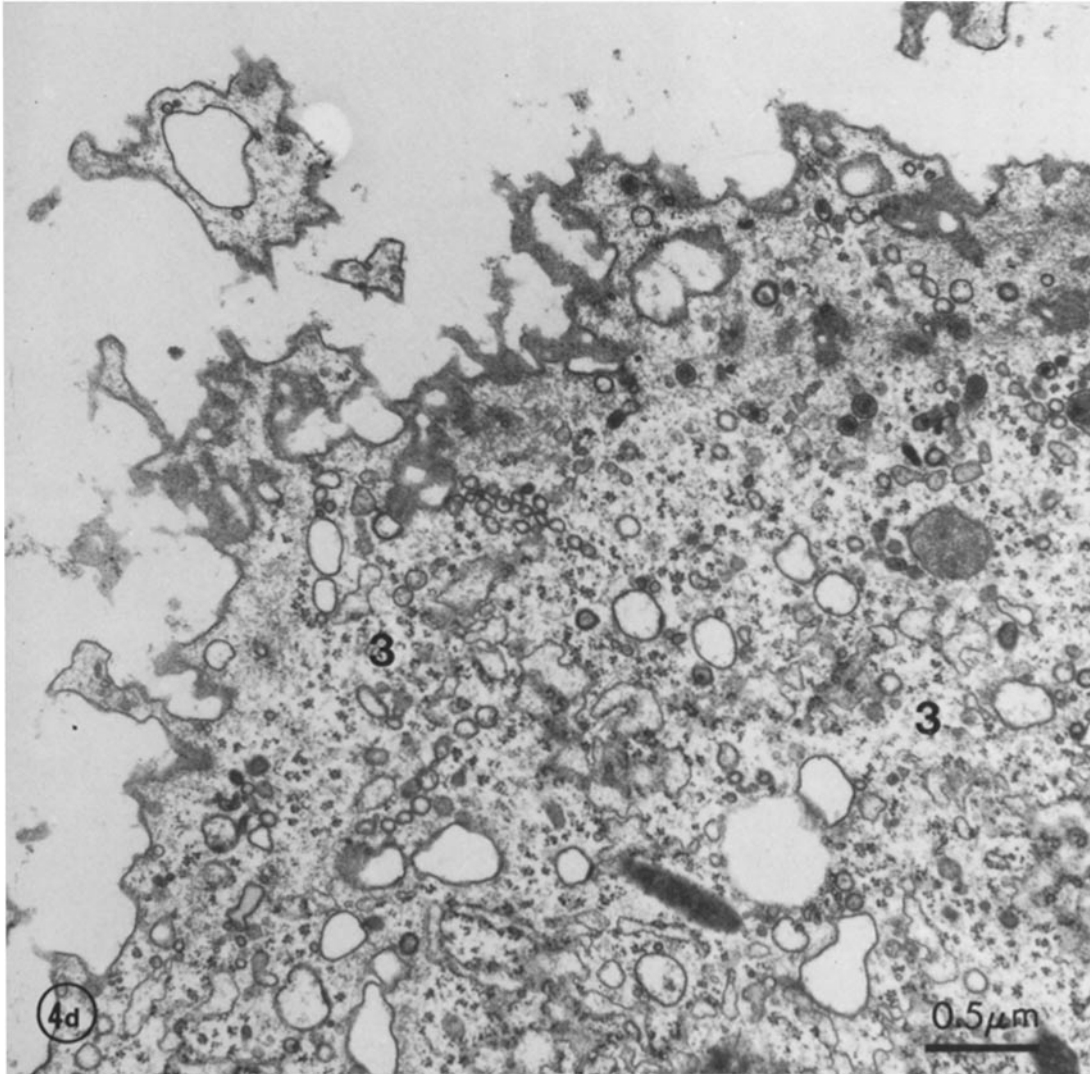


FIGURE 4d Section subjacent to 4 c approximately 2,000-Å interior to the plasma membrane of the attached surface. Section has passed through the subplasmalemmal filament zone and organelles of region 3 are prominent.

plasma membrane to the interior of the cell, traversing both the filamentous network and oriented microfilament zone (Fig. 8). As the individual microtubules extend through the microfilament network they are invariably accompanied on one or both sides by electron-dense, channellike structures. As they penetrate the zone of oriented microfilaments the microtubules often lose these secondary structures and extend unencumbered into the interior of the cell (Fig. 8).

The "microchannels" are bounded by a unit

membrane similar in dimension to that of the macrophage plasma membrane. In favorable sections the apparent continuation of these structures directly from the plasma membrane can be seen (Fig. 9 a). Interconnections between individual microchannels flanking a microtubule were not observed. However, the possibility that these structures are continuous has not been ruled out. The microtubule assumed to accompany the microchannel in Fig. 9 a is seen only deep in the cell (arrow); however, in other sections the close asso-

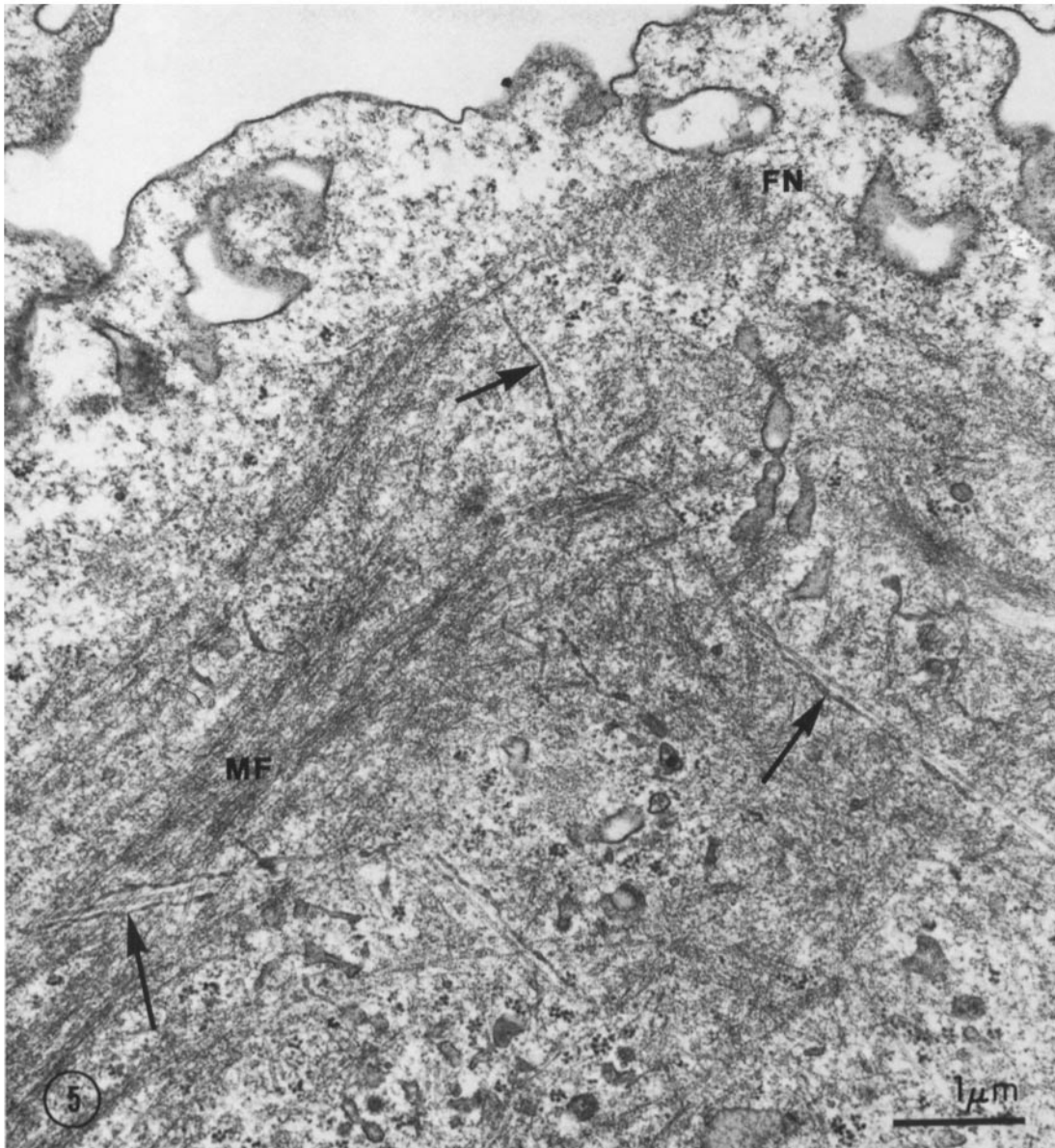


FIGURE 5 Low magnification view of subplasmalemmal microfilament zone of attached surface of resting macrophage. Section was cut parallel to the surface of adhesion of cell to its substratum. Micrograph shows spacing of bundles of microfilaments (*MF*) as well as region of filamentous network (*FN*). Microtubules accompanied by microchannels (arrows) are prominently displayed. Glutaraldehyde fixation. $\times 18,000$.

ciation and possible insertion of microtubules into the plasma membrane itself are apparent (Fig. 9 *b*).

Microtubules of the second type are closely associated with the oriented bundles of microfilaments, and are often oriented in the same

plane (Fig. 10). Microtubules are especially numerous in the region of the oriented 40-Å microfilaments and the 100-Å filaments surrounding phagocytized particles. However, the interrelationship of microtubules with the microfilaments of this region has been difficult to interpret since

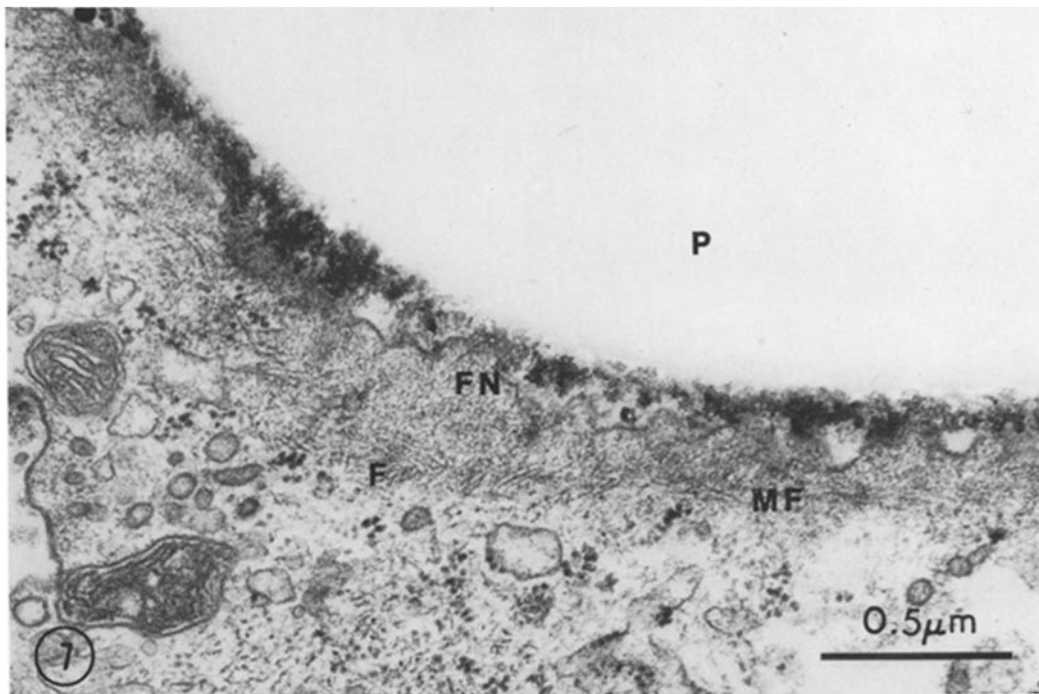
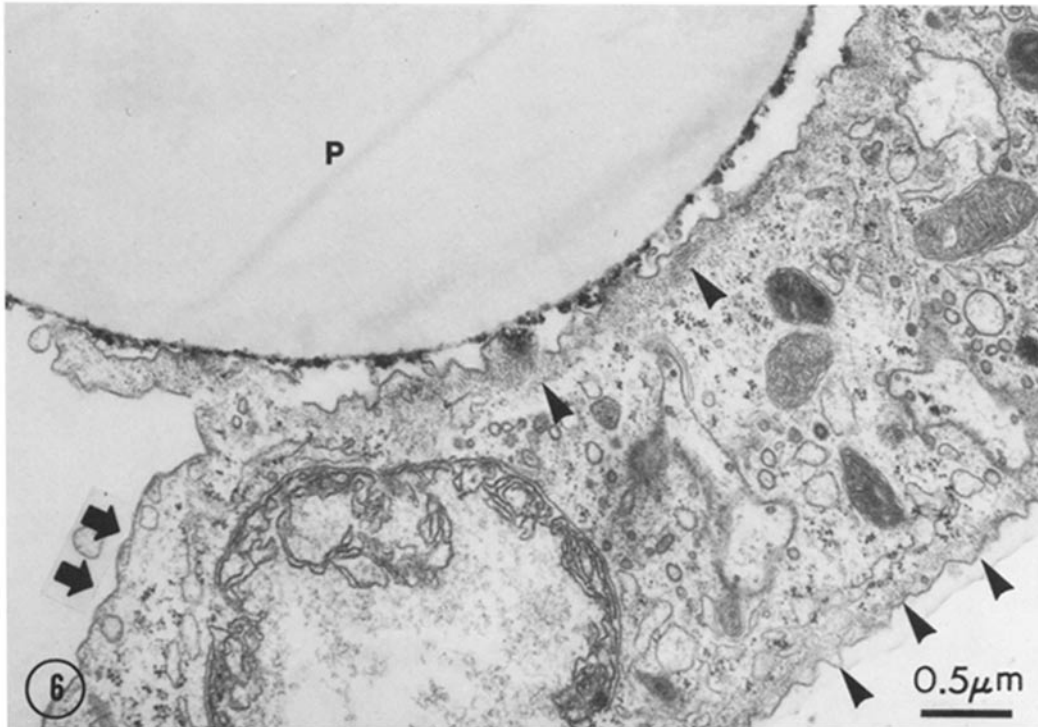


FIGURE 6 Low magnification longitudinal view of macrophage phagocytizing large particle (*P*). Polystyrene particle (*P*) has been dissolved during fixation leaving an electron-dense shell. Free surface of cell not participating in phagocytosis is indicated by arrows. Note the difference in thickness and complexity of subplasmalemmal zone on this surface as compared to filament zone of free surface surrounding particle (double arrowheads) and subplasmalemmal filament zone of attached surface (triple arrowheads). Glutaraldehyde fixation. $\times 24,000$.

FIGURE 7 Details of subplasmalemmal microfilament zone of free surface of cell surrounding large particle (*P*). Section demonstrates filamentous network (*FN*), oriented 40–50-Å microfilaments (*MF*), and basket-weave effect of 100-Å filaments (*F*) with microfilaments. Glutaraldehyde fixation. $\times 50,000$.

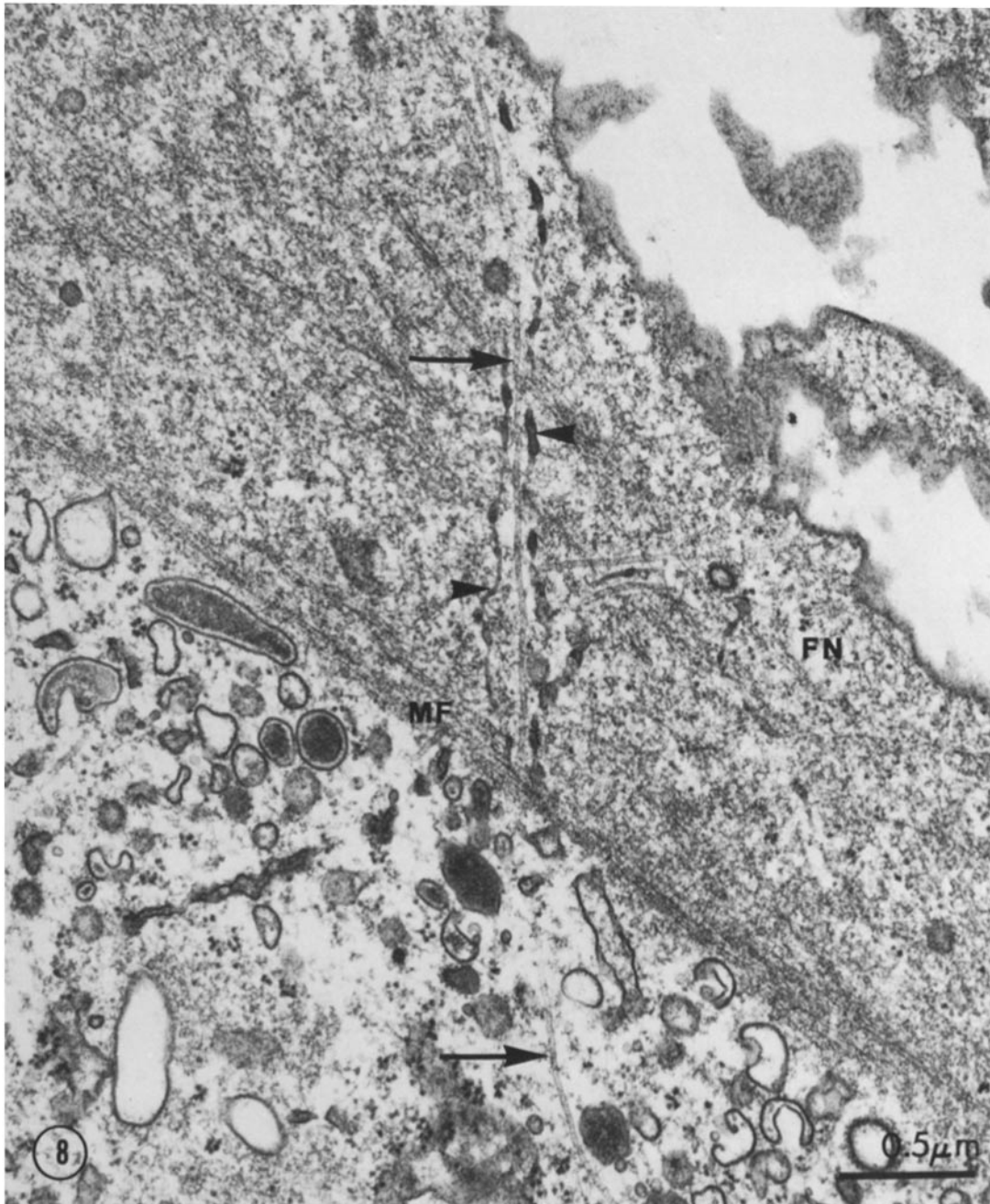


FIGURE 8 Tangential section through subplasmalemmal microfilament zones of attached surface of resting macrophage. Microtubules (arrows) traverse the filamentous network (*FN*) and zone of organized microfilaments (*MF*) accompanied by microchannels (arrowheads). Beyond the zone of organized microfilaments the microtubule enters the interior of the cell without the secondary microchannels (arrow). Glutaraldehyde fixation. $\times 42,000$.

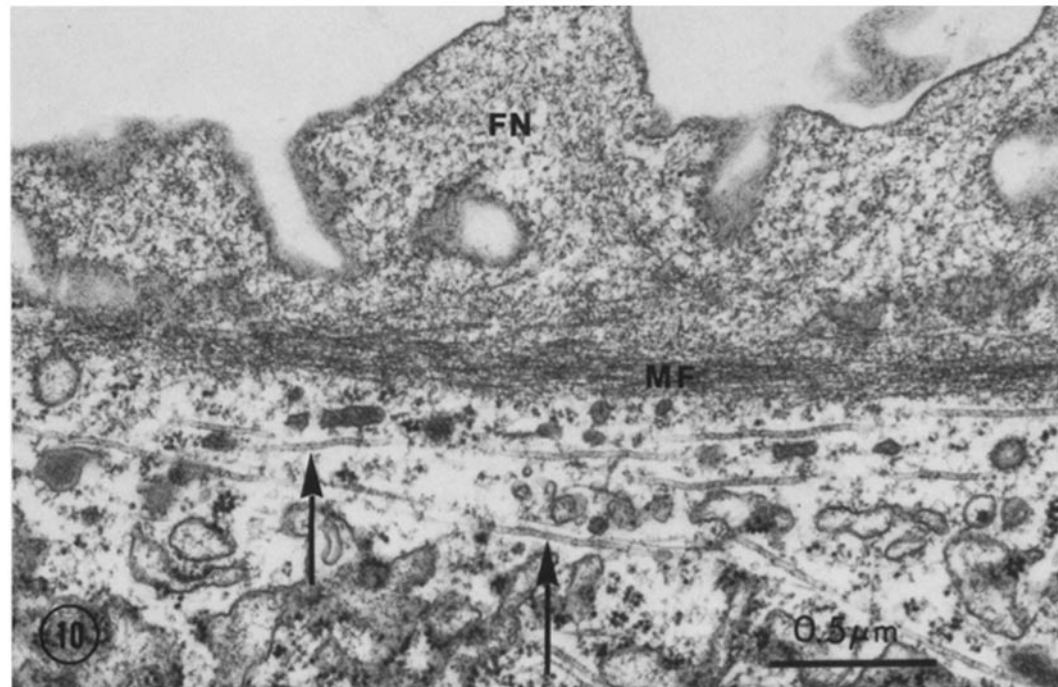
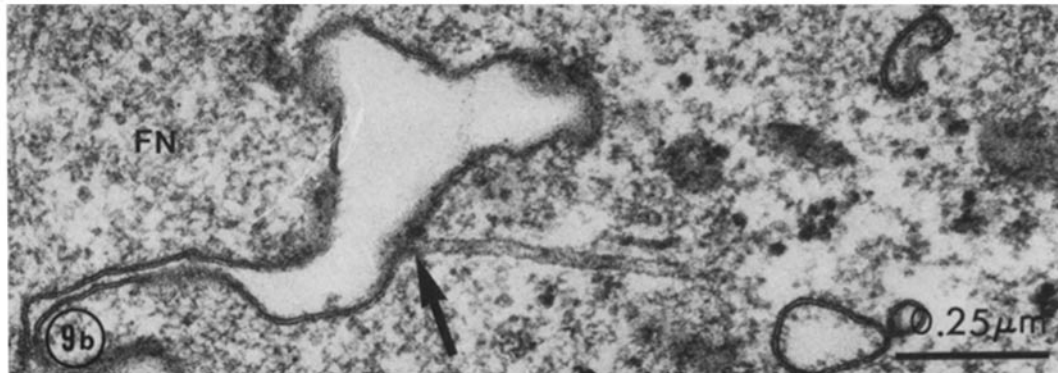
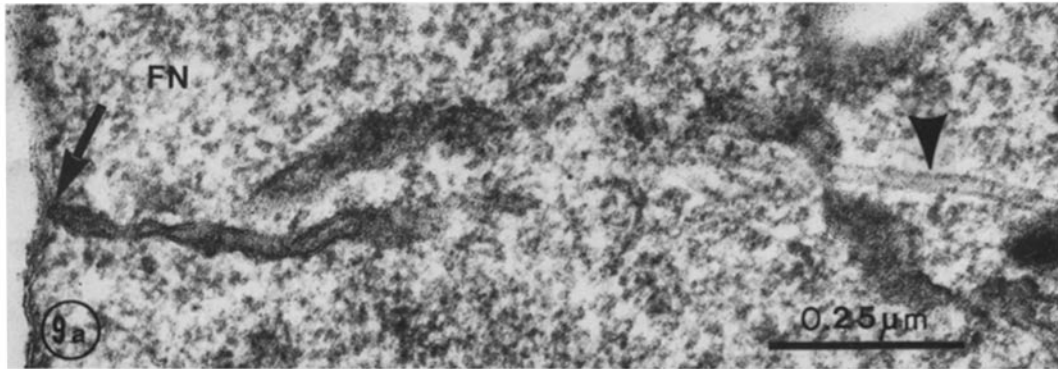


FIGURE 9 Association of microtubules and microchannels with plasma membrane of the attached surface of the resting macrophage. Filamentous network (*FN*). Glutaraldehyde fixation. (a) Limiting membrane of microchannel (arrow) in close association with the plasma membrane. Microtubule assumed to accompany the microchannel(s) is seen on the right (arrowhead). $\times 96,000$. (b) Possible insertion of microtubule into the plasma membrane (arrow). $\times 83,000$.

FIGURE 10 Tangential section through subplasmalemmal microfilament zones of the attached surface of resting macrophage illustrating the relationship of the filamentous network (*FN*), organized bundles of 40-50-Å microfilaments (*MF*), and microtubules (arrows). Glutaraldehyde fixation. $\times 44,000$.

the curvature of the cytoplasm around spherical particles rarely permits the various microfilament and microtubular elements to be present in the same plane of sectioning.

DISCUSSION

A schematic representation of the observed subplasmalemmal features of resting and phagocytizing macrophages cultivated on glass is shown in Fig. 11. The glass-attached surfaces of all outstretched cells have a comparatively complex microfilament, microtubule, and microchannel system. Although the free aspect of these cells appears to consist of a simple microfilament morphology at rest, it too is capable of assembling a more complex microfilament-microtubule organization associated with the ingestion phase of phagocytosis.

It is difficult to assign specific functions to the various components of the subplasmalemmal regions described above. However, it seems that subplasmalemmal regional differences do exist, and they appear to be related to certain activities of the cell. The most unifying explanation of the above phenomenon is that the simplest subplasmalemmal construction, the filamentous network, is intrinsic to the resting surfaces of cells and that more complex arrangements on either surface

appear in association with specialized activity at a given site, such as phagocytosis or cell attachment to a substrate. But this concept is not new (19) and certainly, in regard to the existence of organized bundles of microfilaments, this formulation is consistent with reported generalizations on cell movement in a variety of highly spread cells (4, 5, 16-19, 26). However, the difficulty in the past has been that few workers have been concerned with precisely which surface of which cell exhibits which microfilamentous features. Serial sections of oriented cells have, for the most part, not been previously obtained. From this study it seems reasonable to assume that organized bundles of microfilaments do not occur randomly in subcortical regions but are found specifically in association with those portions of either the free or the attached surface of a cell which is spread on, and/or attached to, a foreign surface.

The functional significance of microchannels associated with peripherally located microtubules in cultivated macrophages is not known. The limiting membrane of these microchannels and their location suggests that they are infoldings of the plasma membrane. It is conceivable that these membrane-lined channels participate in a type of membrane renewal system as has recently been proposed by Wessells et al. (27) and Bray (28). However, the finding that the microchannels are regularly observed on the glass-attached surface of resting macrophages suggests their involvement in a type of structural specialization specifically related to the function of this area of the cell surface. In this regard previous descriptions of various types of cell surface specialization thought to be involved in the adhesion of cells to a substrate may have relevance. Abercrombie et al. (16) have described electron-dense plaques on the attached surface of cultivated fibroblasts which they consider to be cell attachment points. Spooner et al. (18) have described membranous structures through the lower surface of the undulating membrane of glial cells as an "adhesion organelle," and Clawson et al. (29) have found surface-associated micropapillae which they suggest may be involved in cell adhesion. In our own experiments related to this study² the addition of cytochalasin B (5×10^{-6} M) to cultivated macrophages resulted in the abrupt

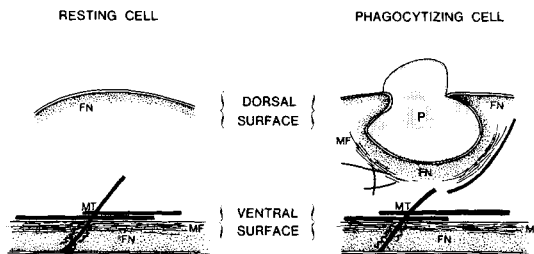


FIGURE 11 Schematic diagram of subplasmalemmal microfilaments, microtubules, and microchannels in resting and phagocytizing cultivated macrophages. The subplasmalemmal region of the attached surface of both cells is composed of a filamentous network (FN), organized bundles of 40-50-Å microfilaments (MF), and microtubules (MT). Some microtubules are accompanied by electron-dense microchannels (wavy lines). The subplasmalemmal region of the free surface of resting cells is composed of a filamentous network (FN), whereas the free surface which participates in the ingestion of large particles (P) is characterized by a thickened zone of the filamentous network as well as organized bundles of 40-50-Å microfilaments, 100-Å filaments, and microtubules.

² S. G. Axline and E. Reaven. Ultrastructural and physiological effects of cytochalasin B on resting and phagocytizing macrophages. In preparation.

retraction of cytoplasmic extensions, leaving only slender microspikes which were firmly attached to the glass. Microchannels were regularly found in the unretracted cytoplasmic microspikes which remained. This association between microchannels and adherent portions of cells leads to the speculation that microchannels may participate in the process of adhesion and, as such, may represent a variant of the surface-associated adhesion organelles described by others (18, 29, footnote 2).

Any hypothesis regarding the role of microchannels should also take into consideration the finding that microchannels in subplasmalemmal regions of the attached surface of macrophages are regularly associated with individual microtubules. It is possible that the microtubules of this region are directly linked to the contiguous microchannels with fine filamentous cross bridges analogous to those described between microtubules and endoplasmic reticulum (30). Peritubular microfilaments were observed but their participation in cross-linking could not be adequately evaluated in the subplasmalemmal region which is composed of a dense microfilament network. An integrated microchannel-microtubule-microfilament assembly could provide structural stabilization to the substrate-adherent portion of the cytoplasm.

The findings that macrophages develop a complex subplasmalemmal system consisting of a filamentous network, organized microfilament bundles, 100-Å filaments, and microtubules on the free cell surfaces during phagocytosis suggest that such a complex system is obligatory for the invagination and/or spreading of cytoplasm around a large object. The mechanism by which such a complex microfilament-microtubule system could alter the cell surface and assist the cell in engulfing a large particle is not clear. In several instances plasma membrane and microtubules were found to form direct communications with microfilaments of different sizes. Whether this represents a standard relay system of communication within cells for the purpose of accomplishing movements of the plasma membrane as proposed by others (4, 5, 17) remains to be determined. However, it should be mentioned that bundles of thick, 100-Å filaments as well as microtubules have previously been described in perinuclear regions of macrophages (5, 31) and other cells (17, 22, 32). It is possible that the 100-Å filaments and microtubules which in this study are seen to communicate with oriented 40-50-Å microfilaments at the base of the large ingested particles are actually

established components of the central regions of the cells which come into contact with the 40-50-Å organized microfilaments only when the latter have been displaced toward the nucleus by virtue of a large phagocytized particle.

In any event, the newly assembled subplasmalemmal system of the free surface in phagocytizing cells resembles the normal complex subplasmalemmal morphology of the attached surface in a number of ways, and perhaps the functioning of the complex subplasmalemmal microfilament-microtubule system on both surfaces of the phagocytizing cell may be similar. In both instances, the plasma membrane undergoes conformational changes prerequisite to the process of cell spreading (or evagination) which appears to be mediated through certain components of a complex microfilamentous and microtubular system.

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REFERENCES

1. NORTH, R. J. 1970. Endocytosis. *Semin. Hematol.* 7:161.
2. COHN, Z. A. 1968. Structure and function of monocytes and macrophages. *Adv. Immunol.* 9:163.
3. DE PETRIS, S., G. KARLBAD, and B. PERNIS. 1962. Filamentous structures in the cytoplasm of normal mononuclear phagocytes. *J. Ultrastruct. Res.* 7:39.
4. ALLISON, A. C., P. DAVIES, and S. DE PETRIS. 1971. Role of contractile microfilaments in macrophage movement and endocytosis. *Nat. New Biol.* 232:153.
5. BHISEY, A. N., and J. J. FREED. 1971. Amoeboid movement induced in cultured macrophages by colchicine or vinblastine. *Exp. Cell Res.* 64:419.
6. BHISEY, A. N., and J. J. FREED. 1971. Altered movement of endosomes in colchicine-treated cultured macrophages. *Exp. Cell Res.* 64:430.
7. DAVIS, A. TODD, R. ESTENSEN, and P. G. QUIE. 1971. Cytochalasin B. III. Inhibition of human polymorphonuclear leukocyte phagocytosis. *Proc. Soc. Exp. Biol. Med.* 137:161.
8. MALAWISTA, S. E., J. B. L. GEE, and K. G.

- BENSCH. 1971. Cytochalasin B reversibly inhibits phagocytosis. Functional, metabolic and ultrastructural effects in human blood leukocytes and rabbit alveolar macrophages. *Yale J. Biol. Med.* **44**:286.
9. 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.
 10. DUMONT, A. 1972. Ultrastructural aspects of phagocytosis of facultative intracellular parasites by hamster peritoneal macrophages. *RES J. Reticuloendothel. Soc.* **11**:469.
 11. ZIGMOND, S. H., and J. G. HIRSCH. 1972. Cytochalasin B: Inhibition of D-2-deoxyglucose transport into leukocytes and fibroblasts. *Science (Wash. D.C.)*. **176**:1,432.
 12. SCHROEDER, T. E. 1969. The role of contractile ring filaments in dividing *Arbacia* eggs. *Biol. Bull. (Woods Hole)*. **137**:413.
 13. TILNEY, L. G., and J. R. GIBBINS. 1969. Microtubules and filaments in the filopodia of the secondary mesenchyme cells of *Arbacia Punctulata* and *Ecinarachnius*. *J. Cell Sci.* **5**:195.
 14. NACHMIAS, V. T., H. E. HUXLEY, and D. KESSLER. 1970. Electron microscope observations on actomyosin and actin preparations from *Physarum Polycephalum*, and on their interaction with heavy meromyosin subfragment I from muscle myosin. *J. Mol. Biol.* **50**:83.
 15. POLLARD, T. D., E. SHELTON, R. R. WEIHING, and E. D. KORN. 1970. Ultrastructural characterization of F-actin isolated from *Acanthamoeba Castellani* and identification of cytoplasmic filaments as F-actin by reaction with rabbit heavy meromyosin. *J. Mol. Biol.* **50**:91.
 16. ABERCROMBIE, M., J. E. M. HEAYSMAN, and S. M. PEGRUM. 1971. The locomotion of fibroblasts in culture. *Exp. Cell Res.* **67**:359.
 17. GOLDMAN, R. D. 1971. The role of three cytoplasmic fibers in BHK-21 cell motility. I. Microtubules and the effects of colchicine. *J. Cell Biol.* **51**:752.
 18. SPOONER, B. S., K. M. YAMADA, and N. K. WESSELLS. 1971. Microfilaments and cell locomotion. *J. Cell Biol.* **49**:595.
 19. WESSELLS, N. K., B. S. SPOONER, J. F. ASH, M. O. BRADLEY, M. A. LUDUENA, E. L. TAYLOR, J. T. WRENN, and K. M. YAMADA. 1971. Microfilaments in cellular and developmental processes. *Science (Wash. D.C.)*. **171**:135.
 20. BARRETT, L. A., and R. B. DAWSON. 1972. Microtubules and erythroid cell shape. *Fed. Proc.* **31**:629.
 21. AXLINE, S. G., and Z. A. COHN. 1970. *In vitro* induction of lysosomal enzymes by phagocytosis. *J. Exp. Med.* **131**:1,239.
 22. HIRSCH, J. G., and M. E. FEDORKO. 1968. Ultrastructure of human leukocytes after simultaneous fixation with glutaraldehyde and osmium tetroxide and "postfixation" in uranyl acetate. *J. Cell Biol.* **38**:615.
 23. BRANSON, S. H. 1971. Epon-embedded cell monolayers. Separating partially polymerized blocks from glass surfaces. *Exp. Cell Res.* **65**:253.
 24. PEACHEY, L. D. 1960. Thin sections: I. A study of section thickness and physical distortion produced during microtomy. *J. Biophys. Biochem. Cytol.* **4**:233.
 25. FEDORKO, M. E., and J. G. HIRSCH. 1970. Structure of monocytes and macrophages. *Semin. Hematol.* **7**:109.
 26. YAMADA, K. M., B. S. SPOONER, and N. K. WESSELLS. 1971. Ultrastructure and function of growth cones and axons of cultured nerve cells. *J. Cell Biol.* **49**:614.
 27. WESSELLS, N. K., B. S. SPOONER, and M. H. LUDUENA. 1973. Surface movements, microfilaments, and cell locomotion. *Ciba Found. Symp.* In press.
 28. BRAY, D. 1973. Role of growth cone in neurite extension in locomotion of tissue cells. *Ciba Found. Symp.* In press.
 29. CLAWSON, C. C., and R. A. GOOD. 1971. Micro-papillae. *J. Cell Biol.* **48**:207.
 30. FRANKE, W. W. 1971. Cytoplasmic microtubules linked to endoplasmic reticulum with cross bridges. *Exp. Cell Res.* **66**:486.
 31. SUTTON, J. S., and L. WEISS. 1966. Transformation of monocytes in tissue culture into macrophages, epithelioid cells and multinucleated giant cells. *J. Cell Biol.* **28**:303.
 32. GOLDMAN, R. D., and E. A. C. FOLLETT. 1970. Birefringent filamentous organelle in BHK-21 cells and its possible role in cell spreading and motility. *Science (Wash. D.C.)*. **169**:286.