

# TRANSFORMATION OF MONOCYTES IN TISSUE CULTURE INTO MACROPHAGES, EPITHELIOID CELLS, AND MULTINUCLEATED GIANT CELLS

## *An Electron Microscope Study*

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### ABSTRACT

The sequential transformation of chicken monocytes into macrophages, epithelioid cells, and multinucleated giant cells *in vitro* was studied by electron microscopy after fixation and embedment *in situ*. The following changes occur. In the *nucleus*, margination of chromatin, evident in monocytes, decreases in later forms. Nucleoli become more complex and nuclear pores increase in number. In *cytoplasm*, a progressive increase in volume of the ectoplasm and endoplasm occurs in culture. *Lysosomes* increase in number and size prior to phagocytosis. During phagocytosis (most active from 1 to 3 days of culture) lysosome depletion occurs. Lysosomes are present in greatest number and show maximal structural variation in the epithelioid and young giant cells. Aging giant cells lose lysosomes. All stages possess variably large quantities of rough-surfaced *endoplasmic reticulum* and free *ribosomes*. The Golgi apparatus, small in monocytes, increases in size and complexity. Massive accumulations of lysosomes within the Golgi apparatus of macrophages and epithelioid cells suggest that lysosomes originate there. In giant cells, multiple Golgi regions occur, often ringing the nuclei. Monocytes and macrophages have few *mitochondria*. Mitochondria of epithelioid cells are larger, more numerous, and may have discontinuous outer membranes. Mitochondria are most numerous in giant cells where they increase with age and become polymorphous. *Cytoplasmic filaments* are approximately 50 to 60 A in diameter and of indeterminate length. They occur both singly and in bundles which touch cytoplasmic vesicles and mitochondria. Few filaments occur in monocytes and macrophages. A large increase in the number of filaments occurs in epithelioid cells, where filaments (90 to 100 A) surround the cytocentrum as a distinctive annular bundle often branching into the cytoplasm. The greatest concentration of filaments occurs in aged giant cells. Pseudopodia are always present. They are short and filiform in monocytes and giant cells, and broad, with abundant micropinocytotic vesicles, in macrophages and epithelioid cells. At every stage, the cell membrane contains dense cuplike structures. These may represent the membranous residue of lysosomes which have discharged to the outside, analogous to merocrine secretion. Contiguous epithelioid cells display elaborate cytoplasmic interdigitation. In places, the plasma membranes break down and epithelioid cells fuse to form giant cells.

## INTRODUCTION

Monocytes undergo a characteristic sequential transformation *in vitro* into macrophages, epithelioid cells, and multinucleated giant cells. The cytology of this transformation has been characterized by light microscopy (1-3) and, to a limited extent, cytochemically and biochemically (4-7). While reports on the ultrastructure of circulating monocytes (8, 9) and macrophages from various sources (e.g., peritoneal and alveolar) (6, 10, 11) have been published, studies of the transformations of these cells have not. This work constitutes such an electron microscope study of the changes observed in blood monocytes in tissue culture. It deals with (a) changes in cytoplasmic organelles, (b) phagocytosis, and (c) the formation of multinucleated giant cells.

## MATERIALS AND METHODS

The material consisted of Leighton tube cultures of leukocytes separated from cardiac blood of Rhode Island Red cockerels by albumin flotation (12) and grown in a medium consisting of 60% Eagle's medium and 40% chicken serum. Cultures were incubated at 37°C in an atmosphere containing 5% CO<sub>2</sub>.

After 24 hr incubation, the majority of the transformed monocytes were firmly attached to the glass substrate. At this time, the culture tubes were vigorously shaken to dislodge unattached cells, and the medium was replaced. Thereafter, the medium was replaced every 3 days. Before fresh medium was introduced, the pH of the old medium was measured with a Beckman pH meter. The pH ranged from 7.0 to 7.5, but the majority of cultures were maintained at a pH of 7.3 to 7.45.

The progress of each culture was followed daily by light microscopy. Appropriate stages of the transformation of monocytes into macrophages, epithelioid cells, and multinucleated giant cells were selected and prepared *in situ* for electron microscopy. For general cytological study, flying cover slip cultures were removed, fixed in absolute methyl alcohol, and stained by Jacobson's modification of the May-Grünwald-Giemsa stain (13). The remainder of the culture growing directly upon the floor of the tube was prepared for electron microscopy.

**ELECTRON MICROSCOPY:** The cultures were prepared for electron microscopy by fixation, dehydration, and embedment in Araldite *in situ*. Portions of the embedment containing suitable cells selected by light microscopy were cut out and affixed to a small metal dowel, sectioned in a Porter-Blum microtome (MI) with glass knives, mounted on bare grids, stained with 1.0% KMnO<sub>4</sub> (26), and studied in a

Siemens Elmiskop I. Methods of fixation, embedding, and mounting were developed for this problem and are reported elsewhere (14).

## OBSERVATIONS

Phagocytosis is most active between 6 and 24 hr. At 24 hr, a uniform monolayer of macrophages covers the floor of the flask. Transformation to epithelioid cells begins by the third or fourth day and is complete by the fifth to sixth day. Giant cells are present at 9 to 15 days, but may appear as early as 2 to 4 days. The stages reported here are the original inoculum and cells cultured for 1, 6, and 24 hr, 3, 7, 15, 26, and 70 days. The observations are presented by stage and organized under the following headings: Nucleus, Cytoplasm, Mitochondria, Endoplasmic Reticulum (ER), Ribosomes, Lysosomes, Cytozentrum, and Cytoplasmic Filaments.

### *Circulating Monocytes (Fig. 1)*

**NUCLEUS:** Chromatin is aggregated. Nucleoli are not evident. Nuclear pores are present.

**CYTOPLASM:** Endoplasm and ectoplasm are not consistently differentiated, but small ectoplasmic pseudopodia are present.

**MITOCHONDRIA:** Mitochondria are few (2 to 8 in a section of a cell), and are small and oval. Their matrix is denser than the hyaloplasm and contains no granules.

**ENDOPLASMIC RETICULUM:** Granular ER is present. Smooth ER is rare. Many sacs are moderately dilated by finely granular material of low density. Centrally, the ER runs circumferentially about the nucleus, whereas, more peripherally, it appears randomly placed.

**RIBOSOMES:** Free ribosomes as well as those on the ER are abundant. Free polyribosomes were not observed.

**LYSOSOMES:** Small, membrane-bounded granules containing a uniformly granular material are present throughout the cytoplasm.

**CYTOCENTRUM:** The cytozentrum is roughly circular in outline and may measure 2.5 to 3  $\mu$  in diameter. It typically contains a diplosome. The Golgi apparatus is well developed and small.

### *Mononuclear Cells, 1 to 6 hr (Figs. 2 and 3)*

**NUCLEUS:** The nucleus is eccentric, but otherwise similar to that of circulating monocytes.

**CYTOPLASM:** A distinct, narrow zone of

ectoplasm is present. Pseudopodia are increased in number and length and display basal microvesicles. Dense cuplike structures with an inward convexity (caveolae) appear in the plasma membrane at this stage. Fat droplets are regularly present.

**MITOCHONDRIA:** Mitochondria show no change.

**ENDOPLASMIC RETICULUM:** Granular ER is abundant. It may be aggregated into parallel rows of flattened, elongated cisternae or occur in short sausage-shaped and vesicular form. Smooth ER is also abundant.

**RIBOSOMES:** Free ribosomes are plentiful.

**LYSOSOMES:** Lysosomes, varying in both density and internal structure, and reaching even  $1.0 \mu$  in diameter, are more numerous and larger than in circulating monocytes. They may contain myelin figures and multiple small vesicles.

**CYTOCENTRUM:** The triple array of flattened parallel sacs, small vesicles, and vacuoles is present. Vesicles are more numerous than in circulating monocytes and may be continuous with granular ER. Lysosomes are present within the cytocentrum.

**FILAMENTS:** Cytoplasmic filaments are first observed after 6 hr. A single bundle containing five to eight parallel filaments often sweeps through the cytoplasm. Filaments touch mitochondria and lysosomes.

#### *Macrophages, 24 hr (Figs. 4 to 6)*

**NUCLEUS:** Chromatin is less marginated and occurs in smaller aggregates than earlier. Nucleoli are now evident.

**CYTOPLASM:** Many large phagocytotic inclusions occur. A rim of ectoplasm is no longer present. Ectoplasm occurs only as pseudopodial extensions. Fat droplets are common and lie next to mitochondria.

**MITOCHONDRIA, ENDOPLASMIC RETICULUM, AND RIBOSOMES:** These elements are not significantly changed from the 6 hr stage (Figs. 4 and 5).

**PLASMA MEMBRANE:** Dense cupped structures occur in the plasma membrane but are less numerous than at 6 hr.

**LYSOSOMES:** Lysosomes are increased in number but decreased in size (Fig. 5). They are particularly numerous in cells containing phagosomes in advanced stages of digestion (Fig. 5). Few lysosomes are seen in macrophages contain-

ing whole cells (presumably recently phagocytosed) and incompletely digested cells.

**CYTOCENTRUM:** The centrosome is larger than before and contains more vesicles. Vesicles may be interconnected, forming a highly branched network (Fig. 5). Continuity between Golgi vesicles and ER is common.

**FILAMENTS:** Single filaments are more numerous. They are absent from the cytocentrum. Bundles of parallel filaments occur. They frequently extend radially toward the plasma membrane, often abutting mitochondria and lysosomes (Fig. 6).

**PHAGOCYTOSIS AND PHAGOCYTOTIC INCLUSIONS:** Phagocytosis is most evident in macrophages cultured for 1 to 3 days. Phagocytized material is present in all stages of digestion (Figs. 4 and 5). Thrombocytes, the most common cell type among macrophages at 24 hr, are frequently phagocytized.

#### *Macrophage, 3 days (Fig. 7)*

**NUCLEUS:** Chromatin is finely dispersed, no longer marginated or clumped. Two or three nucleoli stand out prominently.

**CYTOPLASM:** No distinct division into endoplasm and ectoplasm exists. Phagocytotic inclusions in advanced stages of digestion crowd the cytoplasm. Long, slender pseudopodia with small vesicles linearly arranged at their base are present.

**PLASMA MEMBRANE:** Dense, cuplike indentations are prominent and numerous in the plasma membrane.

**ENDOPLASMIC RETICULUM:** Granular ER takes two predominant forms: (a) long, slender profiles, the membranes flat and parallel, and (b) vesicles of varying diameter containing lightly staining material.

**LYSOSOMES:** Lysosomes are further increased in number over earlier stages and are prominent in every part of the cytoplasm. Most common is the small dense granule. They are radially arranged within and about the cytocentrum.

**CYTOCENTRUM:** The cytocentrum is larger ( $9.0$  to  $12.5 \mu$  in diameter) than in previous stages. Flattened sacs of the Golgi element are arranged radially around the centrioles.

**FILAMENTS:** Filaments are greatly increased in number. Singly, or in small groups of up to three or four, they extend toward the plasma membrane, often touching lysosomes and mitochondria. Filaments, in bundles containing up to ten, en-

circle large phagocytotic vacuoles and occasionally entwine a mitochondrion or vesicle.

**PHAGOCYTOTIC VACUOLES:** Phagocytotic inclusions are present in greatest concentration in cells at this stage.

#### *Epithelioid Cells, 7 days (Figs. 8-17)*

These cells are polygonal and touch one another, assuming an epithelial appearance.

**NUCLEUS:** Chromatin remains finely dispersed. Two or more nucleoli are present. Nuclear pores are numerous.

**CYTOPLASM:** The cytoplasm contains a plethora of cytoplasmic organelles including mitochondria, lysosomes, and large vacuoles (Figs. 8 and 9). The latter vacuoles, larger in diameter than any other cytoplasmic structure, are more or less uniformly distributed in the cytoplasm outside the cytocentrum (Figs. 9 to 11, and 16). Their contents appear fluid with uniformly suspended bits of solid material. Many of these cysts doubtlessly represent terminal stages of phagocytotic vacuoles. Unequivocal phagocytotic inclusions

are rare at this stage of culture. Free ribosomes and polysomes abound.

**PLASMA MEMBRANE:** Cuplike structures in the plasma membrane are especially prominent (Figs. 11, 14, and 17). The rim and contents of these structures are similar in density to those of lysosomes (Fig. 11). Lysosomes in direct contact with invaginations of the plasma membrane suggest that the cupped structures represent lysosomes which discharged their contents extracellularly (Fig. 11; cf. Fig. 3).

Pseudopodia ranging in form from slender, elongate projections to bulky, irregular masses are present. Microvesicles have greatly increased in number within pseudopodia.

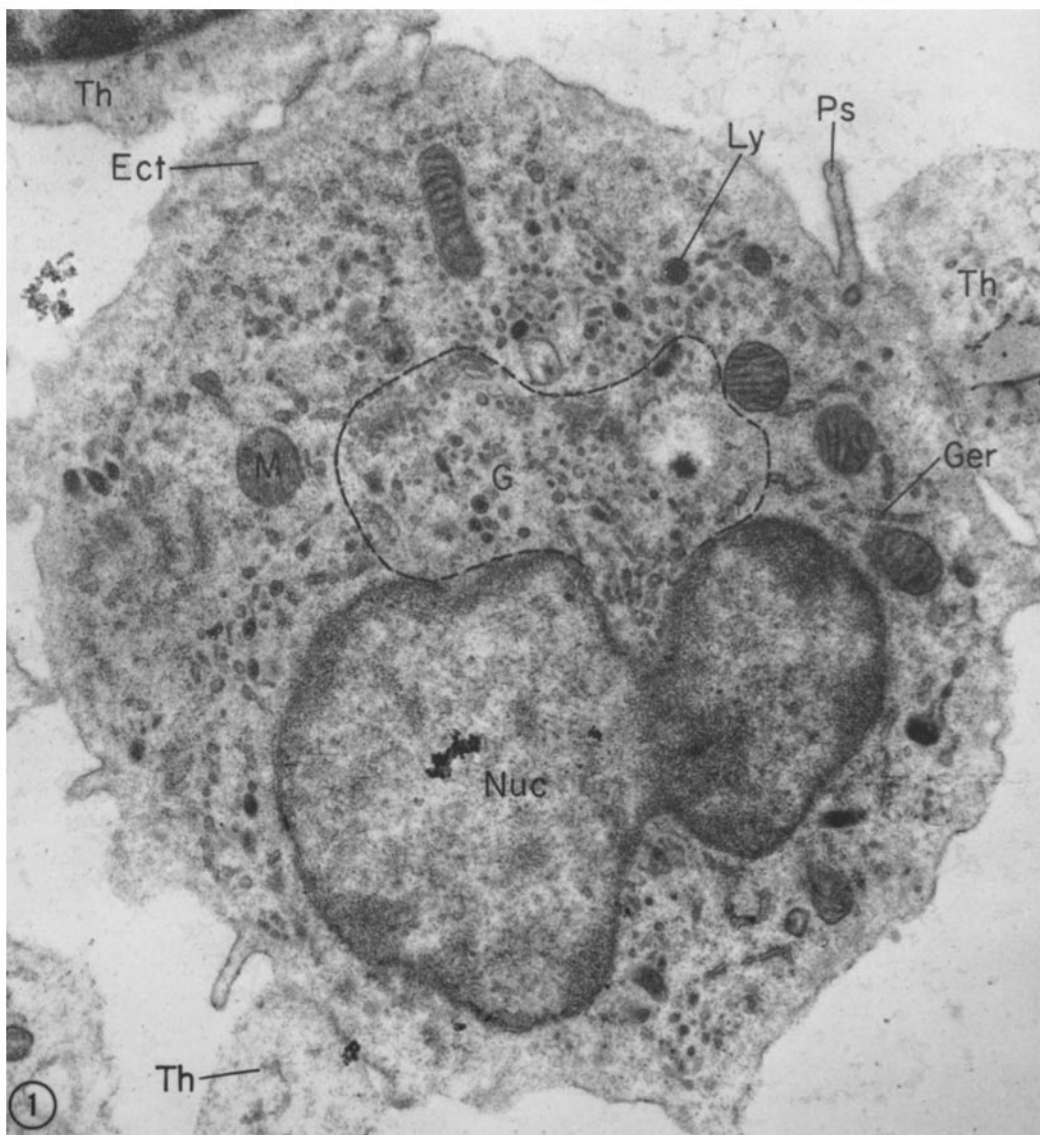
Many binucleated cells are seen by light microscopy. In electron micrographs, these are revealed as fusing epithelioid cells (Fig. 16). Such contiguous epithelioid cells display areas of apposition of plasma membranes. In places, the membranes have apparently broken down and the protoplasm has become confluent (Figs. 17 and 18). Cuplike structures in the cell membrane,

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#### *Key to Abbreviations*

<i>AVac</i> , autophagic vacuole	<i>L</i> , lipid
<i>C</i> , cuplike structure	<i>Ly</i> , lysosome
<i>Ce</i> , centriole	<i>M</i> , monocyte
<i>Chr</i> , chromatin	<i>Ma</i> , macrophage
<i>Cm</i> , cell membrane	<i>MVB</i> , multivesicular body
<i>Cr</i> , crescent-shaped vacuole	<i>My</i> , myelin figure
<i>Det</i> , detachment	<i>Nu</i> , nucleolus
<i>Ect</i> , ectoplasm	<i>Nuc</i> , nucleus
<i>Ect In</i> , ectoplasmic interdigitation	<i>Om</i> , outer mitochondrial membrane
<i>Ep</i> , epithelioid cell	<i>P, NP</i> , pore or nuclear pore
<i>Fib</i> , fibrin	<i>PhV</i> , phagocytotic vacuole
<i>FI, F</i> , filament	<i>R</i> , polyribosome
<i>Flb</i> , bundle of filaments	<i>RNP</i> , ribonucleoprotein, ribosome (free)
<i>G</i> , Golgi region	<i>SER</i> , smooth-surfaced endoplasmic reticulum
<i>Ger</i> , granular endoplasmic reticulum	<i>Th</i> , thrombocyte
<i>Gr</i> , granule	<i>Vac</i> , vacuole
<i>GVe</i> , Golgi vesicles	<i>Ve</i> , vesicle

**FIGURE 1** This electron micrograph illustrates the salient features of a circulating monocyte. A myriad of variably dense lysosomal granules (*Ly*) is present. The Golgi zone (*G*) is small, but well developed; its external limits are demarcated by the dotted line. Rough-surfaced ER (*Ger*), many channels of which are dilated by electron-opaque secretory material, is abundant. Mitochondria (*M*) are distributed randomly. A narrow but distinct zone of ectoplasm (*Ect*) rings the cell and continues into pseudopodia (*Ps*). Neighboring cells (*Th*) are thrombocytes.  $\times 22,500$ .



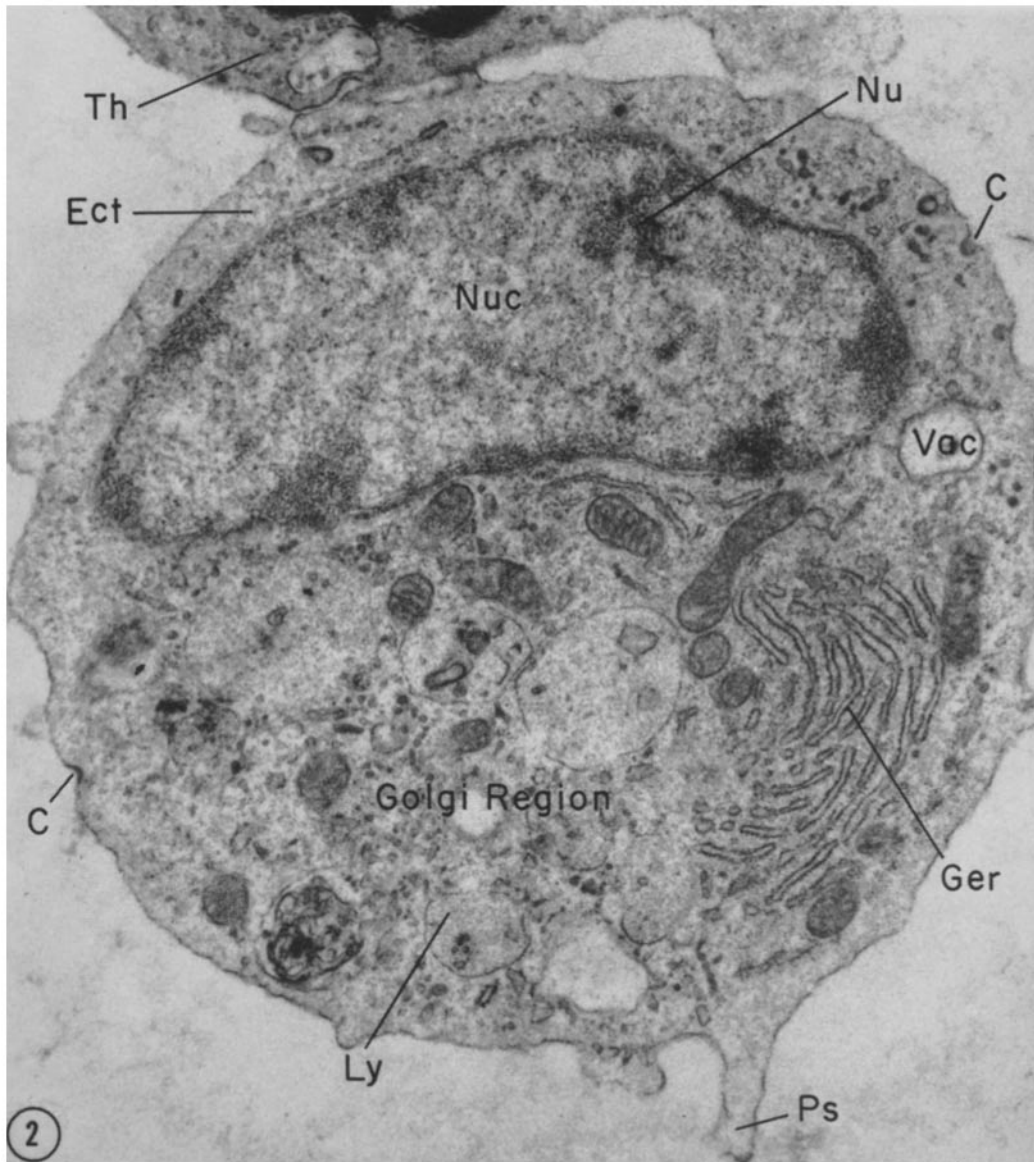


FIGURE 2 This monocyte was cultured for 6 hr. Dense cuplike structures (*C*) appear in the plasma membrane. Lysosomes (*Ly*) are larger and more complex; the largest contain internal membranes and vesicles. Elongate, flattened cisternae of rough-surfaced endoplasmic reticulum, aggregated in the lower right sector of the cytoplasm, contain material of low density similar to the cytoplasmic matrix. Mitochondria are slightly increased in number and a few in length. The cell membrane along the upper border touches a thrombocyte (*Th*). Macropinocytotic vacuoles (*Vac*) are occasionally encountered.  $\times 20,000$ .

lysosomes, and mitochondria are common in the areas bordering regions of fusion (Figs. 16 and 17). Epithelioid cells may touch without fusing. Often, elaborate interdigitations of the plasma

membranes of contiguous cells may be present (Fig. 15).

**MITOCHONDRIA:** Mitochondria are more numerous and larger than in earlier stages (Figs.

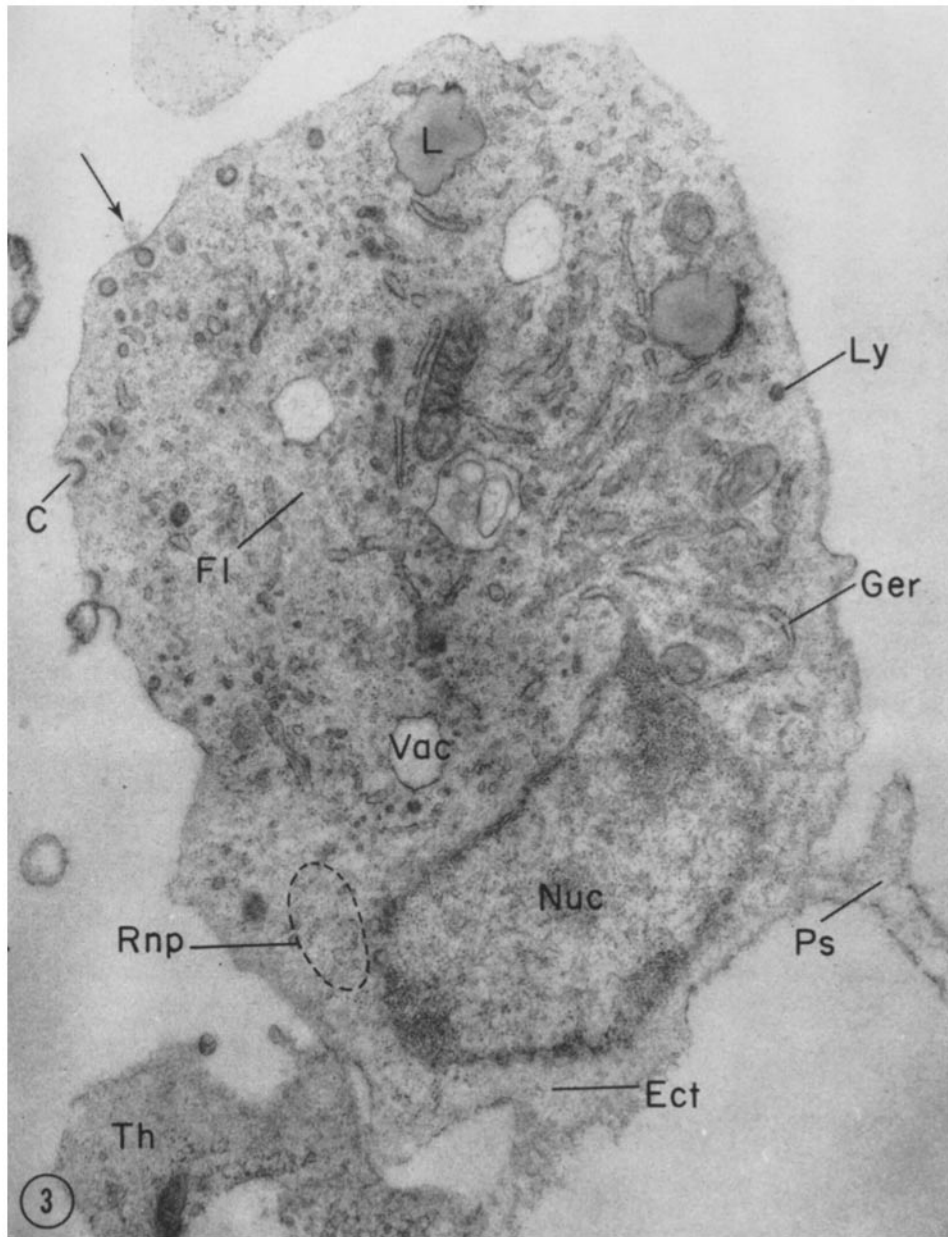
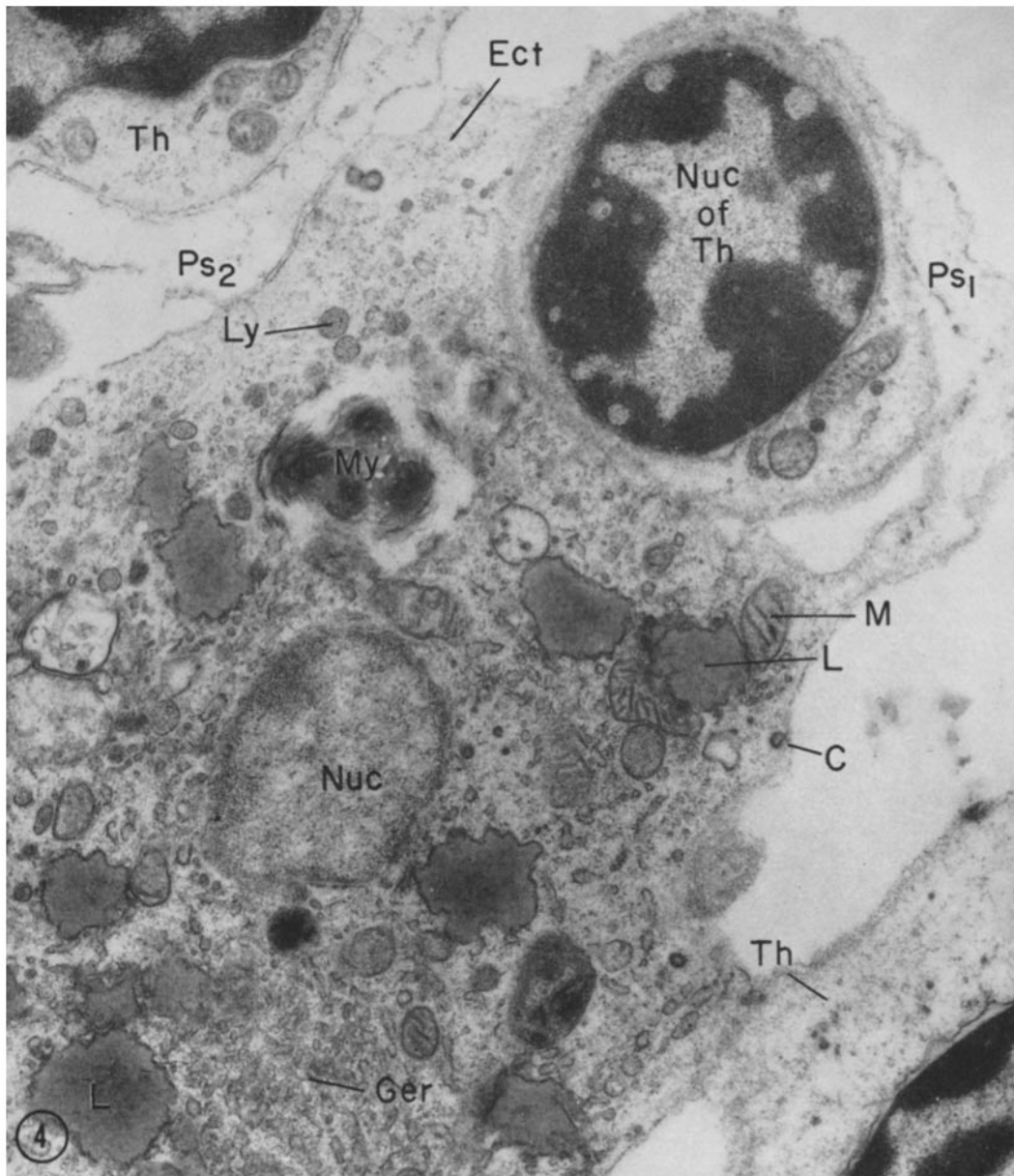
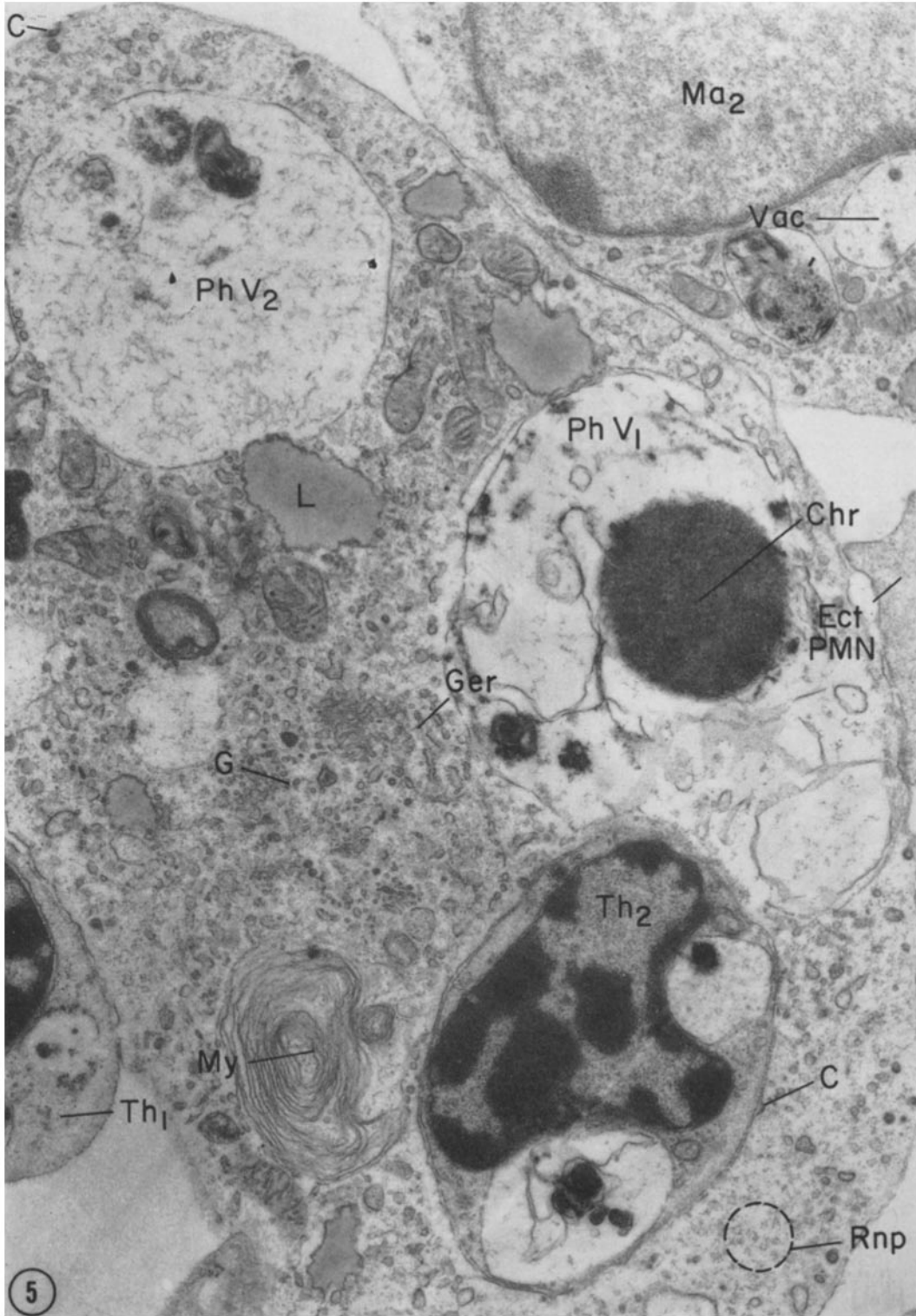


FIGURE 3 This monocyte, cultured for 6 hr, demonstrates several dense, cup-shaped structures (*C*) in continuity with the plasma membrane. Several vesicular structures of similar morphology are located nearby. One of these structures appears to be extruding its contents extracellularly (arrow). Three large vacuoles are present (*Vac*). Rough-surfaced endoplasmic reticulum (*Ger*) and free ribosomes (*Rnp*) are abundant. Cytoplasmic fibrils (*FI*) are present, but sparse.  $\times 19,000$ .



FIGURES 4 and 5 These micrographs depict findings at 24 hr, at which time phagocytosis of thrombocytes is maximal. The phagocyte and thrombocyte make contact with each other by apposition of their plasma membranes (Fig. 5, *Th<sub>1</sub>*). Organelle-free ectoplasm surrounds the thrombocyte (Fig. 4), enclosing it within a membrane-bounded phagocytotic vacuole which is often found in the vicinity of the Golgi apparatus (Fig. 5). In Fig. 5, thrombocytes in several stages of digestion are present. Thrombocyte *Th<sub>2</sub>* lies within a phagocytotic vacuole. A cuplike structure (*C*) continuous with the vacuole membrane likely represents the residual membrane of a lysosome which discharged into the phagocytotic vacuole. Vacuole *PhV<sub>1</sub>* contains a thrombocyte undergoing dissolution. The large, dense, oval mass represents chromatin, whereas the dark granules and membranes are similar to platelet granules (compare with *Th<sub>2</sub>*). Phagocytotic vacuole *PhV<sub>2</sub>* contains mostly unrecognizable bits of a cell in an advanced degree of digestion. A portion of the Golgi apparatus (*G*) is seen in the area enclosed by these phagocytotic vacuoles. The myelin figure in Fig. 4 doubtless represents a portion of a cell undergoing digestion. Juxtaposed macrophages (*Ma<sub>2</sub>* in Fig. 5) show no evidence of fusion.  $\times$  Fig. 4, 18,000; Fig. 5, 16,000.





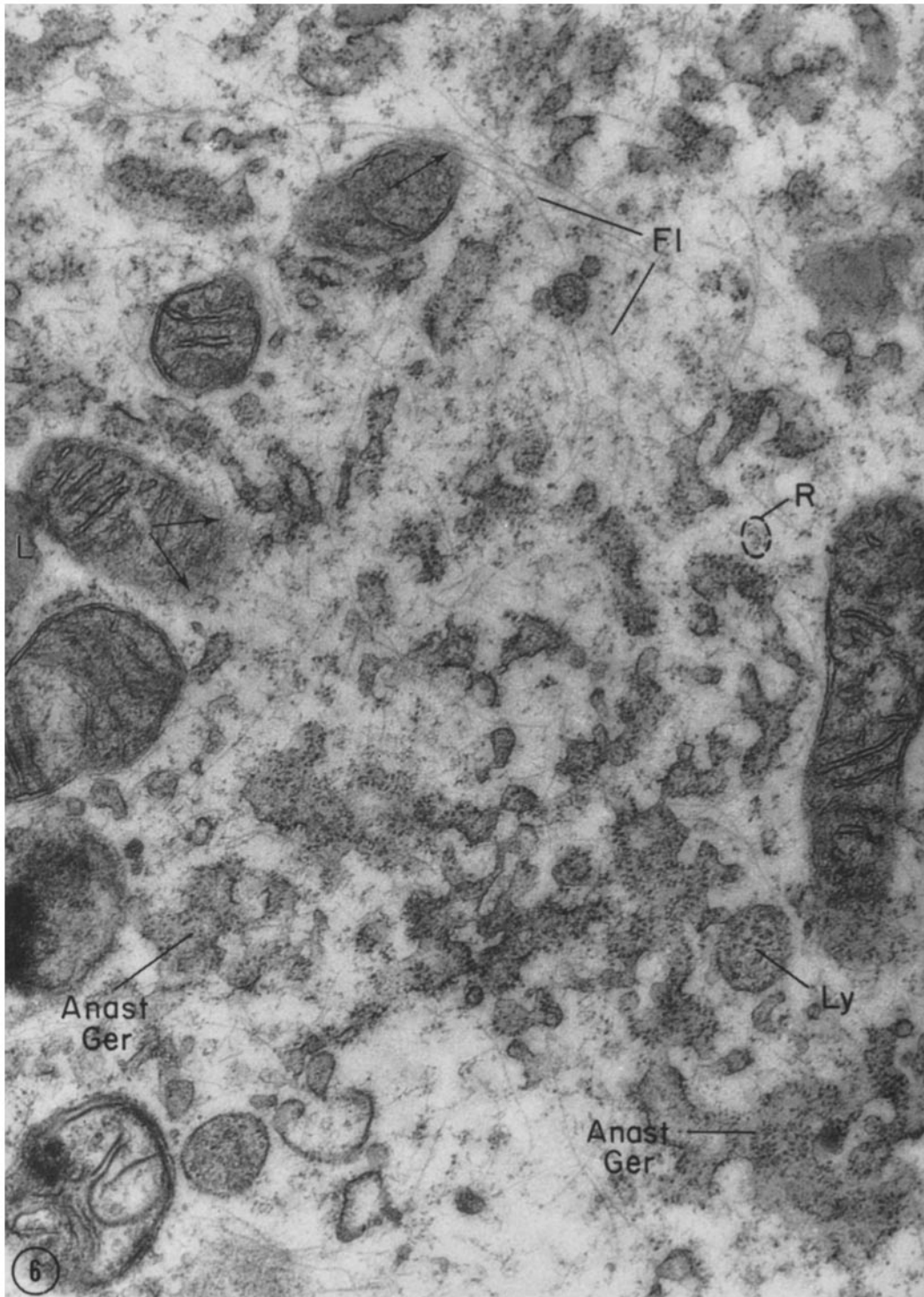


FIGURE 6 Cytoplasmic ground substance shows to advantage in the peripheral portion of a 23 hr macrophage. Bulbous portions of ER (*Anast Ger*) dilated by a granular material of medium density are richly studded with ribosomes. Abundant unattached ribosomes and polysomes are present. Numerous individual filaments (*FI*) run among the cytoplasmic organelles. In two mitochondria sectioned tangentially (arrows within), the outer membrane appears to be either indented or fused with filaments.  $\times 44,500$ .

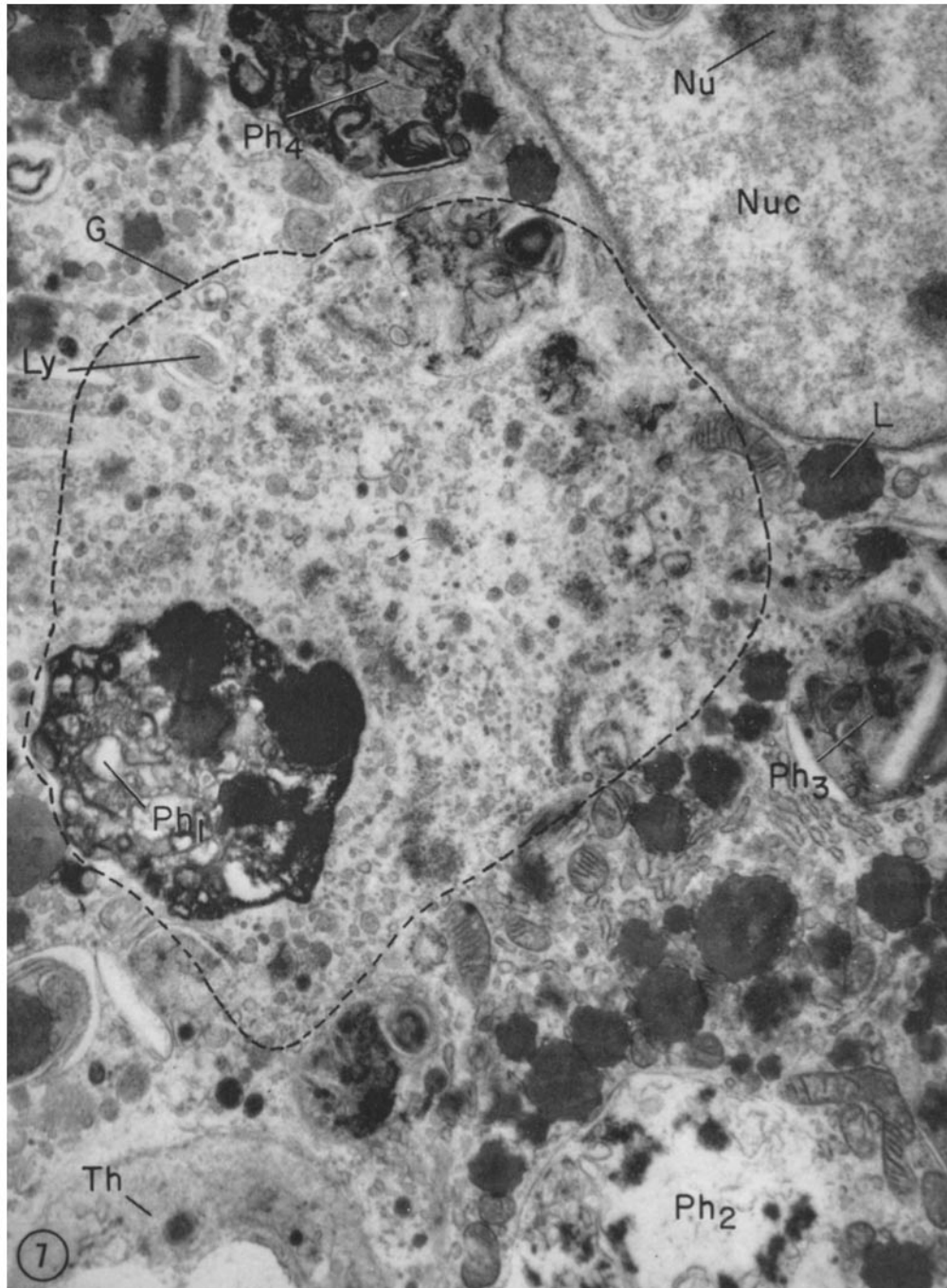


FIGURE 7 This 3-day-old macrophage contains large phagocytotic inclusions ( $Ph_{1-4}$ ), their varied appearance being due to different degrees of digestion. Note the recently phagocytosed thrombocyte ( $Th$ ) and the numerous fat vacuoles ( $L$ ). The cytocentrum ( $G$ , dotted line) occupies the major central portion of the cell. Chromatin is finely dispersed and nucleoli ( $Nu$ ) are prominent. Small lysosomes of medium density are present throughout the Golgi region and cytoplasm. Mitochondria and rough-surfaced ER are abundant.  $\times 22,000$ .

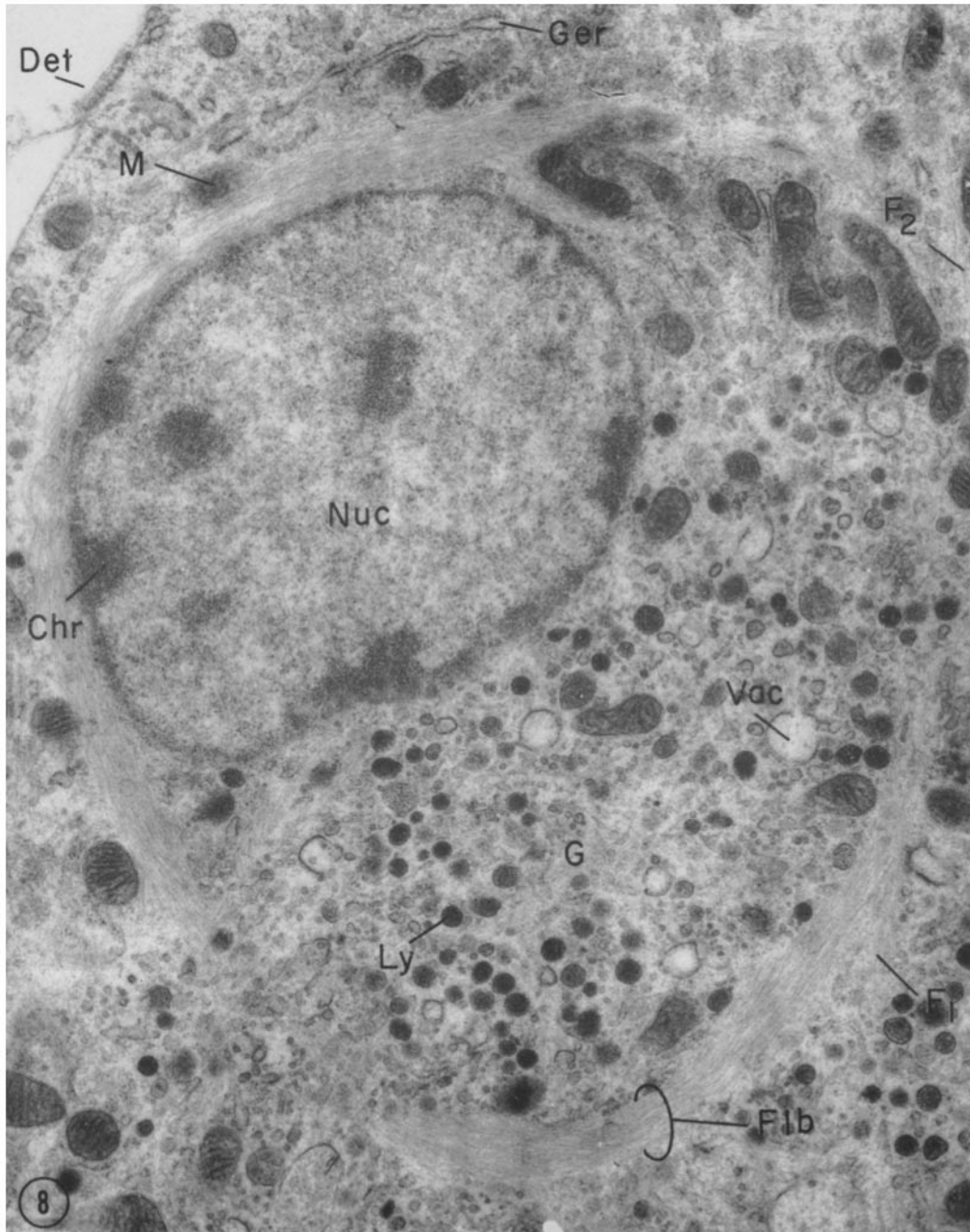


FIGURE 8 A large band of filaments (*Fib*) encircles the nucleus and Golgi zone (*G*) of this 7 day epithelioid cell. Branches of it extend into the cytoplasm (*F<sub>1</sub>* and *F<sub>2</sub>*). Mitochondria and lysosomes are greatly increased in number. A small tag of cytoplasm attached to a large piece of ectoplasm (not shown) appears to have detached along a row of microvesicles (*Det*). Vacuoles (*Vac*) are numerous.  $\times 17,500$ .

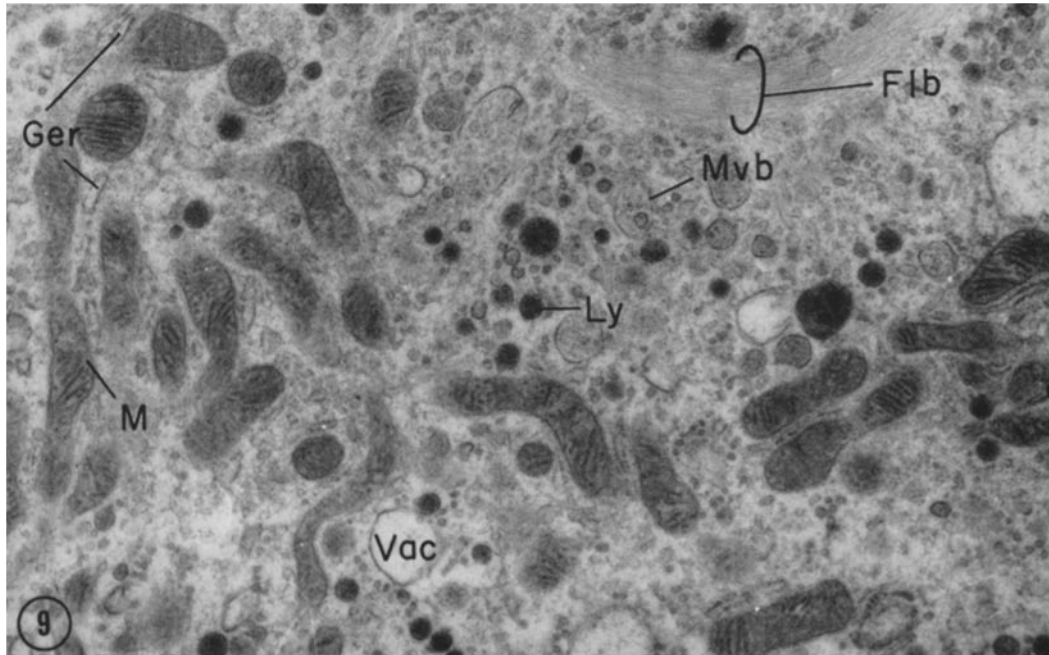


FIGURE 9 This micrograph shows a peripheral area of cytoplasm from the cell illustrated in Fig. 8. Mitochondria are greatly increased in number and length. A profusion of small lysosomes and microvesicles is present.  $\times 17,500$ .

9 and 17). They may exceed  $2.0 \mu$  in length. The outer mitochondrial membrane is thinner than before and is discontinuous in many places (Figs. 9 to 11, and 17). The space between the outer and inner membranes is dilated. The matrix is denser than cytoplasmic ground substance (Figs. 8, 10, and 17).

**ENDOPLASMIC RETICULUM:** Granular ER is present as randomly distributed isolated profiles.

**LYSOSOMES:** A conspicuously large increase in lysosomes occurs (Figs. 8, 12, and 18). Epithelioid cells at this stage exhibit a myriad of multiform membrane-bounded granules which may be classified as follows: (a) homogeneous granules of variable density and diameter, (b) granules of varying diameter and density containing randomly or concentrically arranged membranes, (c) multivesicular-type bodies of variable diameter and density, and (d) large, irregular crystalloid inclusions. Occasionally, compact phagocytotic inclusions which contain recognizable cellular components are present.

The membrane around each granule is distinct (Fig. 12). Indeed, in the majority of forms, its

presence is accentuated by a region of less density immediately subjacent to the membrane.

**CYTOCENTRUM:** The cytocentrum is more voluminous than in previous stages. It contains many granules (Fig. 8).

**FILAMENTS:** Cytoplasmic filaments are massively increased in number (Figs. 8 and 10). Individual filaments measure 50 to 60 A in diameter and are of indeterminate length. They occur singly or in bundles everywhere in the cytoplasm except the cytocentrum. Filaments are almost always seen in longitudinal and tangential section; they are rarely observed in cross-section in sections parallel to the plane in which the cells grow. Lysosomes and mitochondria are often found in the interstices of the larger bundles and between the fine network of fibers (Figs. 8 and 10).

A distinctive annular bundle often encircles the cytocentrum, thus delimiting it from the remaining cytoplasm (Figs. 8 and 10). Filaments within this bundle measure 90 to 100 A in diameter. The nucleus may or may not be included within its circumference. This centrally situated annular bundle often branches peripherally. These

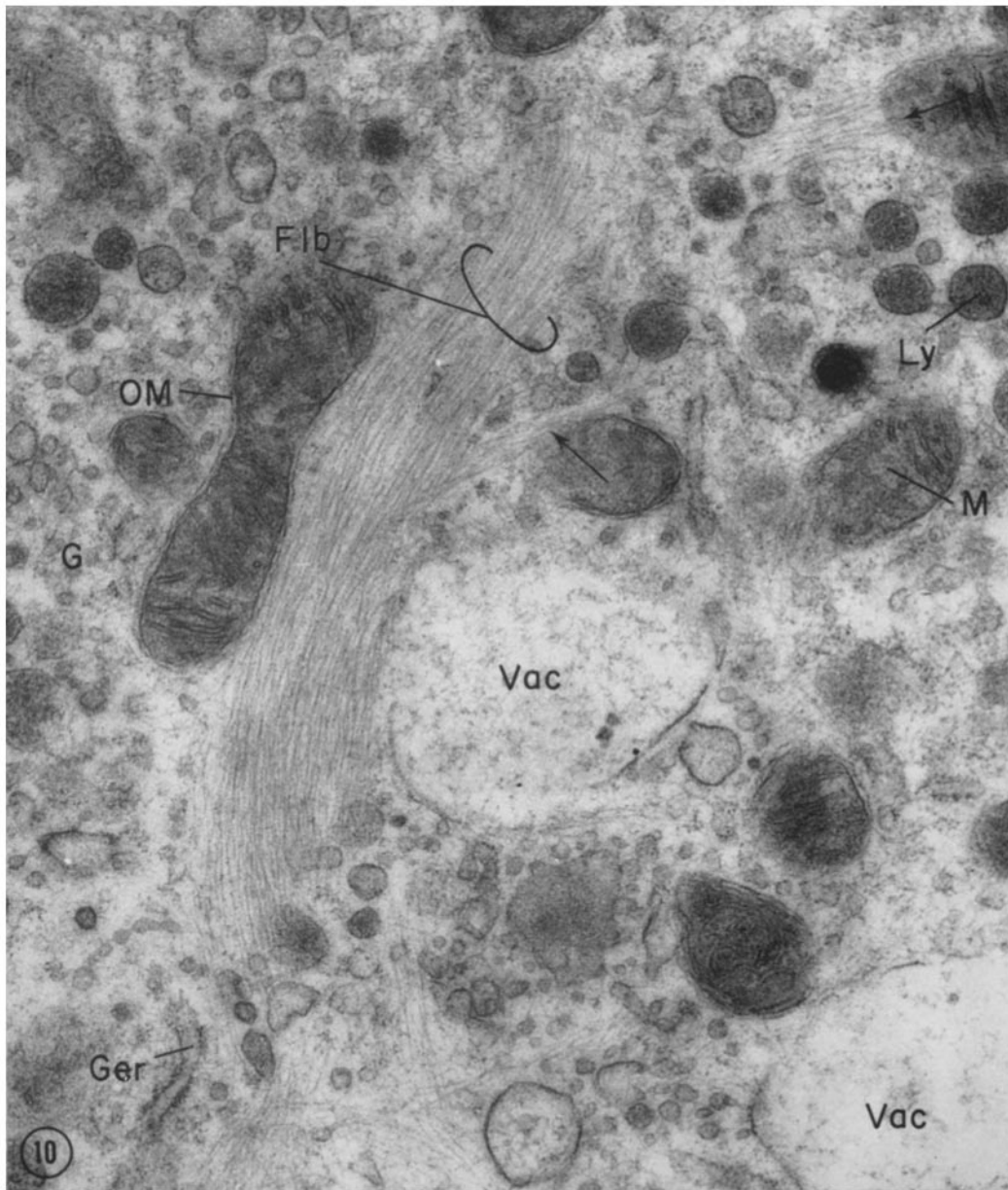


FIGURE 10 A large bundle of filaments separates Golgi zone (*G*) on the left from the endoplasm on the right in this 7 day epithelioid cell. At bottom center, filaments fan out in several directions. Filament appear to be attached to the mitochondrion at the upper right corner (arrow). The large vacuoles (*Vac*) contain the residuum of digested phagocytosed material.  $\times 43,500$ .

branches sweep around among cytoplasmic organelles. They often indent and deform the lysosomal and outer mitochondrial membranes (Figs. 8, 10, and 17).

PHAGOCYTOTIC INCLUSIONS: Terminal stages of phagocytotic inclusions appear to be represented by large clear vacuoles (Figs. 8 and 16). Fresh phagocytotic inclusions are rare.

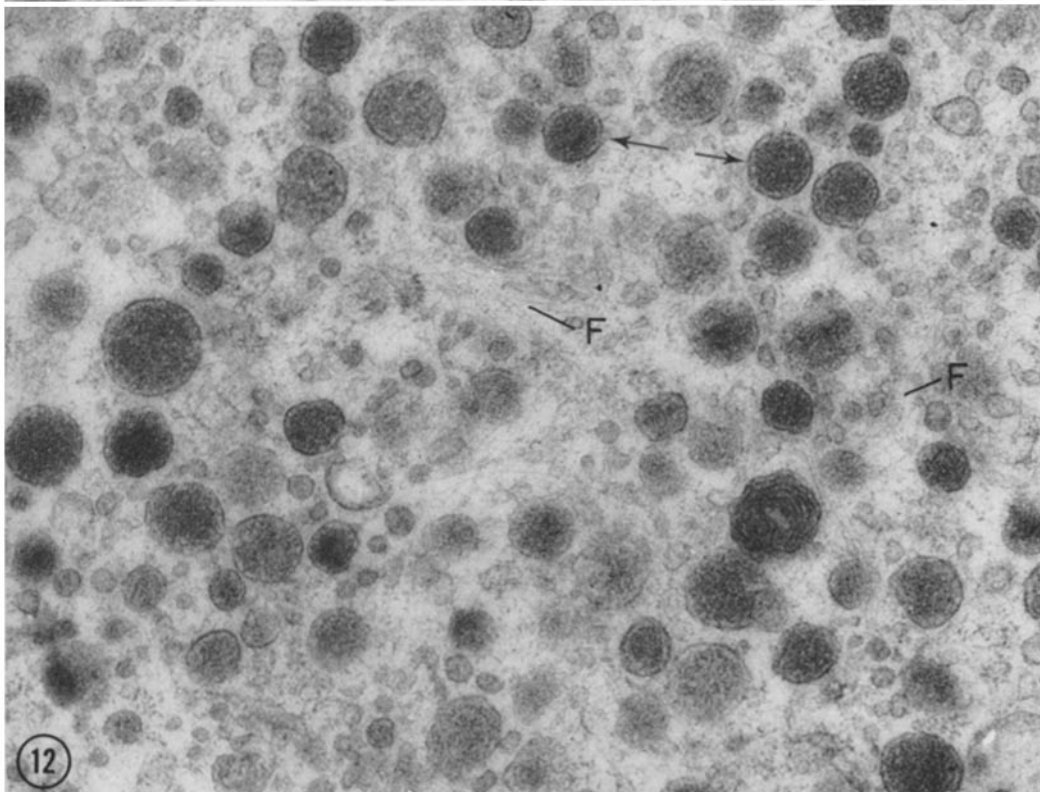
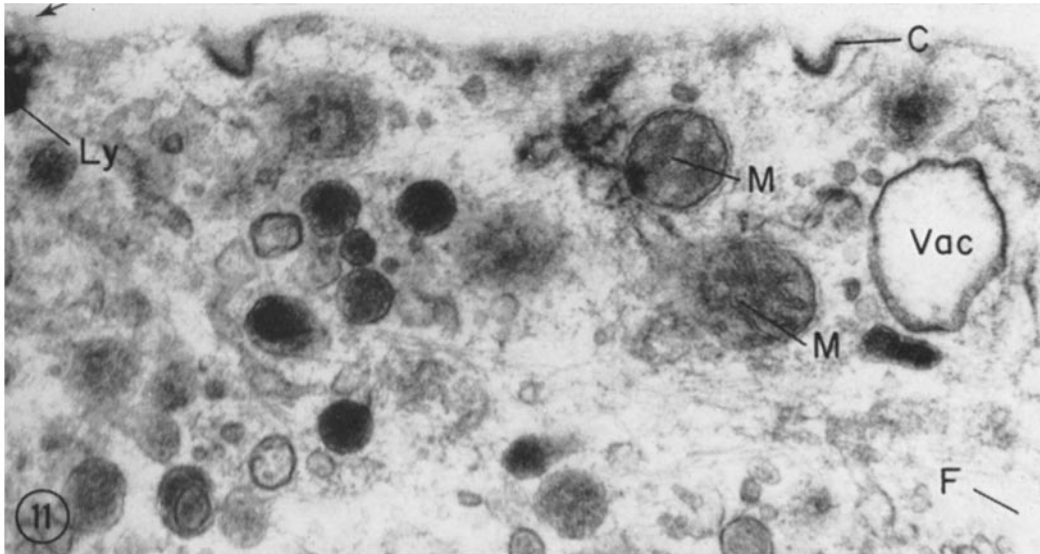


FIGURE 11 The plasma membrane of this 7 day epithelioid cell contains two dense cuplike structures (C). Their concave portions have a density similar to that of nearby lysosomes (Ly). The lysosome at the far left (arrow) is continuous with an invagination of the cell membrane. The vesicle is partially emptied of its contents (clear area in granule near stoma).  $\times 39,000$ .

FIGURE 12 This field of lysosomes is in the Golgi region of a 7 day epithelioid cell. In general, the smaller membrane-bounded vesicles have the least and the larger ones the greatest density. In the majority of lysosomes, an area of less density subjacent to the membrane creates a halo around the granule (arrows).  $\times 40,500$ .

A remarkable structure occurs that consists of a central vacuole, vesicle, or dense granule surrounded by multiple concentric rows of tiny vesicles containing material identical or nearly identical to the central structure. The surrounding vesicles are identical to the clusters of small vesicles in the Golgi region (Fig. 13).

#### *Multinucleated Giant Cells, 15 days (Figs. 18 to 22)*

Giant cells in general consist of large circular-to-oval masses of cytoplasm containing up to 100 or 150 or more nuclei and large clear cytoplasmic vacuoles exhibiting Brownian movement by vital microscopy.

**NUCLEUS:** Each nucleus contains a very thin rim of dense marginal chromatin with focal aggregations and one to three distinct nucleoli (Figs. 18 to 20). The remaining karyoplasm is finely granular and evenly dispersed. Most nuclei in giant cells are round or oval, but others show folds and deep indentations. Nuclear pores are numerous. As in earlier stages, the pores appear open and directly continuous with perinuclear cytoplasm.

**CYTOPLASM:** The cytoplasm is crowded with organelles. Where the cell is free of neighboring cells, a zone of organelle-free ectoplasm, bearing characteristic short, wavy pseudopodia, is present (Fig. 20). An interlocking of long, filiform extensions of cytoplasm between giant cells and neighboring cells frequently occurs (Fig. 15). Groups of cells anchored to one another in such manner would be mistaken, by light microscopy, for syncytial masses of cytoplasm.

**MITOCHONDRIA:** Mitochondria are abundant. In small giant cells, most are short, slender rods. Filamentous forms are present, but not numerous. Annular, angulated, and branched forms are rare. The outer membrane is intact, in contrast to the membrane of 7-day epithelioid cells. The matrix may be similar in density to hyaloplasm, but in many mitochondria it is so dense

that cristae are obscured and granules are not evident.

**ENDOPLASMIC RETICULUM AND RIBOSOMES:** Granular ER is present, throughout the cytoplasm, as flattened channels of variable length.

Smooth ER is represented by a myriad of small vesicles and vacuoles throughout the cell. Small and large vesicles are abundant adjacent to the plasma membrane. Free ribosomes are plentiful.

**LYSOSOMES:** A large number of lysosomes is present, the smaller giant cells having the greatest number (Figs. 18 to 20). Fewer dense concavities in the plasma membrane are present than in earlier stages.

**CYTOCENTRUM:** Cytocentra appear at various locations in the cytoplasm and contain one or two centrioles (Fig. 20). The Golgi interna is packed with variably sized vesicles, canaliculi, and granules.

**FILAMENTS:** Cytoplasmic filaments are few in number, reminiscent of the earliest period of culture, and are usually situated in filiform ectoplasmic extensions. In bi- and trinucleated cells, filaments may surround the Golgi element and nucleus.

#### *Multinucleated Giant Cells, 26 days*

The general features at 26 days are similar to those at 15 days. The major changes are as follows:

**MITOCHONDRIA:** Mitochondria show a slight increase in number, and polymorphism is more marked. They are round, oval, filamentous, branched, angulated, and annular. The matrix is extremely dense, almost obscuring the cristae.

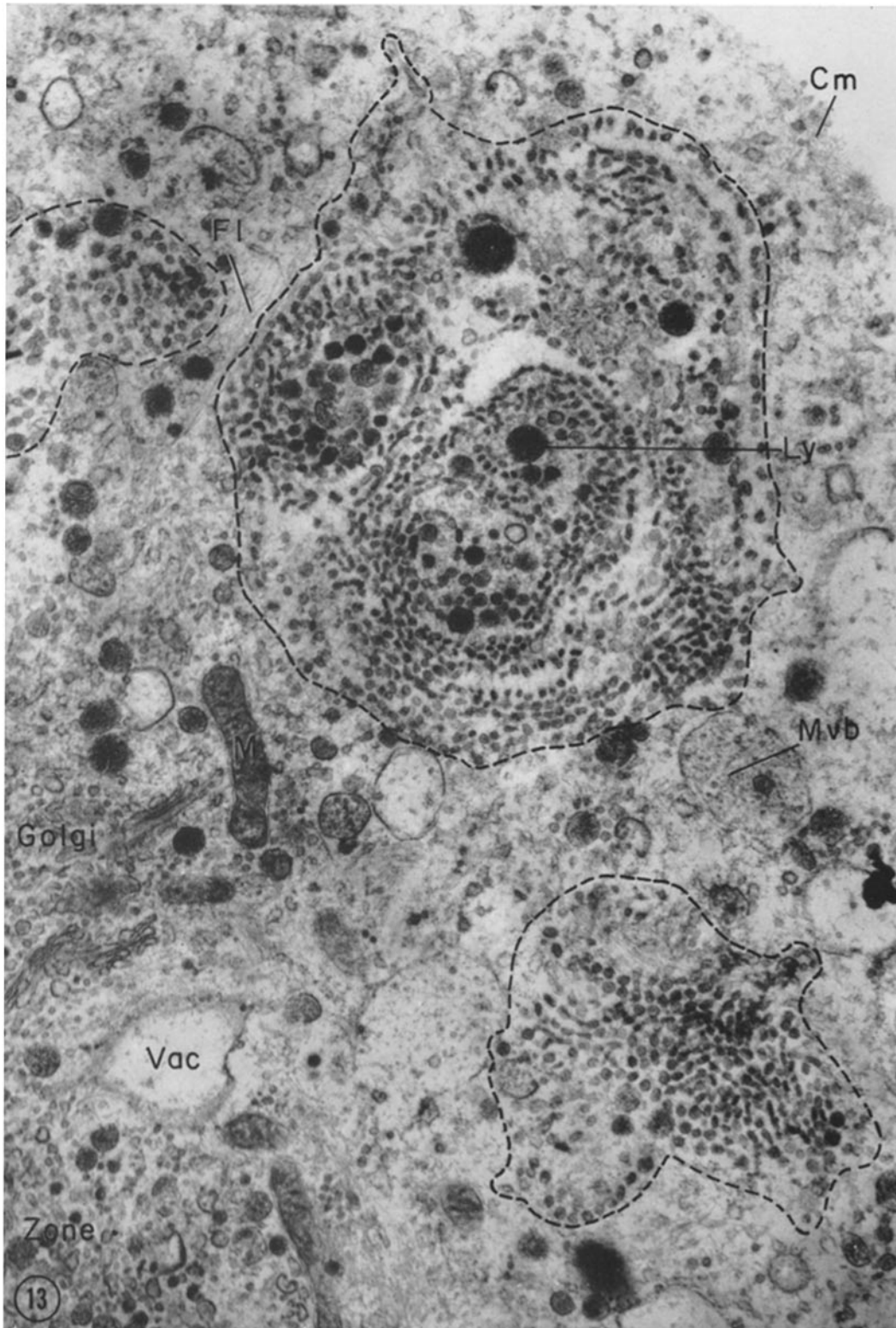
**LYSOSOMES:** Lysosomes are greatly reduced in number and variety. The majority are small, dense, homogeneous granules.

**VESICLES AND VACUOLES:** Micropinocytotic vesicles are increased in number. They are rectilinearly arranged in rows at the base of and within pseudopodial extensions of ectoplasm.

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**FIGURE 13** A remarkable structure (dotted lines) is located in the peripheral cytoplasm of a 7 day epithelioid cell. Plasma membrane (*Cm*) is at the extreme right upper corner. Flattened, elongated sacs and vesicles of the nearby Golgi apparatus are present along the entire left side of the figure. The large dense central granules (*Ly*) are lysosomes. The contents of the small vesicles disposed in concentric rows are similar in density to the central granules.  $\times 25,000$ .





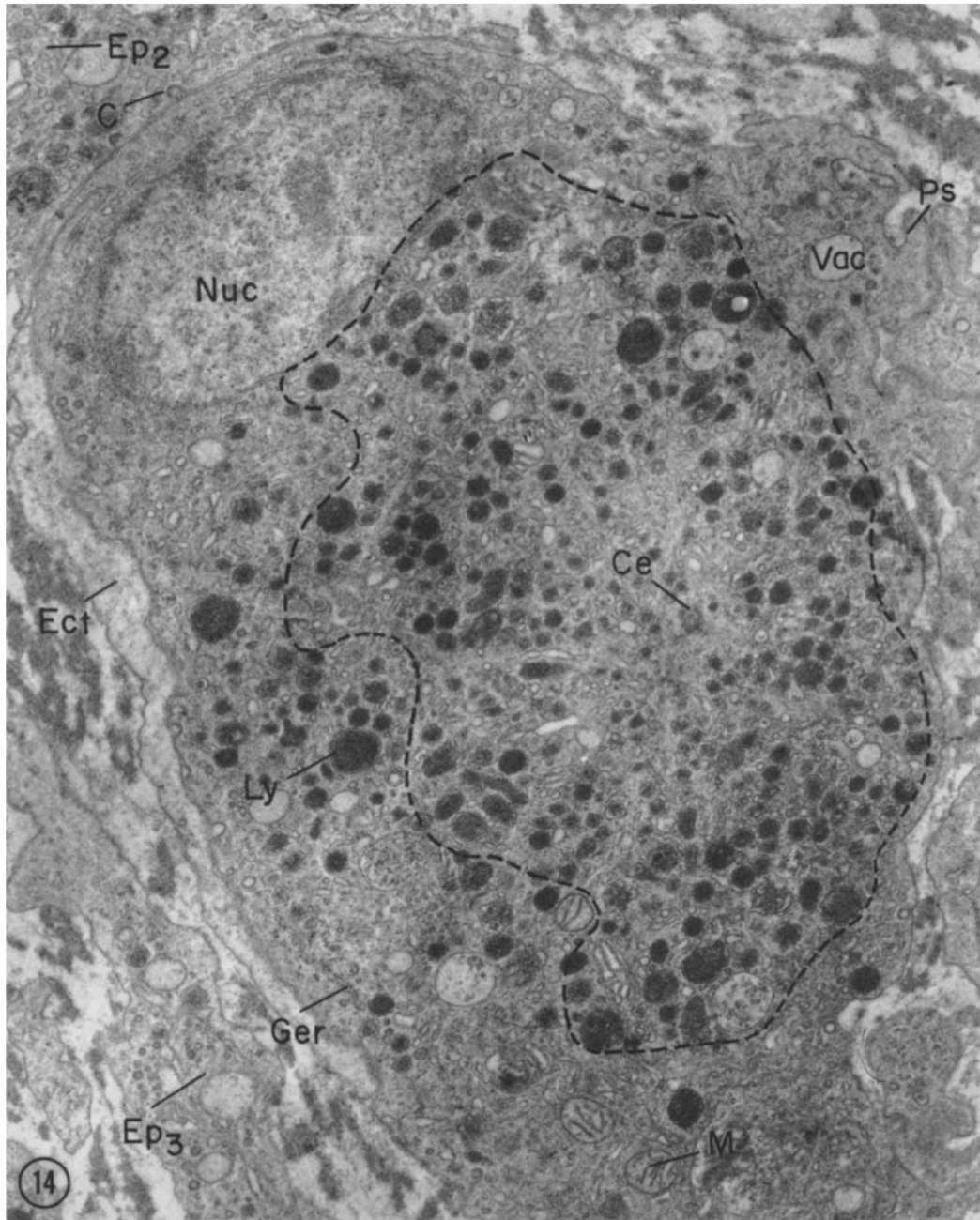


FIGURE 14 In this 15 day epithelioid cell, the cytocentromerous region is conspicuous, owing to the radial arrangement of lysosomes (in the area outlined by dotted line). The smaller lysosomes are centrally located and the larger ones are more peripheral. A centriole (*Ce*) is present. Abundant rough-surfaced ER (*Ger*) and free ribosomes are scattered. Several cuplike structures are present in the plasma membrane (*C*). A zone of ectoplasm from adjacent cell (*Ect*) touches the cell along its left border. The dark condensed material at the upper right corner is fibrin.  $\times 15,500$ .

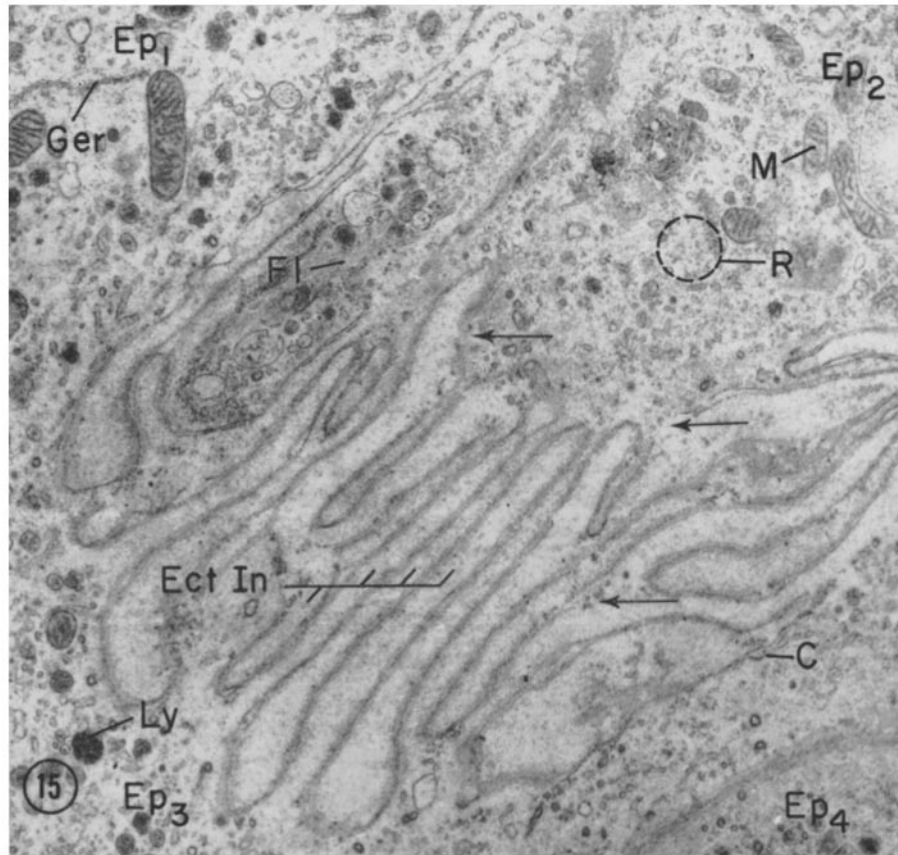


FIGURE 15 This field shows the boundary of a cluster of 4 epithelioid cells ( $Ep_{1-4}$ ) from a 15 day culture. Extensive ectoplasmic interdigitation between adjacent epithelioid cells ( $Ep_2$  and  $Ep_3$ ) is present. The interdigitated ectoplasm lacks organelles. Arrows indicate areas where membranes appear discontinuous, thus establishing cytoplasmic continuity between cells. A small bundle of cytoplasmic filaments ( $FI$ ) is present in cell  $Ep_2$ . The cytoplasm of  $Ep_2$  is rich in polyribosomes ( $R$ ).  $\times 15,000$ .

Endoplasmic vacuoles are greatly reduced in number and size.

**FILAMENTS:** Increased numbers of filaments are present. They are found mainly adjacent to and touching mitochondria. Many small vacuoles are encircled by filaments, and many small bundles course freely in the endoplasm between organelles. They are rare in the ectoplasm.

*Multinucleated Giant Cells, 70 days (Figs. 21 and 22)*

With age, multinucleated giant cells contain huge cytoplasmic vacuoles, many of which bulge from the cell surface as bleblike protuberances (Fig. 21).

**NUCLEUS:** The karyoplasm is homogeneously

finely granular and of low density, usually bearing a single large nucleolus (Fig. 21).

**CYTOPLASM:** A thin band of ectoplasm bearing filamentous pseudopodia encircles the giant cell (Fig. 21).

**MITOCHONDRIA:** Mitochondria are profusely abundant throughout the cytoplasm, reaching their highest concentration at this stage (Figs. 21 and 22). The matrix is similar in density to that of mitochondria at 26 days of culture. Every form observed previously is present, with the long filamentous and branched forms predominating. Outer mitochondrial membranes are intact and complete. Many are touched by filaments.

**ENDOPLASMIC RETICULUM:** ER is predominantly smooth. Short, isolated channels of

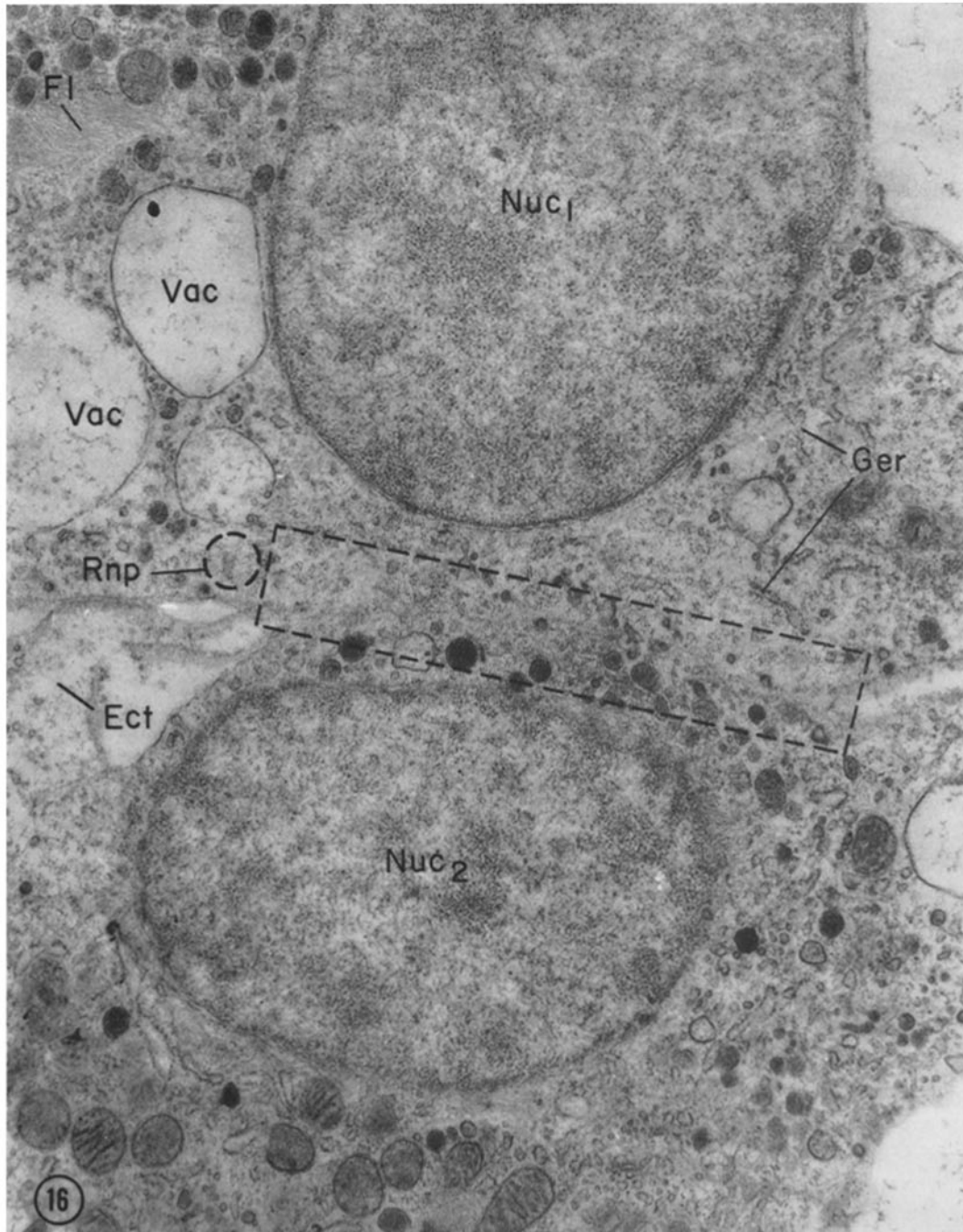


FIGURE 16 These contiguous epithelioid cells from a 7-day-old culture are fusing in the region between their nuclei (dotted line box). A faint, ill defined region, where membranes of the two cells appose each other, is detectable along the left half of the line of fusion. Continuity, evidenced by intermingling of cytoplasmic organelles, exists along the right half. The high concentrations of lysosomes, rough-surfaced ER, and ribosomes in and near the region of fusion are characteristic. Large vacuoles containing fragments of debris (*Vac*), large numbers of lysosomes, and mitochondria are present in both cells. Such a pair of cells would appear as a binucleated cell by light microscopy.  $\times 13,000$ .

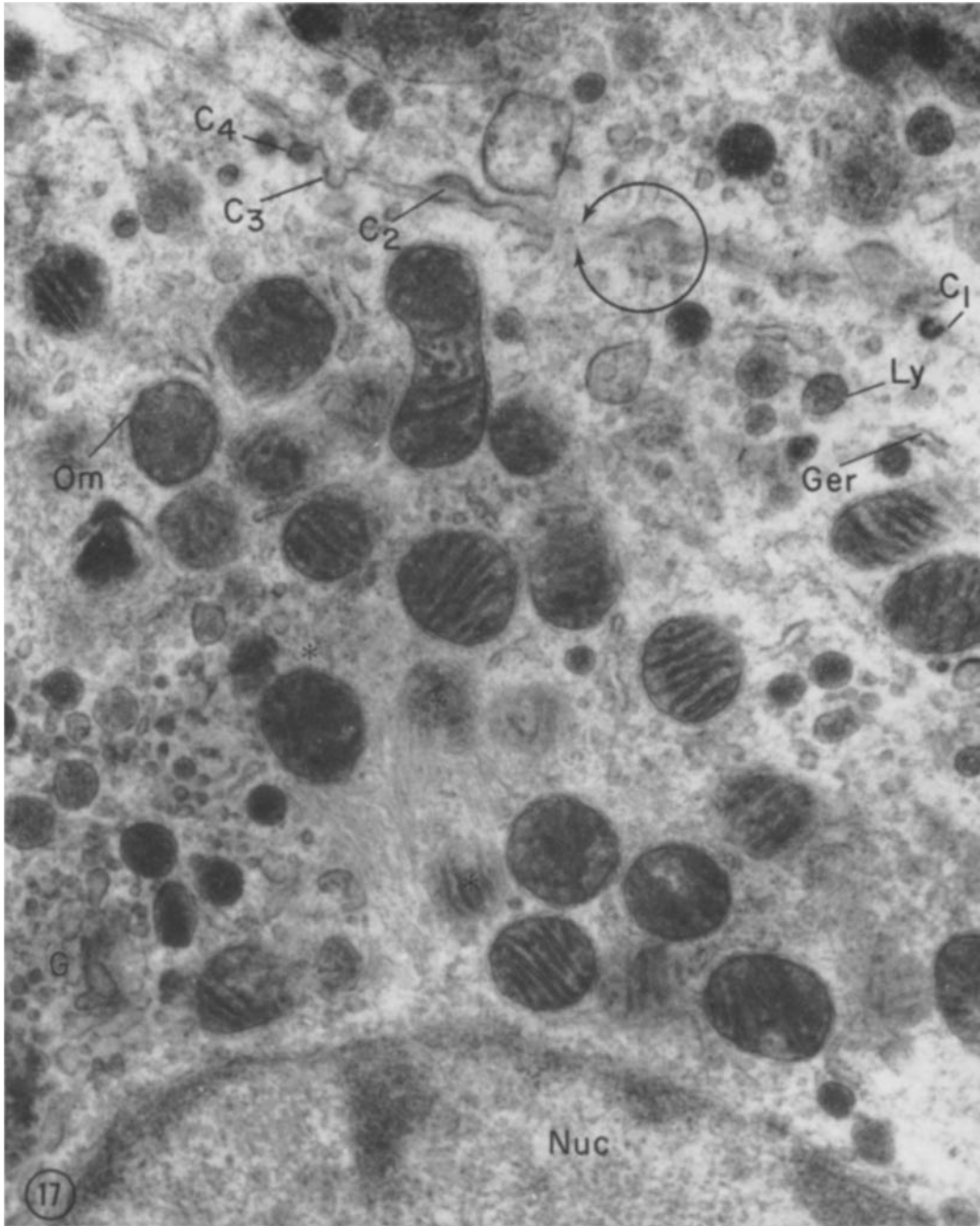


FIGURE 17 This micrograph illustrates at high magnification a region of fusion between two 7 day epithelioid cells. A bridge of cytoplasmic continuity has been established (between double-headed circular arrow). Cuplike structures are present in both cell membranes on each side of the fused area ( $C_{1-4}$ ). In each cell, numerous lysosomes are present near the region of fusion. Mitochondria are present in large number, the majority having unusually thin attenuated outer membranes ( $Om$ ). Mitochondria identified by asterisks appear to have attached filaments.  $\times 31,000$ .

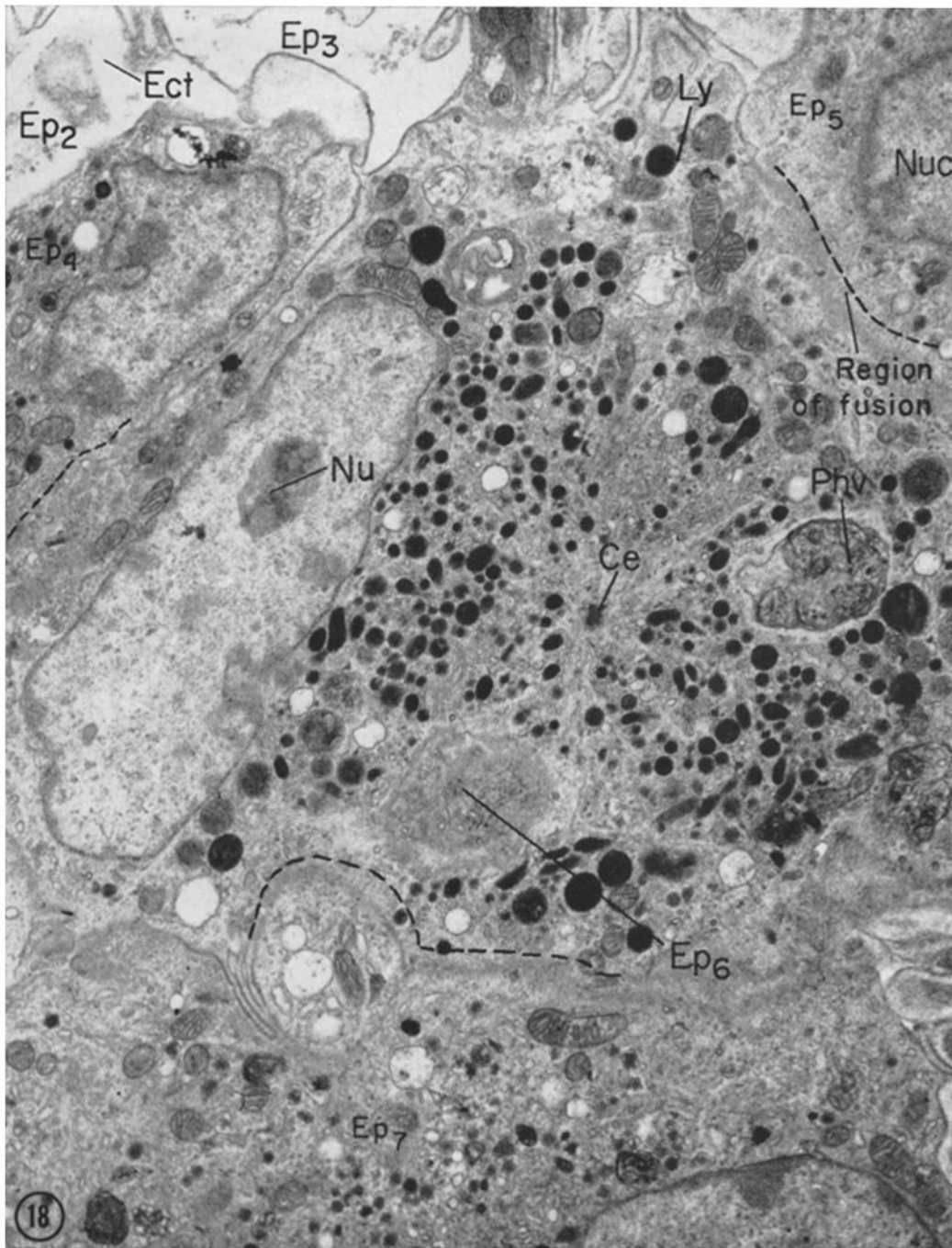


FIGURE 18 A "young" multinucleated giant cell with a characteristic abundance of lysosomes is illustrated in this electron micrograph of a 15 day epithelioid culture. The centrally situated cell (*Ep<sub>6</sub>*) appears to be fusing with neighboring cells (*Ep<sub>4</sub>*, *Ep<sub>5</sub>* and *Ep<sub>7</sub>*) at the dotted lines. Clear areas at the upper left corner are ectoplasm of adjacent cells (*Ect*).  $\times 10,100$ .

granular ER are distributed in the cytoplasm. Free ribosomes are not numerous.

**LYSOSOMES:** Lysosomes are drastically reduced in number. They are *rare* at this stage.

**VESICLES:** The major portion of cytoplasm is occupied by a myriad of uniform-sized microvesicles (Figs. 21 and 22). This pattern is broken only by focal areas of Golgi vesicles and an occasional large vacuole. Numerous electron-opaque fat vacuoles are present.

**CYTOCENTRUM:** The cytozentrum is represented by small multicentric aggregations of characteristic sacs and vesicles (Fig. 21). A peculiar horseshoe-shaped, Golgi complex-like formation found at this stage is represented in Fig. 22.

**FILAMENTS:** Filaments are abundant and reach their greatest concentration in giant cells at this stage (Fig. 21). The close relationship to mitochondria is again observed. Many small, loosely arranged bundles consisting of ten to twenty filaments touch, and, in many instances, appear directly continuous with the outer membrane of mitochondria. Densely packed bundles and sheets of filaments course throughout the cytoplasm and separate the cytoplasmic constituents.

## DISCUSSION

### *Monocytes and Lymphocytes*

Transformation of blood monocytes into macrophages, epithelioid cells, and giant cells occurs in cultures of mixed blood leukocytes (1-3). The lymphocyte has been considered capable of undergoing such transformation. But lymphocytes isolated from the blood and only moderately contaminated with RBC, do not become macrophages in tissue culture whereas monocytes do (15). Labeled lymphocytes have been followed by radioautography with no evidence of transformation into macrophages (16). Small hemal lymphocytes are immunologically competent cells (27), and so their capacity to differentiate into macrophages may be restricted. Monocytes contain the organelles required for protein synthesis (ribosomes, nucleoli, nuclear pores) and those required for bounding the product in membrane (Golgi elements, ER). These cells are cytologically suited to the transformation to macrophages.

Lewis and Lewis (1) showed that the transformation of blood monocytes *in vitro* is characterized by an exuberant development of neutral

red-positive granules in the perinuclear area, often arranged in a rosettelike pattern. We take these granules to be lysosomes. Cohn and Weiner (5) recovered 70% of neutral red, taken up by alveolar macrophages, in the particulate hydrolase fraction, which was composed of stained granules resembling those of the intact cell. Such granules in circulating monocytes are small, measuring up to 0.2  $\mu$  in diameter, and therefore below the limits of resolution of the light microscope. Weiss and Fawcett (4) demonstrated these granules to be the site of acid phosphatase activity. They found monocytes of the blood devoid of stainable acid phosphatase, whereas macrophages and epithelioid cells were strongly positive. Among the most significant features of this transformation are the appearance, in large quantity, of acid phosphatase, an enzyme found in lysosomes, and evidence of intense protein synthesis.

It is a matter of interest that granules in polymorphonuclear leukocytes do not come closer than 40 A to the cell membrane, separation being maintained by a narrow band of ectoplasm (24), while, in monocytes, lysosomes reach and touch the cell membrane. The characteristic dense, cuplike structures in the cell membrane likely represent foci of lysosomal discharge. Appearing after 6 hr of culture, they are present in every subsequent stage. Their appearance coincides with increasing amounts of acid phosphatase in the culture medium. Lysosomes of density similar to that of the residual contents of these cuplike structures are abundant near the plasma membrane and may touch it. These granules appear unrelated to pinocytosis, since microvesicles are rare at this juncture. Release of lysosomes extracellularly by monocytes and cells arising from them may initiate or hasten degradation of material in preparation for phagocytosis. The process may well affect the state of gelation or solvation of extracellular connective tissue. Extracellular discharge of lysosomes appears to represent a normal merocrine function of these cells. It is interesting that cell fusion almost always occurs in the neighborhood of discharging lysosomes. Perhaps the activity of certain hydrolytic enzymes is required for the membrane changes associated with cell fusion. While extracellular granule discharge may not occur in polymorphonuclear leukocytes, it appears to be a normal function of mononuclear phagocytes.

Acid phosphatase-positive granules (lysosomes)

disappear from alveolar macrophages following phagocytosis of yeast cell walls, and acid phosphatase activity accumulates about the ingested product in the phagocytotic vacuole (17). Furthermore, on phagocytosis of yeast cell walls by macrophages, neutral red-staining granules disappear from the cytoplasm, and the dye accumulates within phagocytotic vacuoles. Similarly, in our cultures, lysis of macrophage granules occurs following phagocytosis, and a portion of the granule contents becomes incorporated into the newly formed phagocytotic vacuoles. The most likely explanation for the absence of lysosomes in 70-day-old giant cells is incorporation into phagocytotic vacuoles and discharge into the external environment.

Following phagocytosis, cytological changes indicate intensive protein synthesis. Large nucleoli develop, chromatin becomes widely dispersed, nuclear pores are more frequent, the Golgi apparatus increases in size and complexity, polyribosomes increase in amount, and dilated cisternae of rough-surfaced endoplasmic reticulum containing electron-opaque material are present throughout the cytoplasm.

Lysosomes are undoubtedly the major synthetic product of the cell. Their production proceeds at such a pace that the cytoplasm of epithelioid cells is packed with lysosomes. The configuration of the Golgi apparatus at this stage vividly suggests that lysosomes are formed in a manner similar to that described for the formation of zymogen granules in the pancreatic acinar cell (18).

Cytoplasmic filaments occurring in monocytes in tissue culture appear identical to those described by de Petris et al. (19) and Tanaka (20). Similar filaments are found in a variety of normal and leukemic white blood cells (references 19-21; see Fig. 1 in latter reference), as well as in a variety of pathologically altered cells in diseases of the hematopoietic and reticuloendothelial systems (20).

Additionally, cytoplasmic filaments have been observed in fibroblasts in healing wounds (22), as well as fibroblasts in tissue culture. Such filaments are present after different fixatives (19-21), different embedments (8, 19, 20), and are revealed by a variety of stains (19, 20). We have observed only isolated filaments or bundles of three to four filaments in the avian circulating monocytes; we do not find abundant filaments as reported by de Petris et al. (19) for human and guinea pig monocytes. In our material, the filaments are abundant in macrophages, but attain greatest concentration in epithelioid and multinucleated giant cells. Structural differences between centrally and peripherally located cytoplasmic filaments in epithelioid cells are not noted. Differences of diameter might reflect different physical states in similar filaments or the existence of two different species of filaments.

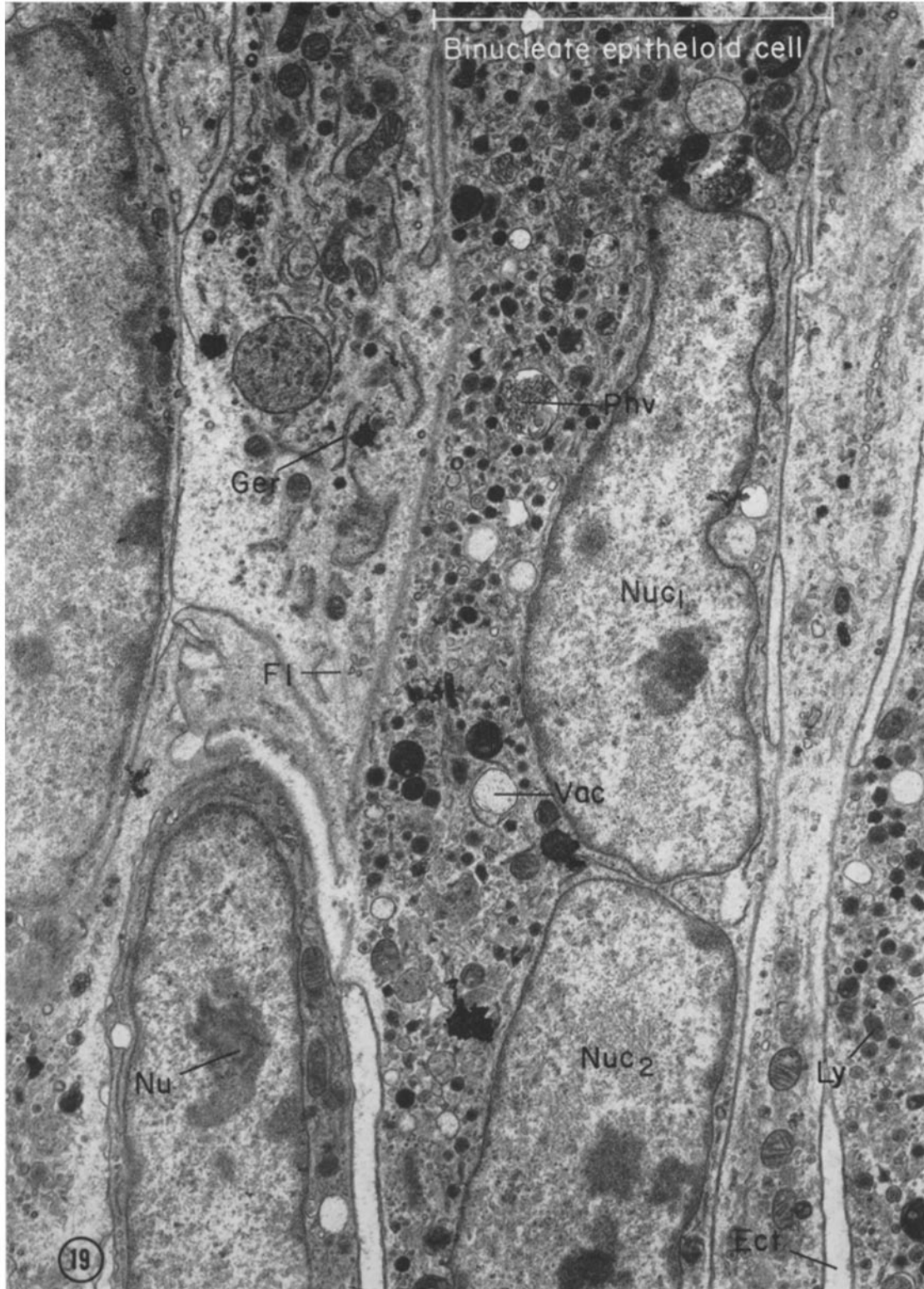
Filaments display a consistent topographical relationship to mitochondria. They appear to originate or insert upon the outer mitochondrial membrane in epithelioid cells. They do not reach the mitochondrial matrix.

Filament formation around phagocytotic vacuoles in 3-day-old macrophages is consistent with the hypothesis of de Petris et al. (19) that filaments are contractile in nature, and are in some way related to the dynamic activity of the cell in the transport of phagocytosed inclusions to definite cytoplasmic regions. In addition to intracellular transport of phagocytotic inclusions, they may also play a "skeletal" role in supporting the membrane around the inclusion and, in fact, in maintaining the configuration of the cell and protecting it, or certain of its parts, against the distortions that phagocytosis or motility may induce. Filaments may serve a transport role in the cell, moreover, providing surface for the movement of colloids. A similar role has been postulated for collagenous fibers in the extracellular connective

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FIGURE 19 This field demonstrates the characteristics of several cells from a 15 day "fibroblastic" culture. A binucleate cell at the right center contains abundant lysosomes, many of which are within Golgi regions situated at the left of each nucleus. Nuclei have finely dispersed chromatin and prominent nucleoli. Thin elongate oval regions lacking organelles represent ectoplasm interposed between cells (*Ect*). Peripheral areas of endoplasm of adjacent cells show abundant mitochondria, channels of rough ER, polysomes, and filaments.  $\times 11,500$ .





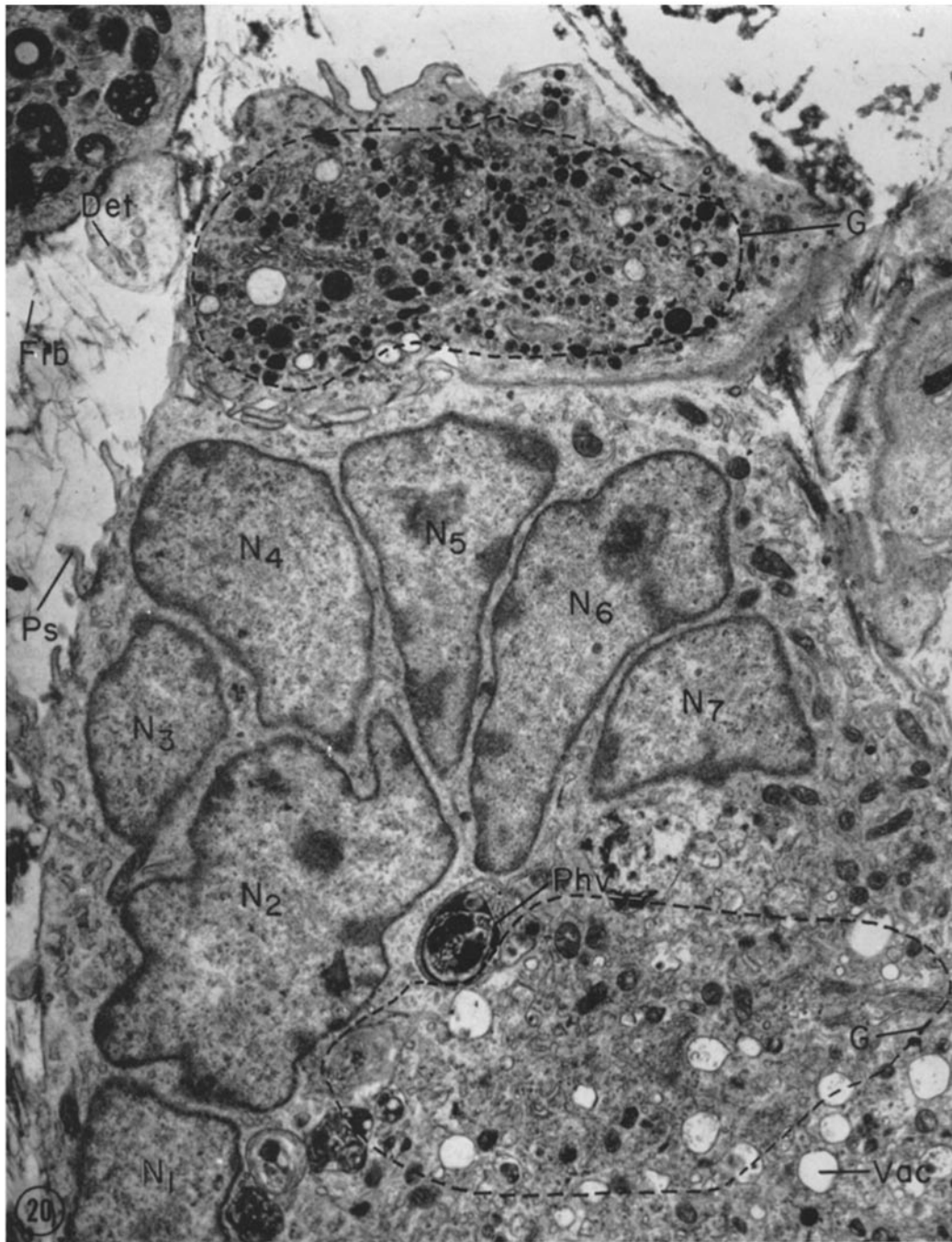


FIGURE 20 This portion of a small, 15 day multinucleated giant cell shows several nuclei ( $Nuc_{1-7}$ ) in lock-and-key relationship. Several short wavy pseudopodia ( $Ps$ ) extend from the left border of the cell, a characteristic feature of giant cells in culture. Below the nuclei are scattered aggregations of Golgi vesicles ( $G$ , dotted line). Several small phagocytotic vacuoles are present ( $PhV$ ). Medium-sized vacuoles of low electron opacity ( $Vac$ ) scattered at the lower right likely represent empty lysosomes. The cytoplasm abutting the apical portion of this giant cell belongs to an epithelioid cell sectioned through its Golgi region ( $G$ ). A distinct boundary of ectoplasm in each cell indicates that fusion of the two cells is not occurring. Along the left half of the apposed membranes, the cells attach to each other by interlocking pseudopodia. Fibrillar extracellular material resembles fibrin ( $Fib$ ). A small bleb of ectoplasm in an attitude of separating from the cell at the upper left corner is labeled  $Det$ .  $\times 8500$ .

tissue (23). Extension into pseudopodia suggests that filaments affect the behavior of the undulating membrane.

The multinucleated giant cell is an extraordinary structure. The salient and unusual features of this cell are the massive concentration of mitochondria, plentiful, folded cell membrane, large vacuoles, and absence of lysosomes. The concentration of mitochondria and plasma membrane suggests that the cell may have active transport as its primary role. It thus must not be regarded simply as a larger cell than those which fused to form it. It is different, as indicated by change in organelle content. This cell may be in a class with the osteoclast and, by movement of calcium in tissues, may play a role in ectopic calcification, as in tuberculosis.

Thus, as monocytes undergo transformation

into macrophages, epithelioid cells, and giant cells, they undergo not only changes remarkable in structure, but changes just as remarkable in function. The function of the monocyte appears to be the synthesis and storage of lysosomes. The macrophage and epithelioid cell function as phagocytes capable of digesting and disposing of their intake. The multinucleated giant cell may engage in active transport in addition to sequestering foreign particles.

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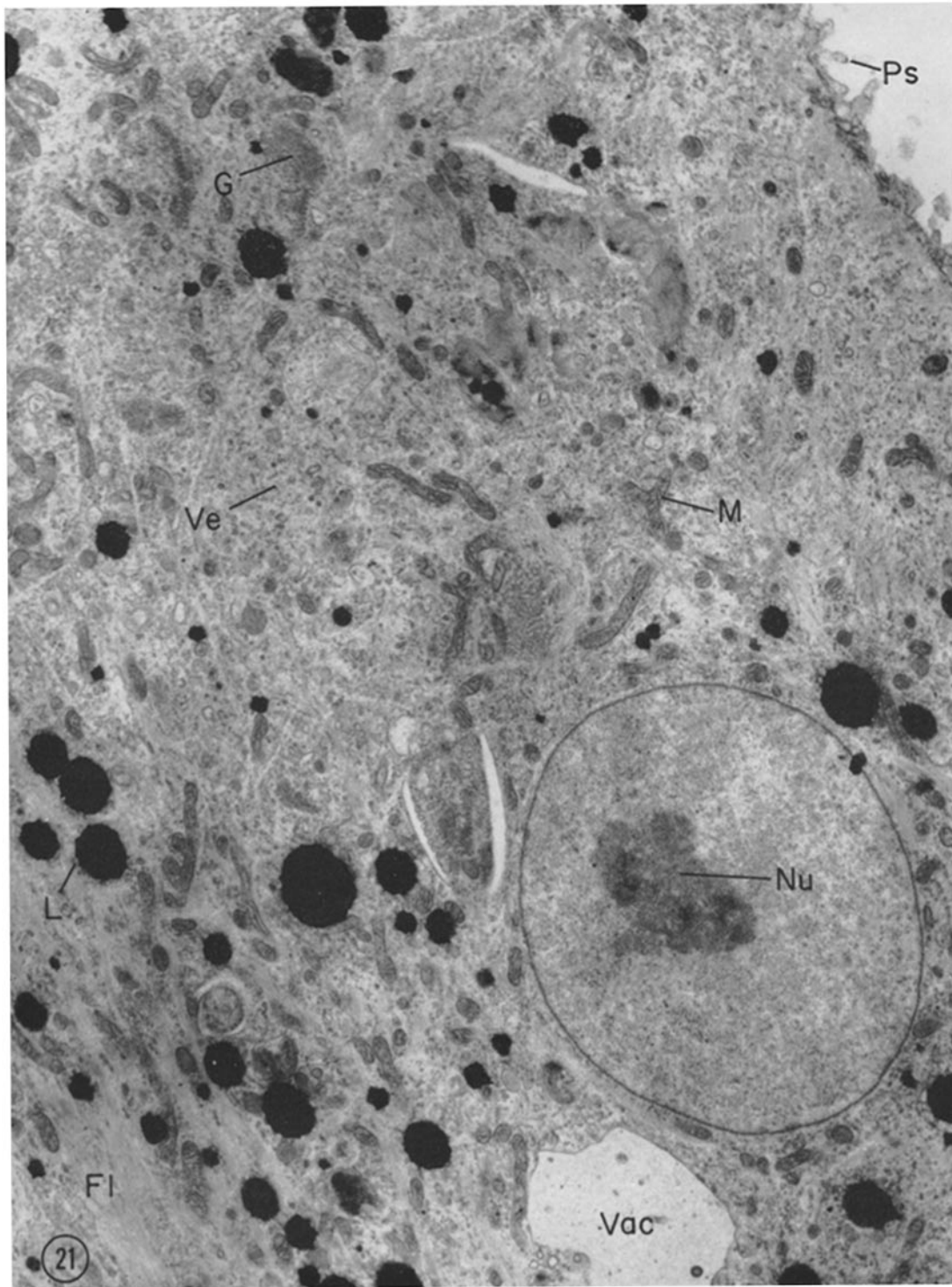
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FIGURE 21 This is a small portion of cytoplasm of a multinucleated giant cell from a 70 day culture and shows only a single nucleus and a prominent nucleolus (*Nu*), finely dispersed granular nucleoplasm, and little chromatin aggregation. Bundles of filaments (*FI*) are characteristically numerous and, by parallel arrangement, form broad sheets. Aggregations of Golgi vesicles are small, numerous, and widely scattered (*G*). Mitochondria are numerous and polymorphous; many are branched (*M*). Lysosomes are virtually absent. The large round homogeneously black bodies are fat vacuoles (*L*). The endoplasmic reticulum consists primarily of tiny smooth-surfaced vesicles. Ribosomes are sparse. The short, stubby pseudopodia (*P<sub>s</sub>*) are characteristic of giant cells. Small vesicles (*V<sub>e</sub>*) pervade the cytoplasm.  $\times 9700$ .



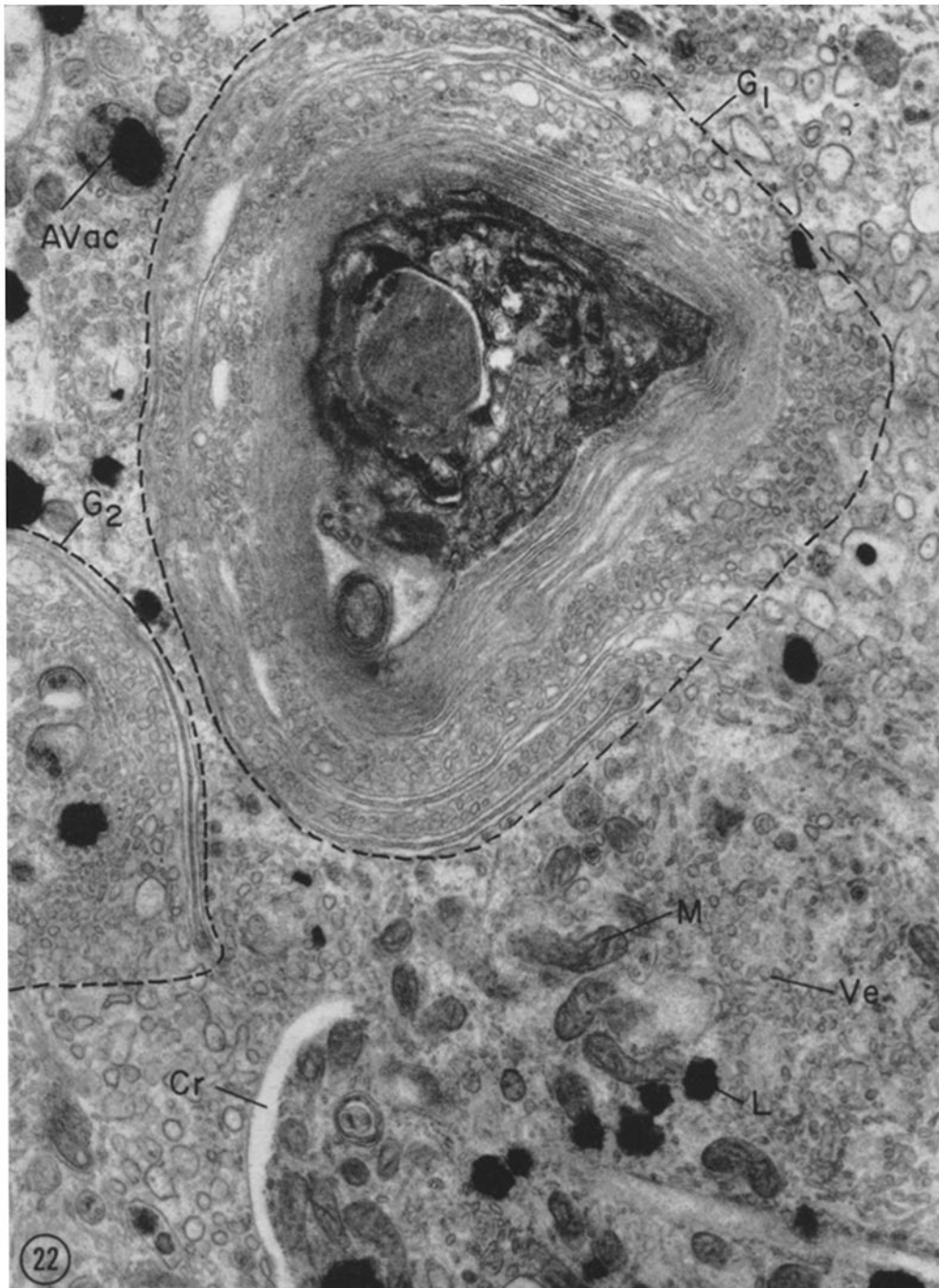


FIGURE 22 Golgi complex-like structures ( $G_1$ ), such as this one encompassing crystalloid material centrally, are frequently observed in 70-day giant cells. Compare this structure with that in Fig. 13. A smaller, similar structure is located at the lower left center of this field ( $G_2$ ). Neighboring vesicles ( $Ve$ ) are smooth-surfaced endoplasmic reticulum. Dense structures represent fat droplets ( $L$ ). Beadlike dilatations in short-length channels within this structure and linear arrangement of vesicles suggest that such structures form by fusion of tiny vesicles within the Golgi region. The crescent-shaped vacuolar structure ( $Cr$ ) most likely represents tangential section of a large vacuole such as are numerous in giant cells.  $\times 10,500$ .