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ULTRASTRUCTURAL CHANGES INDUCED IN
CULTURED HUMAN MONOCYTES BY CONDITIONED MEDIUM

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

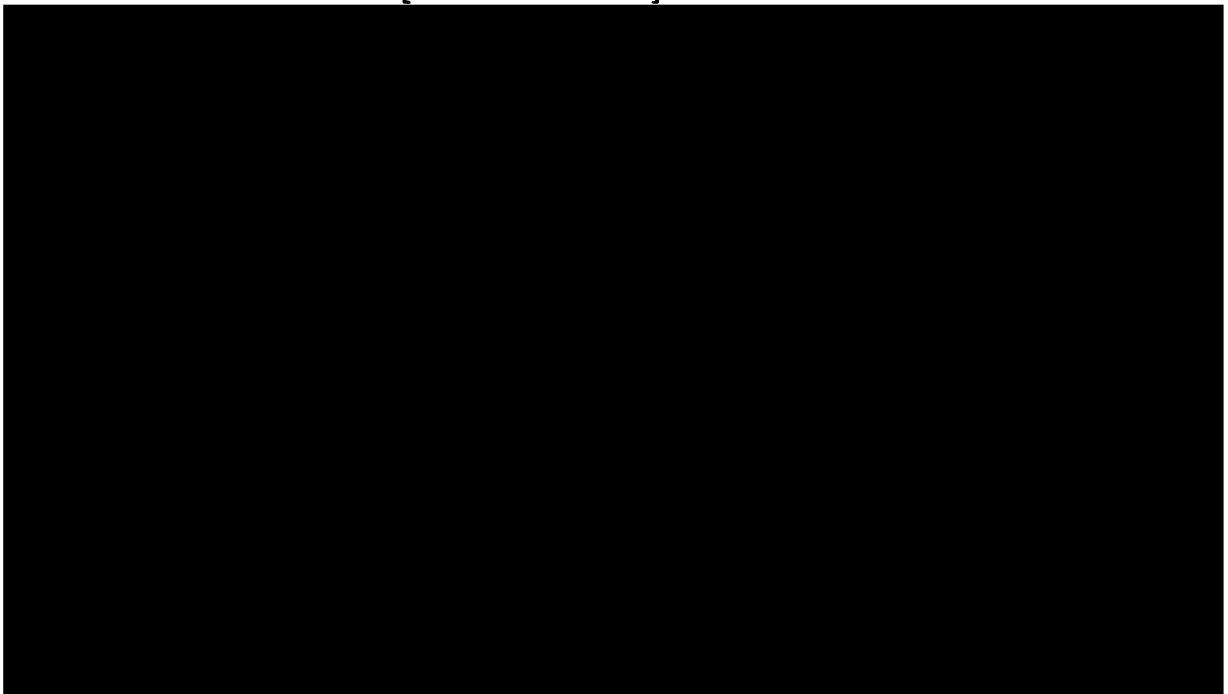
in

Oral Biology

in the

GRADUATE DIVISION

[San Francisco]



Date

Librarian

Degree Conferred: . . . JUNE 22, 1974

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ACKNOWLEDGMENT

I would like to express my deep gratitude to my family, my wife Barbara, Drs. T.M. Christie, Walter McBride and Heynemann and Mrs. Carmen Guillen and Ms. Christina Papanikolas for their patience and cooperation in the preparation of this thesis.

INTRODUCTION

When monocytes are cultured from human blood, multinucleated cells appear in the cultures.^(46, 36) Black, et al.⁽³⁶⁾ noted that monocytes cultured from some subjects produced high counts of multinucleated cells while those from others produced low counts. They were able to increase the numbers of these cells in cultures with low counts by the addition of conditioned media to the culture. This media was obtained from monocyte cultures with high counts.

The purpose of this study was to observe, using the electron microscope, the changes that occur in cultured monocytes producing low counts of multinucleated cells when the conditioned media is added.

Morphology of Monocytes and Macrophages

Monocytes originate from stem cells in the bone marrow.^(1,2,3) The stem cells undergo a process of differentiation and maturation to become mature monocytes, which enter the circulation.^(4,49,50,51,52) After circulating for about one and a half days; they migrate into the tissues where they become macrophages.^(3,40,42,43,44,45)

Monocytes - Monocytes of the peripheral blood are approximately 10 μ in diameter. In light microscopic observation of Giemsa-stained preparations, their nuclei are uniform in shape and filled with deeply stained coarse chromatin. The cytoplasm is granular and may contain clear vacuoles.

In electron micrographs the cytoplasm of monocytes is seen to possess a few peripherally located strips of rough-endoplasmic-reticulum; aggregated ribosomes are rare. The Golgi apparatus is characteristically located in the perinuclear hof and is multicentric.

It is formed of scattered stacks of saccules and a large number of small vesicles. Occasionally, larger pinocytic vacuoles are present near the Golgi zone. A few electron-dense cytoplasmic granules of varying size are scattered in the cytoplasm.

Tissue macrophages - When observed with the light microscope, tissue macrophages contain an eccentrically located horseshoe-shaped nucleus. The margin of the cytoplasm is irregular and the cytoplasm is granular. On Giemsa-stained preparations azurophilic granules and vacuoles are occasionally seen.

Phase-contrast microscopic observations of living peritoneal macrophages of rats show that there is a considerable variation in the size of tissue macrophages, their content of vacuoles, and of phase-dense granules. Except for small vesicles, their peripheral cytoplasm is devoid of organelles. Lipid droplets often can be seen in the peripheral cytoplasm. The plasma membrane is ruffled.

At the ultrastructural level, cisternae of the rough- and smooth-endoplasmic reticulum are scattered at the periphery of the cell or are arranged in stacks. The Golgi apparatus is extensive, multicentric and occupies the perinuclear area adjacent to the centrioles. Granules and mitochondria are located at the periphery of the Golgi zone. These granules have been shown to be lysosomes.⁽⁵⁾

Endocytic Activities of Monocytes and Macrophages

Endocytosis is the process by which the monocytes and macrophages ingest a part of their external environment by enclosing a portion of this environment with invaginations or evaginations of

their plasma membranes. Endocytosis includes phagocytosis and pinocytosis.^(6, 7)

Phagocytosis - Phagocytosis is triggered by an interaction between components of the cell's surface and the particles to be phagocytosed.^(8, 9) Phagocytosis requires metabolic energy. In tissue macrophages, phagocytosis is generally suppressed by inhibitors of anaerobic glycolysis but not by inhibitors of oxidative phosphorylation.^(10, 11) The addition of adenosine triphosphate (ATP), however, enhances the in vitro ingestion of large particles in incubation medium; adenosine monophosphate (AMP) and adenosine have no effect.^(10, 11)

Pinocytosis - The mechanism responsible for triggering pinocytosis is unknown. Nungester⁽¹²⁾ suggested that it may be initiated by differences in electric charges between the cell's surface and the material to be pinocytosed. The in vitro intensity of pinocytosis is influenced by the presence of certain types of molecules in the incubation medium such as anionic proteins, carbohydrates and amino acids.

Pinocytic activity in tissue macrophages can be significantly depressed by the presence of inhibitors of anaerobic glycolysis or oxidative phosphorylation. This depression can be overcome by adding oxygenous ATP to the medium.^(13,14,37,38,39,41,53)

Metabolic Activities of Monocytes and Macrophages

Glycolysis and oxidative respiration are important energy sources.⁽¹⁰⁾ The rate of aerobic glycolysis in circulating blood

monocytes exceeds that of either polymorphonuclear leukocytes or lymphocytes.⁽¹⁰⁾ During in vitro differentiation of monocytes into macrophages, the rate of glucose utilization increases by 40% and lactate production by 200% during the second 24-hour period of incubation.⁽³⁶⁾

Lysosomal Enzymes

Monocytes and macrophages which are grown in vitro are active in protein synthesis.^(15,16,17,18) Most of the newly synthesized protein is condensed and packaged in the Golgi vesicles and subsequently accumulates in the lysosomes.⁽¹⁶⁾

The acid-active hydrolytic enzymes which have been identified in macrophage lysosomes are acid deoxyribonuclease,^(19, 47) acid phosphatase,^(21, 48) acid ribonuclease,⁽²¹⁾ arylsulfatase,⁽²¹⁾ β -galactosidase, β -glucouronidase,⁽²⁰⁾ β -N-acetylglucosaminidase,⁽¹⁹⁾ Cathepsin D,⁽²¹⁾ lipase,^(21, 22) and lysozyme.⁽²¹⁾ Acid phosphatase, β -glucouronidase and Cathepsin D activities increases during the differentiation of monocytes to macrophages by 1900, 450, and 350 per cent, respectively.⁽²²⁾ With the exception of lysozyme, there are no enzymes to which any specific antimicrobial or antitoxic function can be ascribed.⁽²²⁾

The Effect of Conditioned Medium on Cultures of Monocytes

As mentioned above, Black, et al.,⁽³⁶⁾ have studied the effect of a supernatant - the conditioned medium - on monocyte cultures producing low counts of multinucleated cells. They found that the

percentage of multinucleated cells on the fifth day of culture was 6-19% in 29 of the 33 normal subjects whose white blood cells were used in the study. In four of the cultures, more than 20% of the cells were multinucleated.

The addition of supernatant from the high-count cultures to those with low counts led to an increased number of multinucleated cells. The number of multinucleated cells increased between the second and fourth day of culture. The effect began after 30 minutes of incubation and reached a maximum at two hours.

Some of the monocyte cultures were incubated in the presence of tritiated thymidine. Nuclear labelling was rarely observed and the numbers of labelled cells did not increase when the cultures were incubated in the presence of conditioned media. This suggests that the increase in multinucleated cells is the result of cell fusion.

II. MATERIALS AND METHODS

Collection of Monocytes

Fifty ml of blood from normal human subjects was collected in heparinized syringes. Five ml of normal saline containing 6% dextran was added to the samples of blood. (54) Following sedimentation of the red blood cells for one hour at 37°C, the leukocytes were isolated by centrifugation. The final suspension of leukocytes (1.5×10^6 cells/ml in McCoy's 5A medium containing 30% FCS)

was distributed into leighton tubes in which glass cover slips were placed. (54) After incubation for 12 hours at 37°C, when only monocytes were firmly attached to the cover slips, the medium was replaced with McCoy's 5A medium (Grand Island Biological Co., Grand Island, New York) which was supplemented with 30% human AB serum. Thereafter, the medium (pH 7.3 - 7.5) was changed every 2 - 3 days.

Collection of the Conditioned Medium and Experimental Procedure

The supernatant (the conditioned medium) was collected from monocyte cultures that had been shown by light-microscopic observation to have high counts of multinucleated cells. (36) The conditioned medium was millipore filtered and added to monocyte cultures with low counts of multinucleated cells. (36) These cultures were incubated for 30, 60 and 120 minutes. Cultures with low counts were incubated without the conditioned medium to serve as controls.

Electron Microscopy

The specimens, still attached to the glass cover slips, were fixed in a solution of 3% glutaraldehyde and 0.01 M sodium cacodylate for one hour at 0°C. Two groups of specimens were fixed for each of the time periods. Two additional specimens of 5-day old monocyte cultures were fixed as controls.

The cover slips were washed three times (30, 60 and 30 minutes each time) in 0.1 M veronal acetate buffer and postfixed (one hour) in a solution containing 1.2% osmium tetroxide and 0.1 M veronal acetate. Following a 5 minute wash in the veronal acetate buffer,

the monocytes were stained in block with 0.5% uranyl acetate solution (pH 5) for two hours in the dark. (27) After a brief wash (5 minutes) with veronal acetate buffer, the specimens were dehydrated in ethanol.

The specimens were embedded by placing them first in a 1:1 mixture of epon and propylene oxide and then in a mixture of two parts epon to one part propylene oxide. The cover slips were coated on the specimen side with epon and placed specimen side down on wooden sticks that were positioned at either end of a siliconized glass microscopic slide. The sticks were slightly less than the length of a cover slip apart. Epon was also placed on the slide between the sticks. The epon was polymerized in an oven at 60°C for two days.

After polymerization, a trough was formed with walls of dental wax and a base formed by the cover slip. HF acid was placed within the trough in order to dissolve the glass cover slip.

Small blocks were cut from the epon and mounted on plastic rods with epoxy glue. Ultrathin sections were cut with a diamond knife mounted on a Sorval Porter-Blum MT-2 Ultramicrotome. Sections were collected on coated grids and doubly stained with uranyl acetate and lead citrate. The stained sections were examined in a Siemens Elmiskop 1A.

III. RESULTS

Ultrastructures of the Control Group

A sample of mature and immature monocytes from the control culture showed fairly large, condensed aggregates of heterochromatin. A few nuclear pores were present. The cytoplasm and plasma membrane exhibited an occasional, small, finger-like pseudopodium projected from the cellular surface. Some cells touched without fusing. The mitochondria were few, small, and oval-shaped, their matrices denser than the hyaloplasm (the lighter fluid portion of the cytoplasm), with no granules. Some mitochondria showed evidence of degeneration. Granular endoplasmic reticulum was predominant; smooth endoplasmic reticulum was rarely observed. Occasional cisternae were moderately dilated by finely granular material high in electron density. Centrally, the endoplasmic reticulum cisternae surrounded the nucleus. This important organelle complex was small, though well-developed, and circularly arranged. Abundant ribosomes, both free and attached, were apparent, but polyribosomes were uncommon. Small, membrane-bound granules containing a uniform granular substance of high electron density were visible throughout the cytoplasm. The few phagocytic vacuoles distributed in the cytoplasm contained unrecognizable debris or myelin figures and were encircled by an electron-dense membrane. Several large lipid droplets were seen at the apex of the monocytes. Filaments were rarely observed. However, sparse filaments of variable length were present.

Ultrastructural Changes in the 30-Minute Group

Nuclear size was decreased compared to the controls, whereas the cytoplasm was increased. The nucleus was eccentrically located, the amount of euchromatin increased and heterochromatin banding and focal aggregations reduced. The nucleoli were now distinct and conspicuous. The elongated nucleus displayed multiple nuclear pores. Pseudopodia were increased both in number and length. Some cells touched, but no fusion was apparent at this stage. These organelles revealed no visible changes. The granular endoplasmic reticulum was collected into a concentric structure made up of 2 to 3 rows of studded cisternae with electron-dense ribosomes. One of two such arrangements appeared in a single cell close to the cell membrane. The endoplasmic reticulum also was observed in the form of flattened cisternae in the cytoplasm. The Golgi apparatus contained 2 to 3 rows of vacuoles and flattened parallel sacs in the nuclear hof. Occasionally, Golgi vacuoles and the rough endoplasmic reticulum were seen in close proximity. Free ribosomes were plentiful. Electron dense structures (lysosomes) were scattered throughout the cytoplasm and appeared to be more numerous than in the controls. Some were dividing. These organelles appeared to be more numerous and were larger than those in the control group. Some were of variable density, while others were empty, and still others fused. A large number of micropinocytic vesicles surrounded the vacuoles. Centrioles were occasionally seen surrounded by Golgi vesicles. Few lipid droplets were observed in the cytoplasm. Few cytoplasmic filaments

and even fewer microtubules were observed in the cytocentrum. Rugging toward the Golgi apparatus and the endoplasmic reticulum were filiments that formed a loose network in the ground substance.

Ultrastructural Changes in the 60-Minute Group

At 60 minutes the cells were larger than in the previous stages, and all cytoplasmic organelles had increased in number. Heterochromatin was diminished, whereas euchromatin was enlarged, widely dispersed, and finely granular. Nucleoli were prominent. Some filaments were evident in the nuclear substance. A marked increase in number and length of pseudopodia and evidence of ectoplasmic interdigitation between cells were apparent. Multiple fat droplets were found near the mitochondria or plasma membrane. These organelles were slightly increased in number and frequently adjacent to cytoplasmic filaments or the Golgi complex. Granular endoplasmic reticulum was present in two predominant forms: (1) long slender cisternae with flat parallel membranes contiguous with the plasma membrane; and (2) a concentric arrangement consisting of 3 to 4 rows of cisternae studded with electron-dense ribosomes. In addition, this latter arrangement was occasionally found near the plasma membrane. Single and aggregated ribosomes were profusely distributed throughout the cytoplasm. Lysosomes increased still further in size and number compared to the earlier stage and were prominent in the cytoplasm. The small dense granule was the most common organelle, however.

Flattened sacs of the Golgi complex were radially arranged, and empty vesicles and lysosomal granules predominated within the

complex. Centrioles were occasionally juxtaposed against the Golgi elements but were usually located near the nucleus, partially surrounded by the entire Golgi apparatus. Centrioles were arranged at right angles to one another, and were composed of nine triplets of hollow fibrils. At this stage, phagocytic vacuoles reached their greatest concentration in the cell. Cytoplasmic filaments, more numerous than in the 30minute interval preparation, occurred singly or in longitudinally oriented bundles. Occasionally, some filaments were also seen in the nuclear substance. Cytoplasmic microtubules were also frequently observed.

Ultrastructural Changes in the 120-Minute Group

At this stage, the cells were much enlarged. Sometimes a cell contained a number of nuclei and the cytoplasmic organelles were abundant in the extensive cytoplasm. The nucleus was generally round or oval in contour. Heterochromatin was condensed marginally. A single distinct nucleolus usually was apparent. The cytoplasm was crowded with organelles, including mitochondria, filaments, and microtubules. Dense zones of plasma membrane were observed. Interlocking finger-like extensions of cytoplasm occurred between some cells. Occasional disintegration of the plasma membrane was seen. Mitochondria were exceedingly numerous and diverse in form: branched, angulars, and filamentous. The outer membrane of some mitochondria was discontinuous, while in other mitochondria the outer membranes were continuous and intact. Many of these organelles were contiguous with small groups of filaments or lipid droplets. Granular endoplasmic

reticulum was scattered throughout the cytoplasm as isolated, flattened cisternae, with smooth endoplasmic reticulum also abundant. Plentiful free ribosomes and polysomes were frequently seen. Phagocytic vacuoles dominated the cytoplasm, which contained a wide spectrum of different particulate material. Running between these vacuoles were microtubule cylinders. Lysosomal structures, however, were not as frequently encountered as in previous stages. Clear micropinocytic vesicles were found near the plasma membrane. Golgi vesicles and centriole tubules were just as prominent as in earlier stages. An occasional vesicular structure could be identified in the cytoplasm neighboring the nucleus. At this stage, these structures were more abundant than in any previous period. Some filaments were opposed to mitochondria, while microtubules emanated from the centriole tubules to form a randomly dispersed cytoplasmic network of microtubules.

IV. DISCUSSION

Monocytes contain the organelles required for protein synthesis (ribosomes, nucleoli, nuclear pores) and those required for lining the product in membrane (Golgi elements, endoplasmic reticulum). These cells are adapted to transform to macrophages which are responsible for phagocytosis.^(18, 35) Cytological changes in vitro indicate intensive protein synthesis.⁽¹⁸⁾ Large nucleoli develop, chromatin becomes widely dispersed, nuclear pores are more frequent, the Golgi apparatus increases in size and complexity, polyribosomes increase in size, 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, which contain acid phosphatase.⁽²³⁾

In monocytes, the lysosomes reach and touch the cell membrane.⁽¹⁸⁾ The characteristic dense, cuplike structures in the cell membrane likely represent foci of lysosomal discharge. Appearing after 6 hours of culture, they are present in every subsequent stage. Their appearance coincides with increasing amounts of acid phosphatase in the culture medium.⁽²⁴⁾ 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 by cells arising from them may initiate or hasten degradation of material prior to phagocytosis.⁽¹⁸⁾ The process may well affect the state of gelation and solvation of extra-cellular connective tissue.⁽¹⁸⁾ Extracellular discharge of lysosomes appear to represent a normal merocrine function of these cells.⁽¹⁸⁾ 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 that are associated with cell fusion.⁽¹⁸⁾ While extracellular granule discharge may not occur in polymorphonuclear leukocytes, it appears to be normal function of mononuclear phagocytes.⁽¹⁸⁾

After the addition of conditioned media to monocyte cultures, several ultrastructural changes occur -- changes that probably are

induced by the media, since the control group revealed no evidence of modification within the same period of time. Within 30 minutes, ultrastructural alterations were minimal, essentially limited to the euchromatin/heterochromatin ratio, which increased. Otherwise, the cytoplasmic organelles appeared unchanged. It was obvious, however, that the monocytes were actively synthesizing protein, including lysosomal enzymes, as evidenced by the increase in euchromatin in the nucleoprotein substance and by the slight increase in the entire protein-synthesis apparatus, including ribosomes, endoplasmic reticulum, and the Golgi complex.

After 60 minutes, ultrastructural changes were clearly more evident than at the 30-minute period. These modifications consisted of an increase in the size and number of many organelles: pseudopodia, the euchromatin:heterochromatin ratio, nuclear pores, ribosomes, centrioles, Golgi apparatus, mitochondria and filaments, microtubules, lysosomal structures, and phagocytic vacuoles. More interesting, however, is the appearance of a concentric arrangement of the endoplasmic reticulum -- the cytoplasmic whorls. Hicks⁽²⁵⁾ reported that pig and monkey urothelium contain such concentric lamellae of rough endoplasmic reticulum, but in the guinea pig the membranes were nearly devoid of ribosomes and were arranged in a more complex lamello-tubular pattern. The centre of the whorls in the guinea pig were sometimes occupied by large dense granules, similar to granules found in other parts of the cytoplasm.

Membrane whorls in mouse epithelium were first reported by Walker (1960),⁽²⁶⁾ but he was unable to decide whether they should be

regarded as endoplasmic reticulum or Golgi complex. Their general form and the presence of more typical Golgi complexes in the same cells implied that they were reticular, but their positive response to metal impregnation was more indicative that they were Golgi complexes. Since several of the species in Hicks' study⁽²⁵⁾ had ribosome-studded whorls, he concluded that it was highly unlikely that they were Golgi complexes. This was confirmed by the finding that they did not show thiamine pyrophosphatase activity, although high activity was present in typical Golgi complexes in the same cells. Although similar whorls have been seen in a variety of tissues, few workers have ventured to comment on their significance (Palay & Palade, 1955;⁽²⁷⁾ Trier, 1958;⁽²⁸⁾ Nickerson & Curtis, 1969;⁽²⁹⁾ Mori & Matsumoto, 1970;⁽³⁰⁾ Nickerson, 1970⁽³¹⁾). Nickerson and Curtis (1969)⁽²⁹⁾ suggested that they could be sites of assembly of new endoplasmic reticulum.

The distribution of membrane whorls in various animal species offers no help in elucidating their function. Both rodents and primates include forms with and without whorls. Carnivores that have been studies⁽²⁵⁾ are all without whorls, but since this only covers four species (dog, cat, common seal and grey seal), the sample is too small for any general conclusions to be drawn.

Maximal changes occurred in the 120-minute group, where, in addition to the above-mentioned earlier changes, fusion between cells was apparent, as well as further increase in the size of various cytoplasmic organelles (filaments, microtubules, mitochondria, ribosomes, and phagocytic vacuoles). The "new nature" of these cells

is reflected in the appearance of giant cells which are responsible both for phagocytosis and transportation of various particulate matter. Increased frequency of cytoplasmic filaments and microtubules in multinucleated giant cells is generally believed⁽¹⁸⁾ to serve as contractile elements that assist the cell to perform its function of transporting phagocytosed inclusions, in addition to assisting in cell motility and phagocytosis.⁽¹⁸⁾

Sutton⁽¹⁸⁾ reported that in cultured chicken monocytes cytoplasmic filaments displayed a consistent topographic relationship to the mitochondria. They appeared to originate or to insert upon the outer mitochondrial membrane in epithelioid cells, but did not reach the mitochondrial matrix. Filament formation around phagocytotic vacuoles in 3-day old macrophages is consistent with the hypothesis of Depetris, et al.,⁽³²⁾ 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 into specific cytoplasmic regions. In addition to intracellular transport of phagocytotic inclusions, they may also play a skeletal role in supporting the membrane around the inclusions, they may also play a skeletal role in supporting the membrane around the inclusion^(33, 34) and, in fact, in maintaining the configuration of the cell and protecting it, or certain parts of it, against the distortions that phagocytosis or motility may induce. Filaments may serve another transport role in the cell, moreover, providing surface for the movement of colloids. A similar role has been postulated for collagenous fibers in extracellular connective

tissue.⁽¹⁸⁾ As the monocytes undergo transformation into macrophages, and then to giant cells, they not only undergo remarkable changes in structure, but also in function.⁽¹⁸⁾ The fundamental monocyte function appears to be synthesis and storage of lysosomes.⁽¹⁸⁾ The macrophage then can function as a phagocyte able to digest and dispose of its intake. The multinucleated giant cell that is subsequently formed from the fusion of macrophages may engage in active transport in addition to sequestering foreign particles.⁽¹⁸⁾

It appears plausible therefore, as observed by Black⁽³⁶⁾ that the "conditioned medium" (derived from an individual with a high count of giant cells) serves as a stimulus that enhances the syntheses of lysosomal enzymes in the monocyte culture, thereby speeding considerably the onset of its phagocytic activity, fusion between cells, and transformation of monocytes to macrophages and finally to multinucleated giant cells. As in enzyme processes, the effect of the conditioned medium is not to generate a new process, but to speed up greatly an ongoing phenomenon that normally would occur only after a passage of days rather than 1 or 2 hours.

SUMMARY

The purpose of this investigation was to study the possible role of conditioned medium on the ultrastructures of monocytes in vitro. EM observation showed that thirty minutes following the addition of conditioned medium to cultured monocytes, there was an increase in the number of dense lysosomal bodies and Euchromatin/Heterochromatin ration. After sixty minutes there was an increase in the number and length of pseudopods; phagosomes, R.E.R., mitochondria, golgi apparatus and ribosomes increased in size. After 120 minutes the cells increased in size and evidenced fusion. Filaments, microtubules, mitochondria and lipid droplets increased in size and number.

These observations indicate that conditioned medium may be enhancing the synthesis of lysosomal enzymes, phagocytic activity and fusion between cells, thus accelerating the transformation of monocytes to multinucleated giant cells in vitro.

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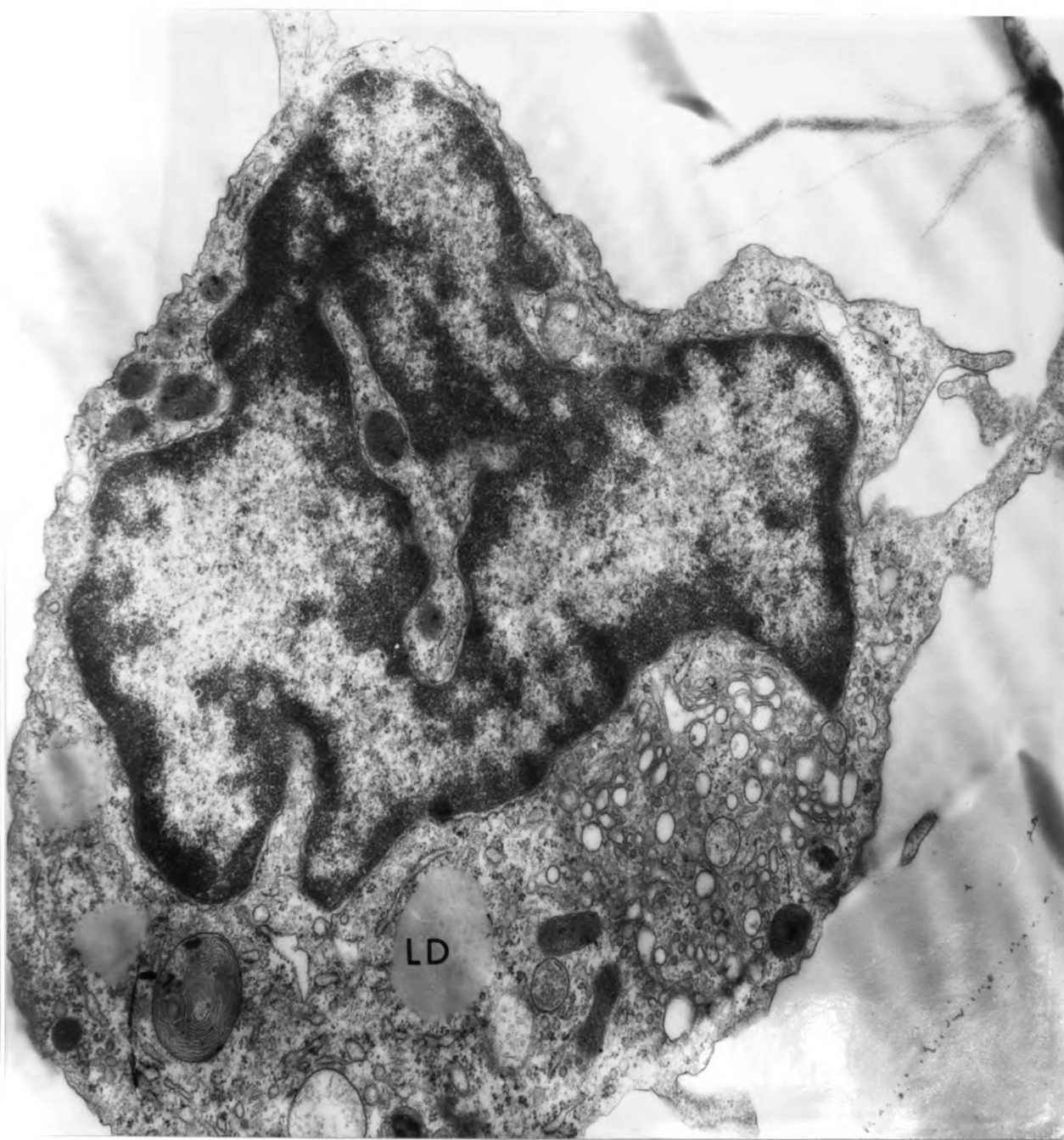
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ABBREVIATIONS FOR PHOTOMICROGRAPHS

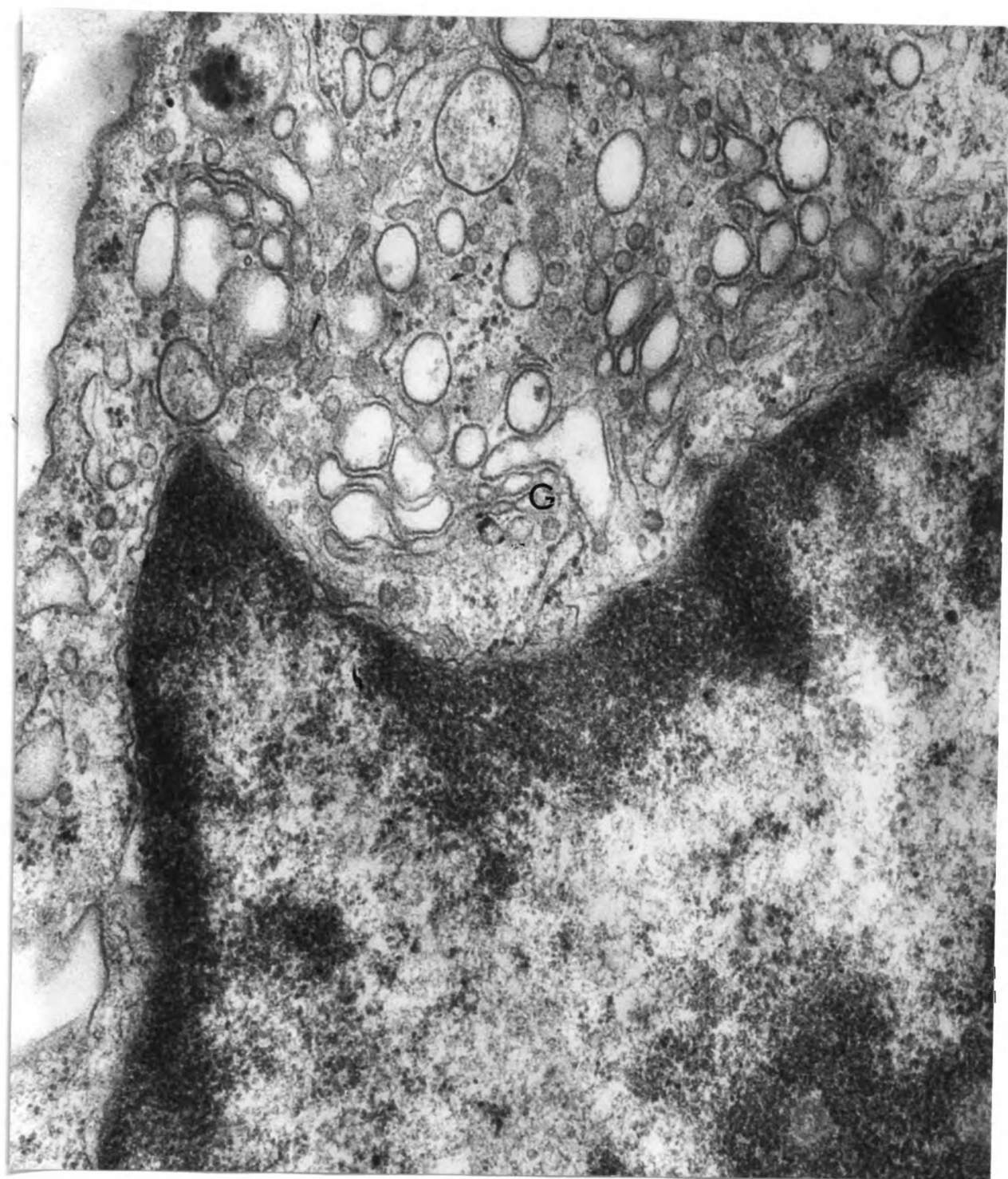
LYSOSOMES	Lys
PHAGOCYTTIC VACUOLES	PhV
LIPID DROPLETS	LD
ROUGH ENDOPLASMIC RETICULUM	RER
NUCLEAR PORES	NP
GOLGI COMPLEX	G
MYELIN FIGURES	My
CENTRIOLES	C
MITOCHONDRIA	M
FILAMENTS	F
PSEUDOPODS	PS
GRANULES	Gr
CAVEOLA	Ca
MICROTUBULES	MT
RIBOSOMES	R
ECTOPLASMIC INTERDIGITATION	Ect. Int.

THE CONTROL GROUP

Electron micrograph of cultured monocytes of the control group showing large nuclear/cytoplasmic ratio, few lipid droplets (LD) and electron lucent cytoplasmic granules magnification.
Magnified (x 52,000)



High magnification (x 70,000) of the Golgi complex (G), showing flattened rounded and angular electron lucent lamellae surrounded by small vesicles.



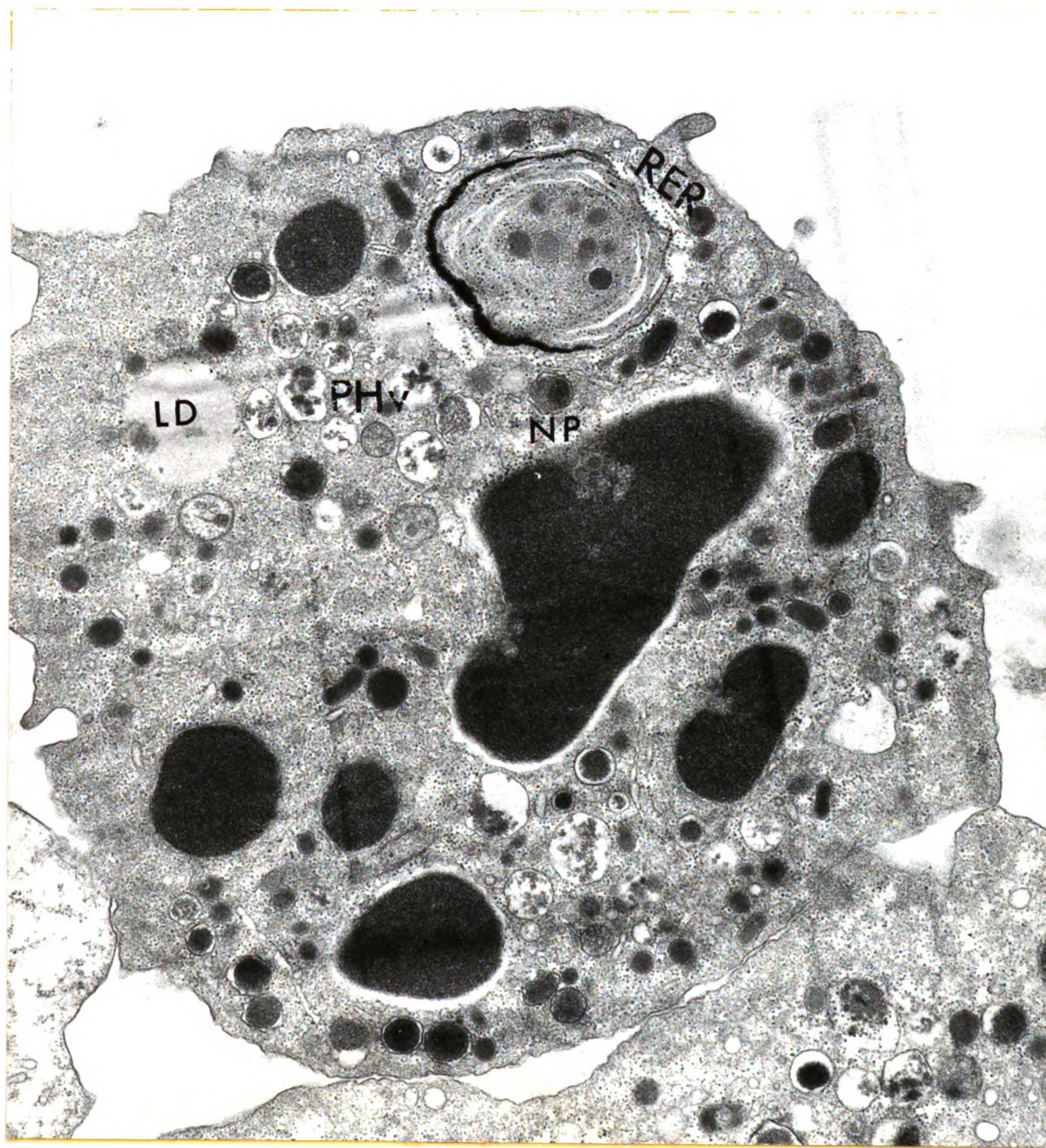
Electron micrograph of a mature monocyte of the control group, showing nuclear Euchromatin/Heterochromatin ratio small, few nuclear pores, scattered electron-dense structures possibly Lysosomes (Lys), small phagocytic vacuoles (PhV) distributed throughout the cytoplasm and two large lipid droplets (LD).

Magnified (x 55,000)



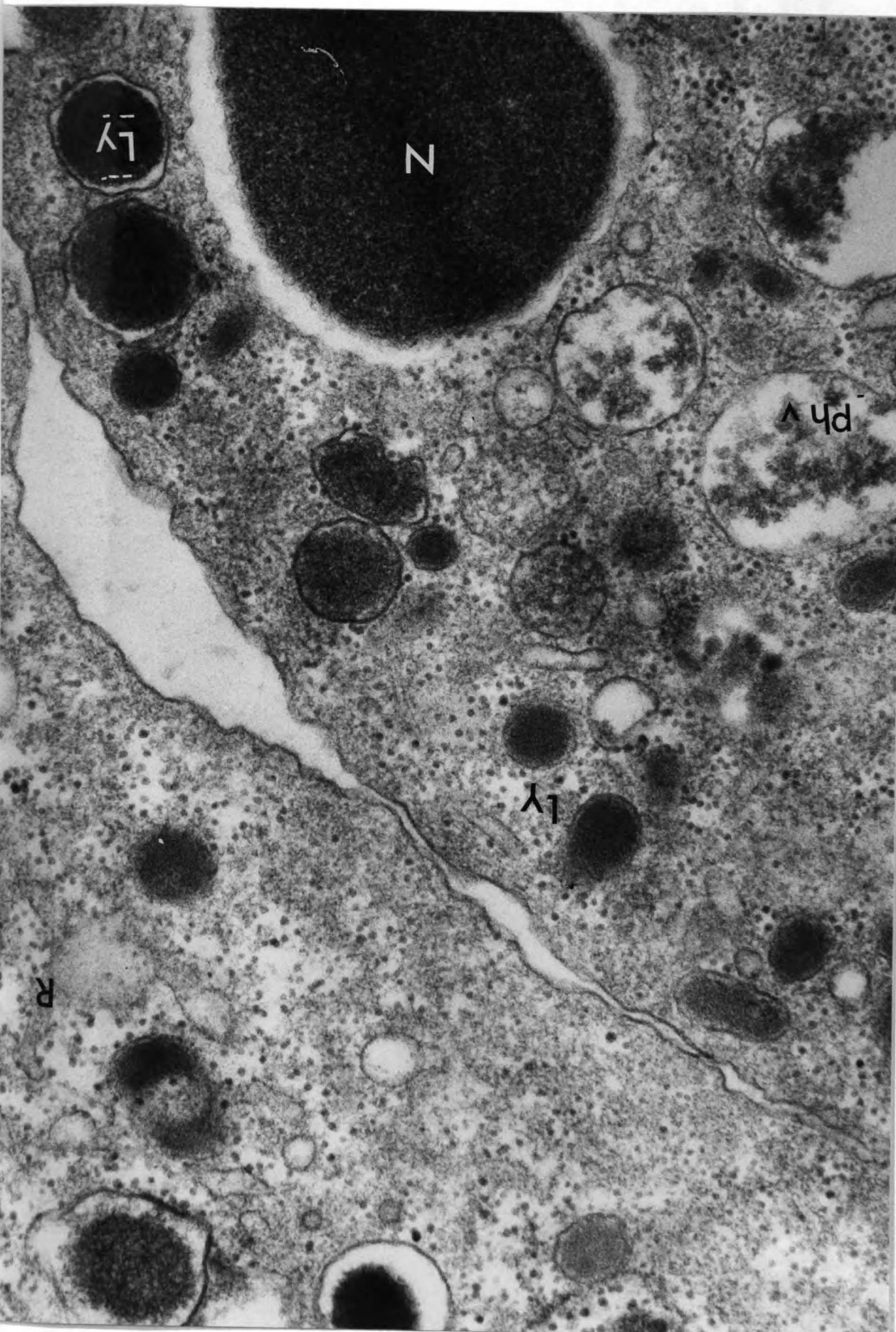
Mature monocyte with short finger like extension (pseudopodia), rough endoplasmic reticulum (RER) are arranged in a concentric pattern. The RER cisternae are studded with electron dense ribosomes, the nucleus is made of dense chromatin with few nuclear pores (N.P) scattered electron dense structures possibly lysosomes (Lys) throughout the cytoplasm.

Magnified (x 55,000)



Electron micrograph of two juxtaposed cell membranes, notice that the two membranes are intact, phagocytic vacuoles (PhV), electron dense rounded structures (Lys) probably lysosomes are peripherally oriented.

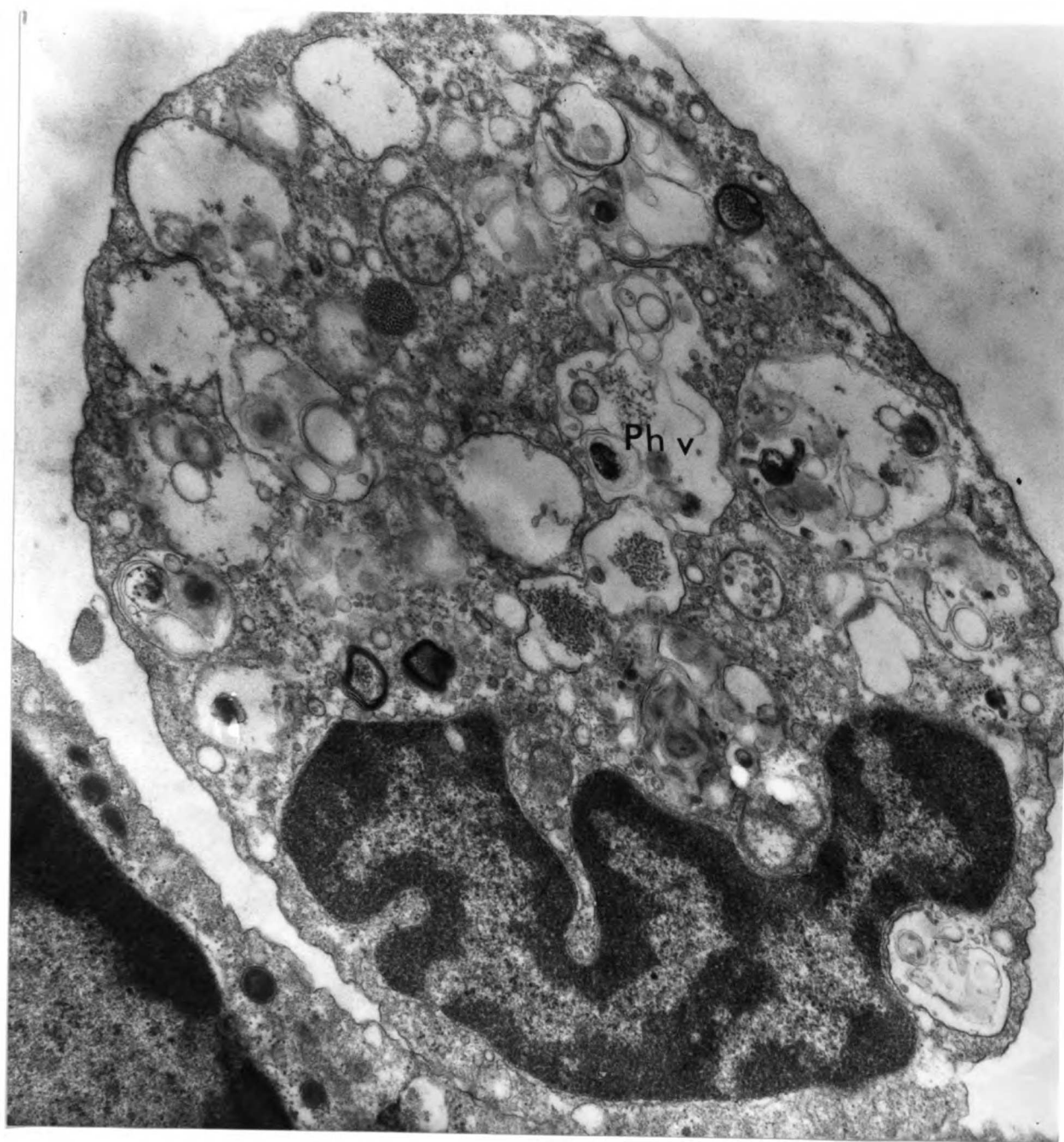
Magnified ($\times 70,000$)



THE 30-MINUTE GROUP

This electron micrograph of 30 minutes monocyte group eccentrically located nucleus, with increasing Euchromatin/Heterochromatin ratio. The heterochromatin appear dense, dark and peripherally aggregated, the cytoplasm is dominated with phagocytic vacuoles (PhV) containing degraded materials.

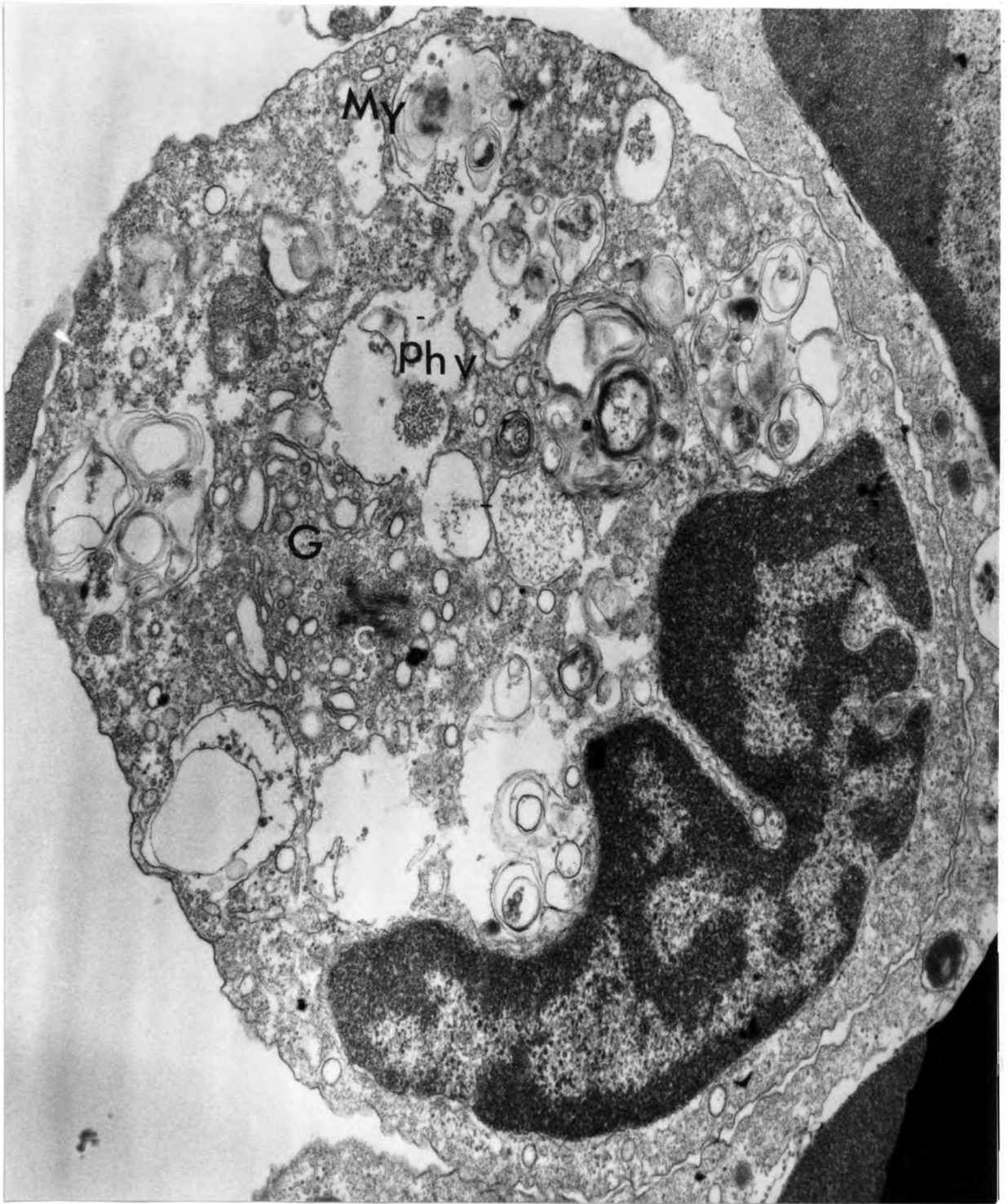
Magnified (x 50,000)



This micrograph illustrates the eccentric location of the nucleus, the Golgi complex (G) and Centriole (C) are located in the center of the cell, multiple phagocytic vacuoles (PhV) are distributed in the cytoplasm including some myelin figures (My).

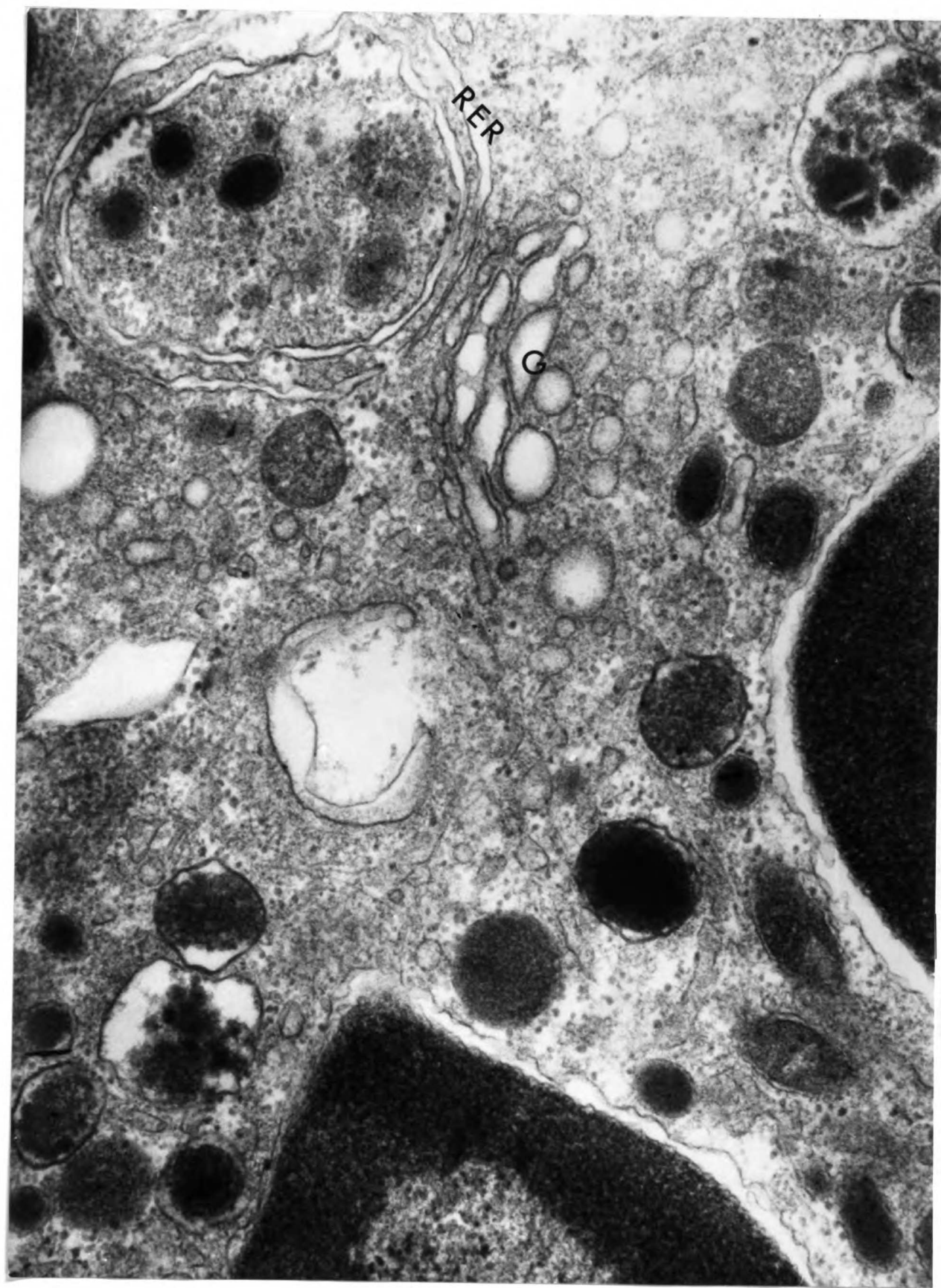
Notice the ectoplasmic interdigitation between the cell and the neighboring one.

Magnified (x 50,000)

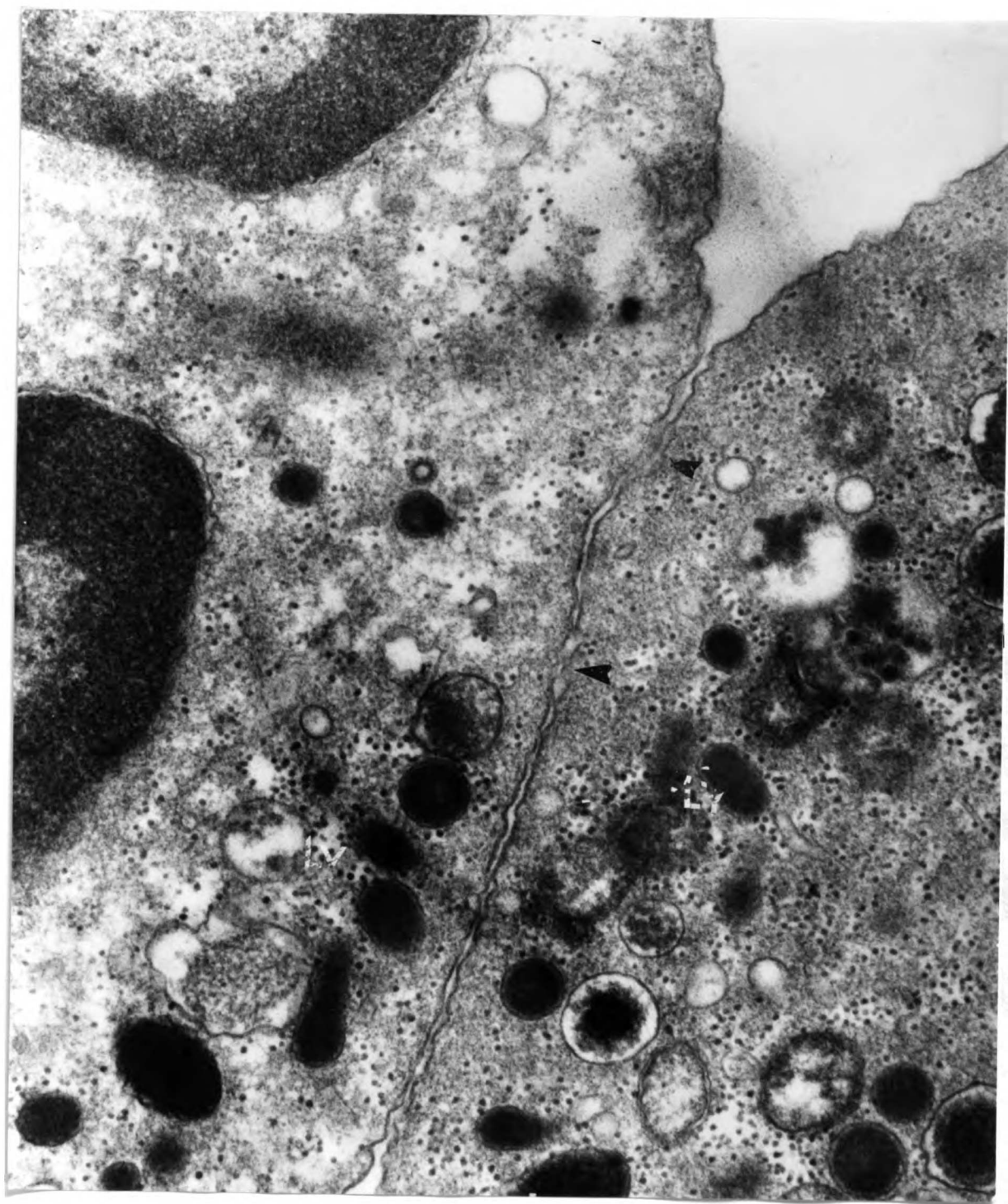


This micrograph shows rough endoplasmic reticulum (RER) arranged into a concentric structure made of two rows of electron dense ribosomes studded cisternae, the Golgi complex (G) is located close to the RER.

Magnified (x 70,000)



High magnification (x 65,000) of two adjacent cell membranes, showing occasional breakage (arrow). Notice the orientation of these dense structures (Lysosomes).



THE 60-MINUTE GROUP

This electron micrograph illustrates the ultrastructure of monocytes following the addition of conditioned medium for 60 minutes. The cell exhibit marked increase in the length and numbers of the pseudopodia and marked increase in the number of mitochondria (M). The appearance of the cell at this stage suggests an active phagocytic function. Magnified (x 50,000)



This electron micrograph shows a marked increase in the number and length of pseudopodia, prominent polysomes and free ribosomes in the cytoplasm.

Magnified. (x 140,000)



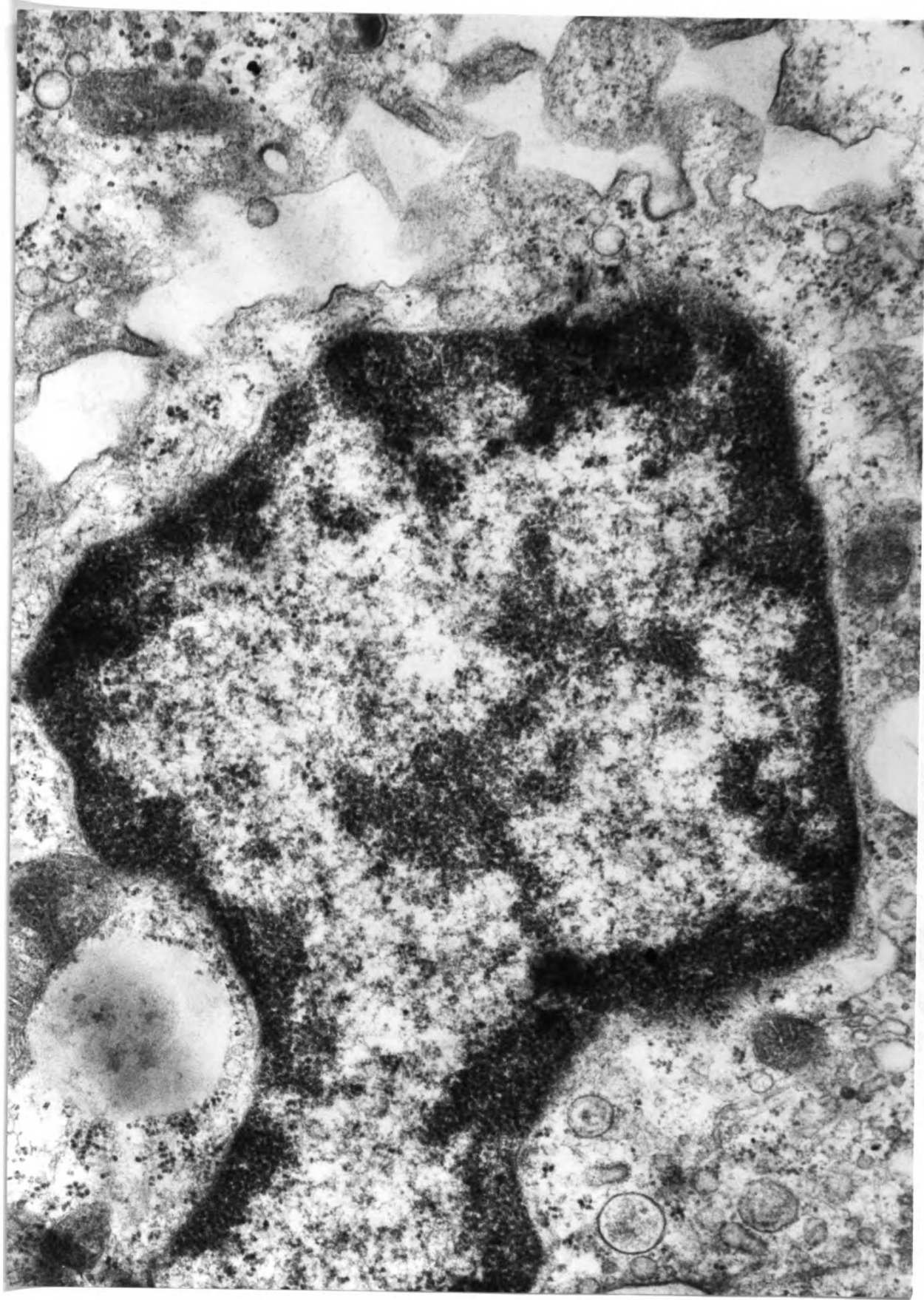
This electron micrograph shows pseudopodia (PS) surrounding the material to be phagocytosed. Marked breakage of the opposing membranes is evident in some areas.

Magnified (x 60,000)



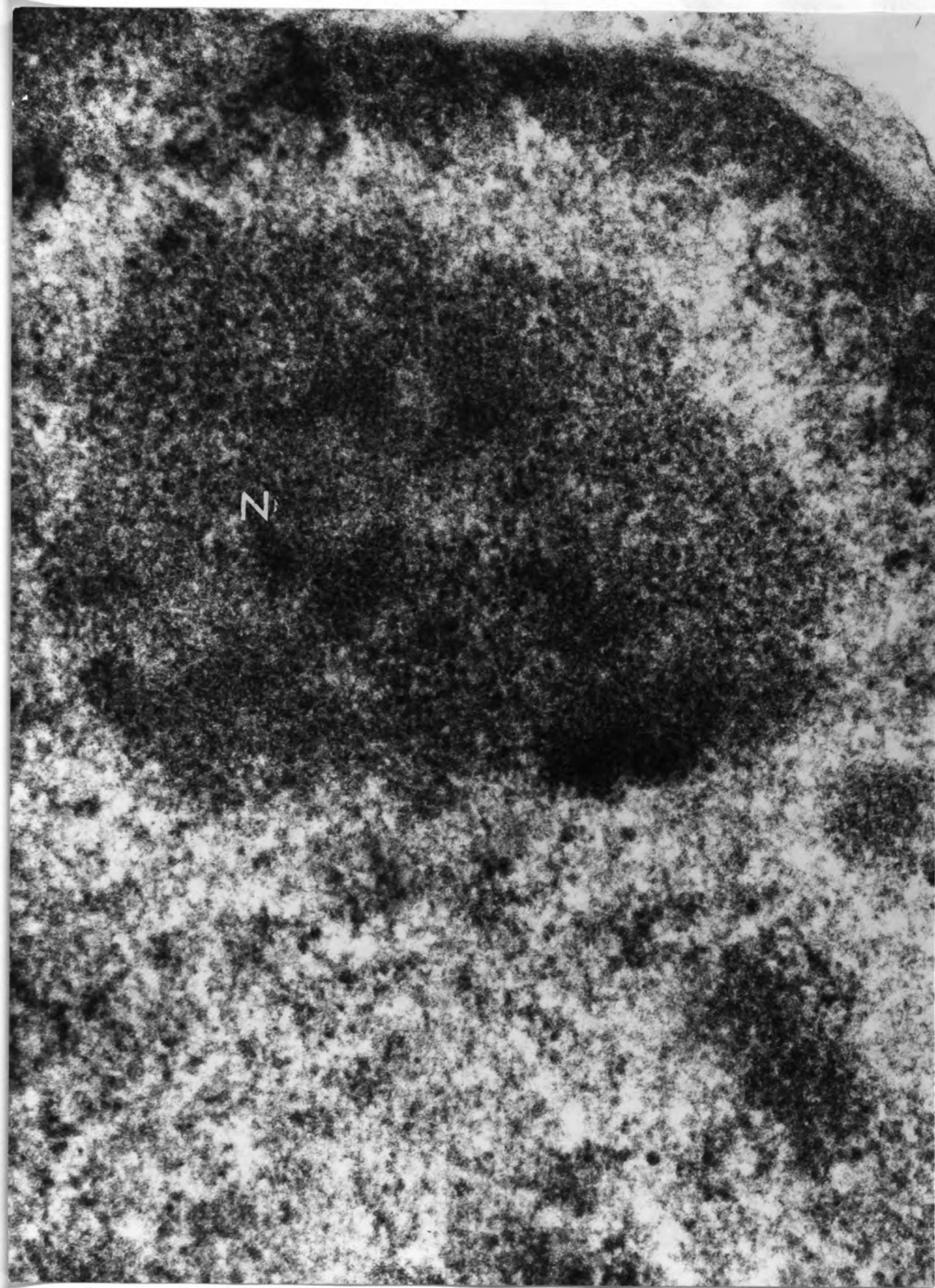
This electron micrograph illustrates the Euchromatin/Heterochromatin ratio to be markedly increased suggesting an active process of protein synthesis.

Magnified (x 80,000)

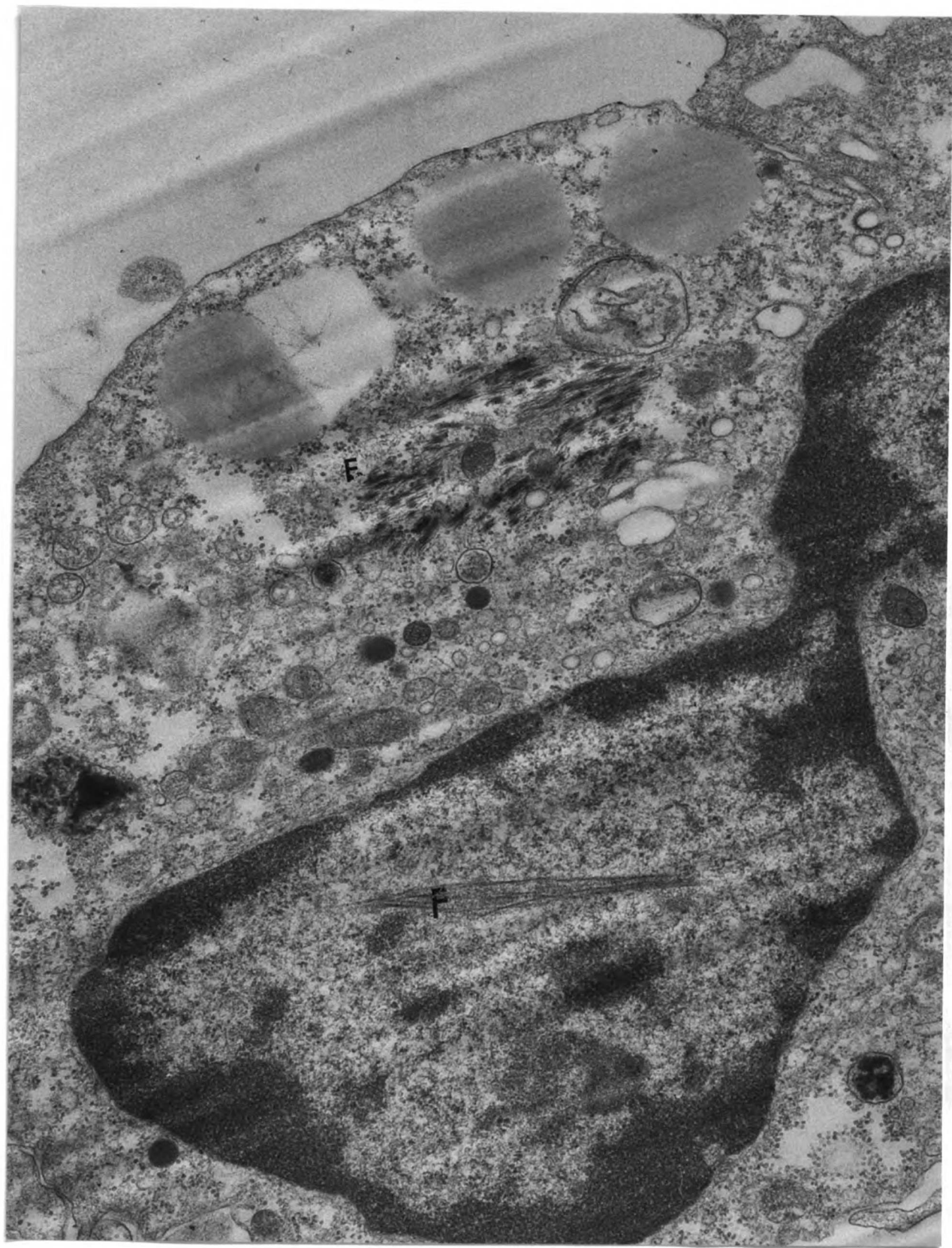


This electron micrograph illustrates that prominent nucleoli at this stage are frequent in cells.

Magnified ($\times 160,000$)

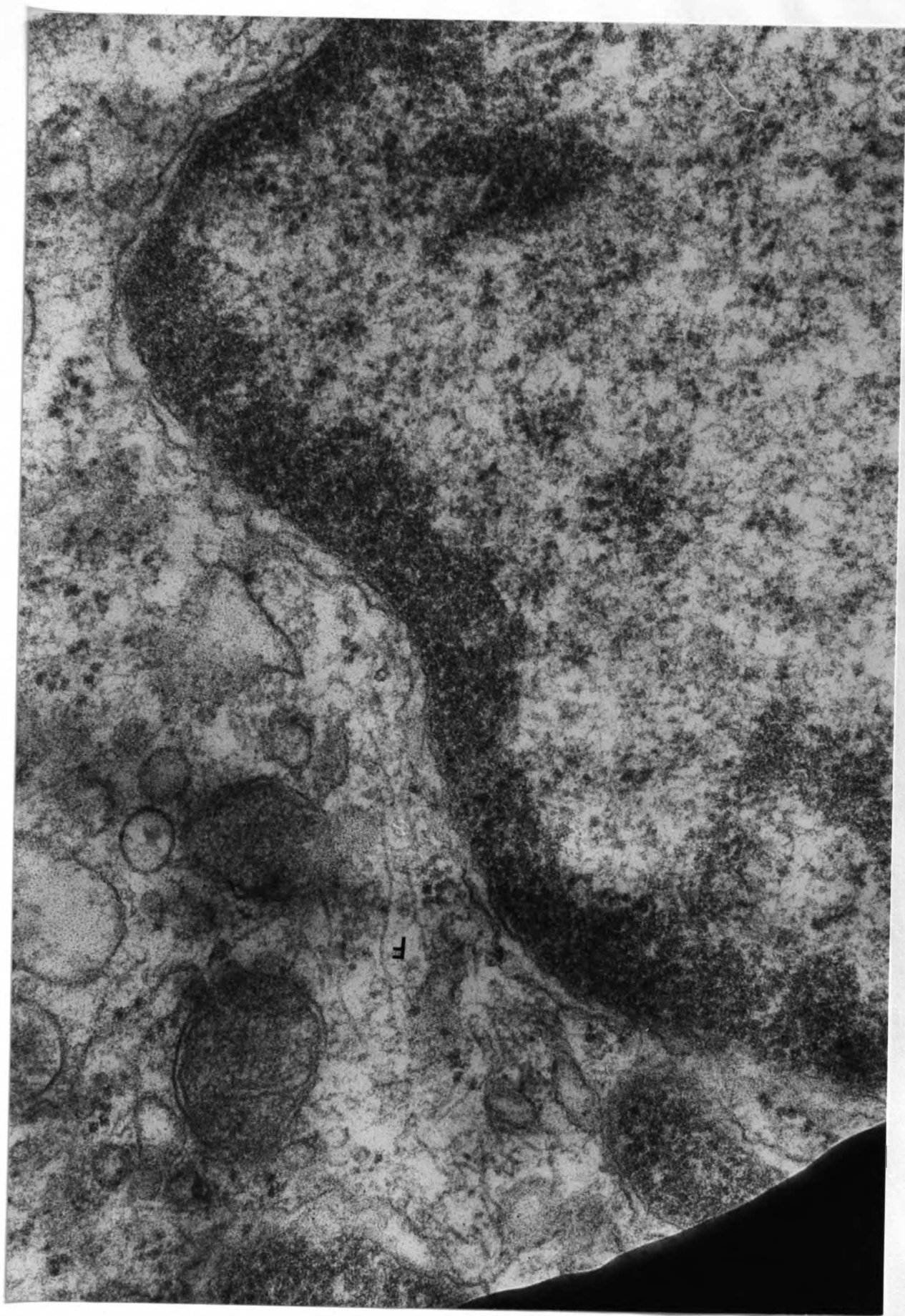


This electron micrograph shows cytoplasmic filaments (F) to be more numerous at this stage. They appear to be arranged in longitudinally oriented bundles, occasionally some filaments were contained in the nuclear substance. Magnified (x 90,000)



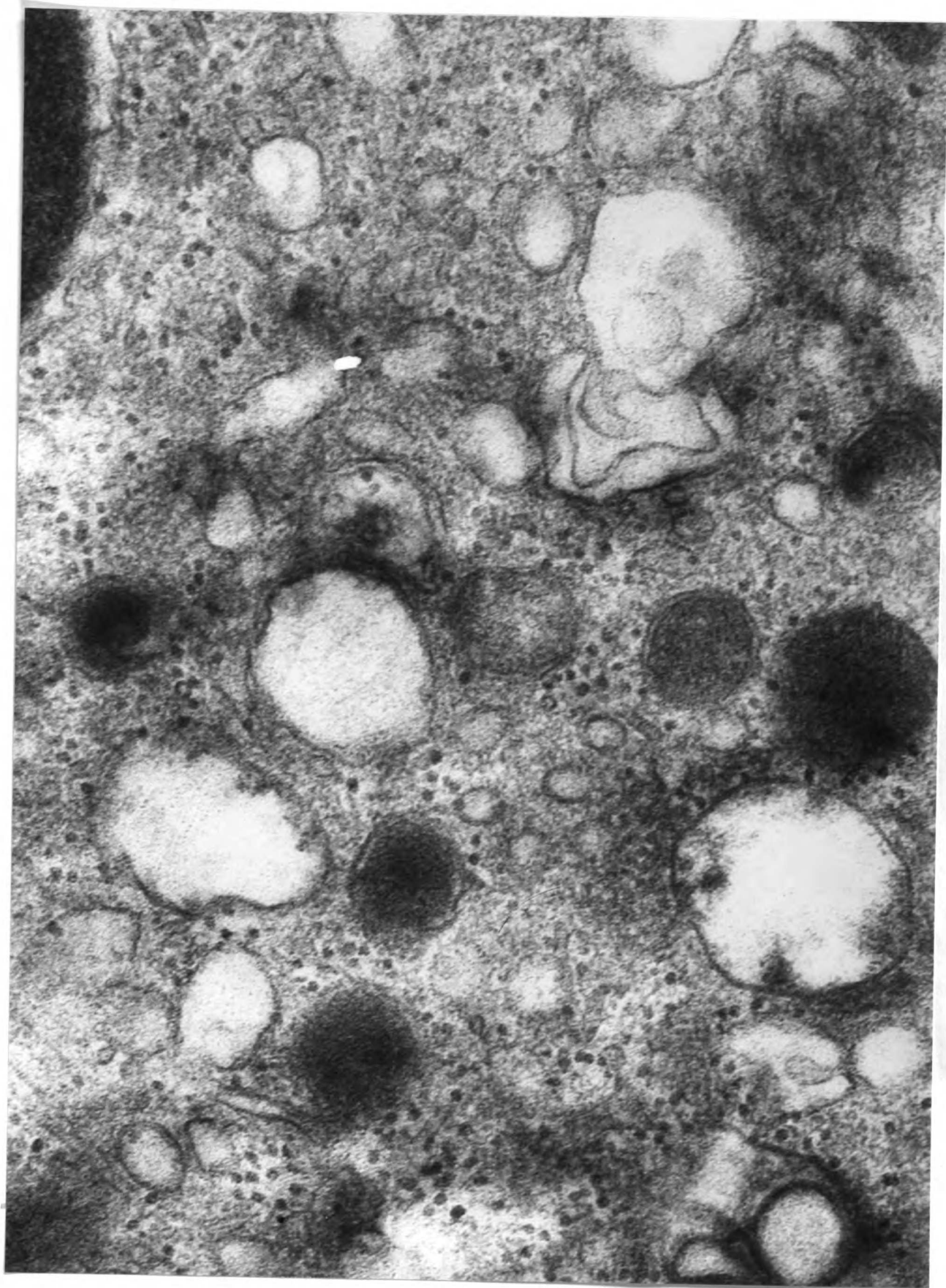
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This electron micrograph illustrates
cytoplasmic filaments (F) arranged juxtaposed
to the mitochondria and the nucleus.
Magnified (x 80,000)



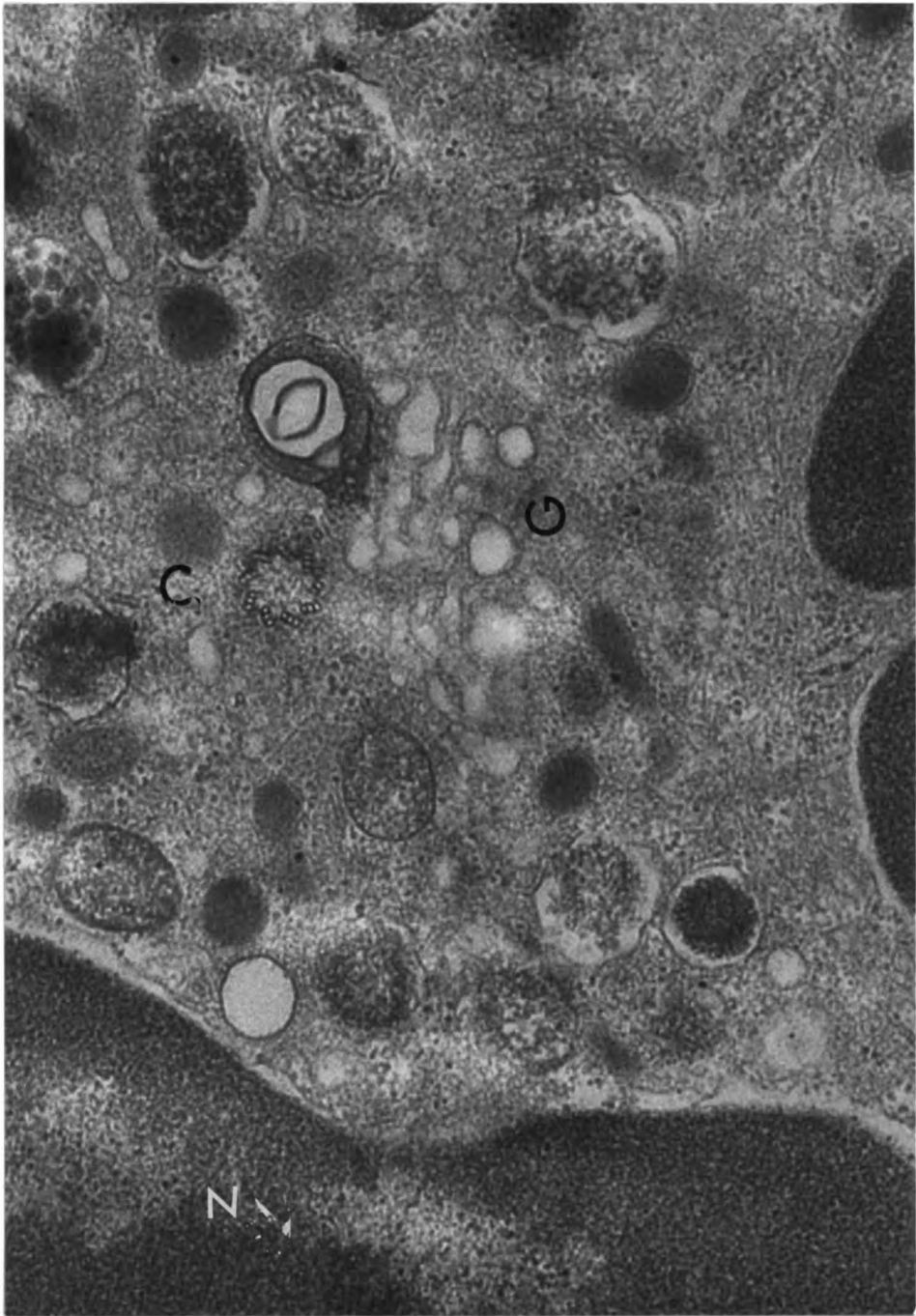
This electron micrograph illustrates monocyte cytoplasm with prominent ribosomes and phagocytic vacuoles and electron dense lysosomal structures.

Magnified (x 80,000)

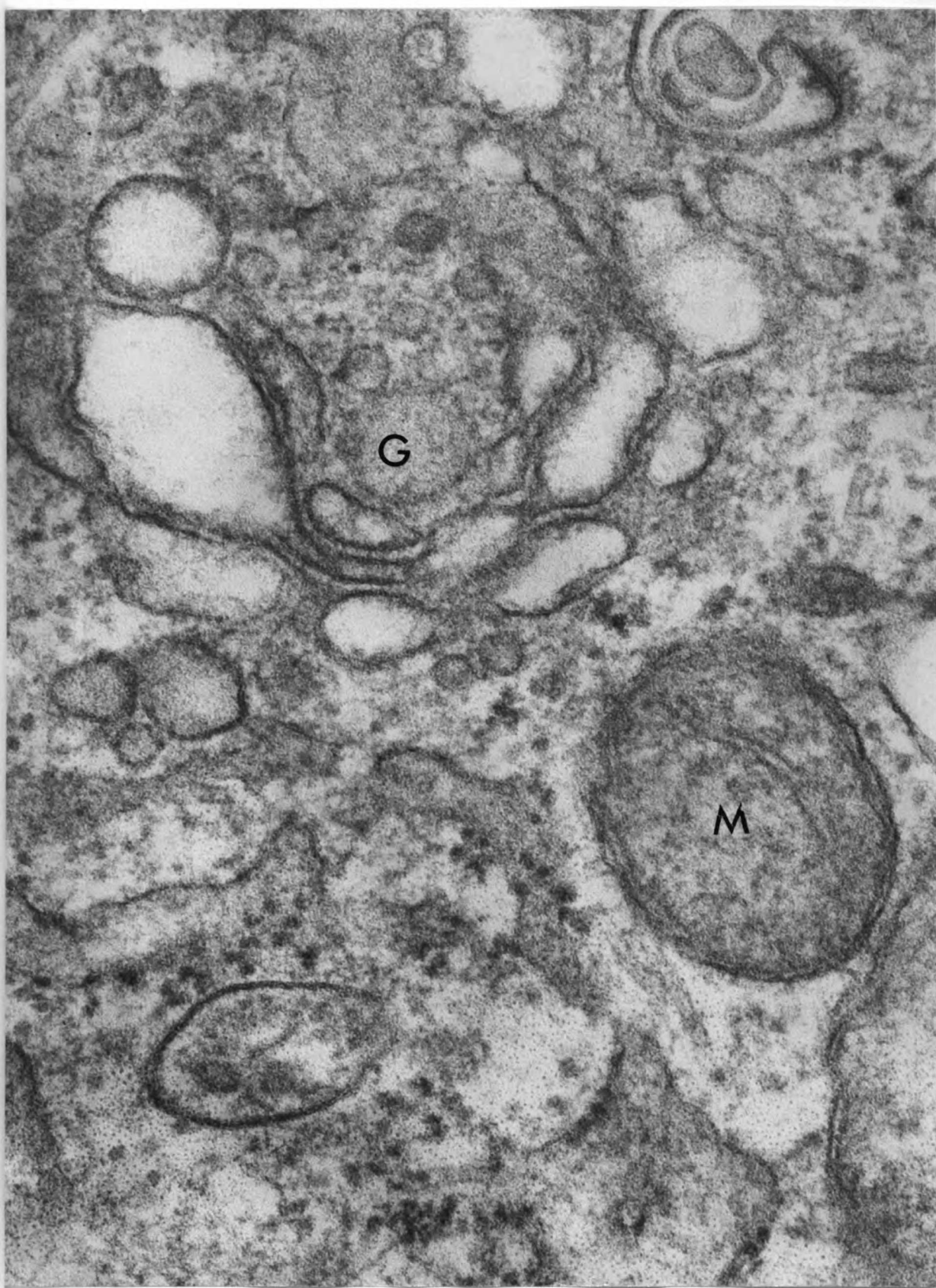


This electron micrograph illustrates in the center of the cytoplasm the Golgi apparatus (G) and centriol (C) cut in cross section. The centriole is a mode of nine triplets of hollow fibrils.

Magnified (x 60,000)

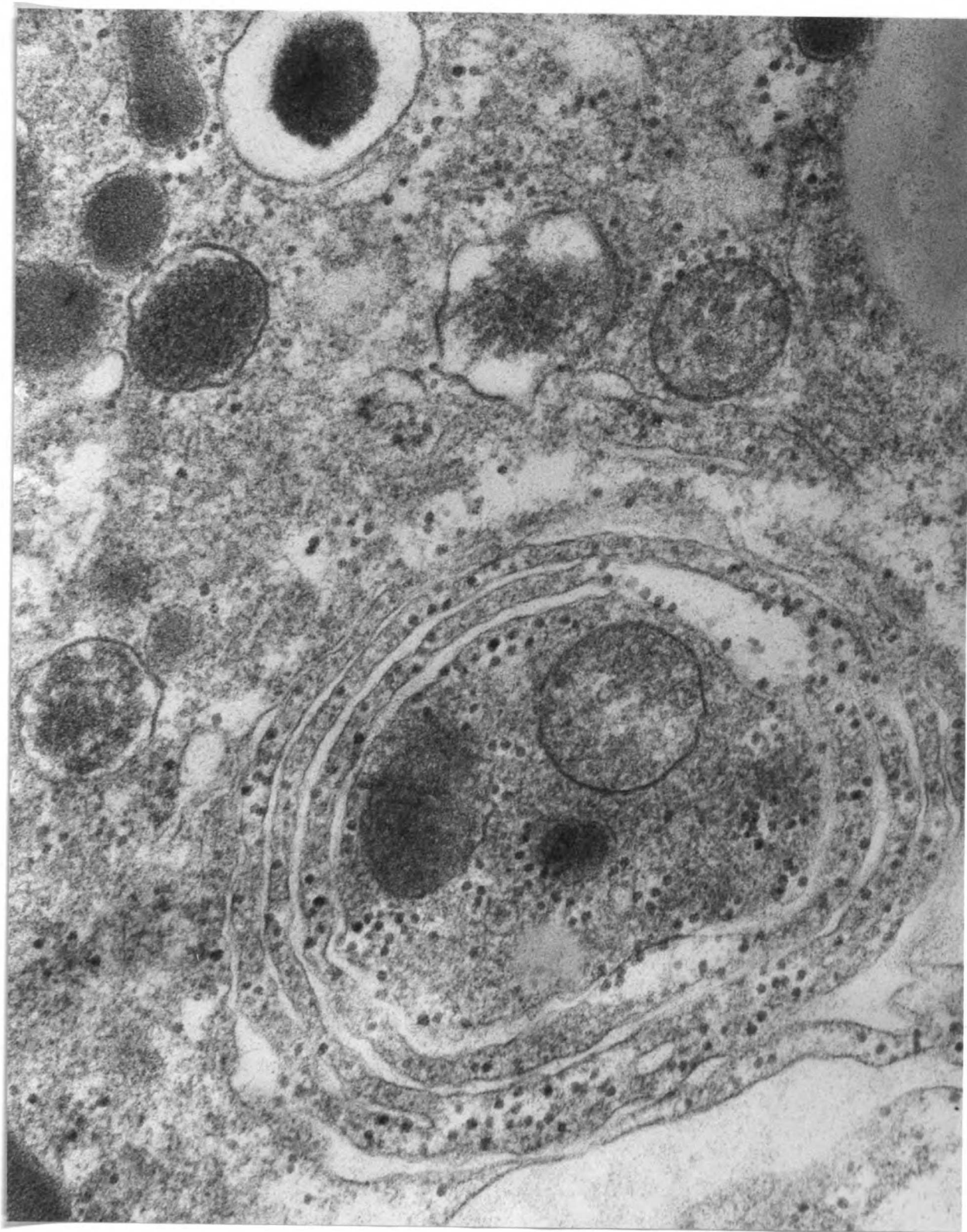


This electron micrograph shows Golgi complex (G)
which is made of flattened sacs radially arranged
surrounded with small vesicles and vacuoles.
Magnified (x 150,000)



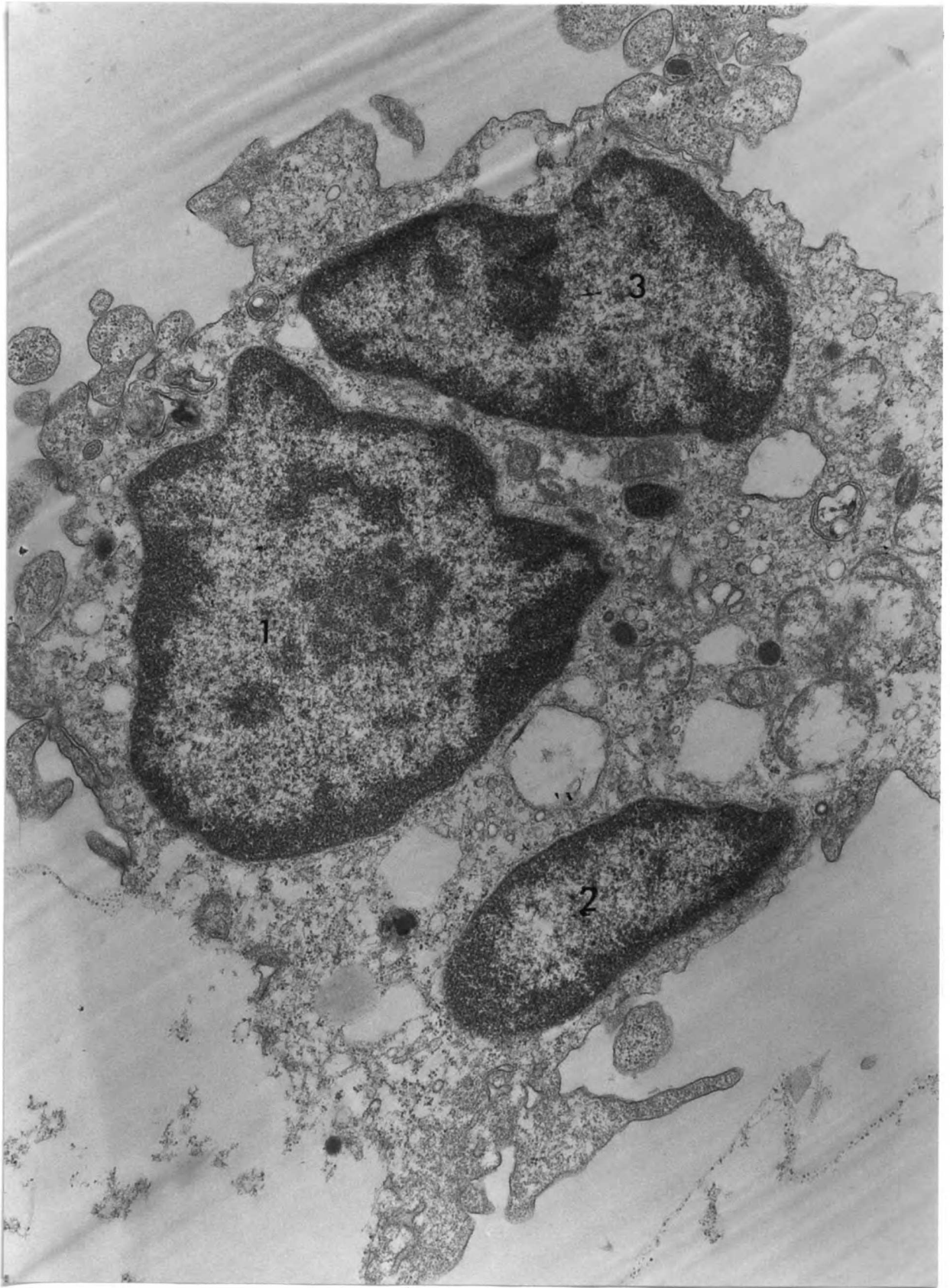
This electron micrograph shows the concentric
pattern of the rough endoplasmic reticulum.

Magnified (x 140,000)



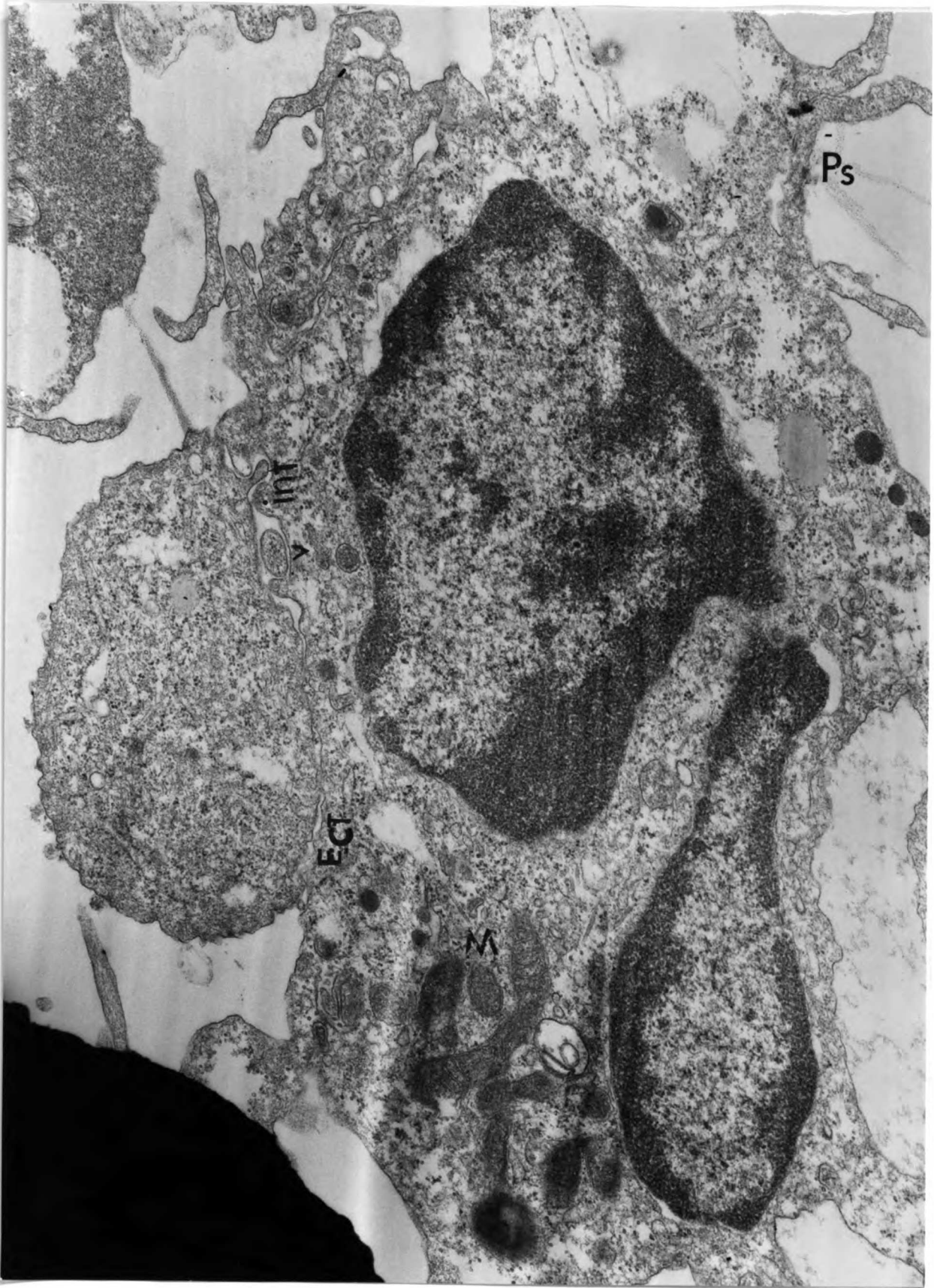
THE 120-MINUTE GROUP

This electron micrograph illustrates a multinucleated cell with 3 nuclei occupying the major portion of the cytoplasm. The nuclei are round to oval in shape with Endhromatin dominating the nucleus material. Magnified (x 50,000)



This electron micrograph illustrates the ectoplasmic interdigitation (Ect. Int.) between the cell and a cytoplasmic mass bound to join the cell.

Magnified (x 65,000)



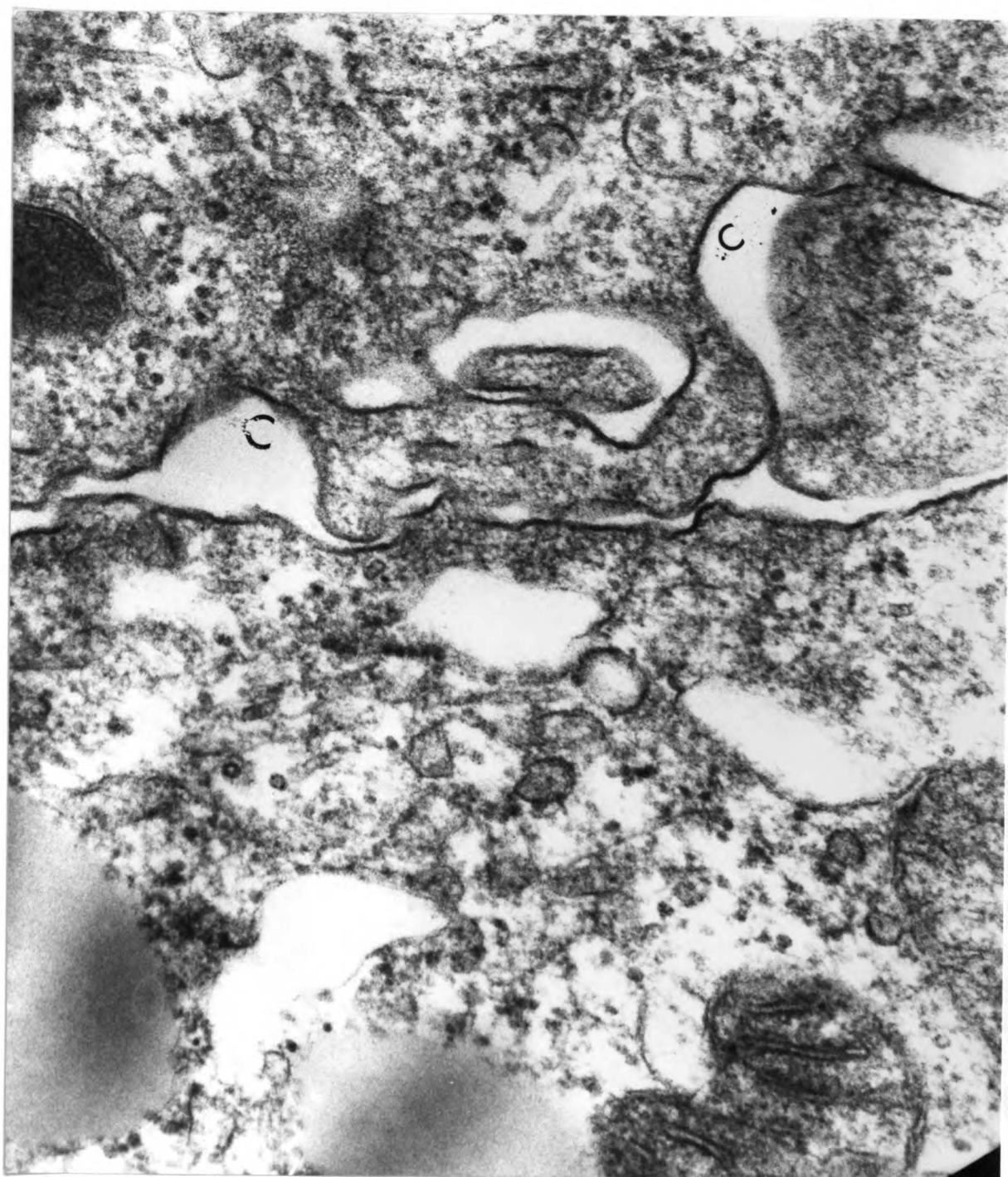
The electron micrograph illustrates marked interdigitation between 3 cells about to fuse, with breakage in the cell membrane in some areas.

Magnified (x 50,000)



The electron micrograph illustrates the dense zone described as caveola (Ca) in the plasma membrane.

Magnified (x 75,000)





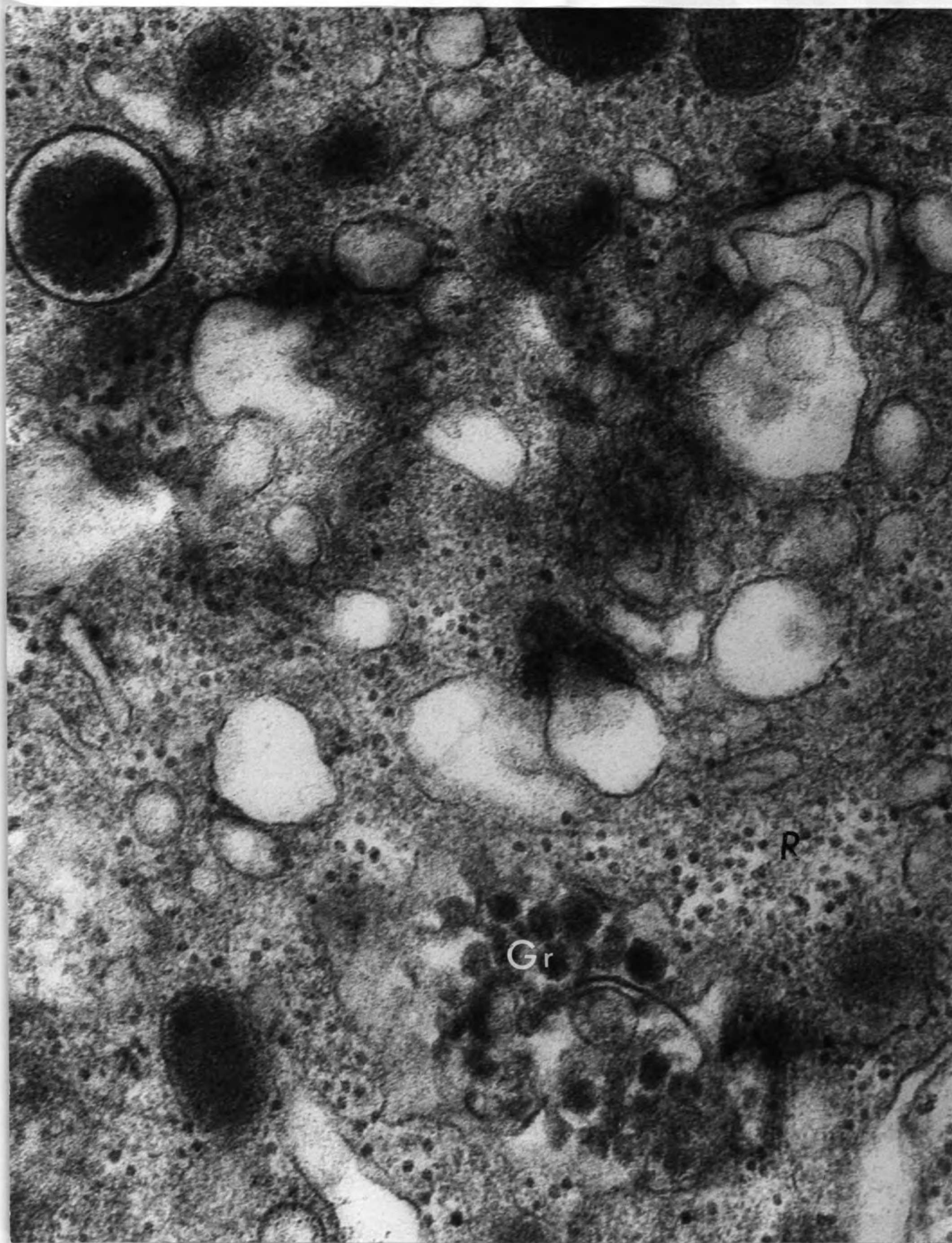
The electron micrograph illustrates evidence
of fusion between opposing cell membrane.

Magnified (x 75,000)



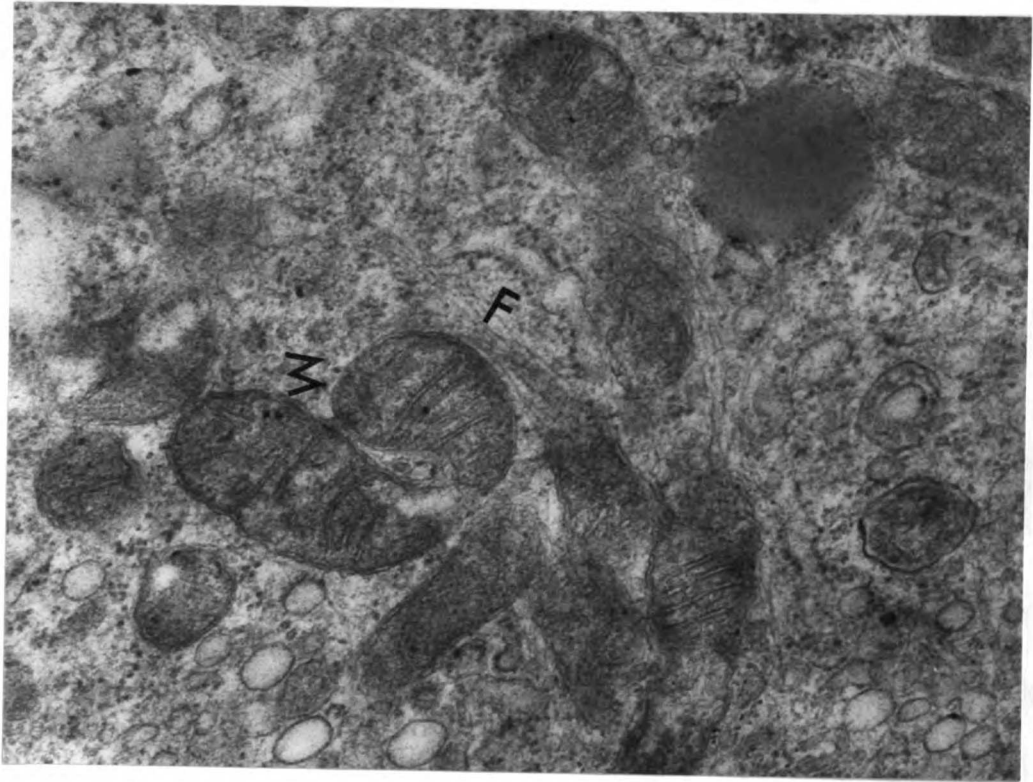
This electron micrograph shows electron dense lysosomal structures and granules (Gr), empty vacuoles and large electron dense free ribosomes (R) to be prominent in some of the cells at 120 minutes stage.

Magnified (x 80,000)



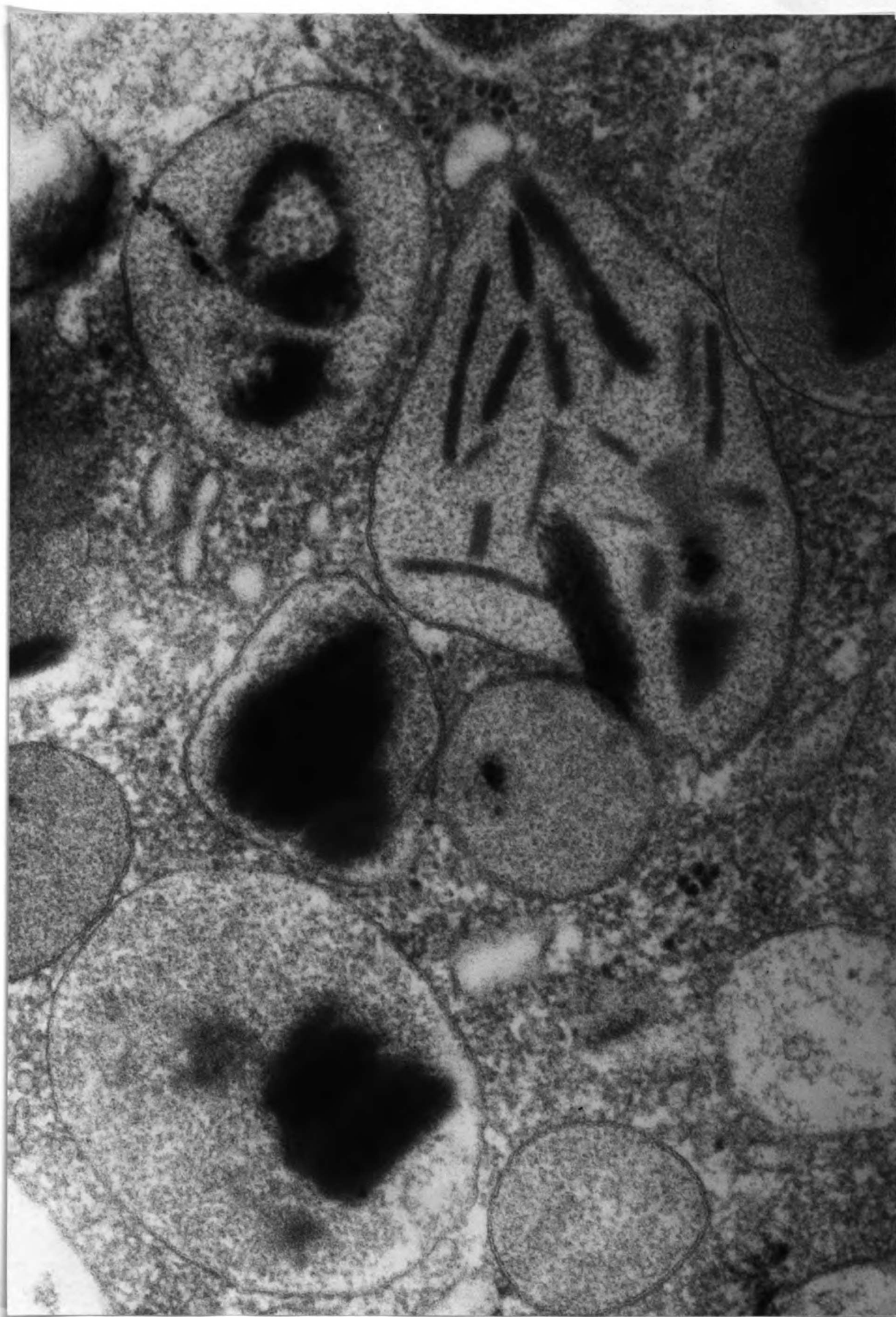
This electron micrograph shows cytoplasmic filaments (F) that appear juxtaposed to the outer mitochondrial (M) membrane probably for support purposes.

Magnified (x 80,000)



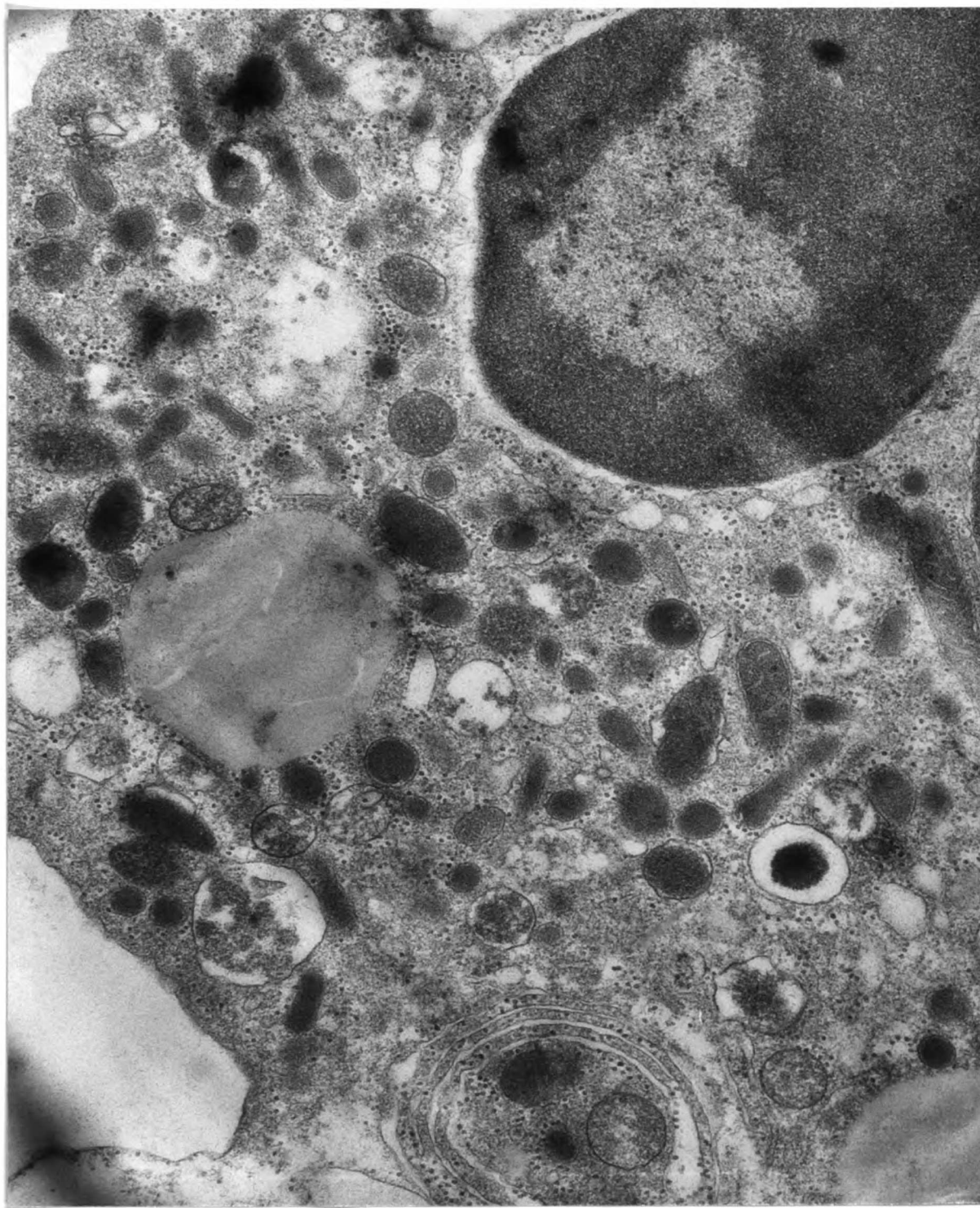
This electron micrograph shows varieties of phagocytic vacuoles containing an array of different particulates.

Magnified (x 90,000)



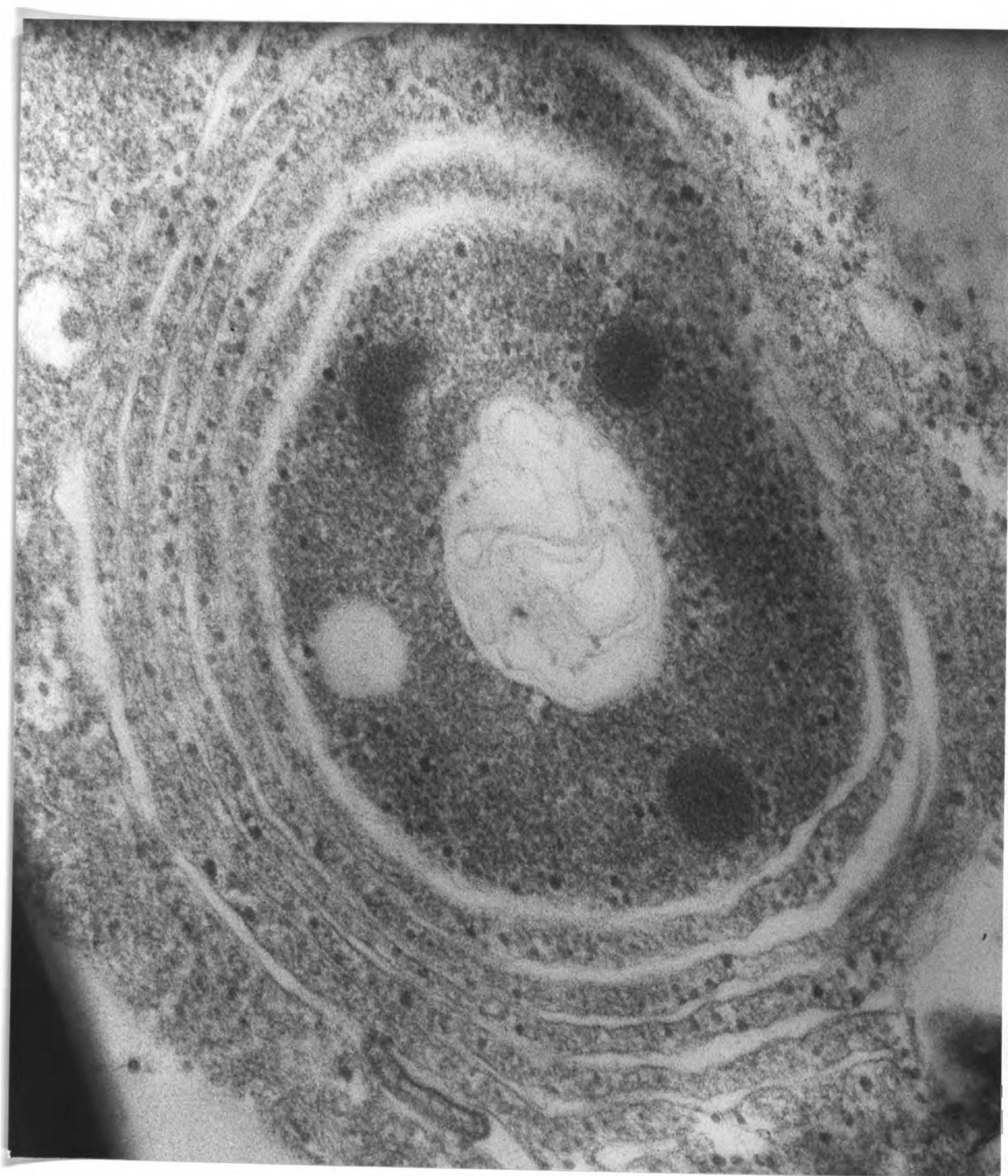
This electron micrograph shows the cytoplasm containing numerous electron dense lysosomal structures, a few large lipid droplets and the concentrically arranged endoplasmic reticulum studded with electron dense ribosomes.

Magnified (x 70,000)



This electron micrograph shows the rough
endoplasmic reticulum with phagocytic
vacuoles superimposed on it.

Magnified (x 150,000)

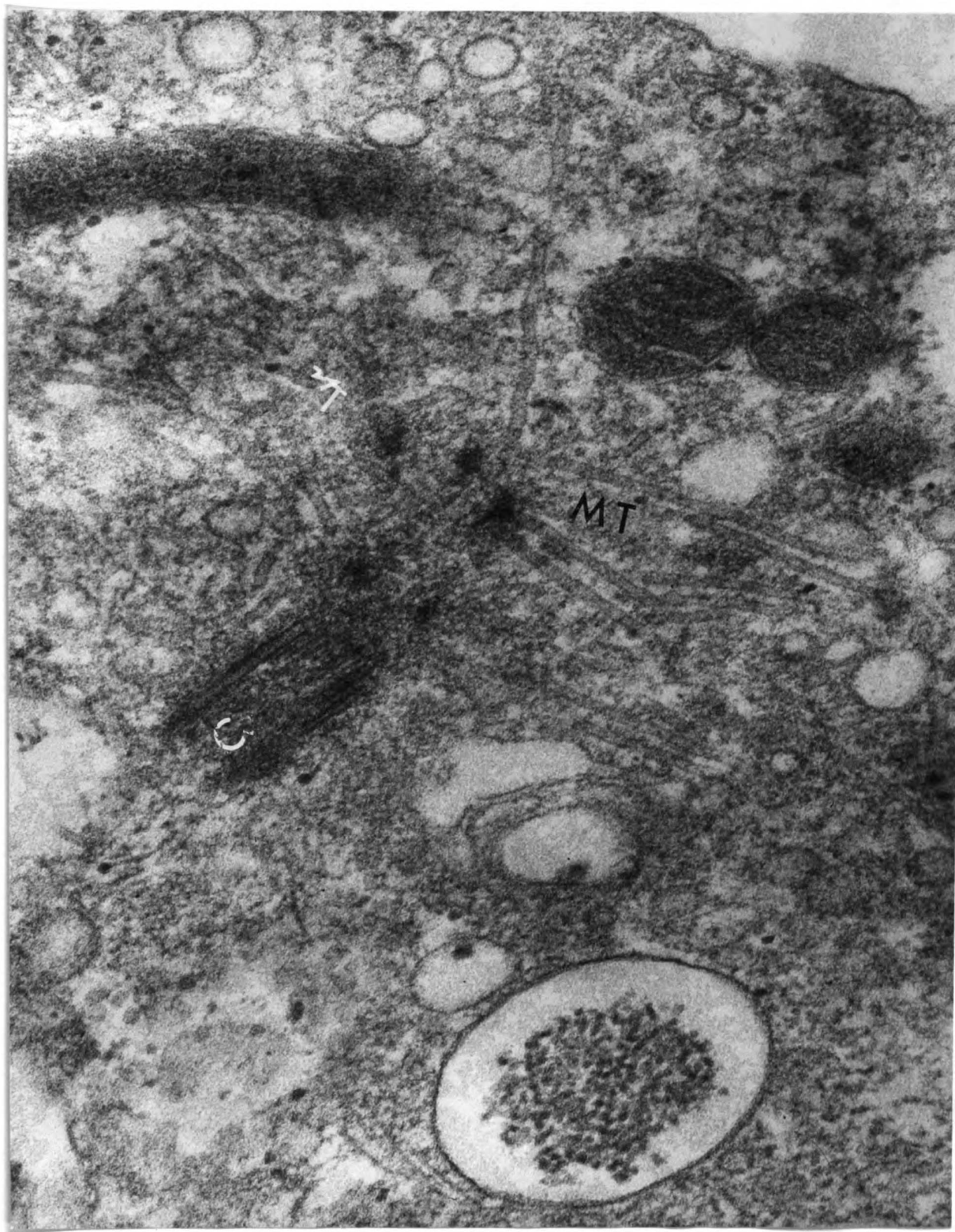


This electron micrograph shows another form of endoplasmic reticulum - long slender cisternae with flat parallel membranes contiguous in the plasma membrane.

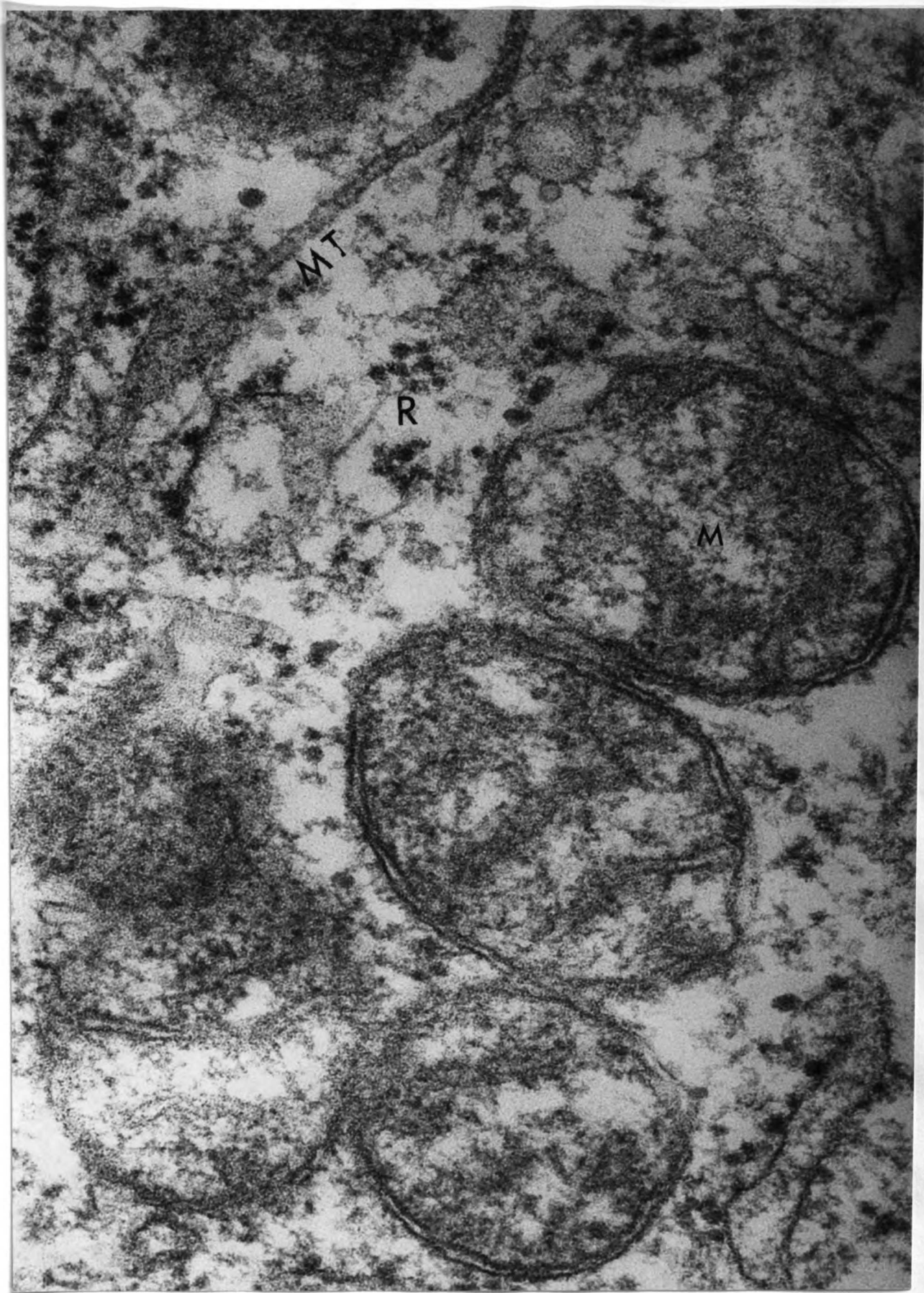
Magnified (x 65,000)



The electron micrograph illustrates abundant microtubules (MT) which seem to be emanating from the centrioles (C) forming a network of microtubules to support the cytoplasmic mass. Magnified (x 95,000)

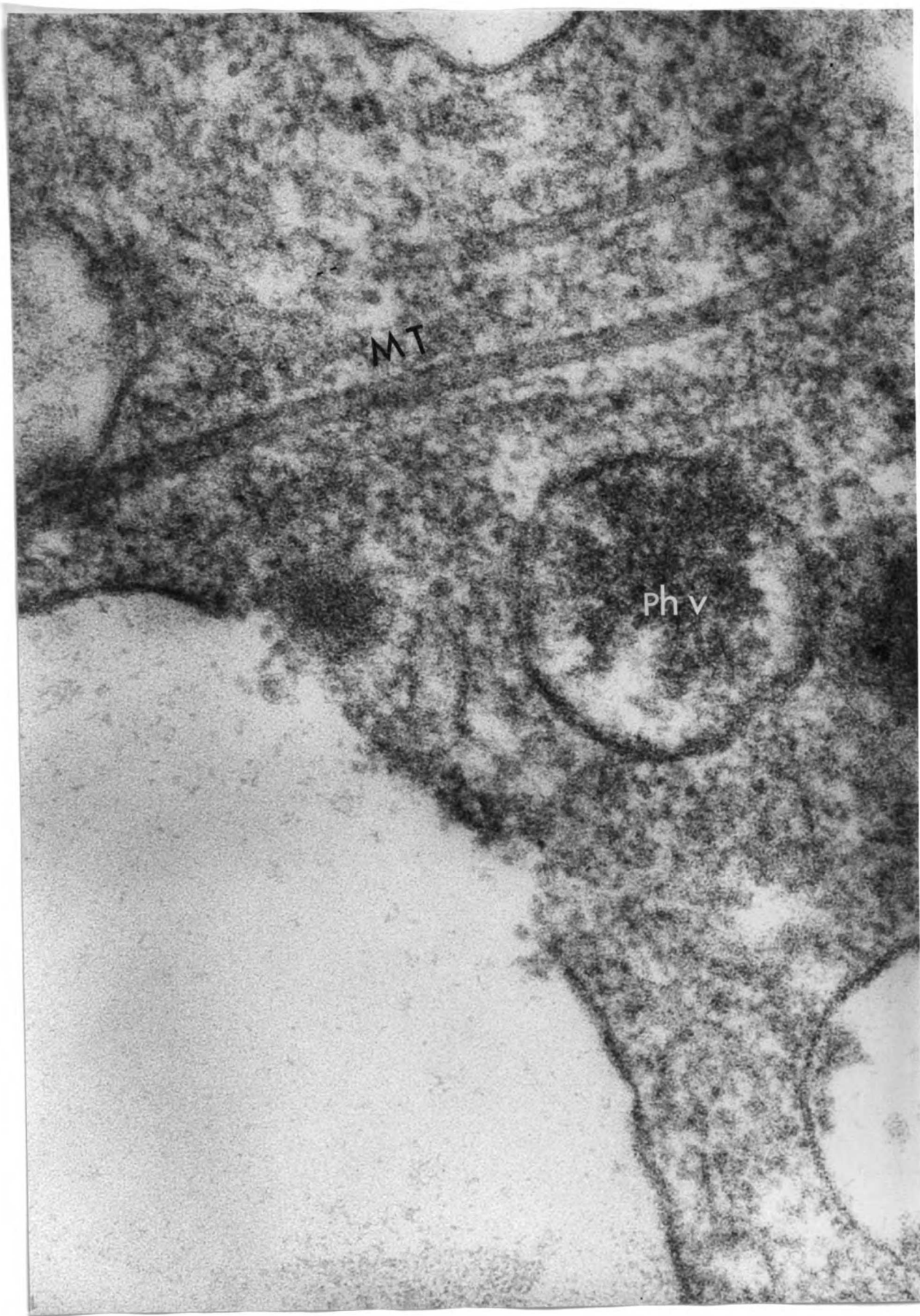


This electron micrograph illustrates
microtubules (MT), filaments, intact
mitochondria (M) and large ribosomes (R)
dominating the cytoplasm at this state.
Magnified (x 90,000)



The electron micrograph illustrates evidence of support to the phagocytic vacuoles (PhV) microtubules.

Magnified (x 120,000)



This electron micrograph illustrates
cytoplasmic filaments forming bundles and
running longitudinally close to the phagocytic
vacuoles.

Magnified (x 120,000)

