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THE ELECTRON MICROSCOPIC BASIS OF THE PATHOLOGY OF LEPROSY[1]

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Electron microscopy combined with ultra-thin sectioning has given us a new approach to the understanding of the interrelationships between *Mycobacterium leprae* and the various kinds of host cells in leprosy lesions. It is now possible to study the details of the infective processes at a practical resolving power of 30Å. With this technologic advancement of morphologic research, we are now faced with the problem of reestablishing the pathology of leprosy based on the new morphology of lesions.

In earlier papers ([22, 23, 24]) I have described the electron microscopic features of lepromatous lesions, both in the skin and in the peripheral nerve trunks. The present paper reports a continuation of that study, and here I have endeavored to elucidate the electron microscopic characteristics of various types of leprosy lesions.

MATERIALS AND METHODS

The tissues used in this study were 25 biopsy specimens, 16 from lepromatous skin lesions, 5 from tuberculoid skin lesions (2 reactional and 5 quiescent), 4 from lepromatous great auricular nerves, and 4 from tuberculoid great auricular nerves.

The specimens were fixed for 3 hours in 1 per cent osmium tetroxide (pH 7.2-7.4) buffered with phosphate buffer or veronal-acetate buffer. For the study of the bacillary morphology, some of the lepromatous specimens were fixed for 30 hours in this fixative. After fixation, the specimens were dehydrated in ethyl alcohol and embedded in 6:4 mixture of n-butyl and methyl methacrylate.

The Shimadzu microtome was used for ultra-thin sectioning. The electron microscopes used were the Akashi Tronscope and the Hitachi HU 10 model.

More than 1,000 electron micrographs of these lesions were made, and all were examined carefully for the detection of the general principles of the pathologic processes of leprosy at the electron optical level.

[1] An outline of this article was read at the VII International Congress of Leprology held at Tokyo in 1958 under the title, "The Differences Between Lepromatous and Tuberculoid Lesions of Leprosy as Observed with the Electron Microscope." Because of the limitation of time for reading the paper, I was unable to discuss the subject sufficiently. Here is presented a fuller description of my findings and ideas on the electron microscopic pathology of leprosy, illustrated with the necessary number of electron micrographs.

FINDINGS AND THEIR SIGNIFICANCE

A. FEATURES OF LEPROMATOUS SKIN LESIONS

1. *Bacilli in the cytoplasm of lepra cells.*—Most of the leprosy bacilli in the cytoplasm of lepra cells group together in a side-by-side arrangement. Very often they are embedded in opaque droplets which seem to correspond to the lipoid degeneration as observed with the light microscope.

As a rule, around the bacilli with electron-dense cytoplasm and distinct nuclei, there are only narrow electron-transparent zones (Fig. 1). On the other hand, around the old bacilli in which bipolar cytoplasmic condensation is distinct and no nuclear apparatus is visible, there are broader electron-transparent zones, which usually lead to intracytoplasmic foamy structures.

The nuclear apparatus of leprosy bacilli is considered to be the index of bacillary vitality ([26]), and the electron-transparent zones around the bacilli result from the metabolic activity during their life in the cytoplasm of lepra cells.

2. *Bacilli in the vascular endothelium.*—Leprosy bacilli are frequently found in the endothelium of the capillaries in the leproma (Fig. 4). In these cases electron-transparent zones and foamy structures have not as yet been observed.

3. *Varieties of opaque droplets.*—As described in former papers ([22, 25]), opaque droplets of various sizes are usually found in lepra cells, and, as the leprosy bacilli grow old, foam spaces appear in the opaque droplets, and finally intracytoplasmic foamy structures are produced.

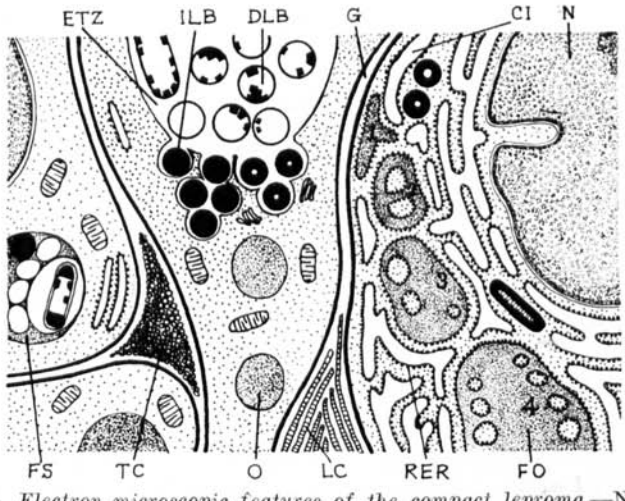
Besides the usual type of opaque droplets, there is another which I propose to designate the "fenestrated opaque droplets" (Fig. 3). Opaque droplets of this kind are found in the cells located in the neighborhood of capillaries in the leproma. These cells have well-developed rough-surfaced endoplasmic reticulum as is seen in plasma cells, but their nuclei have a homogeneous nucleoplasm. Leprosy bacilli are seen in these cells in small numbers, and in such cells which contain bacilli the opaque droplets assume a complicated fenestrated appearance. These fenestrated opaque droplets suggest that these may be formed by the accumulation of the electron-opaque material inside the cisternae of the endoplasmic reticulum as is shown in Text-fig. 1.

The cytoplasm of lepra cells.—In most of the lepra cells found in the leproma, the number of mitochondria is not as much increased as in the epithelioid cells of the reactional tuberculoid lesions which also contain some bacilli in their cytoplasm. A small amount of rough-surfaced endoplasmic reticulum is found in lepra cells, and sometimes it is gathered on one side of the cell, thus forming an organized ergastoplasm. Similar organized ergastoplasm was also observed in epithelioid cells of reactional tuberculoid lesions (Figs. 5, 7), and this fact seems to

suggest that both the lepra cell and the epithelioid cell are derived from the same kind of phagocyte.

Cells as described, packed with the rough-surfaced endoplasmic reticulum and containing a small number of bacilli, are also found in the vicinity of capillaries in the leproma.

In compact lepromas, most of the lepra cells lie side-by-side, parallel to each other, with a constant electron-transparent gap of about 200Å or with collagen fibers between them (Text-fig. 1). This arrangement of lepra cells suggests that the cytoplasmic movement of lepra cells in a compact leproma is quite restricted.



TEXT-FIG. 1. *Electron microscopic features of the compact leproma.*—Neighboring lepra cells are separated by an electron-transparent gap (**G**) of about 200Å, or by interposed collagen fibers (longitudinal section **LC**, and transverse section **TC**, are shown) between adjacent cell membranes. A typical foamy structure (**FS**) is seen in a lepra cell. In another lepra cell in the center of this diagram, ordinary opaque droplets (**O**) and transverse sections of leprosy bacilli are shown. Around intact bacilli (**ILB**) there are only narrow electron-transparent zones, whereas around degenerated bacilli (**DLB**) broader electron-transparent zones (**ETZ**) are seen. In a cell packed with rough-surfaced endoplasmic reticulum (**RER**), there are fenestrated opaque droplets (**FO**, 1-2-3-4). These are formed by the accumulation of electron-dense material inside the cisternae (**CI**) of the endoplasmic reticulum. There is a nucleus (**N**) in the right upper corner.

These findings in the usual lepra cells suggest a dormant state of the defense activity inside those cells.

5. *Extracellular environment of the lepra cells.* — Extracellular edema is seldom seen except in succulent lesions of the infiltrative leproma. Dissolution of the collagen fibers is usually not observed in lepromatous lesions. This finding may explain the presence of the subepidermal collagenous layers free from lepra cells which is commonly observed in lepromatous nodules.

Significance of lipoid material and transparent zones.—According to Mitsuda's opinion ([20]), the lipoid of the lepra cells of the skin lesions is derived from the bacilli, whereas that of the lepra cells in visceral

organs is produced by the biological reaction of the cells as a result of the invasion of the bacilli into the cell cytoplasm.

Artom -- quoted by de Souza and Alayon ([28]) and Campos ([5]) — thought that the bacilli determine "an enlivening of the lipopexic function" of the cells of the reticuloendothelial system, "with a consequent storage of fats and lipoid taken from tissue and internal medium." Ogata ([24]) insists that the lipoid of lepra cells is neutral fat taken from outside the cells.

Campos ([5]) considered that "the source of the lipoids is the leprosy bacillus itself." According to him, "the globi originate by the grouping of bacilli, possibly dead, from the bodies of which there is liberated the wax which was an important constituent of them."

Because of the limited resolving power of the light microscope, there seems to be some confusion in the past discussions of the derivation of the lipoid material of lepra cells.

In the electron micrographs of osmium-fixed lepromas, moderately electron-dense opaque droplets and electron-transparent zones can be distinctly distinguished. The size and number of opaque droplets are quite variable, and it appears as though they surround the groups of bacilli from outside by chance. It seems quite unlikely that the opaque droplets are derived directly from the bacilli. Seeing that the fenestrated opaque droplets are formed by the accumulation of electron-opaque material inside the cisternae of the endoplasmic reticulum, it seems most probable that those droplets may be lipoid material taken from the extracellular medium and afterwards accumulated around clumps of bacilli.

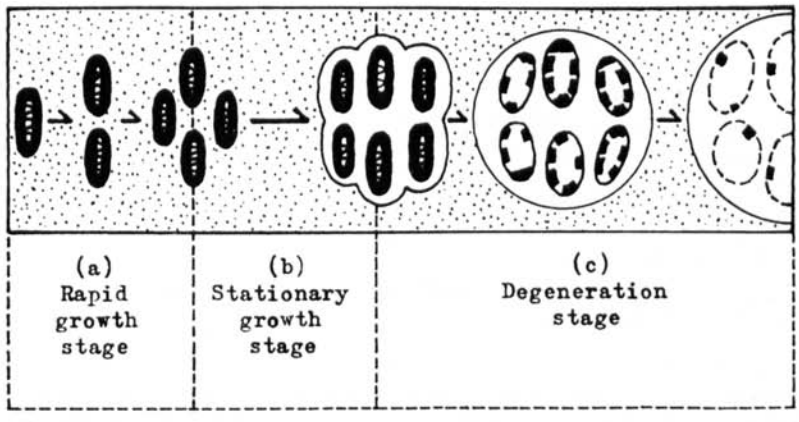
On the other hand, the electron-transparent zones around leprosy bacilli seem to be intimately related to the metabolism of the bacilli in the cytoplasm of the lepra cells. There seems to be a definite relationship between the degree of destruction of the bacillary cytoplasm of the bacilli and the quantity of the electron-transparent material around them; the more marked the destruction, the broader the electron-transparent zone (Fig. 1). This relationship is not a strict one, however, and exceptional cases can be found. But, still this relationship can be observed in most cases of lepromas, and I think the sequence of events may be described as follows, as illustrated in Text-fig. 2.

(*a*) When the bacilli are rapidly multiplying in the lepra cell, the quantity of electron-transparent material around them is not large, because it is not profusely produced under those circumstances.

(*b*) In the stationary stage of bacillary growth, a large amount of electron-transparent material appears to be produced around the bacilli.

(*c*) As a result of an excessive accumulation of electron-transparent material, which begins to interfere with the bacillary metabolism, death of the bacilli may occur. After their death, the destruction of bacillary cytoplasm and the dissolution of decomposing cytoplasm into the surrounding electron-transparent material may follow. I think it well to

designate this stage "the degeneration stage of *M. leprae.*" The cell walls of the bacilli are not broken down very quickly, so they remain very long in the electron-transparent material surrounding them, but finally they also are dissolved into that material.



TEXT-FIG. 2. Representing the relationship between the various stages of growth and deterioration of the leprosy bacillus and the electron-transparent zones. (The dotted background represents the cytoplasm of the lepra cell.)

B. FEATURES OF TUBERCULOID SKIN LESIONS

1. *Bacilli in the cytoplasm of epithelioid cells of the reactional lesions.*—Kooij ([16]) presented some electron micrographs of leprosy bacilli from tuberculoid lesions. However, as the pictures were of crushed tissue material, they gave no information concerning the interrelationship between the bacilli and the various tissue elements of the lesions.

In ultra-thin sections of a very early-stage reactional tuberculoid lesion (7 days after the onset of a tuberculoid macule), bacilli were seen in the cytoplasm of the epithelioid cells, embedded in less-electron-dense droplets (Fig. 8). Most of the intracellular bacilli were swollen, with accompanying fragmentation of their cytoplasm (Figs. 5, 6, 8). This swelling of the bacillary bodies and fragmentation of the cytoplasm may be the first step of the disintegration of the bacilli inside epithelioid cells. Bacilli appear to become fragmented in the epithelioid cells (Fig. 6), but the distinction between such bacillary fragments and lipid particles is often very difficult. Around these bacilli in epithelioid cells there is no electron-transparent zone such as is observed in lepra cells. The absence of that zone (Figs. 6, 8) seems to suggest the suppression of the metabolic activity of leprosy bacilli in the cytoplasm of epithelioid cells. Foamy structures as observed in lepra cells are not seen in epithelioid cells, except very small ones (Fig. 5).

2. *Lipoid material in the epithelioid cells.*—According to Azulay ([2]), lipoid is absent in reactional tuberculoid lesions. "From the moment a

reactional tuberculoid case becomes positive for lipoid'' he classifies the case as lepromatous, and this principle of his classification seems to explain very well why, in his extensive study of lipoid in various types of leprosy, lipoid was not observed in any of the reactional tuberculoid lesions examined.

De Souza and Alayon ([28]) found lipoid dust in the epithelioid cells of reactional tuberculoid cases. In all of his 7 cases of reactional tuberculoid Campos ([5]) found lipoid. According to his description, ''the lipoids occurred in the form of a very fine orange-colored dust, located in the cytoplasm of epithelioid cells. In no case were there large masses such as were seen in the lepromatous granuloma.''

As described, I found less-electron-dense opaque droplets in the cytoplasm of the epithelioid cells in the reactional tuberculoid lesion (Fig. 8), and I think that they correspond to the lipoid dust described by Campos and others. As my reactional tuberculoid case showed complete absorption of the lesions within one year, it is not possible that this case had transformed to lepromatous at the time the specimen was taken. Although the appearance of the fine lipoid material in the epithelioid cells in reactional tuberculoid lesions seems to indicate a slight deviation towards lepromatous nature, I think it should not be considered as a definitive deviation towards lepromatous, as Azulay holds.

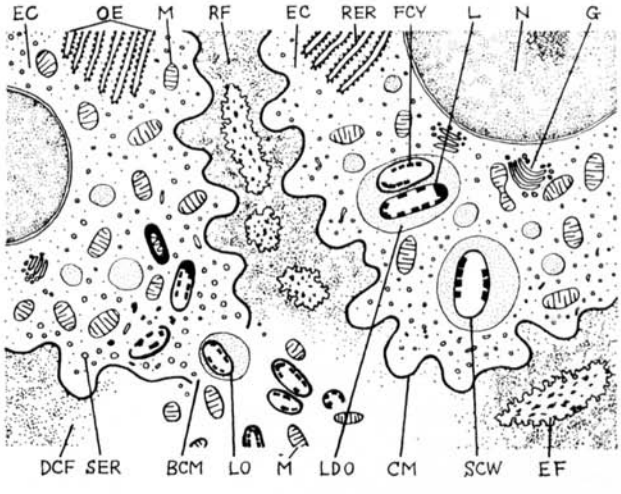
3. *Epithelioid cells of reactional tuberculoid lesions.*—The cell membranes of most of the epithelioid cells in the reactional tuberculoid lesions show undulating edges (Fig. 7), which suggest in appearance the wrinkling, or ruffling, of the cell membranes of epithelioid cells. On observation of a tissue culture of mouse muscle fibroblasts by the interference microscope, Abercrombie and Ambrose ([1]) found that pinocytosis was always connected with the ruffling motion of the cell membrane of those cells. Brandt ([4]) also described in his study of the mechanism of pinocytosis in amoebae, wrinkling of the plasmalemma immediately before pinocytosis. From these observations, I think that the undulating cell membrane of the epithelioid cells, and the less-electron-dense opaque droplets described, may indicate an active pinocytosis accompanying the initial phagocytic phase of epithelioid cells in reactional tuberculoid lesions.

The number of mitochondria is distinctly increased in the cytoplasm of epithelioid cells of the reactional tuberculoid lesion (Fig. 7). The organized ergastoplasm is seen on one side of the cytoplasm of epithelioid cells (Figs. 5, 7). Throughout the cytoplasm of such cells, smooth-surfaced endoplasmic reticulum (large vacuolar and small vesicular forms) is seen.

The above features seem to suggest a lively cytoplasmic response of the epithelioid cells against the bacilli phagocytosed in their cytoplasm. When the cytoplasmic response is too violent, or when the extracellular environment becomes too inadequate, for epithelioid cells their cell

membranes break down, and then various cytoplasmic components such as mitochondria, endoplasmic reticulum, and phagocytosed bacilli are thrown out into the extracellular environment (Text-fig. 3; Fig. 9). Extracellular bacilli are frequently found in the reactional tuberculoid lesion.

In the epithelioid cells of quiescent tuberculoid lesions, the number of mitochondria is not so large as in those of the reactional lesions. This suggests that the cytoplasmic response against leprosy bacilli is calmed down in the quiescent lesions as a result of the complete destruction of the bacilli in them.



TEXT-FIG. 3. *The reactional tuberculoid lesion of the skin.*—The epithelioid cell (**EC**) in this type of lesion is characterized by a markedly undulating cell membrane (**CM**), increased mitochondria (**M**), and smooth-surfaced endoplasmic reticulum (**SER**) of small vesicular form with diameters ranging from 60 mμ to 200 mμ packed in the cytoplasm. (Smooth-surfaced reticulums of large vacuolar form, with diameters ranging from 200 mμ to 450 mμ, are also observed.) Rough-surfaced endoplasmic reticulums (**RER**) which form an organized ergastoplasm (**OE**) on one side of the cell body are frequently seen. Leprosy bacilli (**L**) are embedded in less-electron-dense opaque droplets (**LDO**). The bacilli show swelling of their cell walls (**SCW**) and fragmentation of their cytoplasm (**FCY**).

From one of the epithelioid cells leprosy bacilli and mitochondria are being extruded into the extracellular environment through the broken cell membrane (**BCM**). The bacillus indicated (**LO**) is contained in a less-electron-dense opaque droplet.

In the extracellular environment there are observed transverse and oblique sections of elastic fibers (**EF**), which have electron-dense dots embedded in a homogeneous cement material. In some portions of the environment there is dissolution of collagen fibers (**DCF**), and in other places there is a thin feltwork of reticulin fibrils (**RF**).

4. *Extracellular environment of epithelioid cells.*—In the reactional tuberculoid lesions, dissolution of collagen fibers is quite often observed. Various cytoplasmic components scattered from the broken epithelioid cells infiltrate among the separated collagen bundles.

In both reactional and quiescent tuberculoid cases, characteristic speckled structures simulating the pattern of the leopard's skin were found in the extracellular environment (Fig. 10). In transverse sec-

tions they show an irregular peripheral margin, where they split up into smaller fibers. These structures are the elastic fibers of the extracellular environment, because the appearance of this speckled structure is the same as the elastic fibers presented in the electron micrographs of Dempsey and Lansing ([7]), Rhodin and Dalhamn ([27]), Yokota ([35]), and Karrer ([12]). From these findings the elastic fibers appear to be more stable than collagen fibers in the extracellular environment of tuberculoid lesions.

Comparison of the lepra cells of lepromatous lesions and the epithelioid cells of tuberculoid lesions.—As said by Campos ([5]), the morphologic differences between the Virchow lepra cell and the epithelioid cell are not definitive or irreversible. According to him "both cells arise from the same cell, the monocyte," and "they differ as regards the ability to destroy *M. leprae*, the microorganism being phagocytized by both of them but destroyed only by the epitheliloid cell."

According to Low and Freeman ([17]), the mitochondria of the monocyte are typically small, with an average diameter of 0.2 μ. In the cytoplasm of the monocyte there are abundant profiles of smooth-surfaced endoplasmic reticulum (small vesicular form), whereas rough-surfaced endoplasmic reticulum is never seen in them. Seeing that the rough-surfaced endoplasmic reticulum in the form of organized ergastoplasm is frequently observed in the epithelioid cells of the tuberculoid lesions, it cannot be easily concluded that epithelioid cells are derived from monocytes, as many authors have believed.

Although the derivation of the lepra cell and the epithelioid cell

TABLE 1.—*Differences between the lepra cell in the lepromatous lesion and the epithelioid cell in tuberculoid lesions as observed with the electron microscope.*

	Lepra cell (lepromatous)	Epithelioid cell (tuberculoid)
Cell membrane	Smooth line. Apposition with the cell membrane of neighboring lepra cells with constant gap of about 200Å. No breaking of the cell membrane in the compact leproma.	Wavy indented line. Apposition with neighboring epithelioid cells with irregular gaps. A broken cell membrane is frequently observed.
Leprosy bacilli	Many bacilli in the cytoplasm, in opaque droplets or in intracytoplasmic foamy structures.	Bacilli observed only in reactional tuberculoid and tuberculoid reactivation. In such cases small numbers of bacilli are wrapped in a limiting membrane together with less-electron-dense granular material. Extracellular bacilli are also found. Swelling of bacillary cell wall and fragmentation of bacillary cytoplasm, in both intracellular and extracellular bacilli.

TABLE 1. (*Continued*)

Opaque droplets	Moderately electron dense. Numerous. Large sized.	Similar droplets observed only in reactional tuberculoid lesions, less-electron-dense and of granular appearance. Always with limiting membrane. No opaque droplets in quiescent tuberculoid lesions.
Electron-transparent zone	Almost always present around bacilli. This may be the product of the metabolic activity of the bacilli.	Absent, suggesting suppression of metabolic activity of bacilli in the cytoplasm of epithelioid cells.
Foamy structure	Foamy structures develop in cytoplasm around bacilli and increase in size and number.	In reactional tuberculoid lesions only, small foamy structures seen in some of the epithelioid cells. Relation to bacilli obscure.
Mitochondria	Mitochondria not many, suggesting a sleeping stage of the defensive activity of the cytoplasm.	Number of mitochondria markedly increased. Suggests violent defensive activity of cytoplasm against the bacilli.
Endoplasmic reticulum	Small quantity of rough-surfaced endoplasmic reticulum observed.	Rough-surfaced reticulum accumulates on one side of epithelioid cell, forming an organized ergastoplasm. In reactional tuberculoid lesions, the content of smooth-surfaced endoplasmic reticulum (large vacuolar form) shows slightly increased electron density. Endoplasmic reticulum (small vasicular form) increased throughout the cytoplasm.
Golgi complex	Seldom observed except in early stage of lepra cells, with only few bacilli in cytoplasm.	Frequently observed. Golgi vacuoles not dilated.
Extracellular edema	No edema in compact leproma. Slight edema in succulent loose lesions.	Edema is a constant feature of reactional tuberculoid lesions.
Collagen and elastic fibers	Normal collagen fibers around lepra cells. All the fibers retain fibrillar structure and axial periodicity.	Collagen bundles separated and collagen fibers partly dissolved. Elastic fibers persist in the extracellular environment.
Fate of the cell	After maximum growth of the foamy structures, the lepra cell may break down and the foamy structures accumulate in the lesion.	When the reaction of an epithelioid cell is too violent, or the physicochemical condition of the extracellular medium becomes unfavorable, the cell membrane is broken down and bacilli are thrown out of the cells. Various cytoplasmic organelles are scattered in the extracellular environment.

from the monocyte is problematic, it seems quite probable that both kinds of cells are derived from the same kind of phagocyte. As a new approach to the understanding of the cellular resistance against leprosy bacilli, it seems rewarding to compare the ultrastructure of the lepra cells of the lepromatous lesion and that of the epithelioid cells of the reactional tuberculoid lesion.

Location of the antibacterial factors in the tuberculoid lesions.— Based on the histopathologic finding that bacilli disappear from the reactional tuberculoid lesions soon after their initial flare up, many workers have thought that some bacteriolytic substances active against leprosy bacilli may exist in the epithelioid cells of the tuberculoid lesions.

Investigations are now being made by various authors on the intracytoplasmic bactericidal substance active against various microorganisms other than *M. leprae*. Hirsch ([10]) found a bactericidal substance in the cytoplasm of polymorphonuclear leukocytes of the rabbit which is active against enteric bacilli, and named it phagocytin. According to him this phagocytin is not active against mycobacteria. More interesting results were obtained from the experiments of Fishman and his coworkers ([8, 9]). They examined the location of the bactericidal substance in the rat leukocytes which is active against Gram-positive and Gram-negative organisms, and found that the bactericidal activity of mitochondrial extracts is by far the strongest as compared with that of nuclear, microsomal and soluble fractions. According to them the bactericidal substance in the mitochondrial extracts appears to be a lipid complex, possibly a lipoprotein.

Regarding the actual mechanism of the disintegration of leprosy bacilli in the cytoplasm of epithelioid cells, information at the electron optical level is quite limited, and nothing definite can be said about it. However, it seems very probable that the bacilli become swollen, fragmented and dissolved in the epithelioid cells, because many degenerated bacilli with fragmented bacillary cytoplasm are observed even in an early phase of reactional tuberculoid lesions. The experiment of Fishman and associates on the bactericidal activity of mitochondrial extract may have a bearing on the relationship between the bactericidal activity of the epithelioid cells and the increase of the number of mitochondria in those cells during the active phase of reactional tuberculoid lesions.

Another approach towards the problem of the bactericidal factor against leprosy bacilli in tuberculoid lesions is the opinion of Suter ([29]) that the phagocytes loaded with leprosy bacilli in the tuberculoid lesions are destroyed as a result of a high degree of cellular sensitivity and the bacilli are exposed to an unfavorable extracellular environment. This opinion of Suter was fully confirmed in the electron micrographs of the reactional tuberculoid lesions in which some epithelioid cells have

shown destruction of the cell membrane. In such cases bacilli were found extracellularly, together with various cytoplasmic components extruded through the broken portion of the cell membrane (Text-fig. 3). The exposure of these extracellular bacilli to the natural inhibitor in the tissue fluid could also play an important role in the mechanism of the bacillary disintegration.

No data are available, however, to determine which of these two factors, i.e., the intracytoplasmic bactericidal substance or the extracellular natural inhibitor, plays the more important role in the defense mechanism of the host tissue. It seems probable that these two factors cooperate with each other in the defense activity of the host tissue against leprosy bacilli.

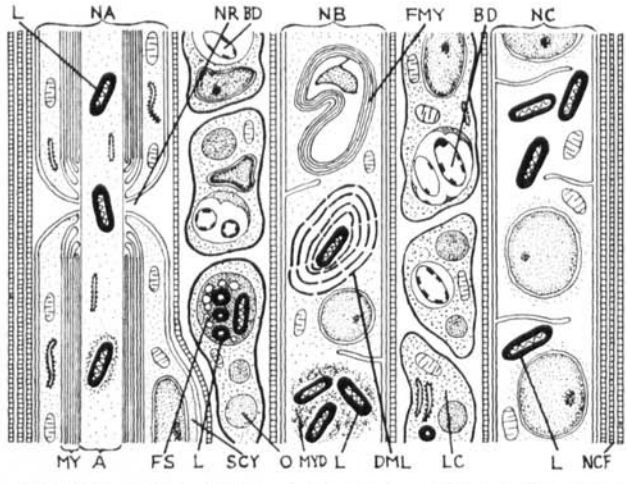
C. FEATURES OF LEPROMATOUS LESIONS

1. *Bacilli in the axoplasm.*—Leprosy bacilli in the axon lie naked or embedded in irregular-shaped electron-dense masses ([22]). I have not yet examined the ganglion cells of leprosy cases with the electron microscope, but according to the work of Mitsuda ([18]) lipid materials together with bacilli were seen in the cytoplasm of various ganglion cells of lepromatous cases. From the finding in electron micrographs of bacilli in the axon, I feel that some bacilli are already covered with lipoid material when they are inside the axon, and that they are transported centripetally to the cytoplasm of the ganglion cells, where they form foamy structures as are observed in light microscope studies. On the other hand, when the bacilli inside the axon are involved in the Wallerian degeneration of the nerve fibers, the lipoid material which surrounds them in that location is gradually digested, together with the myelin sheath by the digestive activity of the Schwann cells. The bacilli themselves, however, resist the lipid-digesting power of the Schwann cell and remain in the cytoplasm of those cells even after the complete disappearance of the axon and myelin sheath debris (Text-fig. 4).

2. *Bacilli in the cytoplasm of Schwann cells of the cord of Büngner stage.*—The Schwann cells ingest leprosy bacilli by a peculiar mode of phagocytosis. As already described in detail ([22]), Schwann cells can receive bacilli through axons, and I think that mode of phagocytosis might be designated "passive phagocytosis." Whether there is an actual phagocytosis by the Schwann cell, in which it actively takes up bacilli floating in the extracellular medium, is not clearly understood at present, but this problem will be clarified by future tissue-culture experiments.

Most of the bacilli in the cytoplasm of the Schwann cells lie naked in the cytoplasm, separated from each other (Fig. 11). They usually have electron-dense cytoplasm, and a distinct nuclear apparatus. Degenerated bacilli have never been encountered in Schwann cells in the materials examined in this study.

Neither the electron-transparent zone nor the opaque droplet is observed around the bacilli in the cytoplasm of Schwann cells of the cord of Büngner stage. Only very small foamy structures, with diameters



TEXT-FIG. 4. *The lepromatous lesion of the nerve.*—Three nerve fibers (**NA, NB, NC**) are shown in the different stages of nerve involvement in lepromatous leprosy. In fiber **NA**, leprosy bacilli (**L**) are seen inside the axon (**A**). The myelin sheath (**MY**) of this fiber is still intact. The cytoplasm of the two neighboring Schwann cells (**SCY**) is separated at a node of Ranvier (**NR**), and for this reason bacilli can be transported centripetally in the fiber only through the axon. In fiber **NB**, folded myelin sheath (**FMY**) and decomposing myelin lamellae (**DML**) are seen, which are the early changes of Wallerian degeneration of nerve fibers. Bacilli are seen in the cytoplasm of the Schwann cell, surrounded by decomposing myelin debris (**MYD**). Fiber **NC** is a cord of Büngner in which axon and myelin sheath have already disappeared, and only Schwann cells remain. Bacilli are seen in the cytoplasm of the Schwann cells, in which they lie naked, separated from each other but not in opaque droplets. Bacilli found in nerve fibers seem to be intact, and no degenerated forms have been found in Schwann cells in the materials examined in this study.

Endoneurial spaces are packed with lepra cells (**LC**), in which many bacilli are within opaque droplets (**O**) or in foamy structures (**FS**). Various stages of bacillary degeneration (**BD**) can be observed in these lepra cells of the endoneurial spaces. Neurilemmal (**NCF**) and endoneurial collagen fibers are seen in the extracellular environment. Edema is seldom observed in lepromatous nerve lesions.

less than 0.7 μ, were observed in a few Schwann cells (Fig. 11). In the course of Wallerian degeneration of nerve fibers there is a stage in which some amount of homogeneous lipid material is seen in the cytoplasm of Schwann cells. It is, however, a very interesting finding that such homogeneous lipid inside Schwann cells does not surround leprosy bacilli except in rare chance arrangement, and this does not lead to the formation of large foamy structures inside the Schwann cells. Seeing that the Schwann cell digests the myelin sheath in the course of Wallerian degeneration, it seems that the lipid material cannot remain very long in the Schwann cells of the cord of the Büngner stage. It may be due to this lipid-digesting ability of the Schwann cell that large foamy structures are never formed inside them. In order to produce a large the electron micrographs, but in the histopathologic specimens of the

intracytoplasmic foamy structure, the bacilli must first be enclosed by an opaque droplet, and then they must have active metabolic activity, as in lepra cells which produce large quantities of electron-transparent material around the bacillary bodies. Therefore, the lack of the large foamy structure and electron-transparent zones means that the bacilli in Schwann cells live in a very low state of metabolic activity, as in a refrigerator. Thus they can be stored here for a very long time without loss of vitality.

3. *Bacilli in lepra cells in the endoneurial spaces.*—The electron microscopic features of lepra cells in the endoneurial spaces of the lepromatous nerve lesions are the same as those of the lepra cells in the skin (Fig. 12). Bacilli are frequently embedded in opaque droplets, and advanced foamy structures are often observed.

4. *Collagen fibers of the neurilemma and endoneurium.* — In the lepromatous nerve lesions the neurilemmal and endoneurial collagen fibers are not dissolved, and extracellular edema is not observed. Even when degenerating nerve fibers are embedded in the endoneurial lepromatous lesions, the cords of Büngner are not destroyed by the lepra cells, and the regeneration of axon and myelin sheath is frequently observed ([22]).

D. FEATURES OF TUBERCULOID NERVE LESIONS

The tissue changes of the tuberculoid lesions of the nerve trunks can be divided as follows: (1) zone of Wallerian degeneration; (2) zone of epithelioid tubercle formation; (3) zone of bionecrosis; and (4) zone of necrosis. The electron microscopic characteristics of these zones are described.

1. *Zone of Wallerian degeneration.*—As a result of the Wallerian degeneration of the nerve fibers, many cords of Büngner are seen (Fig. 13). Leprosy bacilli have not been found in these cords of Büngner in two cases of tuberculoid leprosy, bacilli were found in the Schwann cells of the Büngner cords by light microscopy.

Whether or not the spinal ganglion cells and their axons show active phagocytic activity in tuberculoid and neural cases of leprosy is not well known, although bacilli have been found in nerve fibers of the maculoanesthetic macules ([13]) and in spinal ganglia of neural cases ([19]) by light microscopic studies of some workers. However, when one considers the fact that nerve involvement is most prominent in the tuberculoid and neural types of leprosy, there seems to be some hidden phagocytic activity of axon and ganglion cells, the evidence of which is obscured by the severe destruction of the nerve elements due to the tuberculoid change in the endoneurial spaces. The fact that bacilli are found in the Schwann cells of the cord of Büngner in tuberculoid and neural cases supports the hypothesis that they had been previously phagocy-

tosed by the axon, remaining in the Schwann cells after the destruction of the axon.

2. *Zone of epithelioid tubercle formation.*—Besides the epithelioid tubercles, edema and destruction of the neurilemmal collagen fibers and the cords of Büngner are the characteristics of this zone (Fig. 14).

3. *Zone of bionecrosis.*—In this zone there are large cells which contain many lipid droplets and onion-like bodies (Figs. 15-17). Among these large cells there are some necrotic masses derived from the debris of the broken cells (Fig. 16).

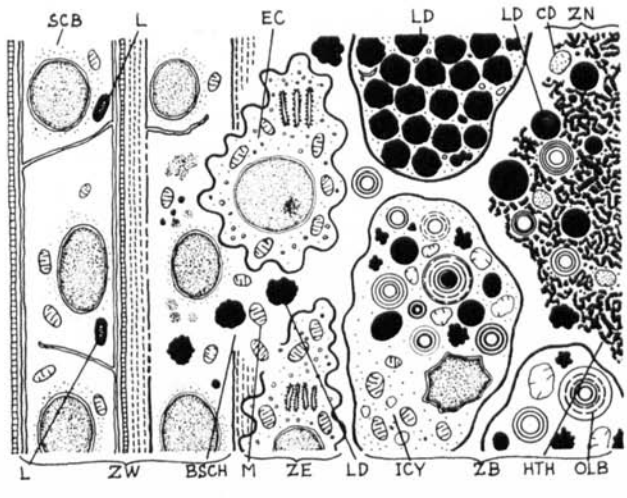
Laminated round bodies are frequently encountered in various types of cells in both normal and degenerated conditions other than leprosy. Kish's "plasmosomes" ([15]), observed in the large alveolar cells, consisted of laminated or tubular elements. Policard and his associates ([26]) named them osmiophilic bodies and considered them to be derived from mitochondria. Cedergren ([6]) examined macrophages infected by tubercle bacilli and found three types of granules in their cytoplasm: A, B, and C granules. The A granule is described as a rounded granule with a finely-granular ground substance. The B granules are rounded clusters of osmiophilic granules, 50-100Å in size. C granules are "a more complicated type of granules about 0.5 to 1 μ," consisting of "a great number of peripheral concentrically layered membranes, approximately 100Å in width and a medulla consisting of small osmiophilic granules." He believed that these granules represent the early stage of the destruction of cells. Although only few such granules can be seen in normal lung-tissue cells, he thought that, normally, cells are to a certain extent degenerating and dying, and that in the infected tissue, where the infecting agent is located in the cell proper, this degeneration is proceeding rapidly and intensively, thus giving rise to very obvious alterations.

Onion-like bodies found in the tuberculoid nerve lesions of leprosy resemble to a certain extent the plasmosome, or the C granules of alveolar cells. Concerning the derivation of the onion-like bodies, two possibilities may be postulated. (*a*) Onion-like bodies may be the remnants of the myelin globules liberated from the degenerating nerve fibers into the extracellular environment by the destruction of the surface membrane of the Schwann cell and ingested in the macrophages of the endoneurial spaces. In an electron microscopic study of Wallerian degeneration, Ohmi ([25]) found small numbers of myelin globules in the macrophages in the later stage of the process, whereas most of the myelin globules were found inside the Schwann cells. In the sections of tuberculoid nerve lesions which have shown the presence of these onion-like bodies, the myelin debris in the remaining Schwann cells of the cord of Büngner had been completely digested and no myelin globules were seen in the Büngner cord or in its immediate vicinity (Fig. 13). If

onion-like bodies were the remaining myelin globules ingested by macrophages, their further disintegration is supposed to have been stopped in the bionecrotic lesion of the peripheral nerve. Many of the onion-like bodies and electron-dense lipid droplets are also observed extracellularly.

(*b*) Most of the intracellular onion-like bodies, however, seem to have been produced in the cytoplasm of macrophages. Similar to the observations on the infected macrophages in lesions of experimental tuberculosis, the production of the onion-like bodies inside the macrophages in the tuberculoid nerve lesions of leprosy seems probable. Some of the bionecrotic cells have shown a partial lamellar change of their cytoplasm, while the rest of the cytoplasm remained relatively intact (Fig. 15), and this means that the lamellar change occurs inside the cytoplasm of the bionecrotic cells.

It is essential to examine both possibilities carefully in further studies of the nerve lesions of tuberculoid leprosy. In any event, these



TEXT-FIG. 5. *The tuberculoid lesion of the nerve.*—The four zones of the tuberculoid lesion of the peripheral nerve trunk (see text) are illustrated here. The zone of Wallerian degeneration (**ZW**) consists of many Schwann cells in the cord of Büngner stage (**SCB**), which sometimes contain small numbers of leprosy bacilli (**L**). (Although bacilli were not observed in Schwann cells of the tuberculoid nerve lesion by electron microscopy, they are frequently found there by light microscopy. For this reason small numbers of bacilli have been drawn inside Schwann cells in this diagram.)

In the zone of epithelioid tubercle formation (**ZE**), epithelioid cells (**EC**) are seen. In this zone many Schwann cells of the cord of Büngner stage are in the course of breaking up (**BSCH**), and in such cases lipid droplets (**LD**) and mitochondria (**M**) of Schwann cells are thrown out into the extracellular environment.

In the zone of bionecrosis (**ZB**), there are large cells packed with onion-like bodies (**OLB**). In some of these cells, a part of the cytoplasm remains relatively intact (**ICY**), while other parts of the cell are changed into piles of the lamellar (onion-like) structures. Cells with lipid droplets (**LD**) are also observed in this zone.

In the zone of necrosis (**ZN**), there are complicated agglomerates of closely coiled helicoidal threads (**HTH**), which form a peculiar network of marked electron density. Lipid droplets (**LD**) and various elements of cellular debris (**CD**) are also observed.

onion-like bodies are the characteristic feature of the bionecrotic part of the tuberculoid nerve lesion. Such bodies are never found in tuberculoid skin lesions, or in any of the lepromatous lesions.

4. *Zone of necrosis.*—In this zone, intricate debris of various cytoplasmic components and nucleoplasm form a kind of complicated thick network of varying electron density (Fig. 18).

The most characteristic necrotic mass is composed of agglomerates of distinctly electron-dense fine threads (150Å threads forming a coil of 600Å diameter). They are coiled together closely, and they present a characteristic appearance in the necrotic lesion (Text-fig. 5). Among these electron-dense coil masses there are many lipid droplets, and debris of the various cellular components.

As a whole the appearance of the necrotic tuberculoid lesion in the peripheral nerves differs very much from that of the tuberculoid skin lesions, where the remnants of various cytoplasmic components found extracellularly retain their original structure relatively well and can be identified very easily.

From these findings, it is quite evident that the cellular destruction is more severe in tuberculoid nerve lesions, whereas in lepromatous nerve lesions cellular destruction, except Wallerian degeneration of nerve fibers, is not observed.

NEUROTROPIC AFFINITY OF THE LEPROSY BACILLUS

1. *The mode of entrance of bacilli into the axon.* — Not much is known at the electron optical level about the problem of how leprosy bacilli enter into nerve fibers. In 1951 Terada ([30]) proposed a hypothesis on the route of entry of the bacilli. Based on his electron micrographs, he reported that the nerve fiber ramifies into "superneurofibrillae," and that "leprosy bacilli were found embedded in the plexus of these superneurofibrillae or entwined among them," and he concluded that "the leprosy bacilli advance into the nerve fiber by way of the bundles of superneurofibrillae."

The "superneurofibrillae" of Terada are about 600Å wide and have an axial repeating period of about 600-700Å. But, according to a recent study of Vial ([31]), the neurofilaments have an axial periodicity of about 200Å with diameters ranging from 100 to 200Å. The differences of the diameters and the axial periodicities of "superneurofibrillae" and "neurofilaments" suggest that the former are actually the neurilemmal collagen fibers or the collagen fibers of the leproma which have an axial periodicity of 640Å. Terada worked with crushed material of human lepromatous nodules, and it seems very difficult to construct from such material any theory on the route of entry of *M. leprae* into the nerve fiber. However, this hypothesis stimulated us to an investigation of the fundamental relationship between the bacilli and nerve elements.

The electron microscopic analysis of ultra-thin sections of leprosy lesions of the peripheral nerves has provided us a clearer understanding of the pathologic process in the nerve lesions. Based on the findings in the lepromatous nerve lesions, I have presented in a previous paper ([22]) a hypothesis that leprosy bacilli are ingested into the axoplasm by the phagocytic activity of the growth cones of the regenerating axons, and are transported toward the spinal ganglion cell body by the centripetal stream of the axoplasm.

In 1951 Khanolkar ([13]) expressed the opinion, based on his microscopic studies, that "the microorganisms seem to have a predilection for migration towards degenerating and regenerating nerve fibrils and the bacilli tend to travel towards the finest nerve twigs in the superficial nerve plexus."

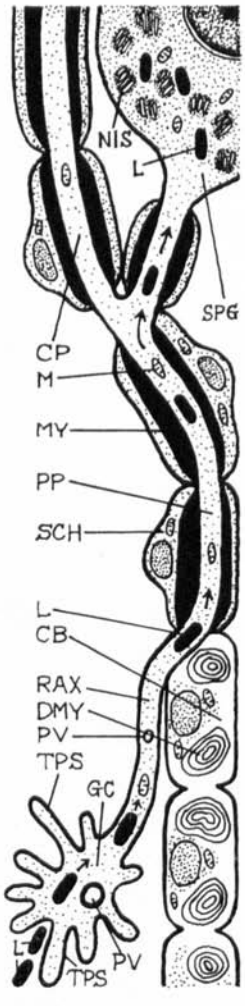
Regenerating axons are frequently observed by light microscopy inside the Schwann cell tube as quite thin threads ([33]), and some authors ([3]) consider that the regenerating axons grow forward inside the cord of Büngner. When examined with the electron microscope, however, there is always a mesaxon connecting the regenerating axon and the surface membrane of the Schwann cell. This mesaxon is evidence that the regenerating axons had been migrating outside the Schwann cells before they were infolded by these cells. The growing tip of the regenerating axon migrates freely in the body fluid until they reach their final position in the skin, although the entire length of the regenerating axon behind the growing tip is infolded by Schwann cells subsequently. It seems to be in this free and naked stage of the regenerating axons that they engulf leprosy bacilli in the skin.

According to Hughes ([11]), "fine pseudopodial processes in constant movement are extended from the terminal expansion" at the tip of a living embryonic neurite, and sometimes "an undulating membrane" is also observed which is "comparable to that of a macrophage." Although quite hypothetical at present, I think it may be an essential property of the ganglion cells that they ingest leprosy bacilli into their cytoplasm, stretching their long arms (axons) and catching the bacilli with their active hands—the growing tips of the regenerating axons. Seeing that nerve degeneration and regeneration are constantly taking place even in normal skin, as shown in the study of Weddell and Glees ([32]), there seem to be many opportunities for the regenerating axons to catch and engulf leprosy bacilli into their axoplasm during their migratory phase in the skin. Nonencapsulated afferent endings which terminate in connective tissue may have a similar phagocytic activity toward leprosy bacilli, if they show a similar movement as that of the growing tip of the regenerating nerve.

2. *Migration of bacilli inside the nerve fiber.*—Based on light microscopic studies, Khanolkar ([14]) has clearly foreseen that leprosy bacilli

travel upwards in the axon. This opinion was confirmed by our electron optical study of the lepromatous nerve trunks ([22]) in which leprosy bacilli were found just inside the axon.

The migration of the bacilli inside the axon must be examined from



TEXT-FIG. 6. *Mode of entrance of the bacilli into axon and spinal ganglion cell.*—This is a hypothetical diagram, the symbols of which signify: **CB**, cord of Büngner; **CP**, central process; **DMY**, decomposing myelin lamellae; **GC**, growth cone; **L**, leprosy bacillus; **M**, mitochondria; **MY**, myelin sheath; **NIS**, Nissl body; **PP**, peripheral process; **PV**, pinocytotic vacuole; **RAX**, regenerating axon; **SCH**, Schwann cell cytoplasm; **SPG**, spinal ganglion cell; **TPS**, terminal pseudopodia.

the standpoint of the behavior of the host cells (ganglion cells, including axons), because it seems unlikely that they swim up-stream in the axoplasm by themselves, like tadpoles. Seeing that the pinocytotic vesicles move centripetally in cinematographs of the regenerating axons ([11, 21]), there seems to be a circulation of the axoplasm in the axon. If leprosy bacilli ingested by the axoplasm are not carried away to a wider space of the axoplasm (i.e., the cytoplasm of the ganglion cell), they might disturb the circulation of the axoplasm and thus cause Wallerian degeneration of the peripheral part of the axon in a few days.

Perhaps it may be the necessary defense process of the axons themselves (a part of the ganglion cell) that they transport the phagocytosed bacilli to a more wide space in the cytoplasm (ganglion cell body) so as to minimize the damage of the axons. The bacilli which could not be transported centripetally and become involved in the Wallerian degeneration of the axons remain in the Schwann cell cytoplasm after the absorption of axon and myelin sheath, as described. I feel that we must concentrate more of our attention on the behavior of ganglion cells and axons, because these ganglion cells and axons seem to be the real lovers of *M. leprae*, which they appear to ingest recklessly. The behavior of the Schwann cells seems to be a rather passive one, as they appear to receive bacilli from the axon which they are infolding in their cytoplasm. However, the bacilli appear to live very long in the Schwann cells, in both lepromatous and tuberculoid lesions of leprosy, and for this reason Schwann cells also play a very important role in the evolution of the pathologic process of leprosy.

CONCLUSION

The electron microscopic features of various leprosy lesions having been described and discussed, I should like to conclude this article with a general view of various phases of the leprosy bacillus in the human body. They show the following three phases, according to the different attitudes of the various host cells.

(*a*) *Hibernation phase.*—The hibernation phase of the leprosy bacillus can be observed in Schwann cells of the cords of Büngner in both lepromatous and tuberculoid leprosy. Those cells seem to act as a biological refrigerator for the bacilli in both types of the disease. In lepromatous nerve trunks the bacilli so located were always morphologically intact, with distinct bacillary nucleus and bacillary cytoplasm. Degenerated bacilli were never encountered in Schwann cells, nor were large foamy structures observed. Here, in the Schwann cells, leprosy bacilli live at a low metabolic level but they can live very long without loss of vitality.

(*b*) *Vigorous multiplication-and-degeneration phase.*—This phase is observed in lepromatous tissues. The lepra cells of these lesions are considered to be a biological incubator for the growth of the bacilli. Here they mutiply, but they also degenerate rapidly after vigorous multiplication. The degeneration is caused by the accumulation of large amounts of electron-transparent material around the bacillary bodies which interferes with bacillary metabolism, kills the bacilli, and causes the dissolution of the bacterial nucleus and cytoplasm.

(*c*) Disintegration phase.—This phase is observed in reactional tuberculoid and tuberculoid reactivation lesions. The epithelioid cells of these lesions are considered to be a kind of biological sterilizer against the bacilli. The bacilli appear to be destroyed in those cells, but extra-

cellular bacilli are also frequently observed. Both intracellular and extracellular bactericidal factors seem to cooperate in the defense mechanism of the tuberculoid tissues.

SUMMARY

Fundamental ultra-structural characteristics of lepromatous and tuberculoid leprosy lesions, in both the skin and the peripheral nerve trunks, have been elucidated by electron microscopy.

There are various gradations in the development of typical foamy structures in the lepra cells of the lepromatous lesions. When the bacilli are multiplying rapidly, there is only a very thin electron-transparent zone around each of them. In the stationary growth stage, thick electron-transparent zones appear around the bacillary bodies. Very often clumps of bacilli in the cytoplasm are embedded in opaque droplets. In such cases, the electron-transparent material takes the shape of foam floating in the opaque droplet, and finally forms a typical mature foamy structure in the cytoplasm of the lepra cells. Another kind of opaque droplet which is found in the cells packed with rough-surfaced endoplasmic reticulum has been designated "fenestrated opaque droplet." This variety of opaque droplet can be easily distinguished from the ordinary foamy structure by the presence of microsomes in every window of the opaque droplet.

Epithelioid cells of the reactional lesions of tuberculoid leprosy are characterized by an increase of mitochondria in their cytoplasm. The bacilli found in the epithelioid cells of such lesions have swollen cell walls detached from the fragmented bacillary cytoplasm. Some of the bacilli lie in less-electron-dense opaque droplets, but the electron-transparent zone is not found around these disintegrating bacilli. The bacilli seem to be broken up in epithelioid cells. The bactericidal activity in the reactional tuberculoid lesions seems to be related to the increase of the mitochondria in the epithelioid cells. In quiescent tuberculoid lesions, the mitochondria of the epithelioid cells are not much increased.

In lepromatous lesions of the peripheral nerve trunks, bacilli are to be found in the axons, in Schwann cells of the cord of Büngner, and in lepra cells in the endoneurial spaces. In all of the bacilli in the Schwann cells referred to the nuclear apparatus is distinct, the bacillary cytoplasm is homogeneous, and the bacillary cell wall is not detached from the cytoplasm. No degenerated bacilli were found in the Schwann cells. On the other hand, in lepra cells many bacilli are degenerated, with swollen cell walls, fragmented cytoplasm, and no distinct nuclear apparatus. This difference suggests that the leprosy bacilli in Schwann cells are living in a state of hibernation at a very low metabolic level, while those in lepra cells are multiplying rapidly and also degenerating rapidly.

In tuberculoid lesions of the peripheral nerve trunks, epithelioid

cells seem to destroy the nerve fibers, and there are many broken Schwann cells of the cord of Büngner stage. In the bionecrotic zone of these lesions, many large cells packed with lamellar onion-like bodies are found. These onion-like bodies have been observed, so far, only in the nerve tuberculoid lesions and not in lepromatous lesions of either the skin or the nerves. The chief component of the necrotic zone of the tuberculoid nerve lesion is an electron-dense helicoidal thread which forms a complicated network.

Electron micrographs of nerve lesions of leprosy show that leprosy bacilli enter into the axon, and their entrance into the axoplasm seems to be effected by the phagocytic activity of the growth cones of the regenerating axons. The Schwann cells of the cord of Büngner which harbor bacilli in their cytoplasm seem to receive them from the axons. The Schwann cells play an important role in the evolution of the pathologic processes of leprosy.

RESUMEN

Por medio de la electronomicroscopia se han dilucidado, tanto en la piel como en los troncos nerviosos periféricos, las características ultraestructurales fundamentales de las lesiones de la lepra lepromatosa y la tuberculoidea.

Existen varios grados en la formación de típicas estructuras espumosas en las células leprosas de las lesiones lepromatosas. Cuando se multiplican con rapidez los bacilos, no hay más que una delgadísima zona electrono-transparente alrededor de cada uno de ellos. En el período de crecimiento estacionario, aparecen gruesas zonas electrono-transparentes alrededor de los cuerpos bacilares. Muy a menudo se implantan grupos de bacilos en el citoplasma en gotillas opacas. En esos casos, las materias electrono-transparentes toman la forma de espuma flotante en la gotilla opaca, creando por fin un típico cuerpo espumosos maduro en el citoplasma de las células leprosas. Otra clase de gotilla opaca que se encuentra en las células henchidas del retículo endoplásmico de superficie áspera ha sido denominada "gotilla opaca fenestrada." Esta variedad de gotilla opaca puede ser diferenciada fácilmente del ordinario tejido espumoso por la presencia de microsomas en todas las ventanillas de la gotilla opaca.

Las células epitelioideas de las lesiones reaccionales de la lepra tuberculoidea se caracterizan por un aumento de mitocondrias en su citoplasma. Los bacilos descubiertos en dichas células muestran paredes celulares, desprendidas del citoplasma bacilar fragmentado. Algunos de los bacilos reposan en gotillas opacas menos espesas electrónicamente, pero no se observa la zona electrono-transparente alrededor de estos bacilos en vías de desintegración. Los bacilos parecen disgregarse en las células epitelioideas. La actividad bactericida de las lesiones tuberculoideas reaccionales se relaciona aparentemente con el aumento de mitocondrias en las células epitelioideas. En las lesiones tuberculoideas quiescentes, no aumentan mayor cosa las mitocondrias de dichas células.

En las lesiones lepromatosas de los troncos nerviosos periféricos, se encontrarán bacilos en los cilindros-ejes, en las células de Schwann del cordón (banda) de Büngner y en las células leprosas de los espacios endoneuriales. En todos los bacilos de las mencionadas células de Schwann, el aparato nuclear aparece bien definido, el citoplasma bacilar es homogéneo y la pared de la célula bacilar no se halla desprendida del citoplasma. No se observaron bacilos degenerados en las células de Schwann. En cambio, en las células leprosas muchos bacilos están degenerados, y hay paredes celulares infladas y citoplasma fragmentado, sin aparato nuclear bien definido. Sugiere esta diferencia que los bacilos leprosos de las células de Schwann viven en un estado invernal a un bajísimo nivel metabólico, mientras que los de las células leprosas se multiplican rápidamente y también degeneran rápidamente.

En las lesiones tuberculoideas de los troncos nerviosos periféricod, las células epitelioideas parecen destruir las fibras nerviosas y hay muchas células de Schwann

desintegradas que corresponden al período de la banda de Büngner. En la zona bionecrótica de esas lesiones, obsérvanse muchas células grandes de cuerpos laminares parecidos a cebollas. Hasta ahora, no se han observado estos cuerpos cebolloideos más que en las lesiones tuberculoideas de los nervios y no en las lesiones lepromatosas ya de la piel o de los nervios. El principal componente de la zona necrótica de la lesión nerviosa tuberculoidea es una hebra helicoidea electrono-espesa que forma una red complicada.

Las micrografías electrónicas de las lesiones nerviosas de la lepra demuestran que los bacilos leprosos penetran en el cilindro-eje y que su penetración en el axoplasma parece efectuarse por virtud de la actividad fagocítica de los conos proliferantes de los cilindros-ejes en vías de regeneración. Las células de Schwann del cordón de Büngner albergan bacilos en su citoplasma parecen recibirlos de los cilindros-ejes. Las células de Schwann desempeñan un importante papel en la evolución de los procesos patológicos de la lepra.

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PLATE 1

FIG. 1. Ultra-thin section of a leproma. The relationship between the quantity of electron-transparent material and the degree of bacillary degeneration can be clearly seen in this picture. Around intact bacilli there is very little electron-transparent material, whereas around degenerated bacilli larger amounts are seen. In the cytoplasm of lepra cells opaque droplets and mitochondria are observed. Magnification 14,300×.

Symbols: **ILB**, intact leprosy bacillus; **DLB**, degenerated leprosy bacillus; **ETZ**, electron-transparent zone; **M**, mitochondria; **O**, opaque droplet.

PLATE 2

FIG. 2. In the cytoplasm of a lepra cell a large opaque droplet is seen which contains one leprosy bacillus. The compact arrangement of the lepra cells together implies limited cytoplasmic movement of those cells. The number of mitochondria is not large, as compared with those in epithelioid cells of tuberculoid lesions. This suggests a dormant state of the defensive mechanism of the cytoplasm of lepra cells against leprosy bacilli. Magnification 16,200×.

Symbols: **L**, leprosy bacillus; **O**, opaque droplet; **M**, mitochondria; **RER**, rough-surfaced endoplasmic reticulum; **N**, nucleus.

PLATE 3

FIG. 3. In the neighborhood of capillaries there are frequently found cells which are packed with rough-surfaced endoplasmic reticulum. They also contain leprosy bacilli. In these cells opaque droplets assume a peculiar fenestrated appearance. This appearance suggests that the opaque material has accumulated in the cisternae of endoplasmic reticulum (Text-fig. 1). Fenestrated opaque droplets can be easily distinguished from ordinary foamy structures by the presence of microsomes in each window. Magnification 19,000×.

Symbols: **FO**, fenestrated opaque droplets; **RER**, rough-surfaced endoplasmic reticulum; **L**, leprosy bacillus; **N**, nucleus.

PLATE 4

FIG. 4. Showing a transverse section of a capillary in a leproma. Bacilli are seen in the cytoplasm of endothelial cells of the capillary, but no foamy structure. Around the capillary there are many cells loaded with bacilli and small fenestrated opaque droplets. Magnification 9,500×.

Symbols: **END**, endothelial cell of the capillary; **L**, leprosy bacillus; **FO**, fenestrated opaque droplet; **N**, nucleus; **M**, mitochondria.

PLATE 5

FIG. 5. Ultra-thin section of epithelioid cells in a reactional tuberculoid lesion which was biopsied 7 days after the lesion started. In the cytoplasm of an epithelioid cell, some of the bacilli are embedded in a small quantity of opaque-droplet material, and a very small foamy structure is seen. In the lower part of epithelioid cell there are endoplasmic reticulums arranged in the form of an organized ergastoplasm. Such organized ergastoplasms accumulated on one side of the cytoplasm are very frequently encountered in the epithelioid cells. Numbers of mitochondria and smooth-surfaced endoplasmic reticulum (small vesicles) fill the whole cytoplasm of epithelioid cells. Magnification 10,600×.

Symbols: **L**, leprosy bacillus; **SF**, small foamy structure; **M**, mitochondria; **OER**, organized ergastoplasm; **SER**, smooth-surfaced endoplasmic reticulum; **N**, nucleus.

PLATE 6

FIG. 6. Ultra-thin section of a reactional tuberculoid lesion. Leprosy bacillus, many mitochondria, and granular electron dense materials (some of which seem to be bacillary fragments) fill the cytoplasm of epithelioid cells. Some of the bacilli show swelling of the bacillary cell wall and fragmentation of the bacillary cytoplasm. These bacilli seem to be disintegrating. Magnification 8,900×.

Symbols: **L**, leprosy bacillus; **G**, granular electron-dense material; **M**, mitochondria; **N**, nucleus.

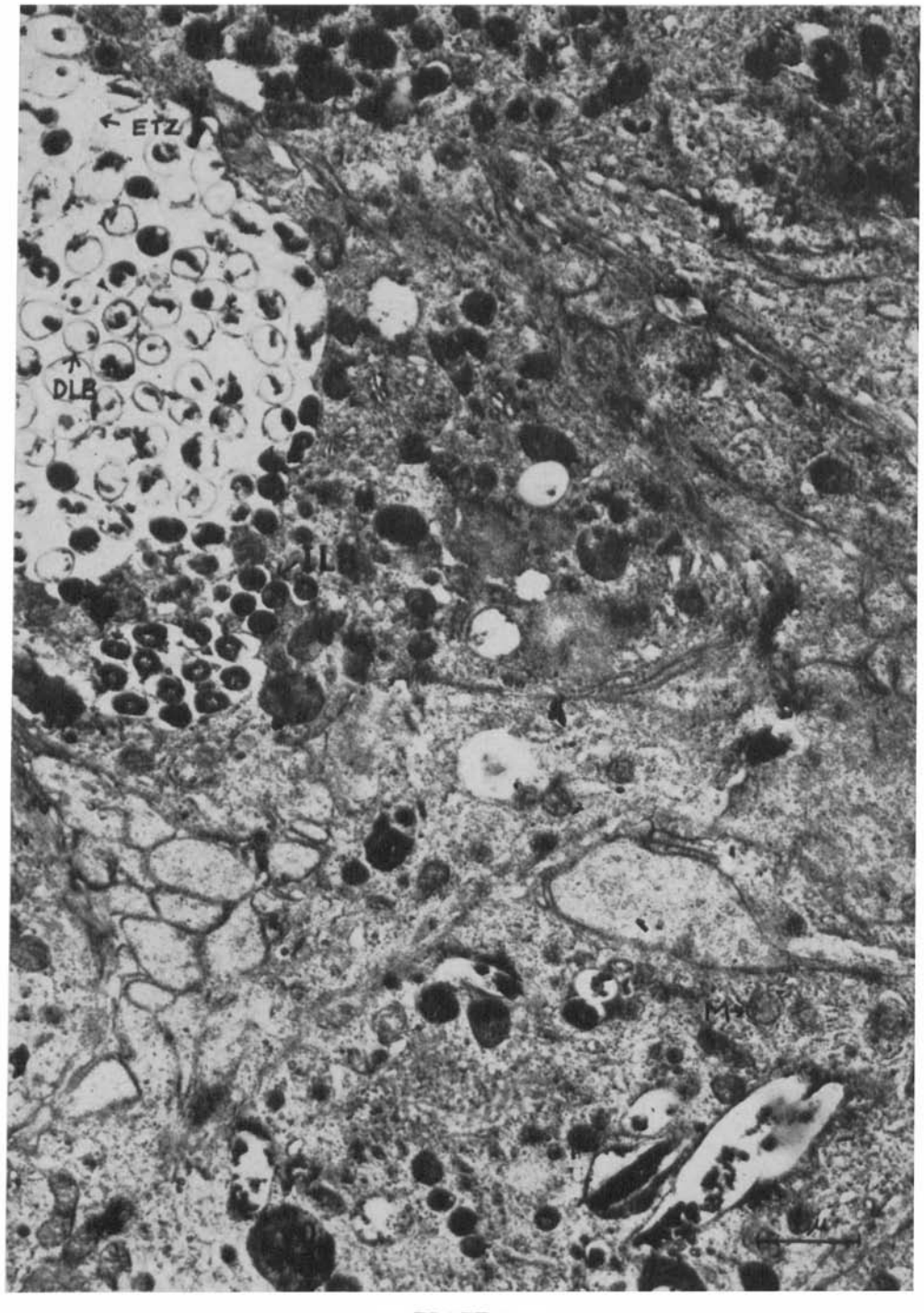


PLATE 1

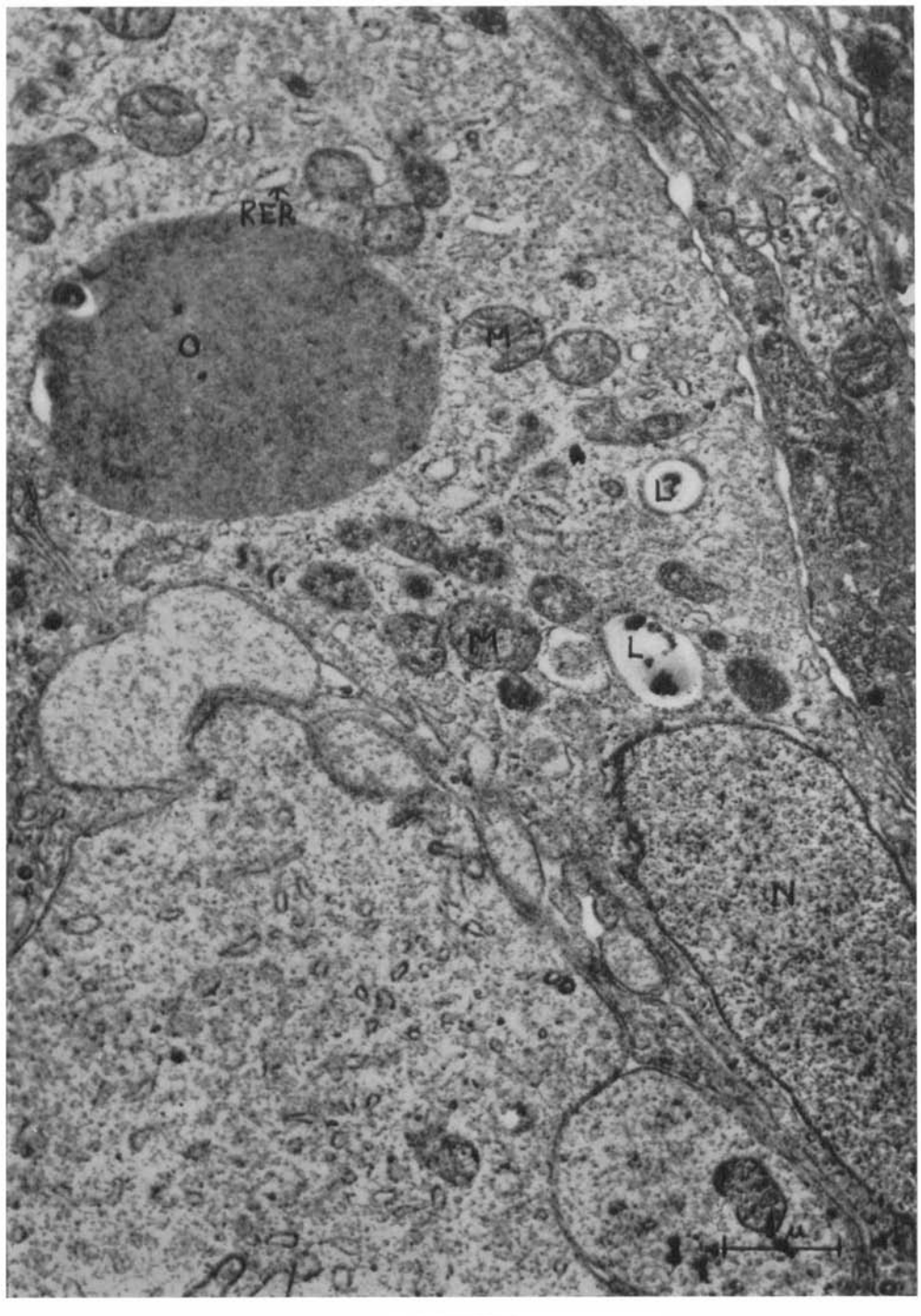


PLATE 2

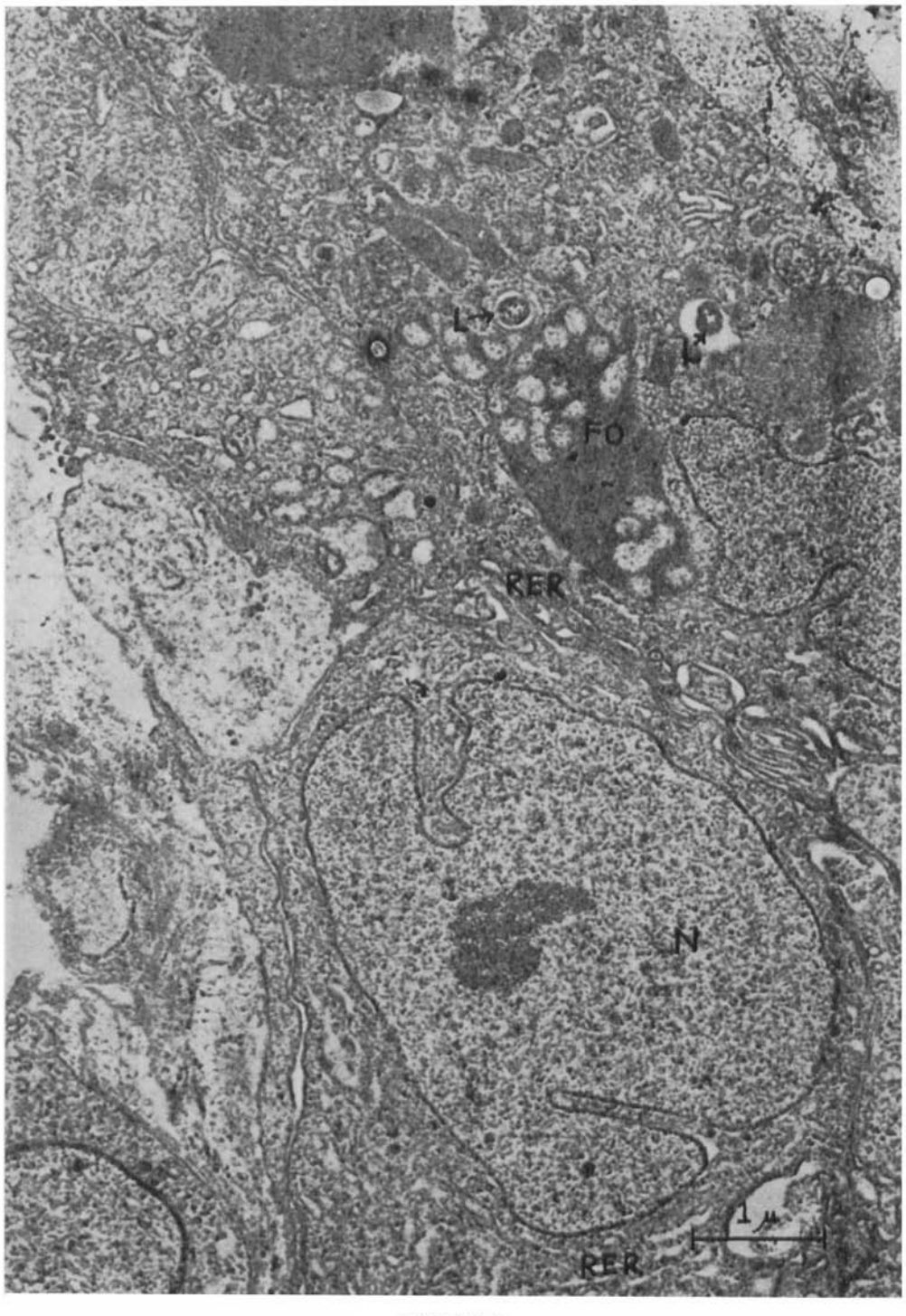


PLATE 3

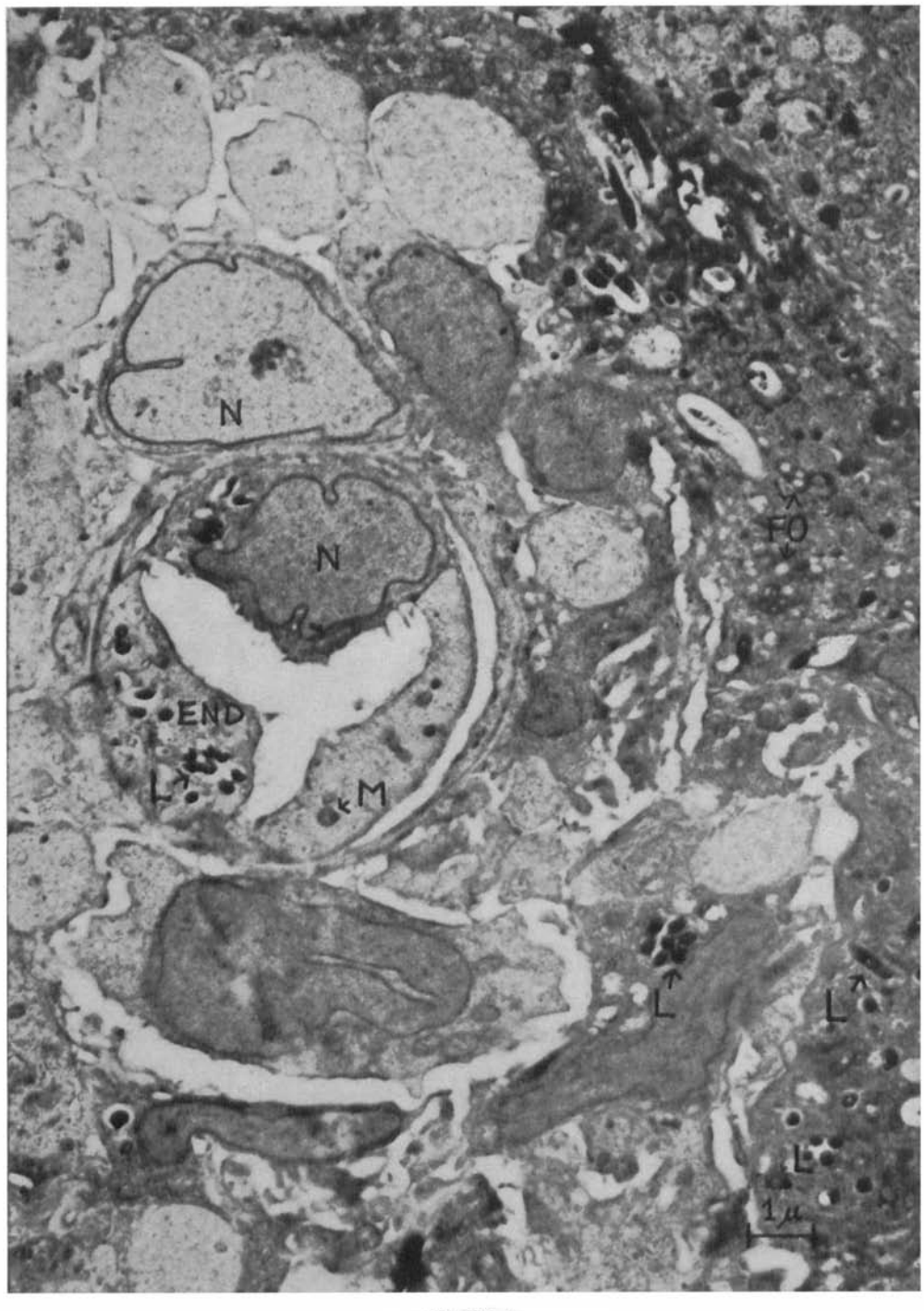


PLATE 4

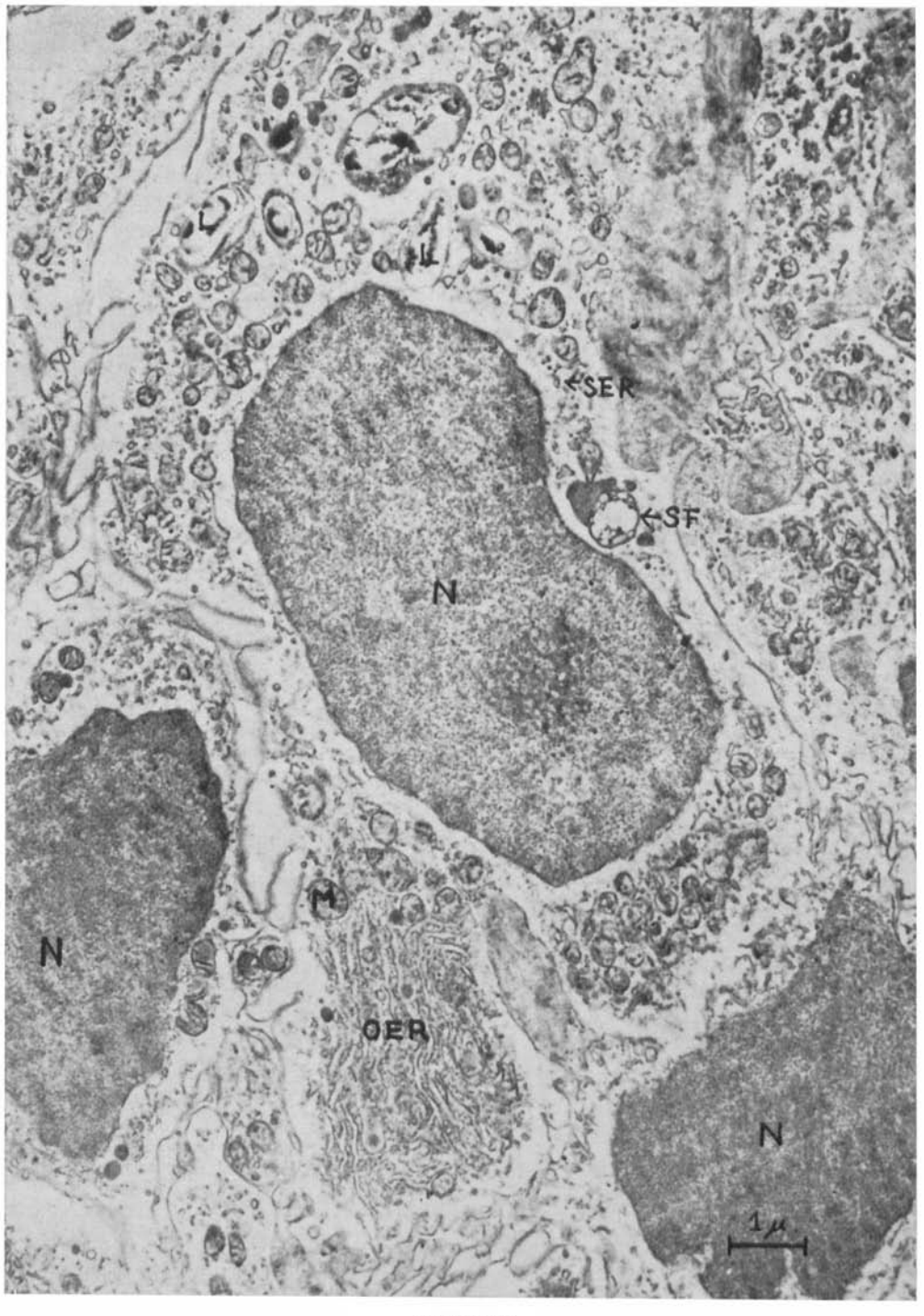


PLATE 5

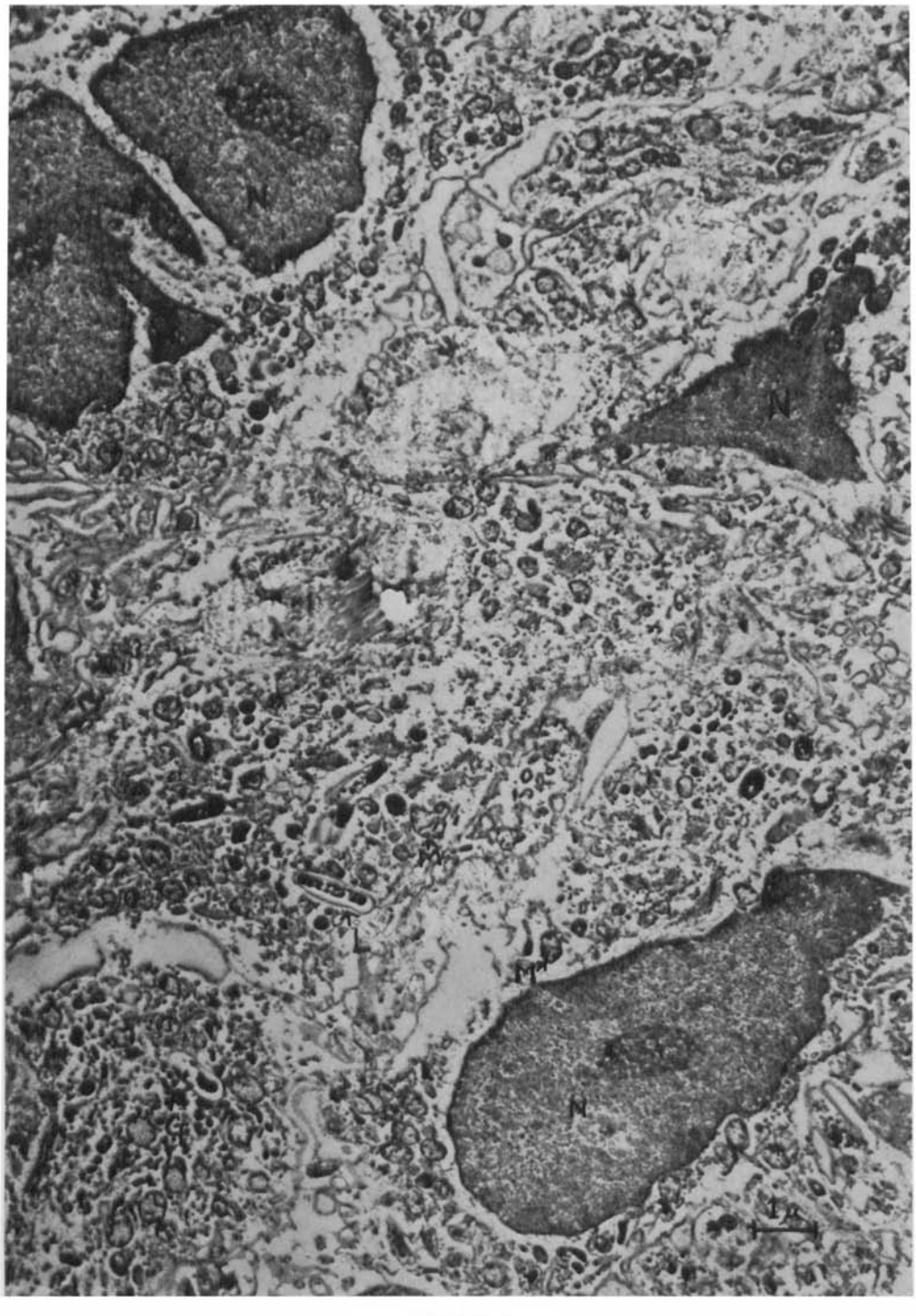


PLATE 6

PLATE 7

FIG. 7. Ultra-thin section of an epithelioid cell in a reactional tuberculoid lesion. Increased mitochondria, accumulated rough-surfaced endoplasmic reticulum, and undulating cell membrane are characteristic of the epithelioid cells of the active phase of reactional tuberculoid lesions. Also the cytoplasm is packed with smooth-surfaced endoplasmic reticulum appearing as small vesicles about 60-200 mμ in diameter. These characteristics of the cytoplasm of epithelioid cells are considered to indicate active defense activity of those cells against the leprosy bacilli. Magnification 15,600×.

Symbols: **RER**, rough-surfaced endoplasmic reticulum forming an organized ergastoplasm; **SER**, smooth-surfaced endoplasmic reticulum; **M**, mitochondria; **UCM**, undulating cell membrane.

PLATE 8

FIG. 8. Cytoplasm of an epithelioid cell in a reactional tuberculoid lesion. Bacilli are seen within less-electron-dense opaque droplets. The opaque droplets of this type have a distinct limiting membrane, but sometimes this limiting membrane fades away. The material in these less-electron dense opaque droplets seem to have been ingested at the time of the phagocytosis of leprosy bacilli or by pinocytosis of the epithelioid cells. By ordinary light microscopy dust-like lipid droplets may be found in the epithelioid cells of reactional tuberculoid lesions, and these less-electron-dense opaque droplets are considered to be those dust-like lipids. Magnification 22,000×.

Symbols: **L**, leprosy bacillus with swollen cell wall and fragmented cytoplasm; **LDO**, less-electron-dense opaque droplet; **M**, mitochondria; **G**, Golgi complex; **SER**, smooth-surfaced endoplasmic reticulum (large vacuolar form with slightly electron-dense content); **N**, nucleus; **CM**, cell membrane.

PLATE 9

FIG. 9. Ultra-thin section of a reactional tuberculoid lesion. In places where the tissue response against the leprosy bacilli seems to be most violent, the boundaries of the cells have disappeared and there is a complicated mixture of nuclei, mitochondria and vesicular endoplasmic reticulum scattered from the broken cells. However, the original forms of various cellular elements such as mitochondria, endoplasmic reticulum and nuclei are well preserved, and usually there is no necrotic mass as is seen in the necrotic tuberculoid nerve lesions. Magnification 9,200×.

Symbols: **N**, nucleus; **SER**, smooth-surfaced endoplasmic reticulum; **M**, mitochondria.

PLATE 10

FIG. 10. Ultra-thin section of a reactional tuberculoid lesion. In the extracellular medium, several transverse sections of elastic fibers are seen. Elastic fibers have a moderately electron-dense homogeneous matrix with electron-dense speckles embedded in it. The outer margins of elastic fibers show complicated indentation. The rest of the extracellular environment is occupied by a thin feltwork of reticulin fibrils. Magnification 31,500×.

Symbols: **MEF**, moderately electron-dense matrix of elastic fiber; **SEF**, speckles of elastic fiber; **RF**, thin feltwork of reticulin fibrils.

PLATE 11

FIG. 11. Shown here is a Schwann cell in the cord of Büngner stage in a lepromatous great auricular nerve. Cytoplasmic components such as mitochondria and endoplasmic reticulum are accumulated in the vicinity of the nucleus. In the peripheral part of the cytoplasm of Schwann cells of the cord of Büngner the cytoplasmic organelles are very scarce, and usually the numbers of mitochondria and amount of endoplasmic reticulum are very small.

Leprosy bacilli lie separated from each other, and there are no electron-transparent zones around them. Only a few little foamy structures are observed, but their relationship to the bacilli is obscure. The bacilli found in the cytoplasm of Schwann cells have distinct nuclear structures, and degenerated bacilli are not observed. From these findings it seems probable that leprosy bacilli live in Schwann cells at a lowered metabolic level, and for this reason there is no accumulation of electron-transparent material around them. The paucity of cytoplasmic organelles in the cytoplasm of Schwann cells suggests their lack of bacterial activity against leprosy bacilli. Magnification 27,300×.

Symbols: **N**, nucleus; **L**, leprosy bacillus; **FS**, small foamy structure; **M**, mitochondria; **SER**, smooth-surfaced endoplasmic reticulum.

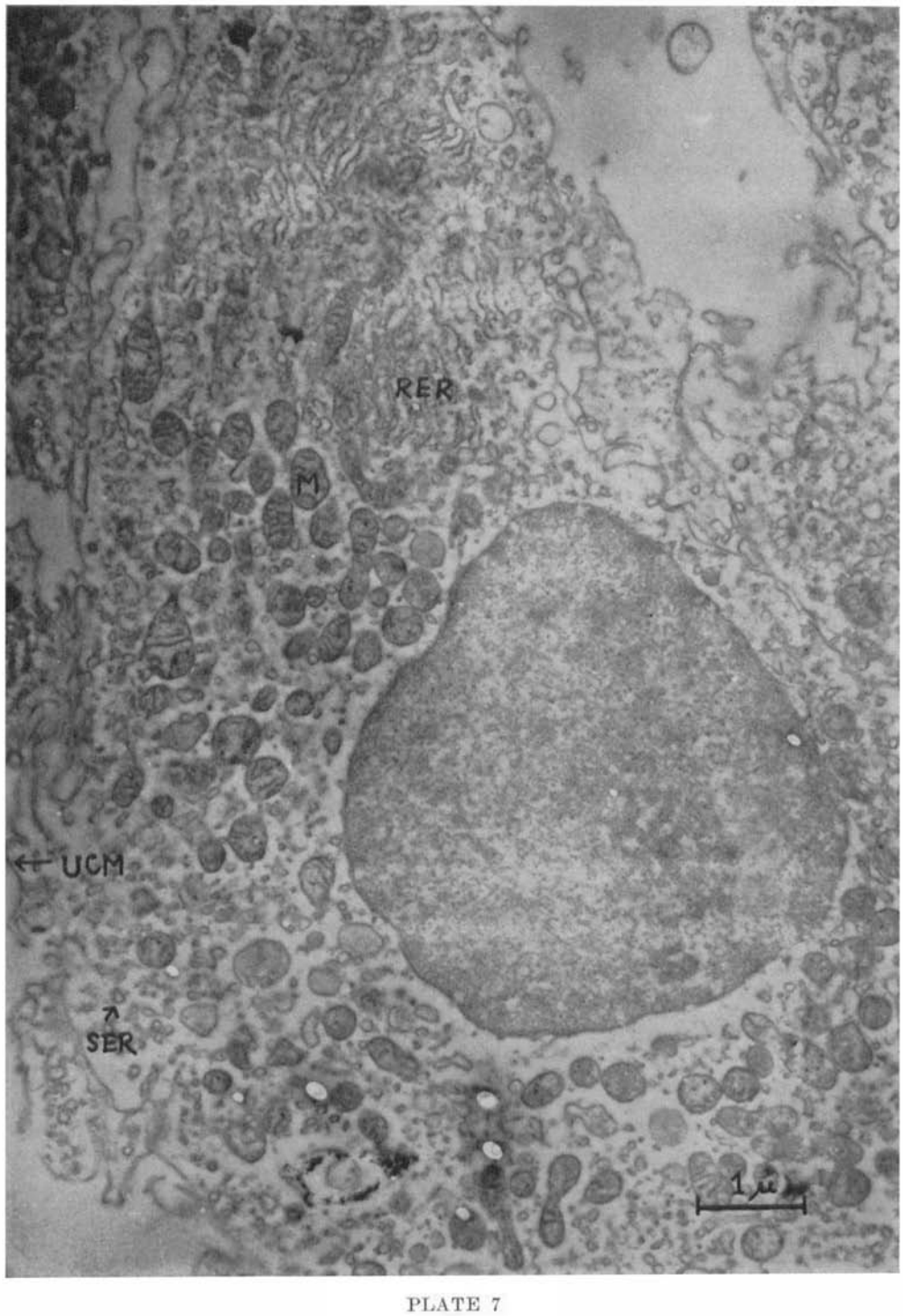
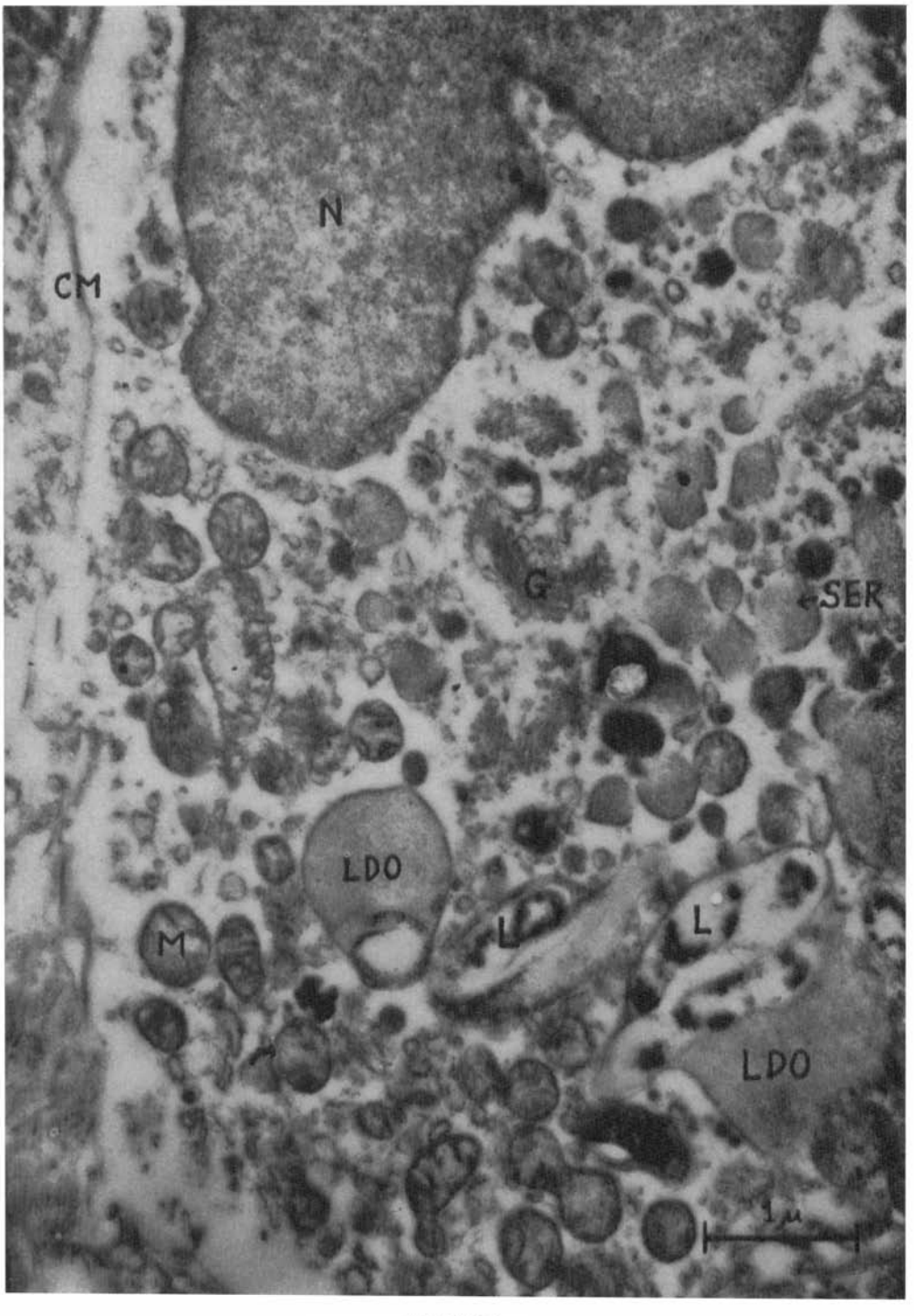


PLATE 7



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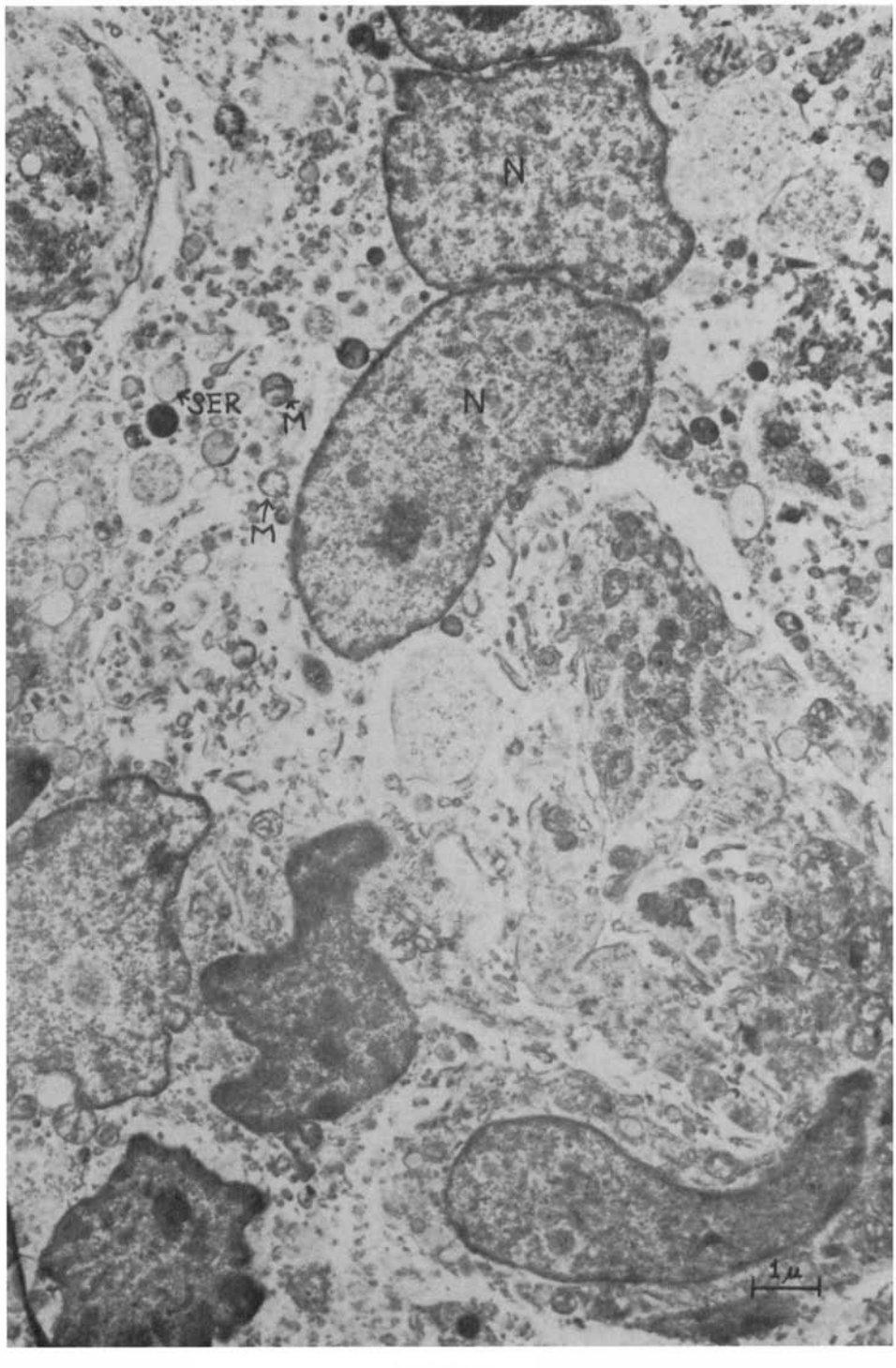
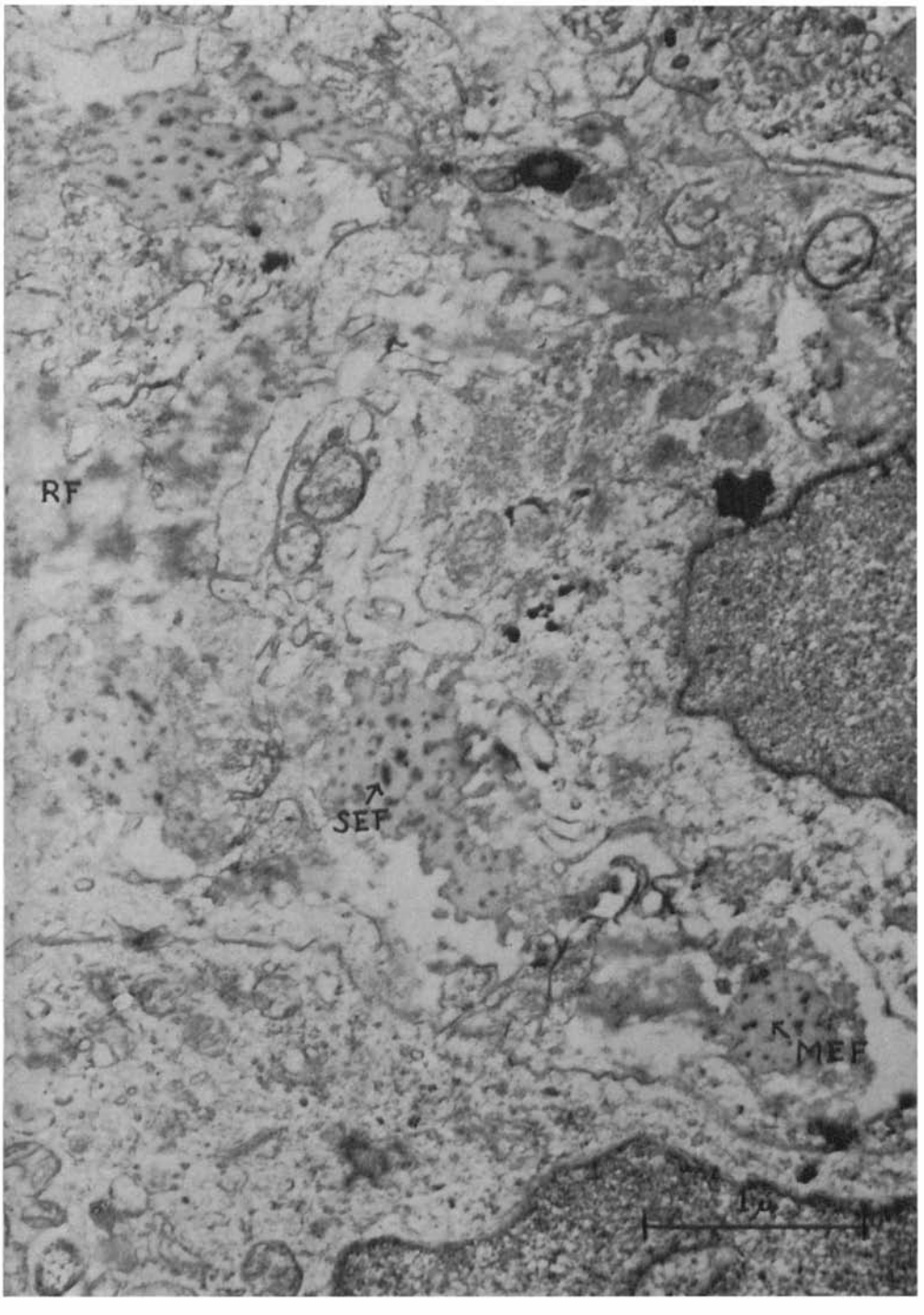


PLATE 9



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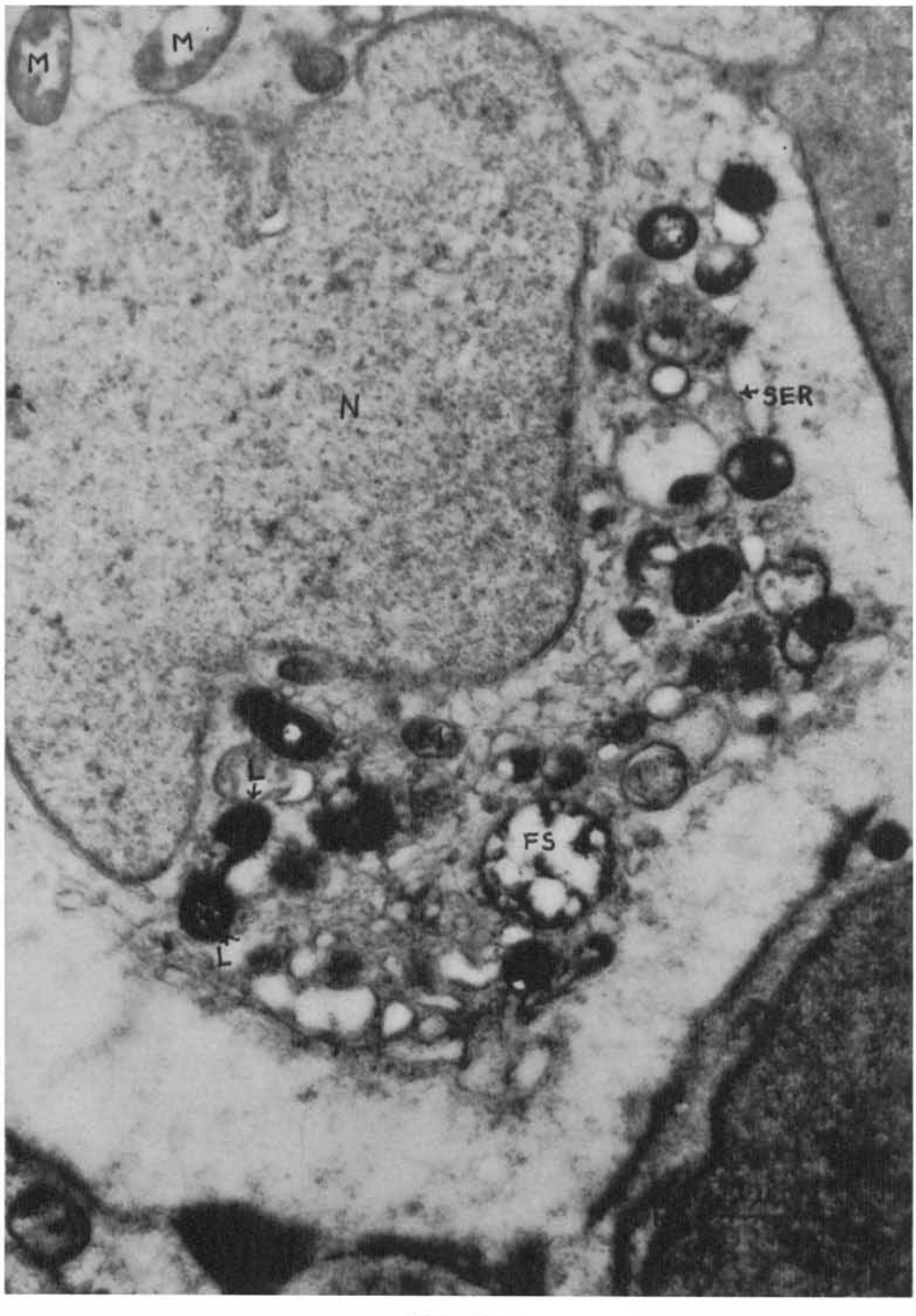


PLATE 11

PLATE 12

FIG. 12. Lepra cell in the endoneurial space of a lepromatous great auricular nerve (the same nerve as Fig. 11). The arrangement of bacilli in the cytoplasm of these cells is quite different from that of the bacilli inside Schwann cells. Most of the bacilli are enclosed in foamy structures. Also, the cytoplasmic texture of the lepra cell is different from that of Schwann cytoplasm. Vesicular change inside mitochondria is seen. Magnification 40,000×.

Symbols: **L**, leprosy bacillus; **FS**, foamy structure; **M**, mitochondria; **VM**, vesicular change in mitochondria; **O**, opaque droplet; **N**, nucleus; **CF**, collagen fibers.

PLATE 13

FIG. 13. Zone of Wallerian degeneration in a tuberculoid great auricular nerve. Parallel ribbons of the cord of Büngner are observed on the right half of this picture. At the left bottom there is a small amount of exudate.

In the zone of Wallerian degeneration the changes of the nerve elements are almost like to those of lepromatous lesions, but the number of leprosy bacilli in Schwann cells is very small as compared with what is found in lepromatous nerves. There are no bacilli in this electron micrograph. Magnification 4,750×.

Symbols: **N**, nucleus; **SCH**, Schwann cell of the cord of Büngner stage; **M**, mitochondria; **CF**, collagen fibers.

PLATE 14

FIG. 14. In tuberculoid nerve lesions of leprosy, the Schwann cells of the cord of Büngner stage are very often destroyed, and various cytoplasmic elements and lipid droplets are discharged outside the Schwann cells. Magnification 13,200×.

Symbols: **SCH**, Schwann cell of the cord of Büngner stage; **LD**, lipid droplets; **M**, mitochondria; **CF**, collagen fibers.

PLATE 15

FIG. 15. Ultra-thin section of a tuberculoid great auricular nerve. The zone of bionecrosis in such lesions is characterized by the presence of the peculiar concentric onion-like bodies in the cytoplasm of large cells. In the upper part of this picture the cytoplasmic texture including mitochondrial morphology is still normal, whereas in the lower part the cytoplasm is changing into lamellar degeneration. By this half-normal and half-degenerated appearance, these large cells with onion-like bodies are considered to be in a transitional stage to utter necrosis, and for this reason these cells are called bionecrotic cells. Magnification 20,500×.

Symbols: **OLB**, onion-like body; **LD**, lipid droplet; **M**, mitochondria; **N**, nucleus.

PLATE 16

FIG. 16. Ultra-thin section of a tuberculoid great auricular nerve. Between the bionecrotic cells with onion-like bodies there are necrotic masses which are composed of closely-coiled, electron-dense helicoidal threads. Also seen is a cell packed with lipid droplets. Magnification 10,700×.

Symbols: **OLB**, onion-like body; **LD**, lipid droplet; **NM**, necrotic mass composed of closely-coiled electron-dense helicoidal threads; **N**, nucleus.

PLATE 17

FIG. 17. Onion-like bodies and lipid droplets in the cytoplasm of a bionecrotic cell (the same nerve as Fig. 16). Some of the onion-like bodies seem to have been ingested from outside the cells, but most of them are believed to have been produced in the cytoplasm of these cells. Magnification 14,000×.

Symbols: **OLB**, onion-like body; **LD**, lipid droplet; **M**, mitochondria; arrow, an onion-like body which looks as if it were being ingested by the cell.

PLATE 18

FIG. 18. Ultra-thin section of a tuberculoid great auricular nerve. The zone of necrosis is composed of aggregates of closely-coiled helicoidal threads. These necrotic masses are considered to be the debris of cell cytoplasm in the stage of acid coagulation. Magnification 17,900×.

Symbols: **HTH**, helicoidal threads; **NM**, necrotic mass; **LD**, lipid droplet.

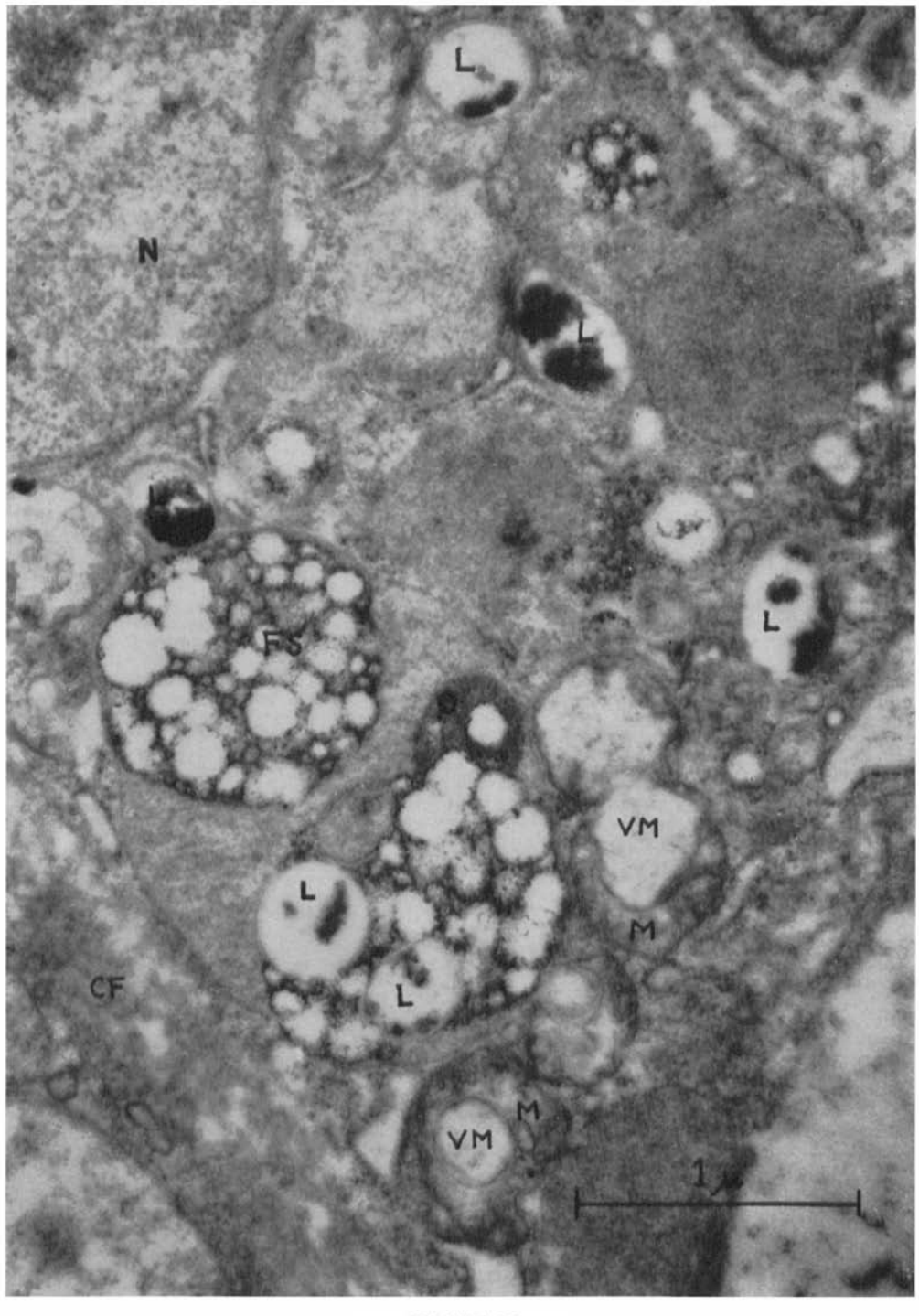


PLATE 12

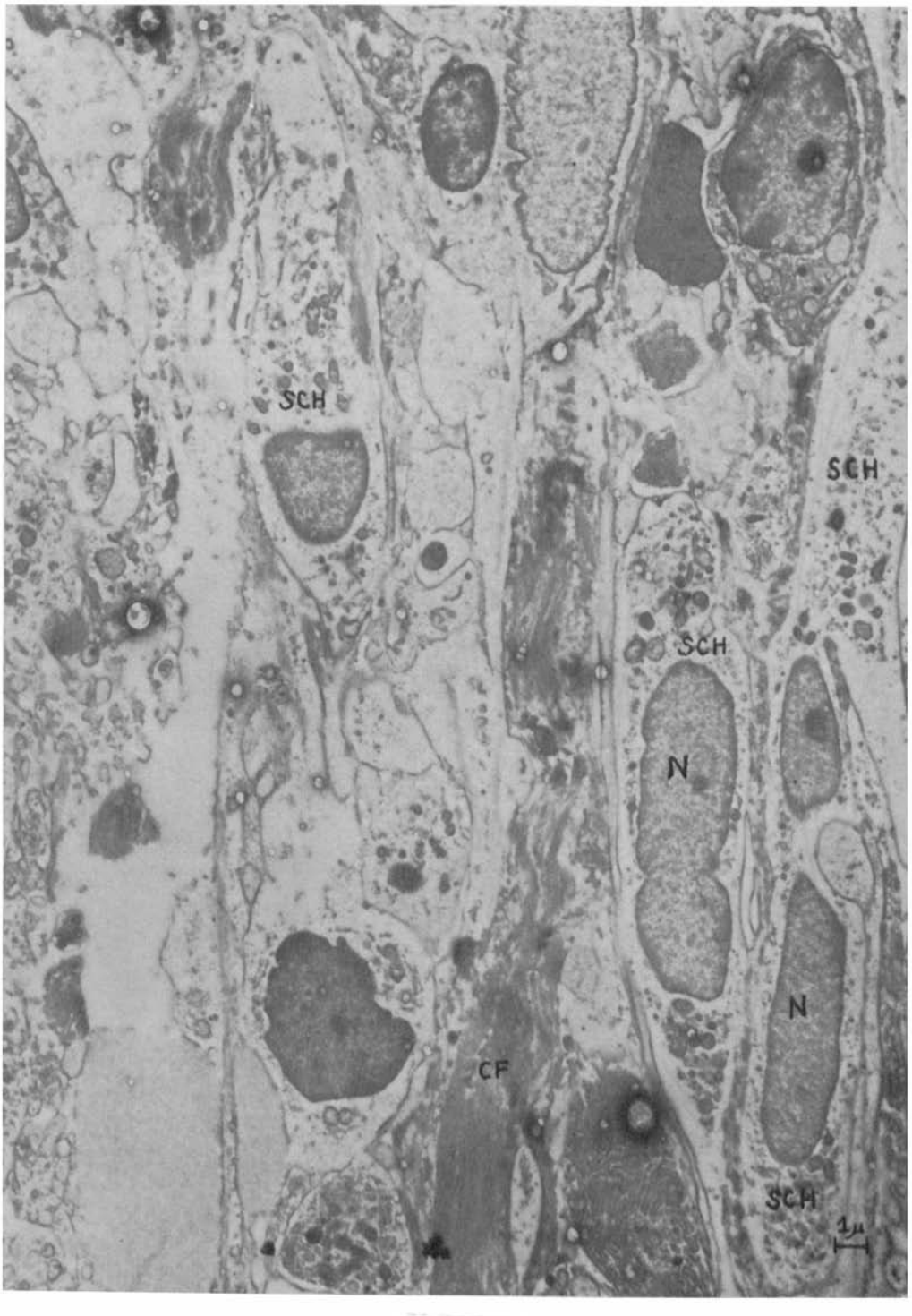


PLATE 13

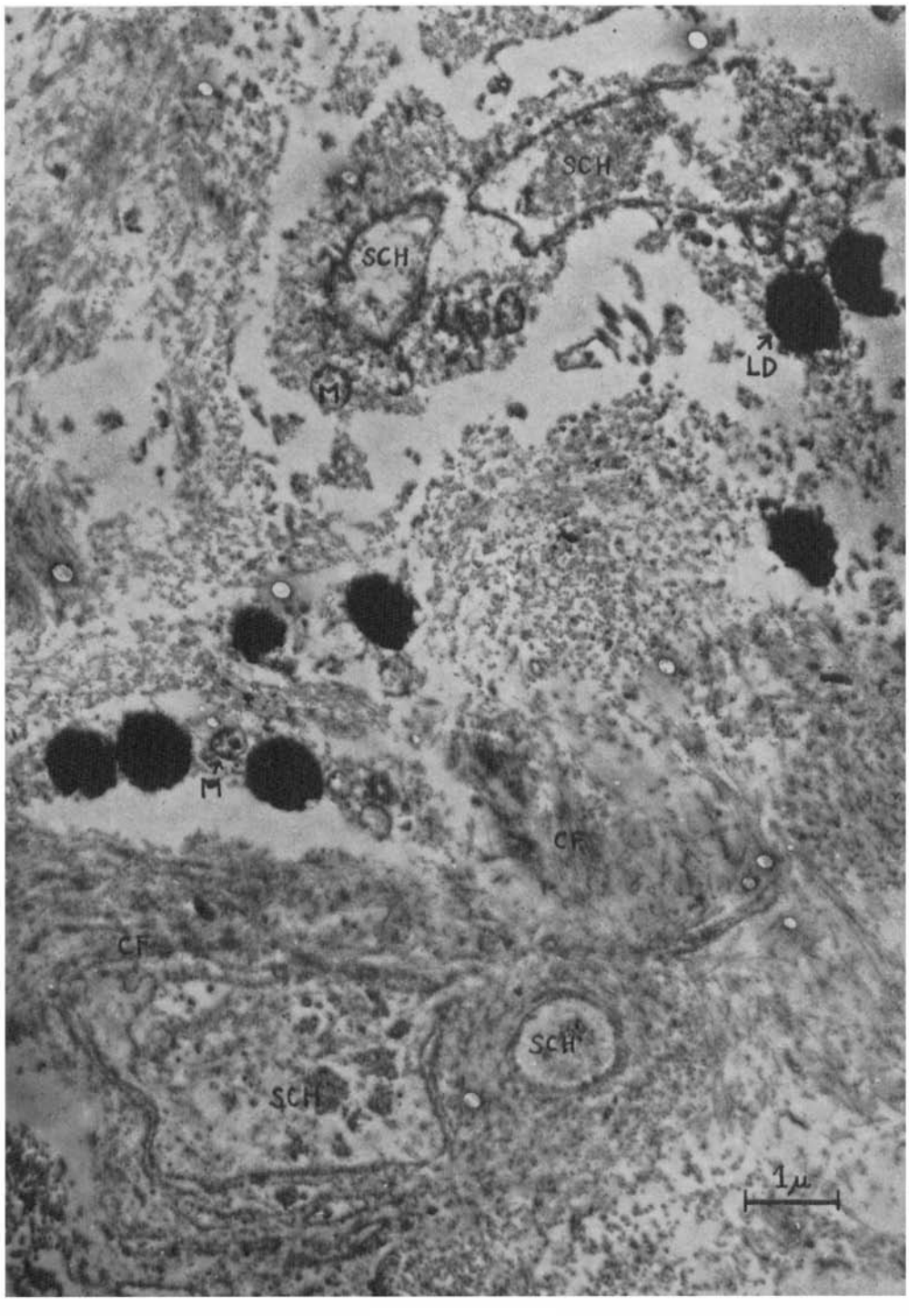
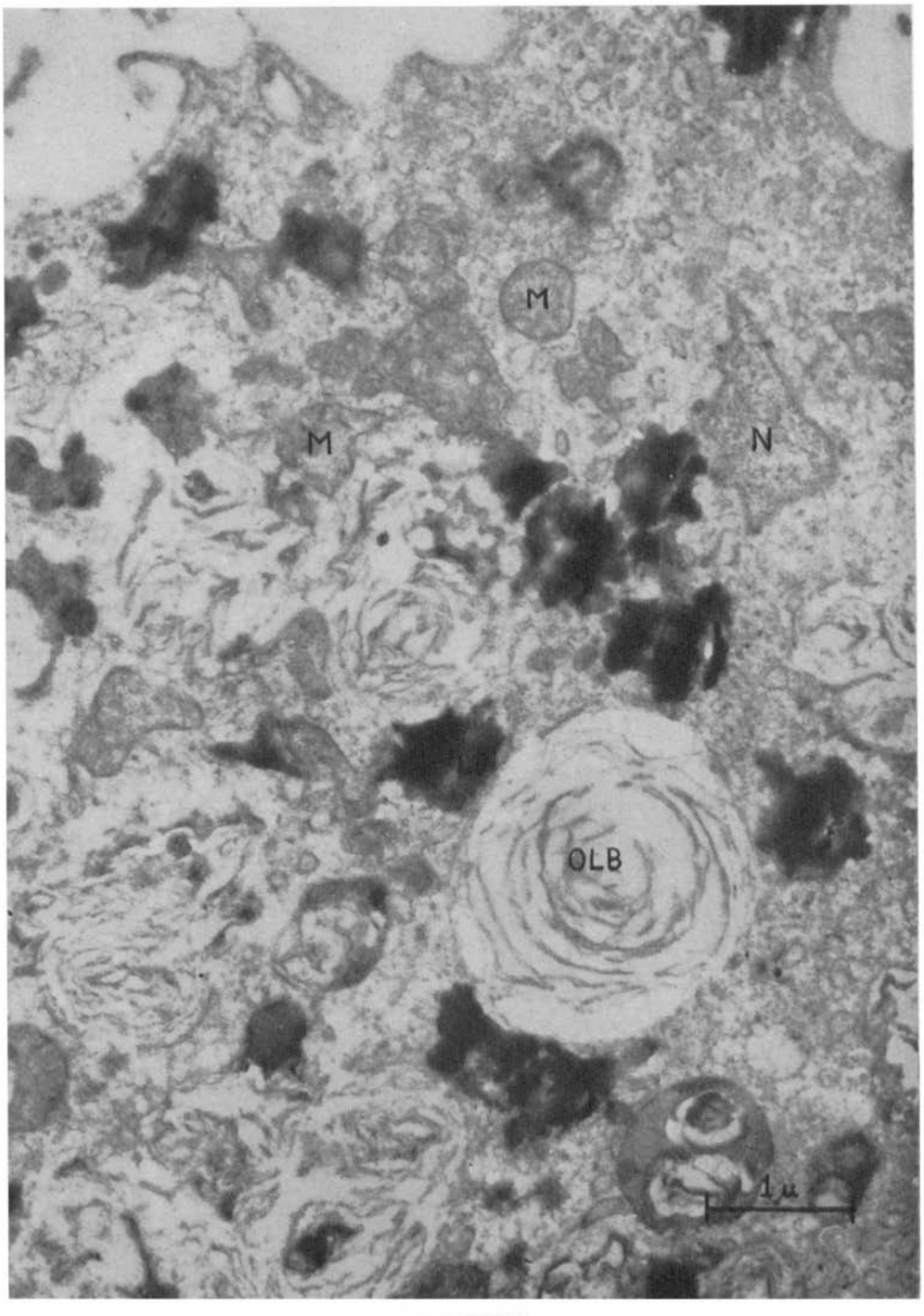


PLATE 14



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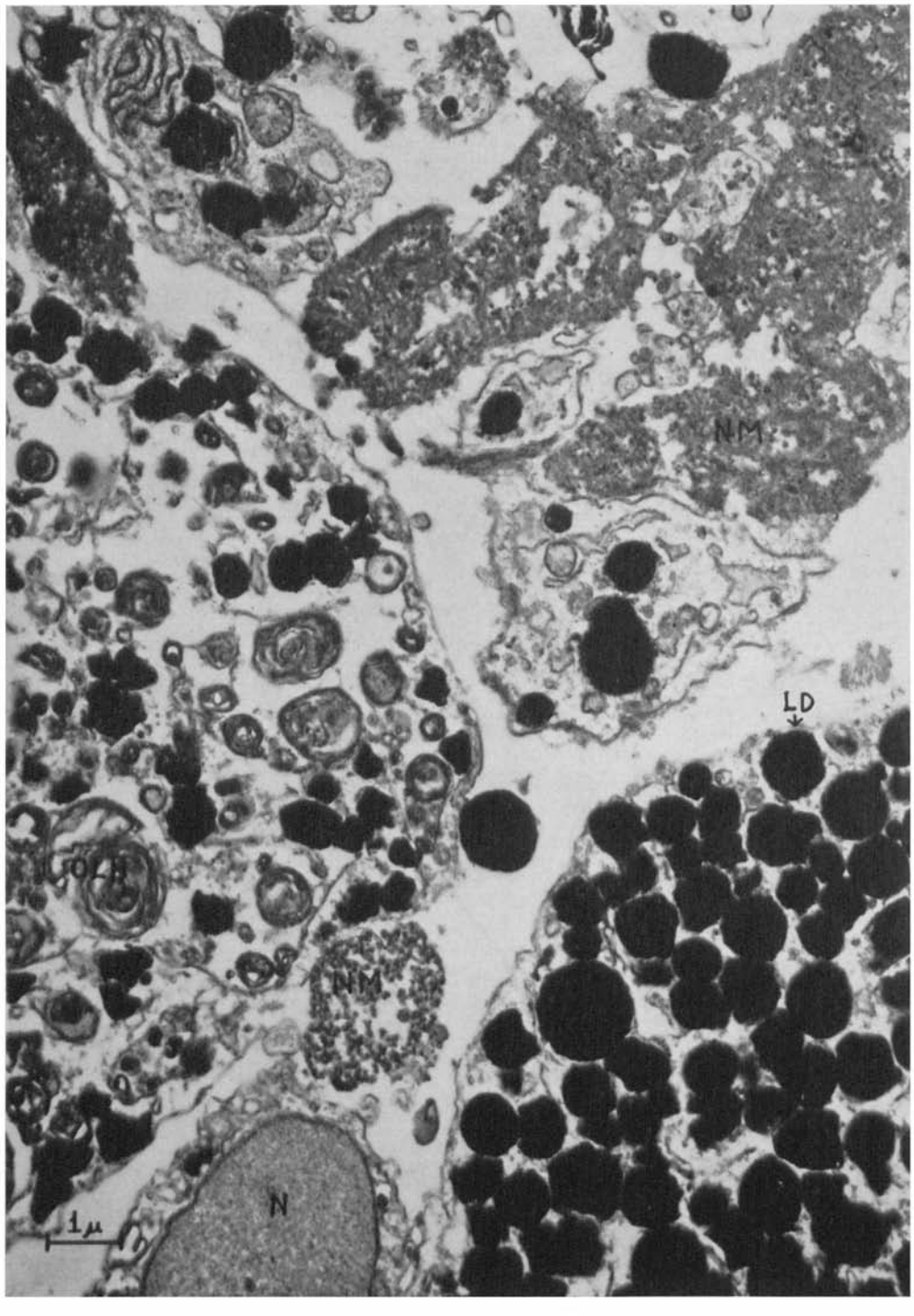

PLATE 16

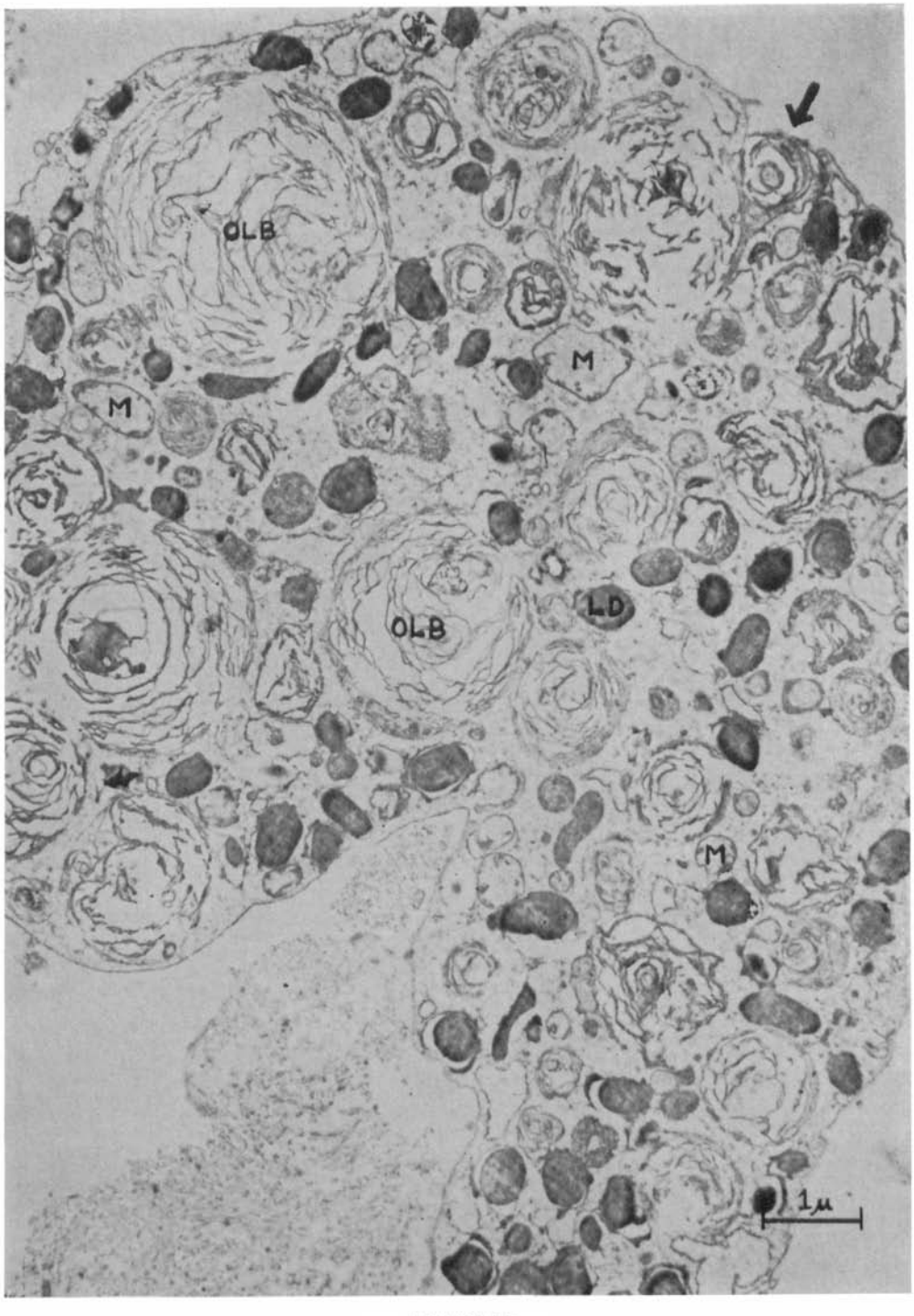

PLATE 17

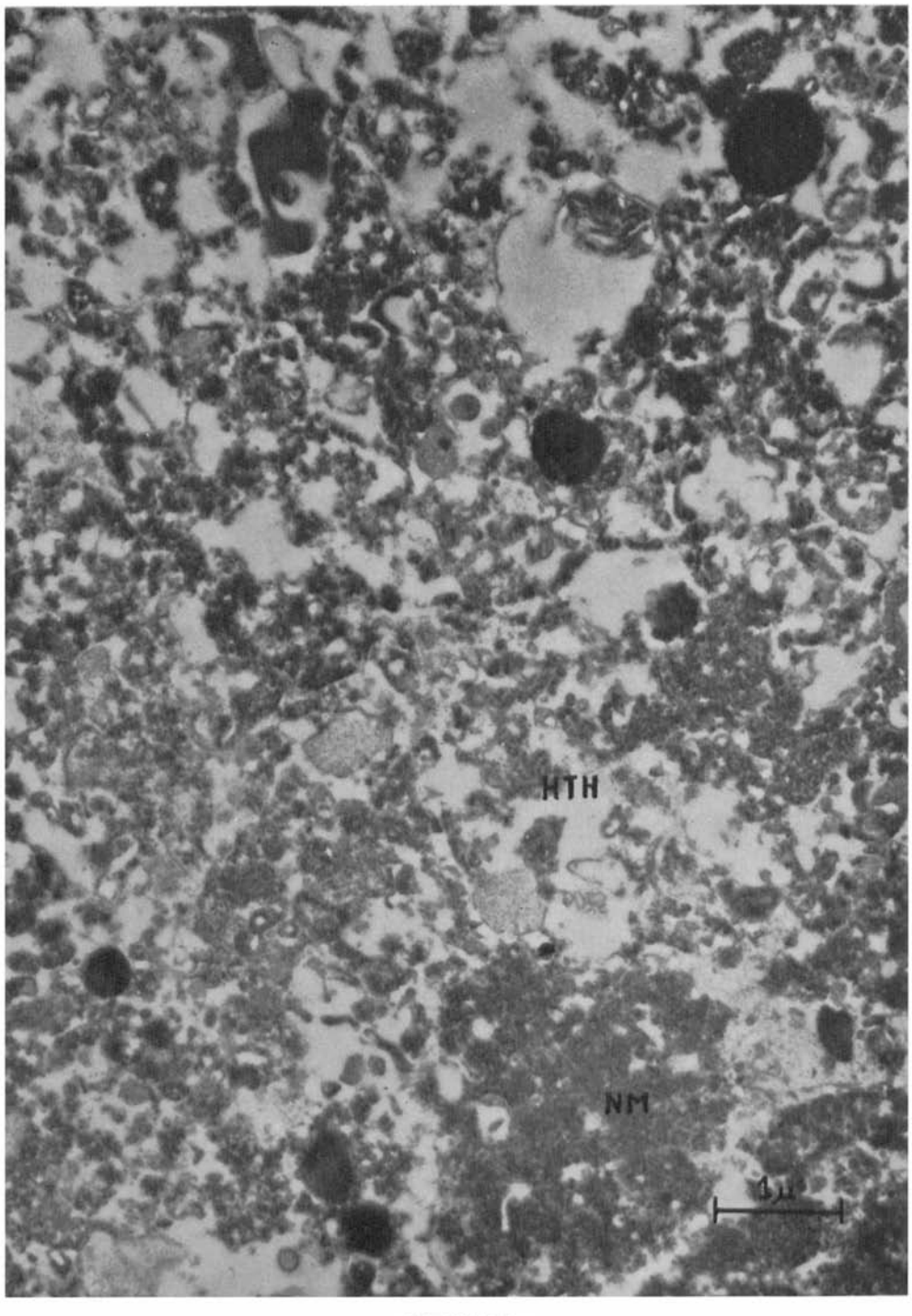

PLATE 18