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ELECTRON MICROSCOPY OF ULTRA-THIN SECTIONS OF LEPRA CELLS AND *MYCOBACTERIUM LEPRAE*

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Several investigators have studied *Mycobacterium leprae* with the electron microscope ([1, 5, 11, 12]) but it was only after the introduction of ultra-thin sectioning that the internal structure of the leprosy bacillus could be shown clearly in electron micrograms. In 1956 Brieger and Glauert ([3]) reported on electron microscopic study of ultra-thin sectioned *M. leprae*, and described the details of the internal structure of the bacilli and the lepra cells.

In the present article we report on the result of our study with the electron microscope of ultra-thin sectioned lepra bacilli and cells. In this study we were able to clarify some aspects of the "nuclear apparatus" of the bacillus. Furthermore, the mechanism of the growth of foamy structures in the cytoplasm of lepra cells was studied.

MATERIAL AND METHODS

Leproma specimens were obtained from six patients with lepromatous leprosy and studied with the electron microscope.

Leproma No. 1. One of the nodules on the left leg of a 24-year-old female patient. The specimen was taken 2 weeks after the commencement of DDS treatment (100 mgm. a day).

Leproma No. 2. One of the lepromas on the right forearm of a 52-year-old male patient. The specimen was taken before the commencement of DDS treatment.

Leproma No. 3. One of the lepromas on the left forearm of a 50-year-old male patient. The specimen was taken after 18 months of treatment with DDS.

Leproma No. 4. Taken from a lepromatous plaque of a 83-year-old female patient after 6 months of DDS treatment.

Leproma No. 5. Taken from the face of a 72-year-old male patient who had repeatedly shown erythema nodosum leprosum reactions and in whom the sulfone treatment, given for 40 months, was not very effective.

Leproma No. 6. Taken from a 46-year-old male patient before the commencement of sulfone treatment.

The specimens when removed were sectioned into small chips (about 1 cubic millimeter) and fixed in veronal-buffered 1 per cent osmium tetroxide (pH 7.2) or phosphate-buffered 1 per cent osmium tetroxide (pH 7.2), for 4 to 30 hours in the refrigerator. After washing and dehydrating, the tissue chips were embedded in 6:4 butyl-methyl methacrylate.

The ultra-thin sections were made with the Shimadzu ultra-microtome. The thickness of the sections ranged from 20 mμ to 100 mμ. The electron microscopes used were the Hitachi HU 10 model, Akashi Tronscope, and Philips.

ELECTRON MICROSCOPIC FINDINGS

THE BACTERIAL CYTOLOGY OF *M. LEPRAE*

Cell wall.—The cell wall of *M. leprae* is about 6 mμ thick. It consists of outer and inner layers which are electron-dense, and a central layer which is less dense (Fig. 9). The cytoplasmic membrane was not distinct in our electron micrograms.

Cytoplasm.—The cytoplasm of *M. leprae* is moderately electron-dense and has a relatively homogenous appearance. In the lepromas from well-treated cases, the bacilli show terminal condensations of the cytoplasm (Fig. 3). These terminal cytoplasmic condensations simulate the Type A body of avian tubercle bacilli, but they are not clearly defined as a Type A body.

Nuclear apparatus.—The No. 1 leproma contained numerous bacilli in which the nuclear apparatus was clearly visible (Fig. 6). This nuclear apparatus is composed of moderately electron-dense threads about 9 mμ wide which show distinct coiling in some of the bacilli (Figs. 1 and 2). The nuclear threads appear to be embedded in an electron-transparent matrix. In the lepromas from well-treated patients (Nos. 3, 4 and 5), no clear threads were found in most of the bacilli.

THE STRUCTURE OF LEPRA CELLS

In the following description we use the term "lepra cell" to indicate the cells which contain leprosy bacilli in their cytoplasm. Single bacilli are embedded in the ground substance of the cytoplasm together with various normal cytoplasmic components such as microsomes, mitochondria, lipid granules and endoplasmic reticulum. Single bacilli and small groups of bacilli are sometimes tightly surrounded with a limiting membrane which seems to be the cell membrane of the same lepra cell indented deeply during the phagocytic process (Fig. 8). Bacilli are often embedded in opaque droplets (Figs. 10 and 11). In the well-developed lepra cells there are aggregates of well-circumscribed foamy structures which contain numerous bacilli and occupy a large part of the space of the cell cytoplasm. In lepromas Nos. 3 and 4, each unit of the foamy structure has a single layer of a markedly electron-dense membrane which seems to be a network of electron-dense micelles each about 20 mμ long and 3 mμ wide (Figs. 4, 7 and 12).

The leprosy bacilli in the foamy structures of lepra cells have the characteristics of lying side by side or aggregated in groups in the electron micrograms of the ultra-thin section, as seen by ordinary light microscopy. This

side-by-side arrangement is quite different from the arrangement of *M. leprae murium* in the murine lepra cells (observation in press).

In most cases the leprosy bacilli can be easily differentiated from other cell components, but sometimes it is very hard to distinguish transversely-sectioned bacilli.

The nucleus of the lepra cell is usually pressed aside by the foamy structures of the cytoplasm. The cell membrane is a continuous single structure, but in the fully-developed lepromas it is often very hard to find the boundary of each lepra cell.

DISCUSSION

The nuclear apparatus of M. leprae.—Because of the development of the technique to demonstrate the DNA of the bacterial nucleus ([2, 6, 15, 16]), and the progress of phase contrast microscopy ([13, 17]), a nuclear apparatus has been found in many bacteria. Various names—nuclear chromatin, chromosome, chromatinic bodies, etc.—have been given to the nuclear apparatus of bacteria.

Recently, electron microscopy of the ultra-thin sections of bacteria has revealed the detailed internal structures of bacterial cells ([4, 8, 14]). A cord-like structure inside tubercle bacilli was reported by Koike and Hiraki ([10]). Higashi ([7]) has found helicoidal threads in *Escherichia coli* and *Micrococcus aureus* which he considered to be the chromonema of the bacterial cells.

Brieger and Glauert ([3]) examined ultra-thin-sectioned leprosy bacilli with the electron microscope and found dense threads and granules in transverse sections, and they also noticed that there are indications of lighter regions within the dense areas of the bacilli not sufficiently well-defined to be identified conclusively with the "nuclear apparatus."

In our electron micrograms of the No. 1 leproma there were found threads 9 mμ wide that showed distinct coiling along the longitudinal axis of the bacillary bodies. The Feulgen reaction of the smears of the bacilli of this patient has shown the presence of Feulgen-positive granules or rods in the middle of the bacillary bodies. From these findings, these minute threads in leprosy bacilli appear to be the chromosome or chromonema of this mycobacterium. For the present we would like to designate their structure the "nuclear thread," although the final decision concerning the function of these structures awaits further studies.

The nuclear threads here described are observed only in bacilli of actively growing lepromas, and this fact suggests that this thread formation in the bacillary nucleus is closely associated with the cell division of the bacilli. On the contrary, in the quiescent lepromas which are undergoing gradual absorption under sulfone treatment the nuclear apparatus is destroyed, and in most of the bacilli in such lepromas there is no morphological element which suggests a bacillary nucleus.

The cytoplasmic condensation of M. leprae.—It is a well-known fact that as a result of intense sulfone treatment the morphology of the leprosy

bacilli in the lesions undergoes some changes. According to Malfatti and Jonquieres ([12]), the pattern of their disintegration under sulfone treatment is bipolar staining, or granular cytoplasmic condensation.

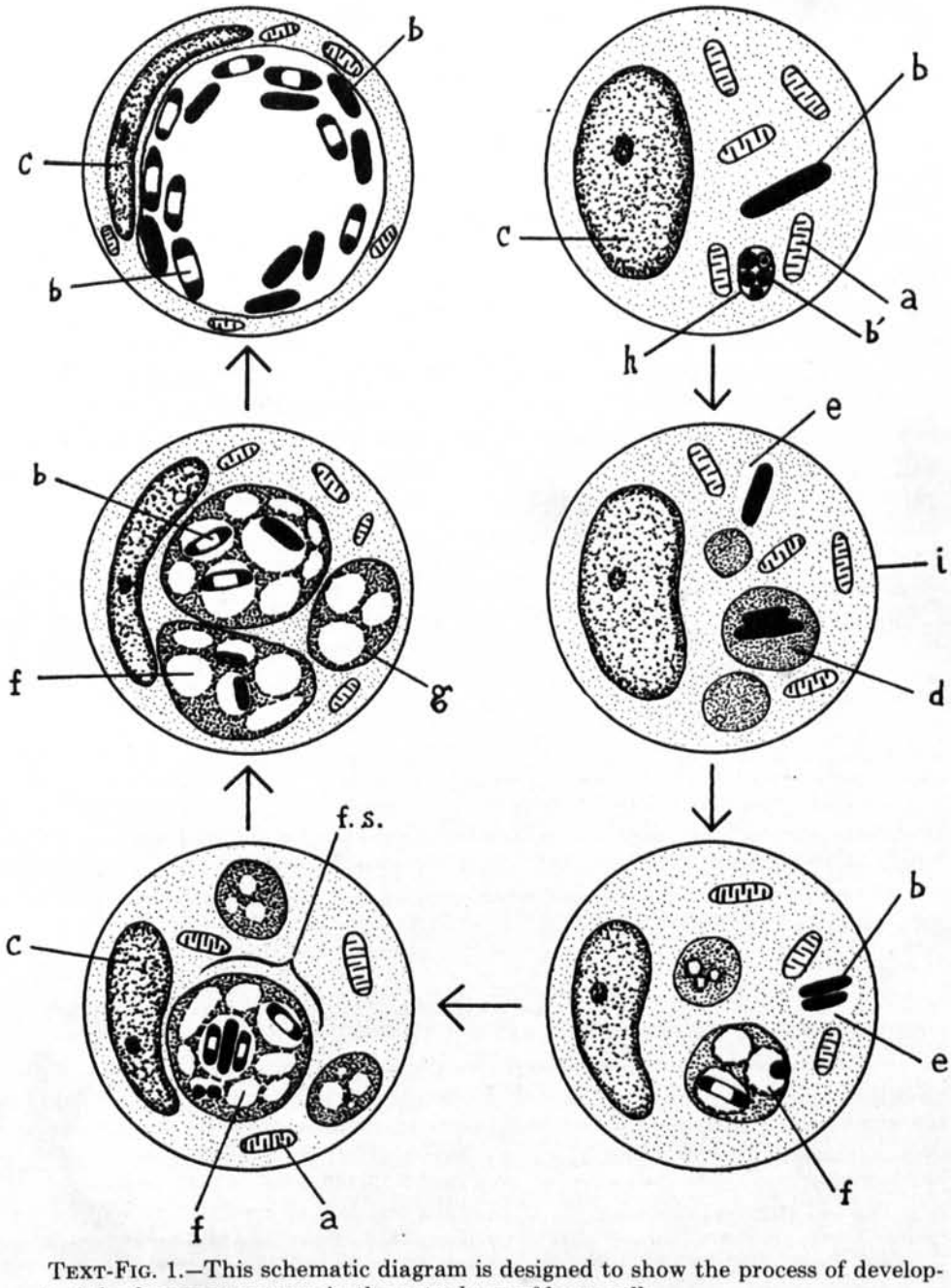
Polar cytoplasmic condensation is easily confounded with the Type A body which appears in tubercle bacilli. Knaysi and associates ([9]) believed that this Type A body in tubercle bacilli is of nuclear nature, and Bishop *et al.* ([1]) seem to agree with this view concerning *M. leprae*. As definite nuclear structure is found in our electron micrograms in the central part of the bacillary body, we do not regard this Type A body as the nucleus of the leprosy bacillus. Although small granules (13-36 mμ) were often found in the bacilli, clearly-defined dense bodies like the Type A body were seldom encountered.

Chatterjee and associates ([5]) expressed the view that a slow phase of multiplication results in solid, homogeneously dense bacilli, while a rapid phase results in forms possessing alternate light and dark regions. It might be possible that in the granular bacilli both the aggregated nuclear apparatus and the cytoplasm of the bacilli show the granular appearance, but since the sharpness of the electron micrograms of unsectioned material is very poor, we feel we should base our views on the findings in ultra-thin sections of the leprous lesions.

Besides polar cytoplasmic condensation of the bacilli, swelling of the central part of bacilli was noted by Malfatti, and we also have found swollen bacilli in our electron micrograms (Fig. 12). Whether this swelling of the bacillary body is due to the sulfone treatment or is only an artefact due to the conditions of fixation, we could not decide in this study.

Opaque droplets and foamy structure in the lepra cells.—Brieger and Glauert described clumps of *M. leprae* as having a definite boundary composed of dense granular material which sometimes formed an unmistakable membrane. They expressed the opinion that the limiting membrane which they showed in their pictures represented the cell membrane of a disintegrating leucocyte. It seems to us, however, that there are two kinds of such limiting membranes. One is the cell membrane of the same lepra cell surrounding tightly the ingested bacilli, after the indentation of the cell membrane during the process of phagocytosis. The other is the remnant of the opaque droplet in which the foamy structure developed, the dense granular material of the opaque droplet being extended extremely in the form of a limiting membrane. The latter kind of limiting membrane surrounds clumps of bacilli loosely, and it can be easily distinguished from the tightly-fitting limiting membrane of the other kind.

Electron microscope study of ultra-thin sections of lepromas reveals a foamy structure in the cytoplasm of lepra cells as found by Brieger and Glauert. In the analysis of our electron micrograms we have found that these foamy structures are closely associated with a kind of opaque droplet. The following is our hypothesis concerning the mechanism of the growth of the foamy structures in the cytoplasm of lepra cells, illustrated in Text-fig. 1.



TEXT-FIG. 1.—This schematic diagram is designed to show the process of development of the foamy structures in the cytoplasm of lepra cells.

Symbols: *a*, mitochondria; *b*, leprosy bacilli; *b′*, transverse section of bacilli; *c*, cell nucleus; *d*, opaque droplet; *e*, electron-transparent zone; *f*, foam space of the foamy structure; *f.s.*, foamy structure; *g*, single layer of electron-dense membrane; *h*, limiting membrane tightly surrounding a group of bacilli; *i*, cell membrane.

1. In the early stage after leprosy bacilli are ingested into a phagocyte, there is no change of the texture of the cytoplasm around the phagocytized bacilli. Sometimes bacilli are surrounded with a limiting membrane (Fig. 8). 2. As the bacilli begin to multiply in the cytoplasm of the lepra cell, they become surrounded with electron-transparent zones which seem to be the gloea (Fig. 5). 3. In other cases the bacilli in the cytoplasm become surrounded by a finely granular material which is moderately dense to the electron beam, i.e., the opaque droplets (Fig. 10). 4. In these opaque droplets, small foam spaces appear just around the bacillary bodies. The inner wall of these spaces consists of a single layer of electron-dense membrane, as has been described (Fig. 7). 5. These foam spaces gradually become larger, and they coalesce with each other. The electron-dense outer boundary which is the remnant of the opaque droplets become thinner (Figs. 11, 12). 6. The foamy structure thus produced may occupy almost the whole space of the cytoplasm of the lepra cell. 7. The lepra cell changes into a round ball which contains numerous leprosy bacilli. The nucleus is pressed aside and becomes very thin (Fig. 13).

Further studies are necessary for the determination of the chemical components of the opaque droplets, the electron-dense single membrane of each foam space, and the electron-transparent content of these spaces. It seems probable, however, that the opaque droplets and the moderately electron-dense outer boundary of the foamy structures contain lipids stainable with Sudan III or Sudan Black B.

Description of Text-fig. 1.—The first of the six circles representing cells shows (upper right), besides a few mitochondria (*a*), present in all, a normal cell nucleus (*c*), a single longitudinal bacillus (*b*) lying naked in the cytoplasm, and a transversely-cut group of bacilli (b′) surrounded by a tightly-fitting limiting membrane (*h*), which we regard as probably derived from the cell membrane during the process of phagocytosis.

In the second circle a lone bacillus is shown with an electron-transparent halo (*e*), while two others lie together inside an opaque droplet (*d*). Two smaller opaque-droplet elements are shown, with no visible enclosure in the plane of the section.

Next, a large opaque droplet is undergoing foamy change, with two of the three foam spaces (*f*) containing bacilli, one longitudinal and one transverse. In a second opaque droplet tiny foam spaces are seen.

The fourth circle (bottom left) shows progression of the process to where the large mass (*f.s.*) is a foamy structure of some complexity, which is shown further developed in the fifth circle. The nucleus in the fourth circle is compressed by the structures in the cytoplasm, and that process continues in other cells (fifth and sixth circles) until in the last one (upper left) the nucleus is flattened in the narrow band of cytoplasm.

The last circle represents a late stage of the process, not regularly attained, where many unit foamy spaces have coalesced to form a large globus. In this development the material which constituted the opaque droplets has disappeared—at least as regards the level at which the cell is shown as having been cut.

The index of vitality of M. leprae.—According to Malfatti, the peripheral envelope surrounding the isolated bacillary units and the globi is an index of the vitality and virulence of the bacilli. He believes that the outer envelope

disappears with the death of the bacilli, and the germs appear to be free in the medium as cellular remains, and this permits direct contact of the germs with the medium which facilitates the production of natural antibodies. In our electron micrograms the peripheral envelope of Malfatti seems to be identical with the electron-transparent zone around single bacilli, or foamy structures around clumps of bacilli.

We agree with Malfatti that the peripheral envelope (identical with the electron-transparent zone or foamy structure) is an index of cellular vitality of the bacilli in the lesion, but we think that the presence of the nuclear apparatus in the bacillary bodies is the surest index of cell vitality, because even after the death of the bacillus the peripheral envelope still remains. In the mature lepromas the peripheral envelopes (foamy structures) coalesce and form large electron-transparent masses in the cytoplasm of the lepra cells. As a result, it looks as though the peripheral envelope had disappeared when examined without ultra-thin sectioning. The precise topographical relationship between the bacilli and the various cytoplasmic elements can only be observed clearly by electron microscopy of ultra-thin sectional lepromas.

SUMMARY

1. Ultra-thin sections of lepromas taken from six cases of lepromatous leprosy were examined with the electron microscope.

2. Leprosy bacilli which exhibited a nuclear apparatus were found. The nuclear apparatus of *M. leprae* is composed of moderately electron-dense threads, about 9 mμ wide, which show a coil-like arrangement in the bacillary bodies.

3. The nuclear apparatus was not found in the bacilli of the retrogressive lesions of well-treated cases.

4. A hypothesis of the mechanism of the growth of foamy structures in the cytoplasm of lepra cells is offered.

RESUMEN

1. Cortes ultradelgados de lepromas obtenidos de seis casos de lepra lepromatosa fueron estudiados con el microscopio electrónico.

2. Se descubrieron bacilos leprosos que ostentaban un aparato nuclear. El aparato nuclear del *M. leprae* está compuesto de hebras moderadamente del grueso de electrones, de unos 9 mμ de ancho, que muestran una disposición espiral en los cuerpos bacilares.

3. No se descubrió el aparato nuclear en los bacilos de las lesiones retrógradas de los casos bien tratados.

4. Ofrécese una hipótesis del mecanismo de la proliferación de tejidos espumosos en el citoplasma de las células leprosas.

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DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Longitudinal section of a leprosy bacillus, showing coiling of nuclear threads. The breadth of the nuclear threads in this electron micrograph is about 9 mμ. Magnification, 99,000×.

FIG. 2. Longitudinal section of another bacillus. Magnification, 103,000×.

Symbols of Figs. 1 and 2: cyt., cytoplasm; nuc.th., nuclear thread.

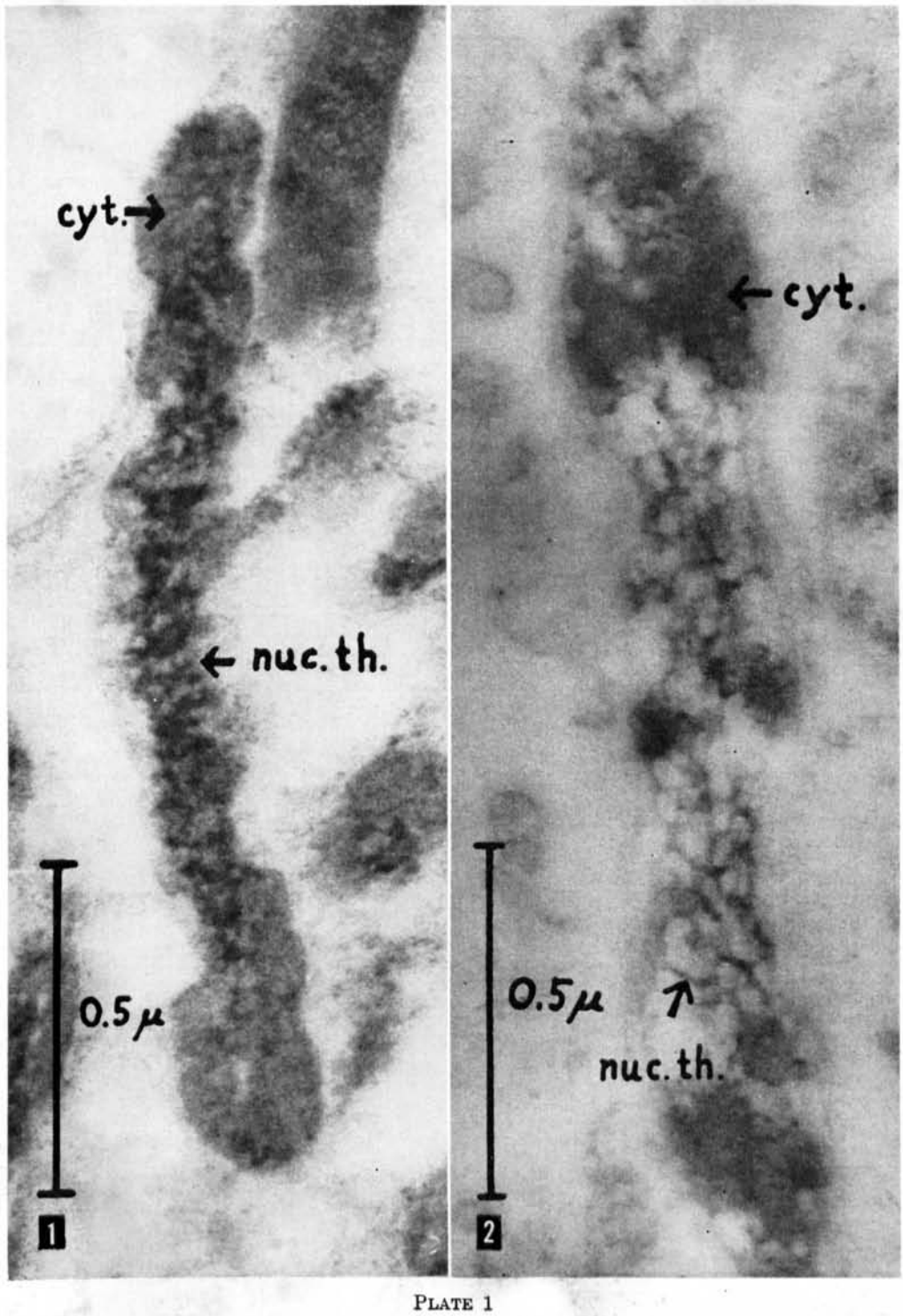


PLATE 1

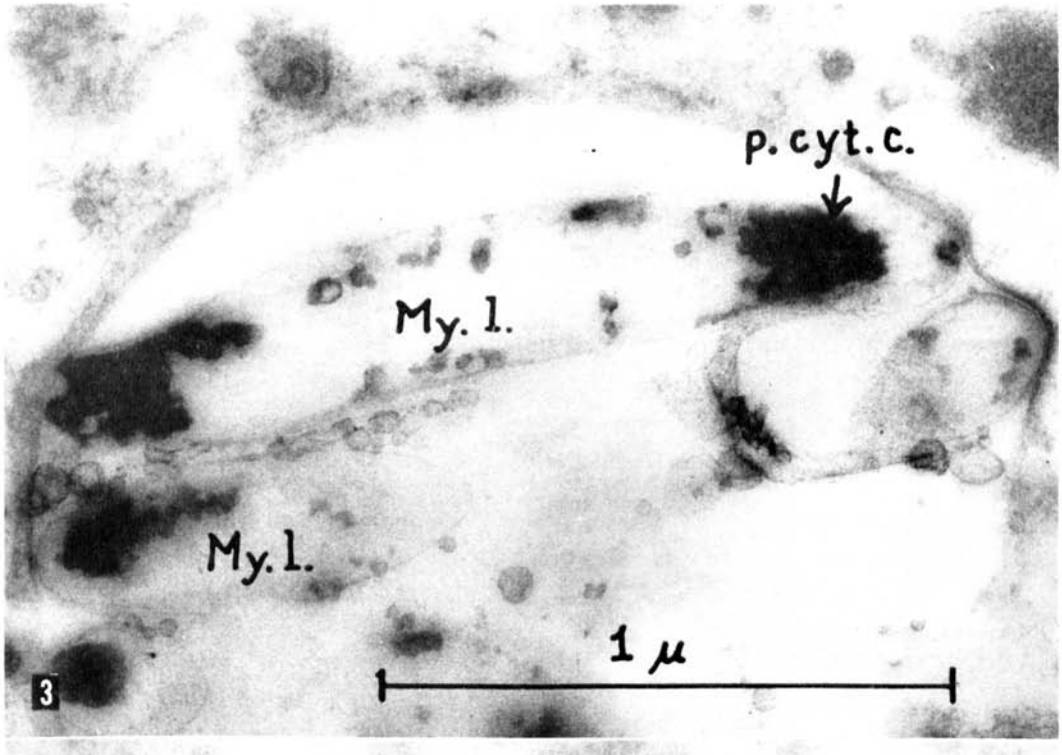
PLATE 2

FIG. 3. Leprosy bacilli in a lepromatous plaque of a well-treated case. The bacilli have no nuclear element, but there is polar cytoplasmic condensation. Magnification, 75,000×.

Symbols: My.1., leprosy bacilli; p.cyt.c., polar cytoplasmic condensation.

FIG. 4. Longitudinal sections of bacilli in leproma from a well-treated case. Cytoplasmic condensations and absence of nuclear elements are noted. In the lower part of the picture there are several foam spaces lying side by side, each of which has a thin electron-dense membrane about 3 mμ in thickness. This electron-dense membrane has the same nature as that of those to be seen in Figs. 7 and 12. Magnification, 73,000×.

Symbols: f, foam spaces with electron-dense membrane; My.1., leprosy bacilli.



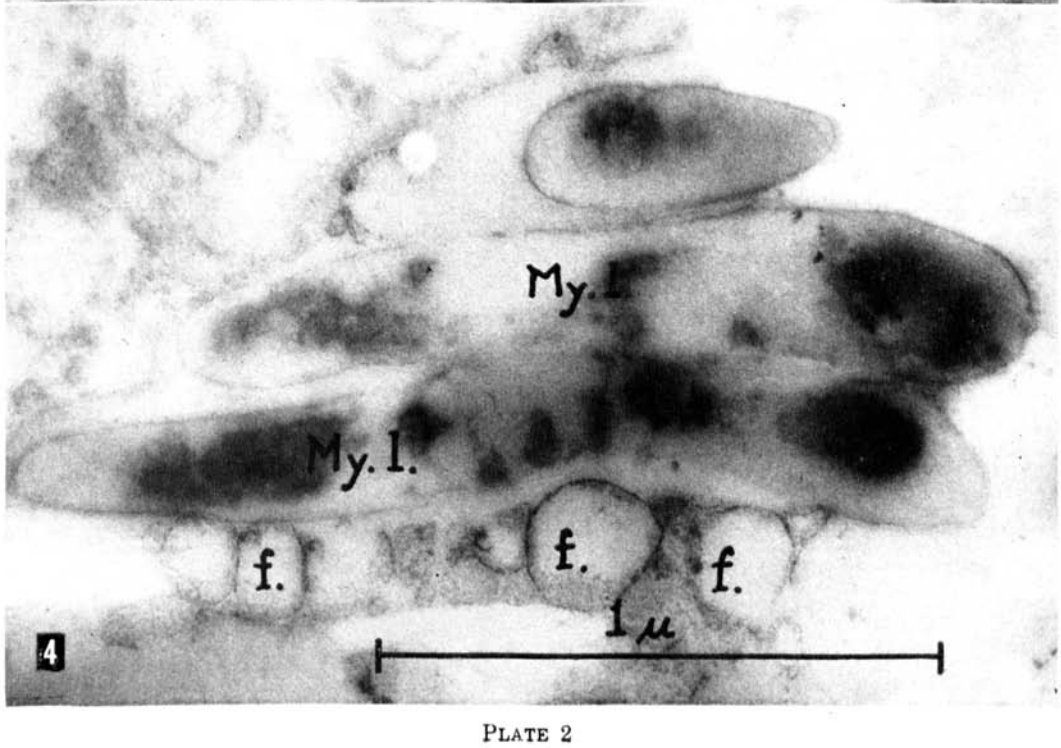


PLATE 2

PLATE 3

FIG. 5. Ultra-thin section of a leproma of the chin. Among many other things there are round structures, marked by a query (?), which for the present are inexplicable for us. Magnification, 11,400×.

Symbols: nuc., nucleus; My.l., leprosy bacilli; E.T.Z., electron-transparent zone; op.d., opaque droplet; c.f., collagen fibers, transverse sections; mit., mitochrondria.

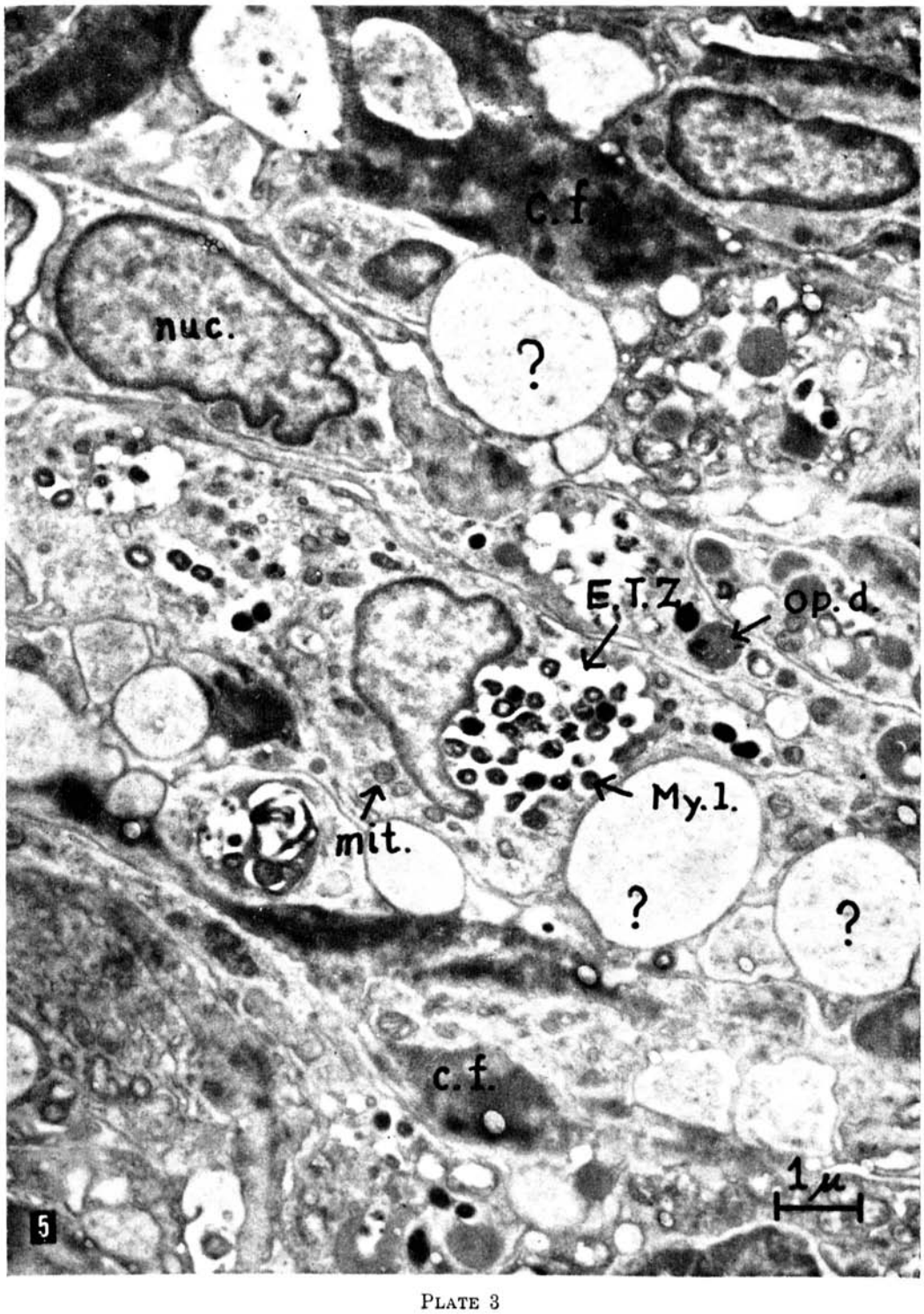


PLATE 3

PLATE 4

FIG. 6. Ultra-thin section of a relatively new leproma. Nuclear apparatus is visible in every bacillus. The bacilli are surrounded by electron-transparent material. Magnification, 33,000×.

Symbols: My. l., leprosy bacilli; b.nuc., bacillary nucleus; E.T.Z., electron transparent zone.

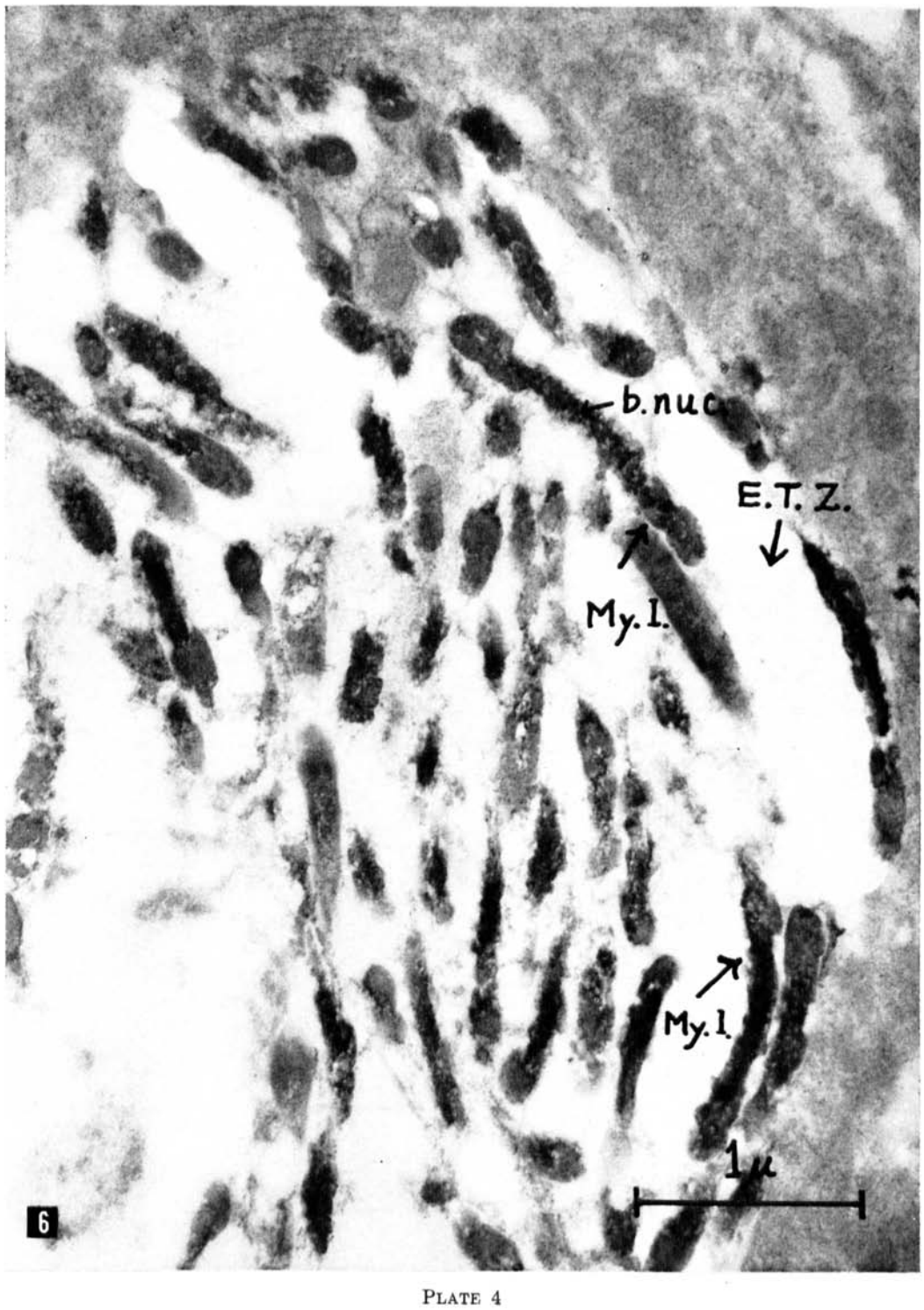


PLATE 4

PLATE 5

FIG. 7. Massed bacilli in an ultra-thin section of a leproma from a well-treated case. Around the bacilli there are many small foam spaces each of which has a single layer of electron-dense membrane. This membrane is composed of small rod-shaped electron-dense micelles. Bacilli in this leproma are devoid of nuclear apparatus. Magnification, 105,000×.

Symbols: f., foam spaces with electron-dense membranes; My.l., leprosy bacilli.

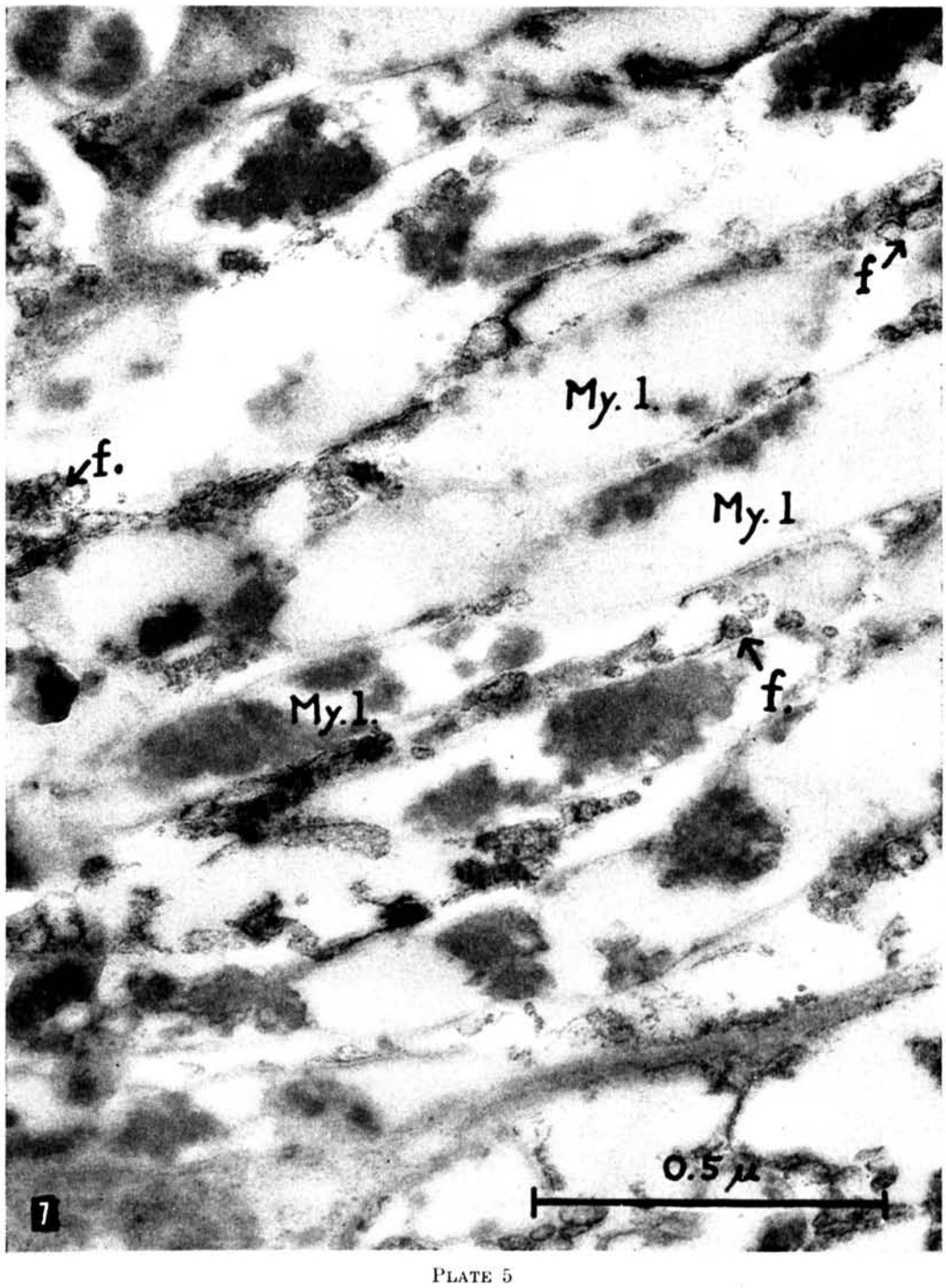


PLATE 5

PLATE 6

FIG. 8. An ultra-thin section of a leproma. Transversely-sectioned bacilli are seen in the cytoplasm of two cells. We think this picture shows the early phase of lepra cells. Magnification, 17,000×.

Symbols: My.1., leprosy bacilli; mit., mitochondria; nuc., nucleus; lim., a limiting membrane tightly surrounding a group of bacilli.

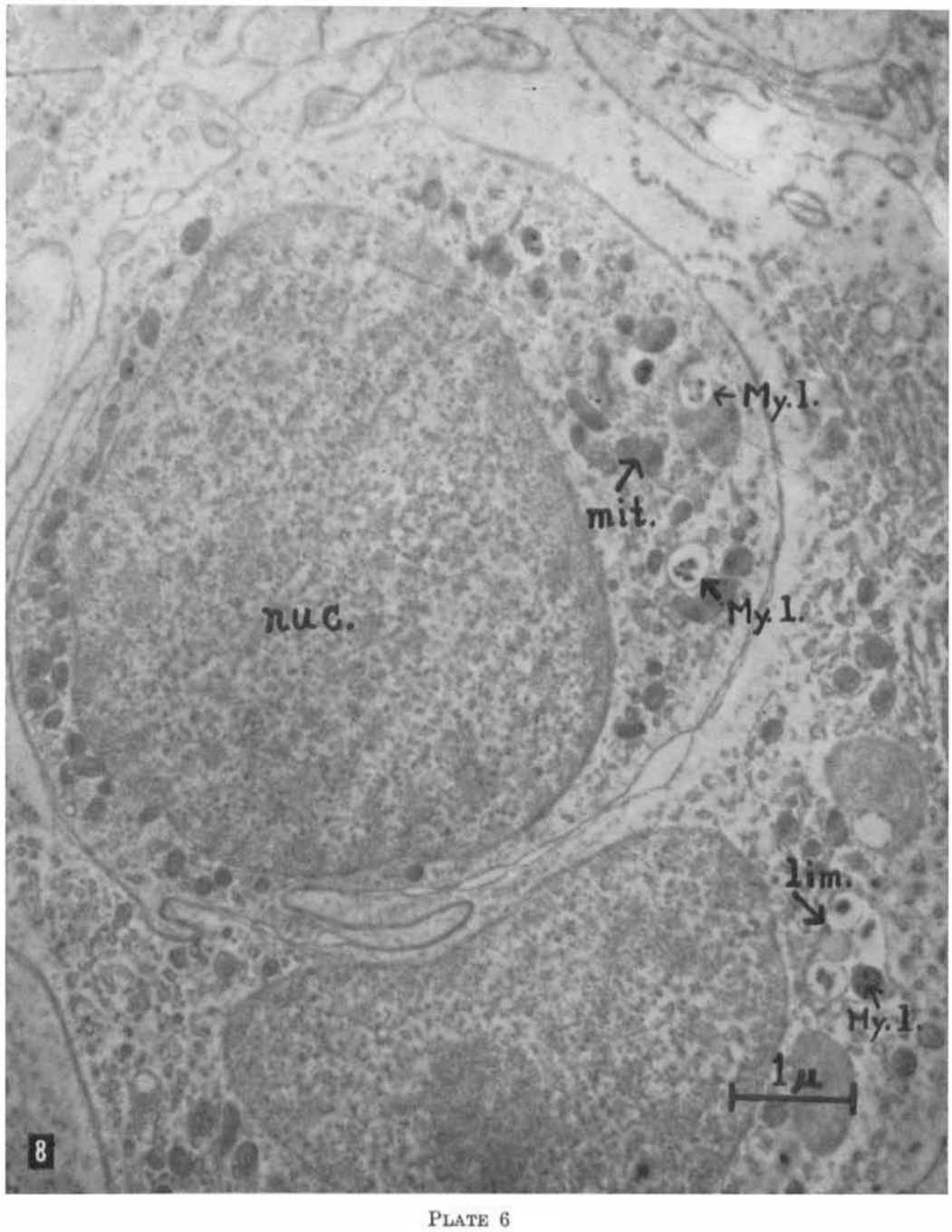


PLATE 6

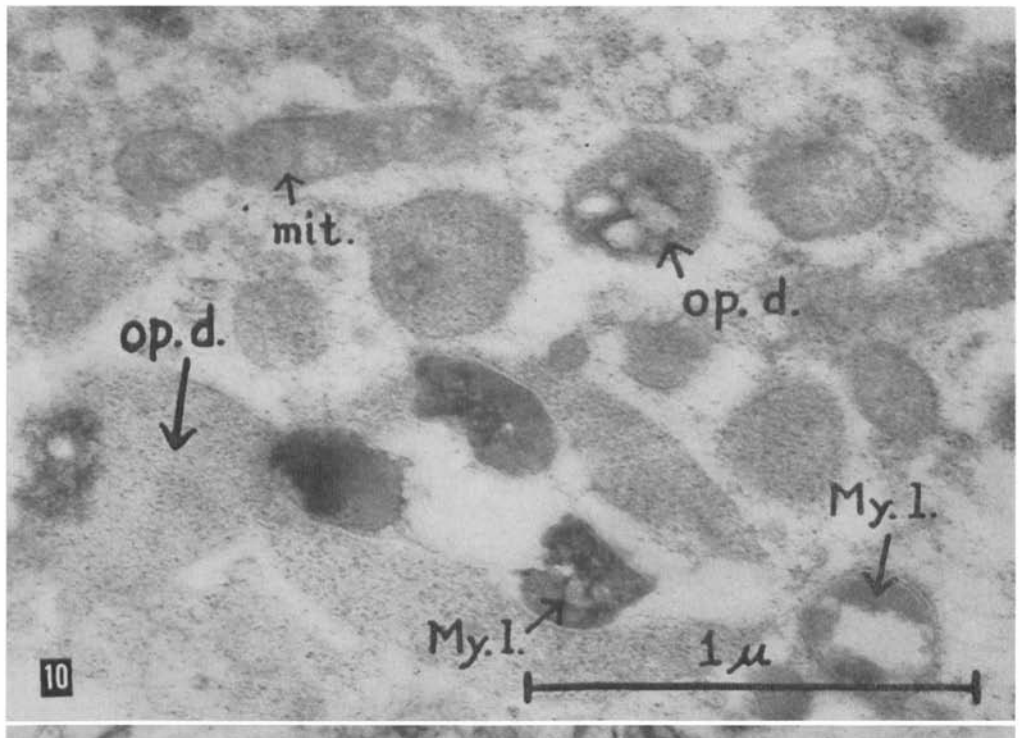
PLATE 8

FIG. 10. Leprosy bacilli in the cytoplasm of a lepra cell, surrounded by opaque droplets. The commencement of foamy development is seen in one of the droplets. Magnification, 59,000×.

Symbols: My. l., leprosy bacilli; op.d., opaque droplet; mit., mitochondria.

FIG. 11. Ultra-thin section of a lepra cell. An opaque droplet containing bacilli is observed in the cytoplasm. Magnification, 30,800×.

Symbols: op.d., opaque droplet; My. l., leprosy bacilli; mit., mitochondria.



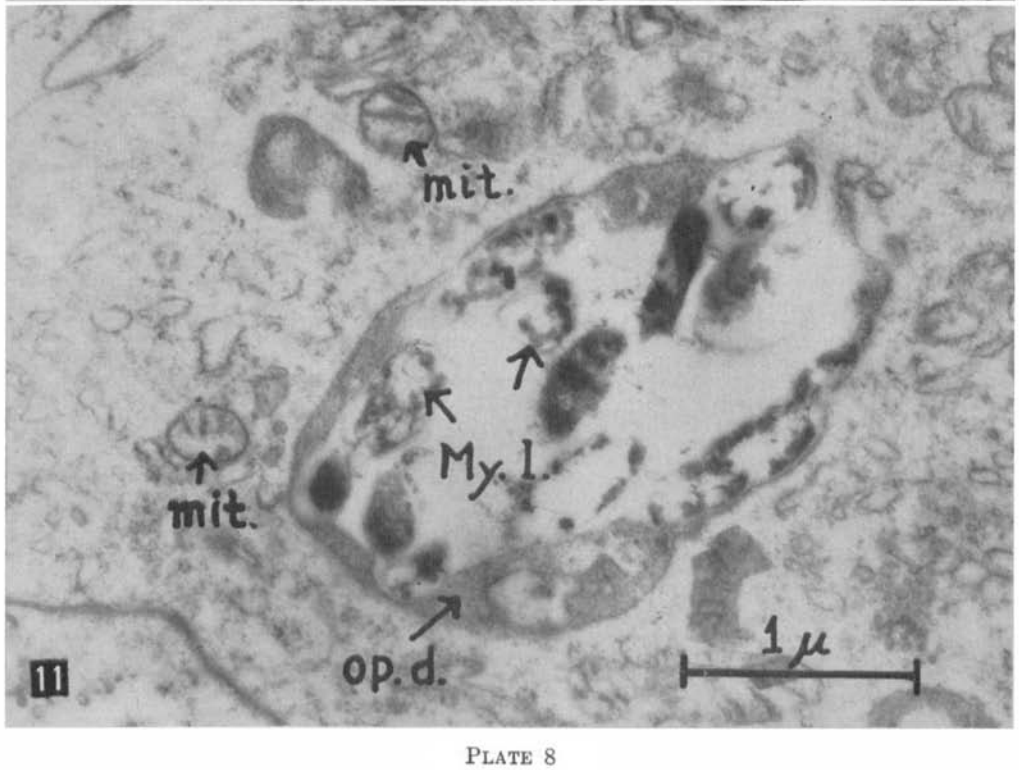


PLATE 8

PLATE 9

FIG. 12. Ultra-thin section of a leproma from a well-treated case. Degenerated bacilli and foamy structure in the cytoplasm of a lepra cell. Each space of the foamy structure is surrounded by a single layer of electron-dense membrane. Magnification, 37,800×.

Symbols: My.1., leprosy bacilli; f., foamy structure; e.d.m., electron-dense membrane of the foam space.

FIG. 13. Foamy structures in a lepra cell. The nucleus is pressed to one side and greatly thinned. Magnification, 7,000×.

Symbols: My.1., leprosy bacilli; nuc., nucleus.

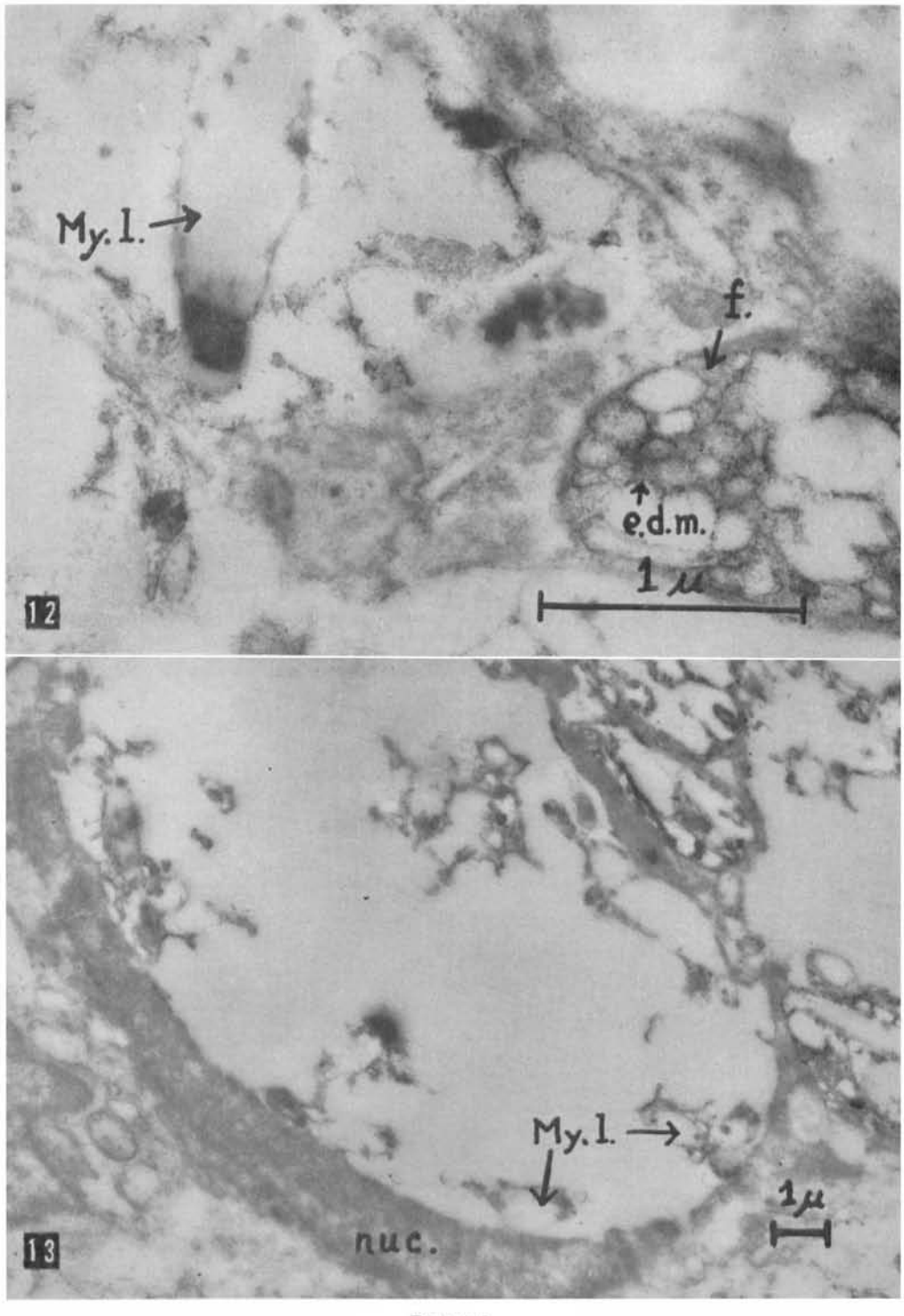


PLATE 9