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ELECTRON MICROSCOPIC STUDY OF BORDERLINE LEPROSY[1]

TAMOTSU IMAEDA, M.D.

Instituto Venezolano de Investigaciones Cientificas (IVIC)

JACINTO CONVIT, M.D. and PEDRO LAPENTA, M.D.

División de Dermatologiia Sanitaria
Ministry of Health
Caracas, Venezuela

Borderline leprosy, since Wade and Rodriguez ([53, 55]) and Wade ([56]) noted its importance in clinical leprosy, has been recognized as a transitional form between tuberculoid and lepromatous leprosy. This transformation occurs after repeated reactions in tuberculoid leprosy, which suggests the possibility of desensitization. Later, Cochrane ([5]) stated that tuberculoid turns into lepromatous leprosy and therefore proposed the term "transitional" as explaining better the development of this type lesion, although previously he had not supported the idea of this transformation ([3, 4]).

These accounts were based on clinical and histopathologic findings, which differ from those of the two distinct types of leprosy, tuberculoid and lepromatous. After a discussion that lasted more than ten years, this form of leprosy was classified as a group in the Madrid Congress ([6]), which stated that it represents an unstable phase of leprosy evolution. In 1960 borderline leprosy was again interpreted as a special condition, with supporting evidence presented by the Brazilian group ([44, 47]). However, it seems that this phase of leprosy has not yet been completely recognized as a separate group, because of a lack of definite elements distinguishing the unique condition of borderline leprosy, although histopathologic and clinical disclosures have defined its characteristics ([3, 4, 6, 7, 12, 31, 39, 44, 57]).

Since the period when British and Japanese groups applied electron microscopy to leprosy investigation, many new facts have been revealed in histopathology and bacteriology, and consequently two polar types of leprosy have been characterized by their particular ultrastructures ([22, 23, 42, 61, 62]). For this reason we made biopsies of

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tissues diagnosed as borderline leprosy clinically and histopathologically, in order to discover new data which would determine whether or not borderline leprosy really is a specific form of leprosy infection. Special attention was also applied in clarifying the host-parasite relationship in the lesion. This paper is a preliminary report on the subject, covering the period from 1959 up to 1963.

TABLE 1.—*Clinical data on patients.*

Patient	Lepromin	Treatment	Clinical aspect		Time onset to biopsy	Histology
			General	Biopsy region		
A. M.[a]	slight	none	plaques nodules	plaque	5 years	mixed
J. P.	slight	DPT 3 mos	plaques	plaque	5 years	mixed
E. C.[a]	negative	DPT 6 mos	plaques depigment. macules	plaque	3 years	mixed
E. R.[a]	negative	DPT 2 mos	nodules plaques	nodules plaques	unknown	mixed giant cells foamy cells
R. C.	negative	none	plaques depigment. macules	plaques	1 year	mixed
J. E. Q.	negative	none	plaques bands macules	plaque macule	unknown	mixed
R. V.	positive	none	plaques	plaque	4 years	mixed
T. R.[a]	negative	none	nodules ulcers	nodules ulcers	unknown (reactional)	non- vacuolated bacillated cells
D. P.[a]	negative	none	nodules macules	nodules macules	15 years	mixed
D. M. B.	negative	DDS 3 mos	macules plaques	plaque	unknown	mixed
J. R. M.[a]	negative	none	nodules plaques	nodules plaques	1 year	mixed clumped
A. G.	negative	none	nodules plaques macules	plaque	10 years	mixed
A. L.	slight	DDS 2 mos	macules	macule	14 years	mixed
M. L.	negative	none	macules plaques	macule	unknown	mixed
I. M. A.	negative	none	plaques macules nodules	plaque	1 year	mixed
C. M.	negative	none	bands	band	16 years	mixed
M. A.	negative	none	plaques macules	plaque	unknown	mixed

[a]Biopsies of these patients were used in this publication.

MATERIALS AND METHODS

Seventeen patients with borderline leprosy, diagnosed clinically and histopathologically, were selected for electron microscopic examination. The majority had received no treatment, but a few had had chemotherapy for a few months. These patients had various skin lesions, such as plaques, bands, reddish succulent macules and nodules. The latter usually were found in the ear lobes. "Immune areas" were sometimes observed, their border eruption frequently being abrupt. In one case we found small infiltrative lesions in which small vesicles had developed, followed by a superficial ulcer. This case may correspond to reactional borderline state ([2]). More data about each patient are summarized in Table 1.

Biopsy specimens usually were taken from two different skin lesions, especially when the lesions from individual patients were stained in varied strength with methylene blue ([7, 8]). In order to compare borderline leprosy lesions with lepromatous lesions, a newly formed leproma not treated by chemotherapy also was studied by biopsy.

Tissue chips were fixed with 1 per cent osmium tetroxide solution, buffered with s-collidine ([18]) and embedded with methacrylate after dehydration with alcohol. Ultrathin sections were made with a diamond knife on a Leitz Ultramikrotom and stained with saturated uranyl acetate solution ([59]), and 10 per cent phosphotungstic acid ([59]) or lead citrate ([45]) in order to obtain contrast and also to demonstrate fiber elements. Thin sections, approximately 0.5 μ in thickness were cut serially and stained with Giemsa solution. These sections were observed with a light microscope to compare them with electron microscopic findings. The electron microscopes used were Siemens Elmiskop I at 60 KV and Hitachi HS-6 at 50 KV.

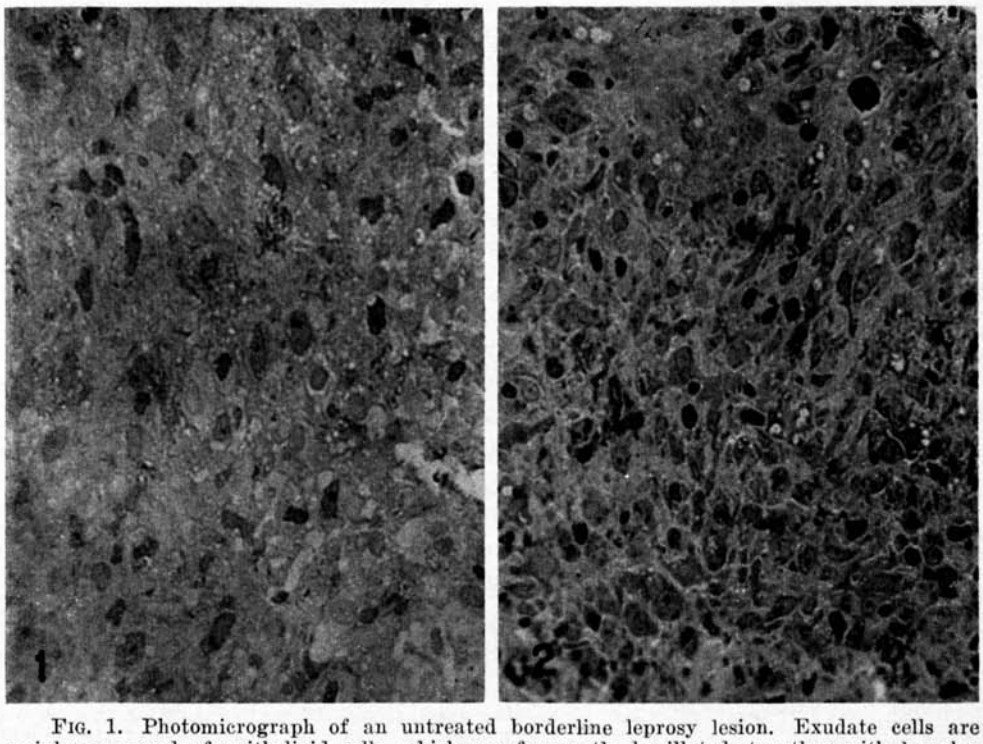


FIG. 1. Photomicrograph of an untreated borderline leprosy lesion. Exudate cells are mainly composed of epithelioid cells, which are frequently bacillated, together with lymphocytes.

FIG. 2. Photomicrograph of a treated borderline leprosy lesion consisting of epithelioid-type cells, foamy cells and lymphocytes.

OBSERVATIONS AND DISCUSSION

Cellular constituents of lesions.—Light microscopic observation of biopsy specimens from borderline cases demonstrates two types of lesion. One is composed of epithelioid cells, bacillated cells of epithelioid appearance and infiltrating leucocytes, mostly lymphocytes (Fig. 1). This is usually revealed in the lesions of untreated patients. Dis-

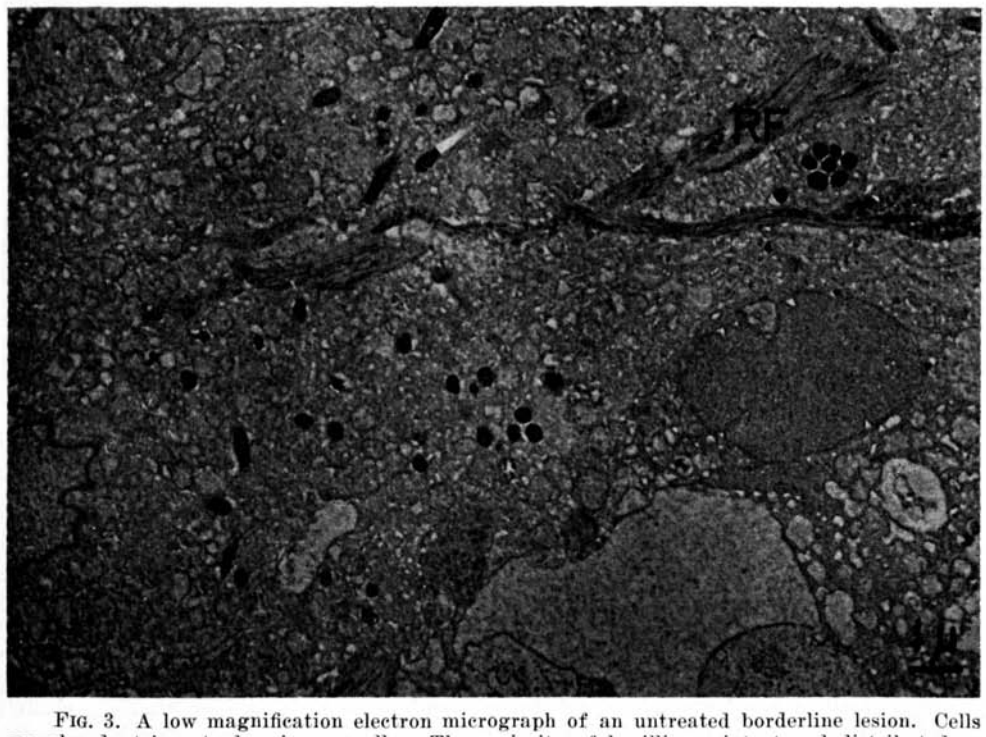


FIG. 3. A low magnification electron micrograph of an untreated borderline lesion. Cells are abundant in cytoplasmic organelles. The majority of bacilli are intact and distributed as single individuals and small groups. In intercellular spaces, moderately developed reticular fibers **(RF)** are embedded in the less dense reticulum. Uranyl acetate staining.

cernible globi do not exist in this type of lesion; i.e., gloea or "Schleim" substance is not evident around the bacilli. This type of lesion has been noted as a lesion composed of nonvacuolated lepra cells ([46, 56]) and has also been referred to as a lesion in which no foamy cell is found ([31]).

The other type of lesion consists of foamy cells, epithelioid cells and leucocytes (Fig. 2); it is generally found in the patient who received chemotherapy for a few months. This type corresponds to the lesion in which tuberculoid and lepromatous elements coexist in the same lesion, as reported by several authors ([1, 7, 44, 46, 48, 57]).

Figure 3 exhibits the electron micrographs of the same lesion as Figure 1 (non-treated case). Cells are characterized by abundant cytoplasmic organelles and frequently by an undulatory cell membrane containing bacilli as single individuals or small groups, the majority of which show intact morphology. Moderately dense droplets and foamy

structures are extremely rare in these bacillated cells.

In a reactional borderline lesion, in which a necrotic process occurs, bacillated cells show an appearance similar to that of the bacillated cells previously described with a tendency to disintegrate, thus forming small cavities in the lesion (Figs. 4a, 4b, 5 and 6).

The cytologic appearance of these bacillated cells in untreated cases is similar to that of epithelioid cells in tuberculoid lesions ([24, 28, 33, 42, 62]) and may indicate that intracellular reaction to bacilli also occurs in the borderline condition. Neither moderately dense droplets nor foamy structures, which presumably represent lipid material extracted by various solvents used in ordinary light microscopic examination, are distributed in these bacillated cells. In previous observations by other investigators these cells are described as nonfoamy lepra cells ([31, 46, 56]).

In biopsy specimens from treated patients, ultrastructures of lesions are slightly different from the lesions of untreated patients. The components of the cellular exudate are epithelioid cells and leucocytes (Figs. 7, 8, 10 and 12). The epithelioid cells frequently contain bacilli showing contracted cytoplasm, possibly representing bacterial degeneration, together with electron-transparent substance, foamy structures and moderately dense droplets. These additional intracellular structures are identical with those in lepra cells in lepromatous lesions ([22, 42, 61, 62]).

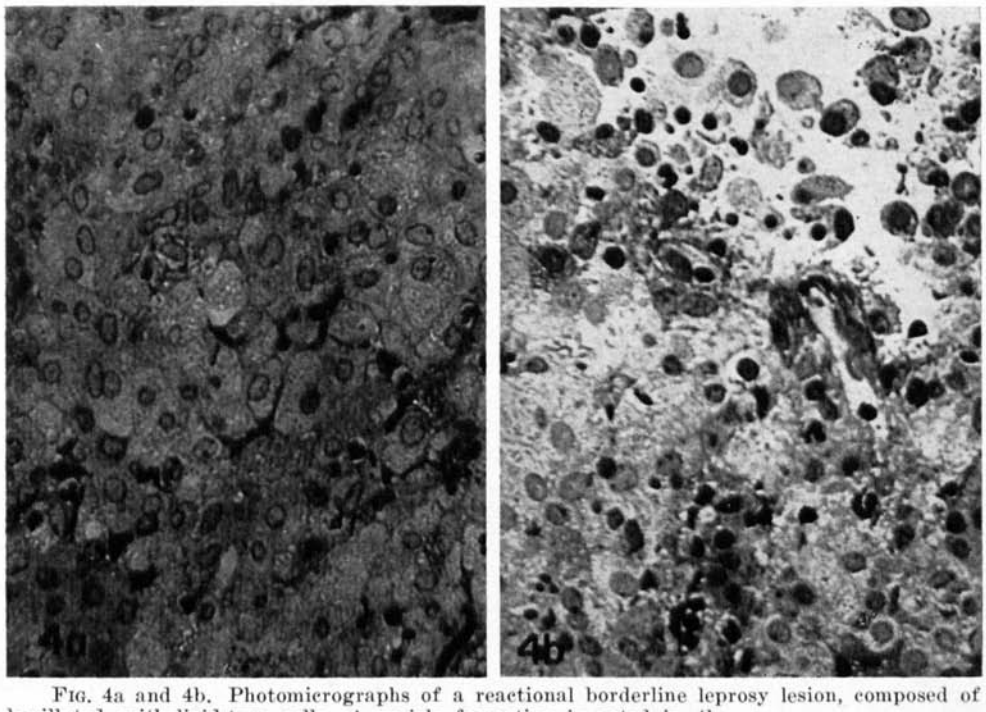


FIG. 4a and 4b. Photomicrographs of a reactional borderline leprosy lesion, composed of bacillated epithelioid-type cells. A vesicle formation is noted in 4b.

Lepra cells in a leproma are usually characterized by scanty cytoplasmic organelles and regularly contoured cell membranes, together with distributed opaque droplets, foamy structures and electron transparent substance around numerous clumped bacilli. These typical lepra cells are never encountered in borderline lesions. It is possible that the

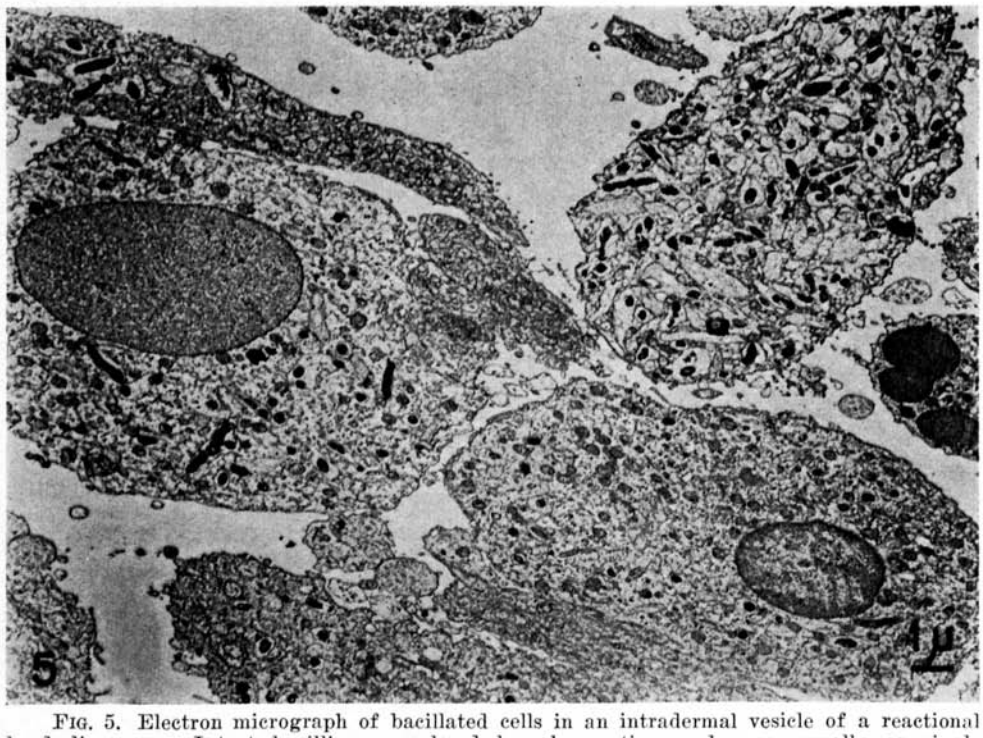


FIG. 5. Electron micrograph of bacillated cells in an intradermal vesicle of a reactional borderline case. Intact bacilli are enclosed by phagocytic membranes, usually as single individuals.

foamy cells observed in borderline leprosy lesions by light microscopy are not true lepra cells, but cytologically epithelioid cells modified by some elements of lepra cells.

In widened extracellular spaces, representing intercellular edema, round membrane-limited structures of various sizes are observed (Figs. 5, 6, 7 and 10). Their contents are fine granules, and occasionally mitochondria are included. Some of them may correspond to the transverse sections of cytoplasmic prolongations of infiltrating cells, especially lymphocytes in which cytoplasmic organelles are not well developed (Fig. 7). However, the intracellular vacuole, with fine granular matrix, is identical with the extracellular vacuole; it is seen also in epithelioid cells, representing the intracellular edema as explained by ordinary histopathology ([44, 46]). It is a natural conclusion that cellular destruction may occur following intracellular edema; this view is supported by the fact that endoplasmic reticulum and mitochondria also are found in extracellular spaces (Fig. 6). From these

findings, it appears that membrane-limited granular substance in extracellular space not only represents cytoplasmic prolongation but also corresponds to the intracellular vacuole discharged from edematous cells after cell destruction.

Formation of the lesion.—Sometimes intricately contoured cells

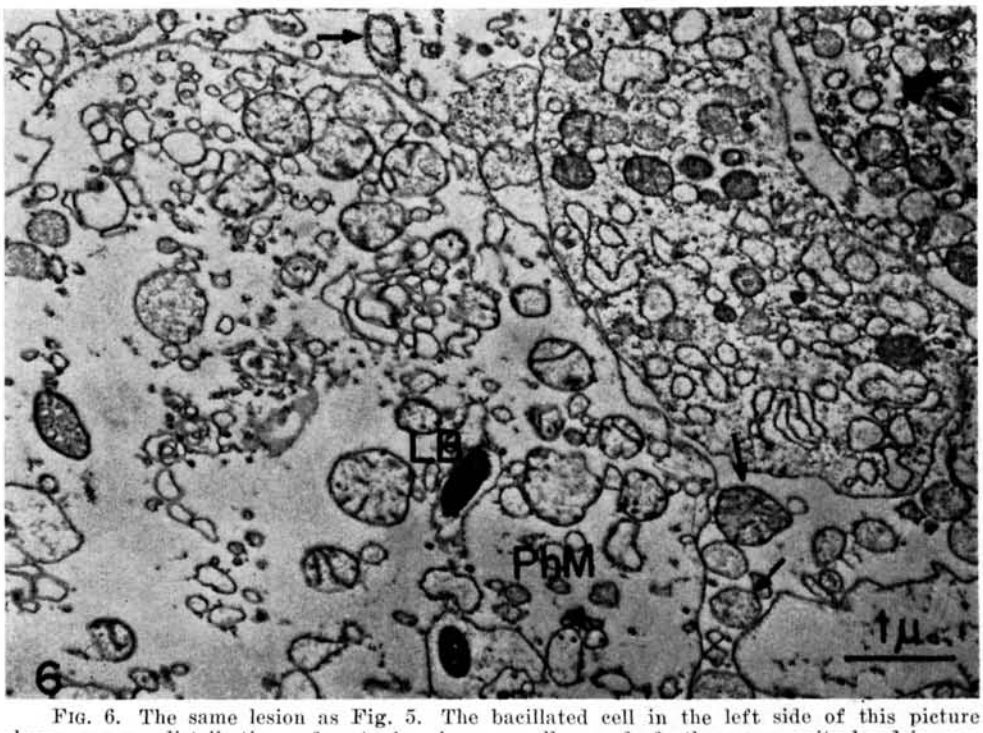


FIG. 6. The same lesion as Fig. 5. The bacillated cell in the left side of this picture shows sparse distribution of cytoplasmic organelles and furthermore, mitochondria are swollen, suggesting advanced intracellular edema. Note that mitochondria, rough surface endoplasmic reticulum and vesicles with granular content are seen in the extracellular space (arrows). **LB**: *M. leprae*, **PhM**: Phagocytic membrane.

with dense cytoplasm due to abundant distribution of endoplasmic reticulum of rough surface, free ribonucleoprotein particles (RNP), and vesicles, lie among epithelioid-type cells (Fig. 11). Occasionally they contain bacilli.

Concerning the formation of lepra cells, Cowdry ([10]) stated that they may be formed from macrophages, but he did not confirm the transformation of fibroblasts to lepra cells. In inflammatory conditions, however, it is believed that immature mesenchymal cells develop mainly into histiocytic cells or macrophages, especially in microbial infections ([34]). In the present study, small mitochondria with dense matrix and other cytoplasmic appearance of dense cells, may indicate the immature nature, as described by Galindo and Imaeda ([14]) in a study of mouse spleen. In addition, cells with cytoplasm of variable density are found (Fig. 11), which appear to be transitional forms between dense cells and epithelioid-type cells. It is suggested therefore that the

dense, irregularly contoured cells may represent immature reticular cells (young histiocytes), which perhaps develop into histiocytic cells, and later on, become bacillated epithelioid-type cells. In normal conditions, these immature reticular cells may develop into fibroblasts producing collagen fibers. This view of the transformation of immature reticular cells to bacillated cells is supported by similar findings in our study of hamster lesions caused by mycobacteria isolated from human leprosy ([30]).

On the other hand, pericytes adjacent to capillary endothelial cells and surrounded by a basement membrane, tend to vacate their original site adjacent to the capillary for more distant locales, as seen in Figure 12. A similar tendency is found also in lepromatous lesions ([25]). Imaeda ([21]) stated that pericytes may develop into fat cells, suggesting that they may be mobilized to form other types of cells. Taking this into consideration, pericytes are probably changed into histiocytic cells which by phagocytosing bacilli become host cells in borderline lesions.

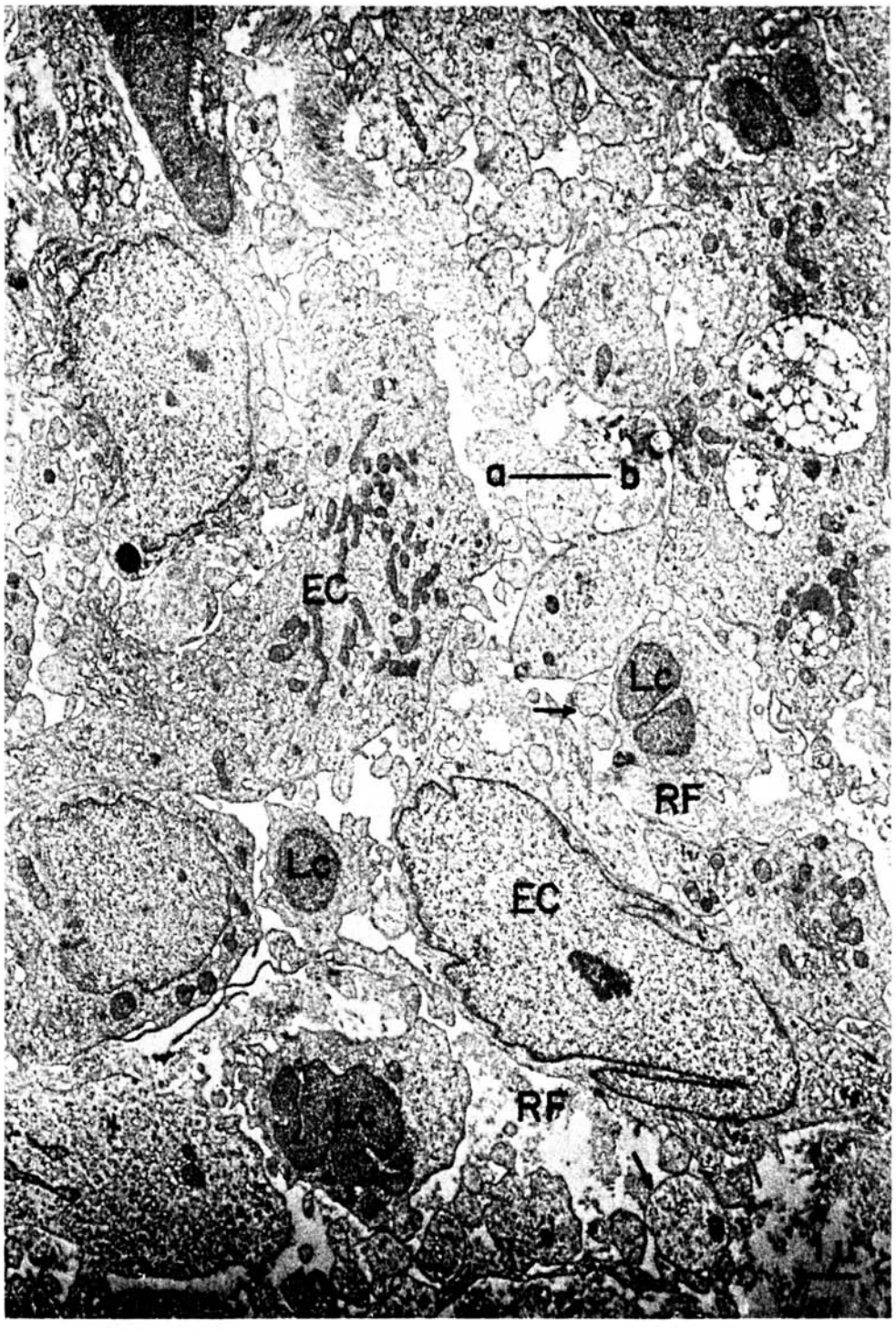
From these findings, two interpretations are possible regarding the origin of bacillated cells, viz., from immature reticular cells in dermis, and from pericytes, although both cells belong to the reticuloendothelial system of the dermis.

Reticulum and reticular fibers.—Striated fibers stained by PTA and uranyl acetate are distributed in intercellular spaces in the lesion (Figs. 3 and 13). These fibers, the diameter of which is approximately 500 Å and the axial periodicity 600 Å, are embedded in a fine fibrillar or homogeneous, amorphous matrix. Sometimes these fibers do not form bundles, but are arranged irregularly in the matrix mentioned above (Fig. 13). It is generally believed that normal skin shows a scarce distribution of reticular fibers, because reticular fibers are condensed to collagen. In pathologic conditions, reticular fibers are contained abundantly in granulomatous tissues resulting from the active formation of young mesodermal cells, such as reticular cells and histiocytes, which are capable of producing reticular fibers ([34]).

On the basis of electron microscopic study of the mouse spleen, Galindo and Imaeda ([14]) asserted that small bundles of fibers with an axial periodicity of ca. 600 Å and a diameter of 500 Å may be an integral part of the reticulum. The latter is a homogeneous amorphous matrix, possibly corresponding to the reticular fibers and not collagen fibers. In the light of these considerations, the intercellular amorphous

DESCRIPTION OF PLATE

FIG. 7. A low magnification of the treated borderline lesion. Cellular exudates are composed of epithelioid cells **(EC)** and lymphocytes **(Lc)**. Epithelioid cells are characterized by abundant distribution of cytoplasmic organelles and also by intricate cell membranes. One of them contains degenerated bacilli together with foamy structures and electron-transparent substance (the right side). Lymphocytes contain a few mitochondria and display cytoplasmic prolongations. Depending on the sectioning angle **(line a-b)**, these prolongations appear to be extracellular vesicles **(arrows)**. Reticular fibers **(RF)** exhibit the discontinuous arrangement due to intercellular edema represented by widened intercellular spaces.


a
b
EC
Lc
RF
Lc
EC
RF

or fibrillar substance observed in the present study, may represent the reticulum which turns into reticular fibers, after first showing irregular arrangement and later forming small bundles. Borderline lesions include considerable numbers of immature reticular cells which actively produce the reticulum. In addition, bacillated cells and nonvacuolated epithelioid cells, which are the main constituents of the lesion, also form the reticulum and subsequently the reticular fibers, because of the slight influences of intracellular edema and bacterial parasitism.

In ordinary histopathology, reticular fibers in lepromatous lesions show a fine network around exudate cells, while in tuberculoid lesions they are fragmented. Borderline lesions display an intermediate structure ([32]). In electron micrographs, nonedematous borderline lesions are packed with exudate cells and both reticulum and reticular fibers are located in the narrow extracellular space (Figs. 3, 11 and 13). On the other hand, moderately edematous lesions demonstrate dispersed fiber elements in broad spaces (Figs. 7, 8, 10 and 12), suggesting that intercellular edema causes the rupture of continuous reticulum and reticular fiber arrangements. This being so, the distribution of intercellular fiber elements in borderline lesions varies according to the intensity of intercellular edema of the lesions.

Morphology of M. leprae.—In untreated patients' lesions, the majority of bacilli exhibit their intact cytoplasm. These intact bacilli lie in epithelioid-type cells, as single individuals or groups. They are revealed also in cutaneous nerves in borderline cases ([28]). Imaeda and Convit ([26]), Imaeda and Ogura ([27]) and Imaeda ([29]) reported the ultrastructure of *M. leprae*, referring to the bacilli in borderline cases. In this structure the bacterial surface consists of an outermost diffuse layer, overlying an intermediate coating layer of low density, and an innermost moderately dense cell wall (Figs. 15a and 15 b). The diffuse layer may not be an actual structure of the bacterial surface but a substance adsorbed on the coating layer, the latter probably being a wax substance.

In a study of other mycobacteria, Imaeda and Ogura ([27]) pointed out that the low density substance occurs in the dividing surface of the moderately dense cell walls between daughter cells, suggesting that the coating substance is one of the components of the mycobacterial surface. Possibly it corresponds to the microcapsule of mycobacteria.

In certain areas, the double plasma membrane, the outer layer of which adheres to the inner surface of the cell wall, penetrates the cytoplasm, forming a complex membranous configuration called an "intracytoplasmic membrane system" (Figs. 15a, 15b and 16). Three dimensionally, this system is believed to be composed of tubular infoldings of invaginated plasma membrane. Consequently it appears to be a parallel membranous, or round vesicular structure, depending on the sectioning angle (Figs. 15a and 15b).

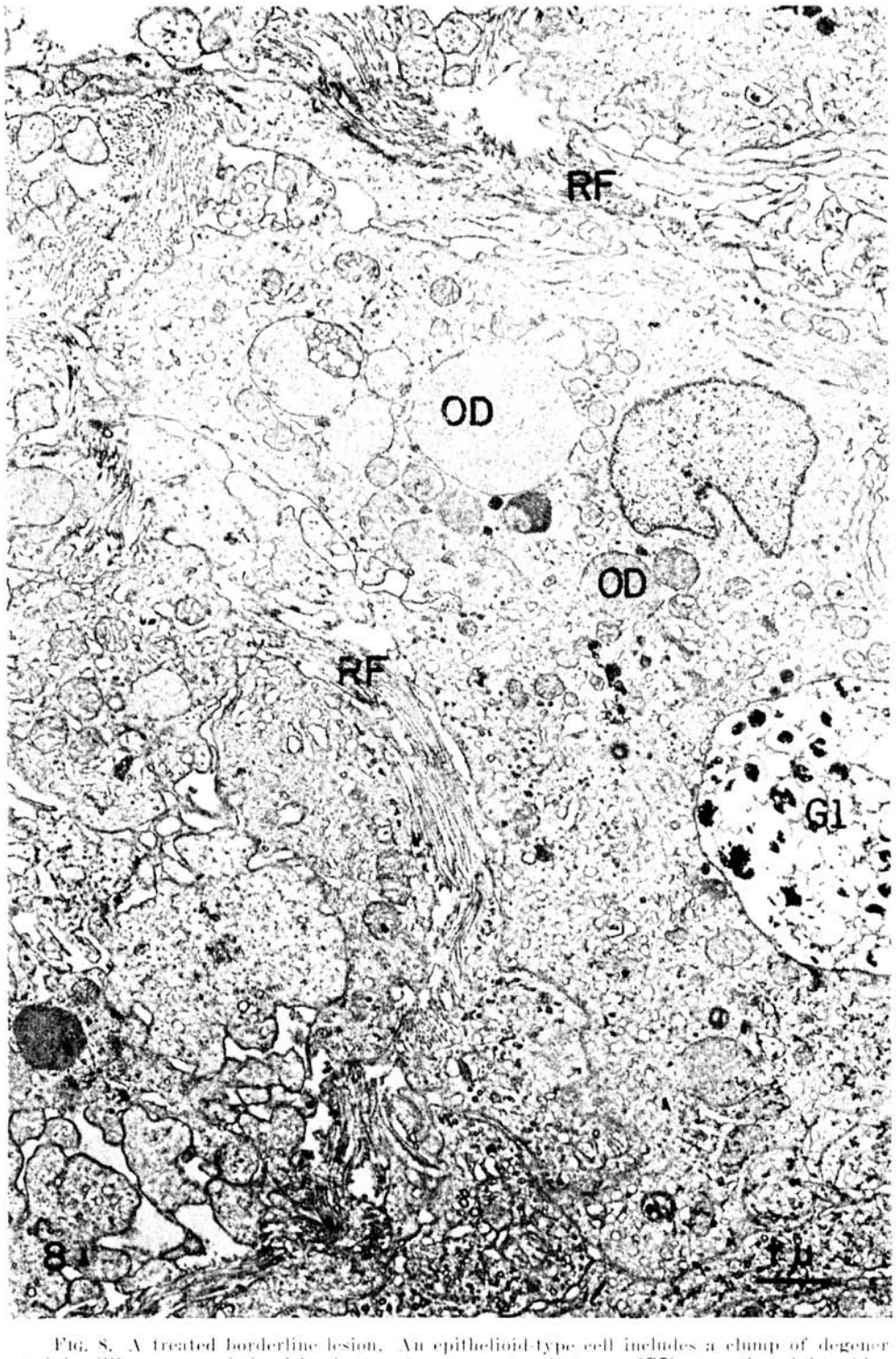


FIG. 8. A treated borderline lesion. An epithelioid-type cell includes a clump of degenerated bacilli accompanied with electron-transparent substance **(GI)** (on the right side). Opaque droplets **(OD)** are also evident and they show a tendency to coalesce with each other. In moderately widened intercellular spaces, reticular fibers **(RF)** are sparsely distributed.

In a well-developed intracytoplasmic membrane system of rapidly growing mycobacteria, a moderately dense substance appears to be sandwiched between two membranes derived from the outer layer of the plasma membrane, fusing into the cell wall in the connecting region of the system. Thus, each unit of the system consists of four layers: the outermost layers from the inner plasma membrane and the inner layers from the outer plasma membrane. Between the latter layers lies a moderately dense substance. Furthermore, the first occurrence of cellular division is noted in the region that connects this system to the cell wall. On the basis of this appearance Imaeda and Ogura ([27]) suggested that the system plays an important role in cellular division, while the moderately dense substance represents the cell wall precursor.

Occasionally, as seen in Figure 15c, the membranous structure is comprised of four layers, the moderately dense substance lying between the two inner layers. This structure is identical with the unit of the intracytoplasmic membrane system of other mycobacteria. In addition, it is closely related to the dividing surface. This explains the fact that the system of *M. leprae* may also produce cell wall precursor during cellular division. We presume that the relationship between the system and the cellular division of *M. leprae* may be clarified more easily in lepromatous cases, especially in the early stage of leproma formation, because bacilli multiply relatively rapidly at this stage of lepromatous lesions.

In the bacterial cytoplasm, there are two types of bodies with homogeneous content, one dense and the other less dense ([29]). These bodies are not limited by any distinct membrane. The dense bodies may represent polyphosphate structures, as already observed in other mycobacteria ([37, 38, 50]). The less dense bodies are believed to be poly-β-hydroxybutylate ([17]). It is noted in the present study that polyphosphate bodies are rarely found in the intact bacilli, while they are frequently encountered in degenerating bacilli. Ebel *et al* ([13]) asserted after study of lower organisms that polyphosphate may be formed more actively when the organisms undergo degeneration. With reference to his observation, it is possible that there is a close relationship between the occurrence of polyphosphate bodies and bacterial activity in *M. leprae*. This problem will be elucidated further in another paper.

The bacterial nucleus of *M. leprae* appears to be a dense irregularly shaped substance. Sometimes fine threads are revealed. This nuclear apparatus disappears when the bacillus shows the first sign of degeneration, as described by Zapf and Wahn ([63]) in a study of *M. tuberculosis*.

Rath de Souza ([44]) reported that bacilli in borderline lesions are usually short, seldom longer than 4μ. In their study of *M. leprae murium*, Hilson and Elek ([20]) substantiated the claim that a transient but significant increase in average bacillus length occurs during the

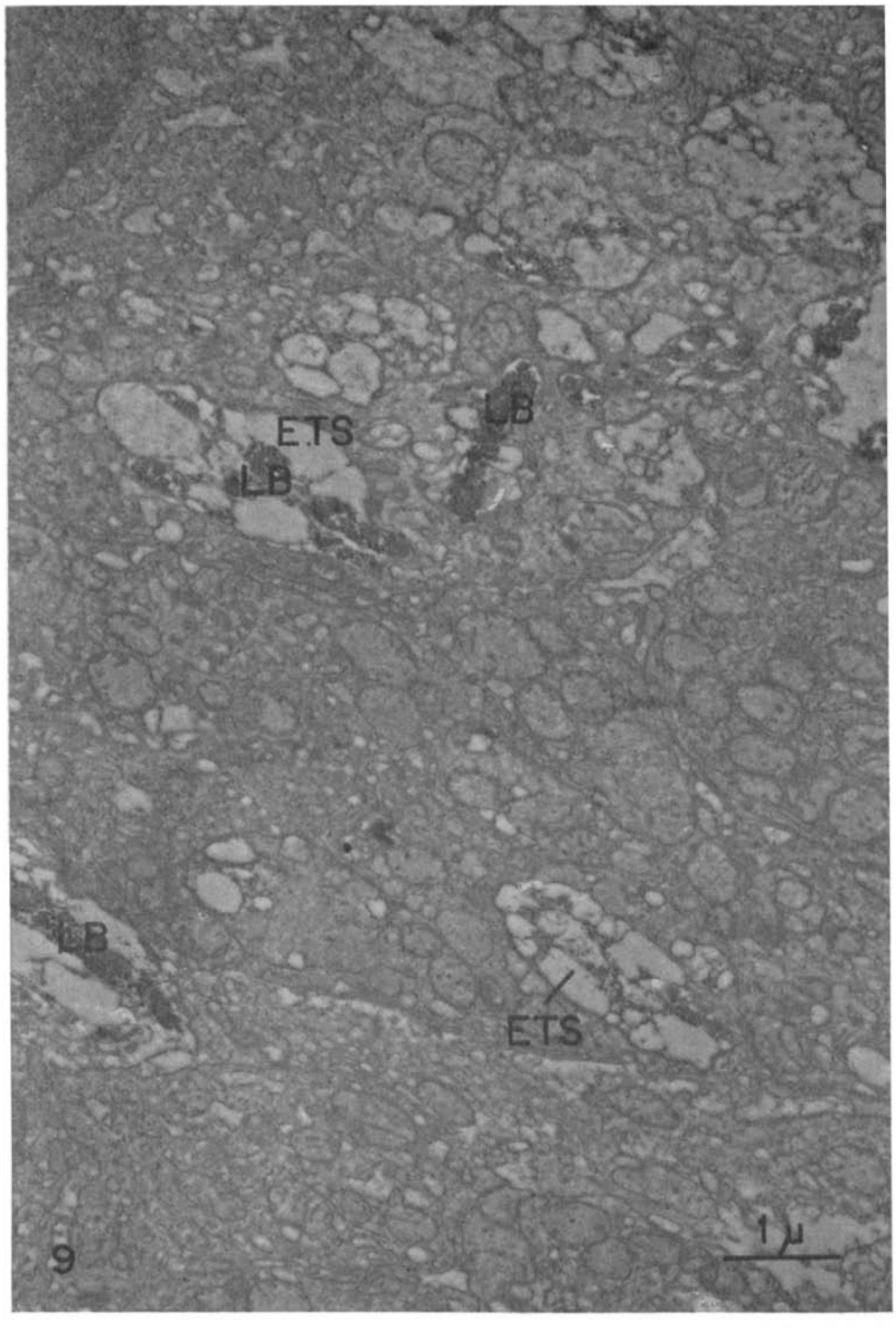


FIG. 9. An untreated borderline lesion. The bacilli **(LB)** are distributed as single individuals or small groups in epithelioid-type cells. They are encased in electron-transparent substance **(ETS)** and some contain contracted bacterial cytoplasm, implying bacterial degeneration.

lag phase of multiplication. In electron microscopy it is very difficult to determine whether or not bacilli in borderline lesions are shorter than in lepromas, especially in ultrathin sections. However, it is possible to say that relatively long bacilli contain a well-developed intracytoplasmic membrane system, indicating that they may undergo

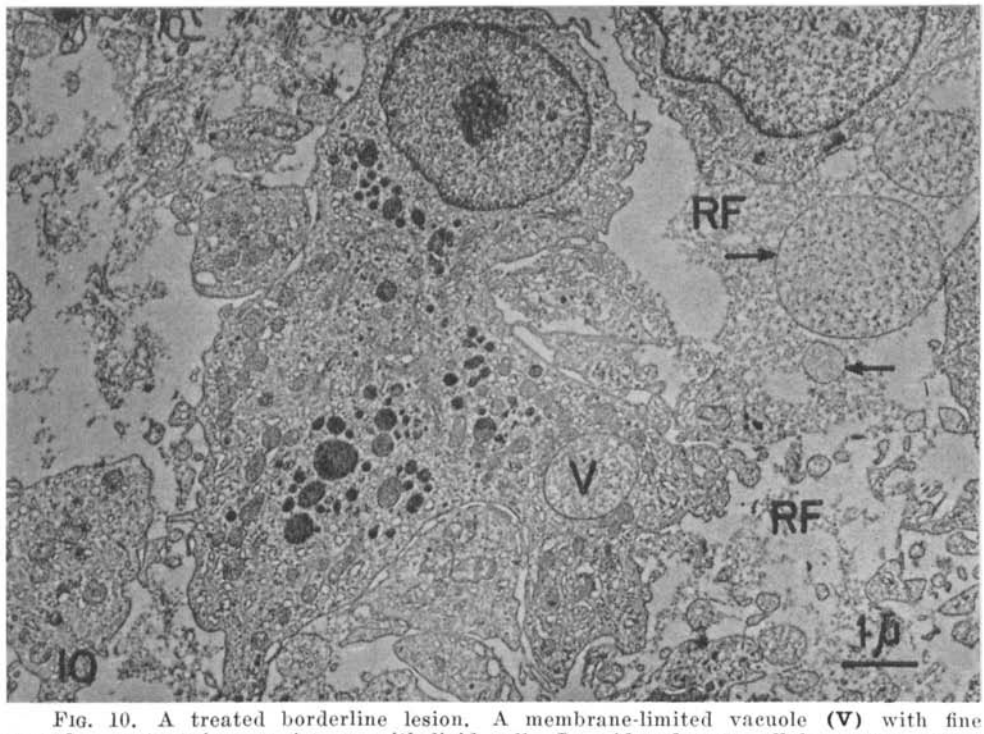


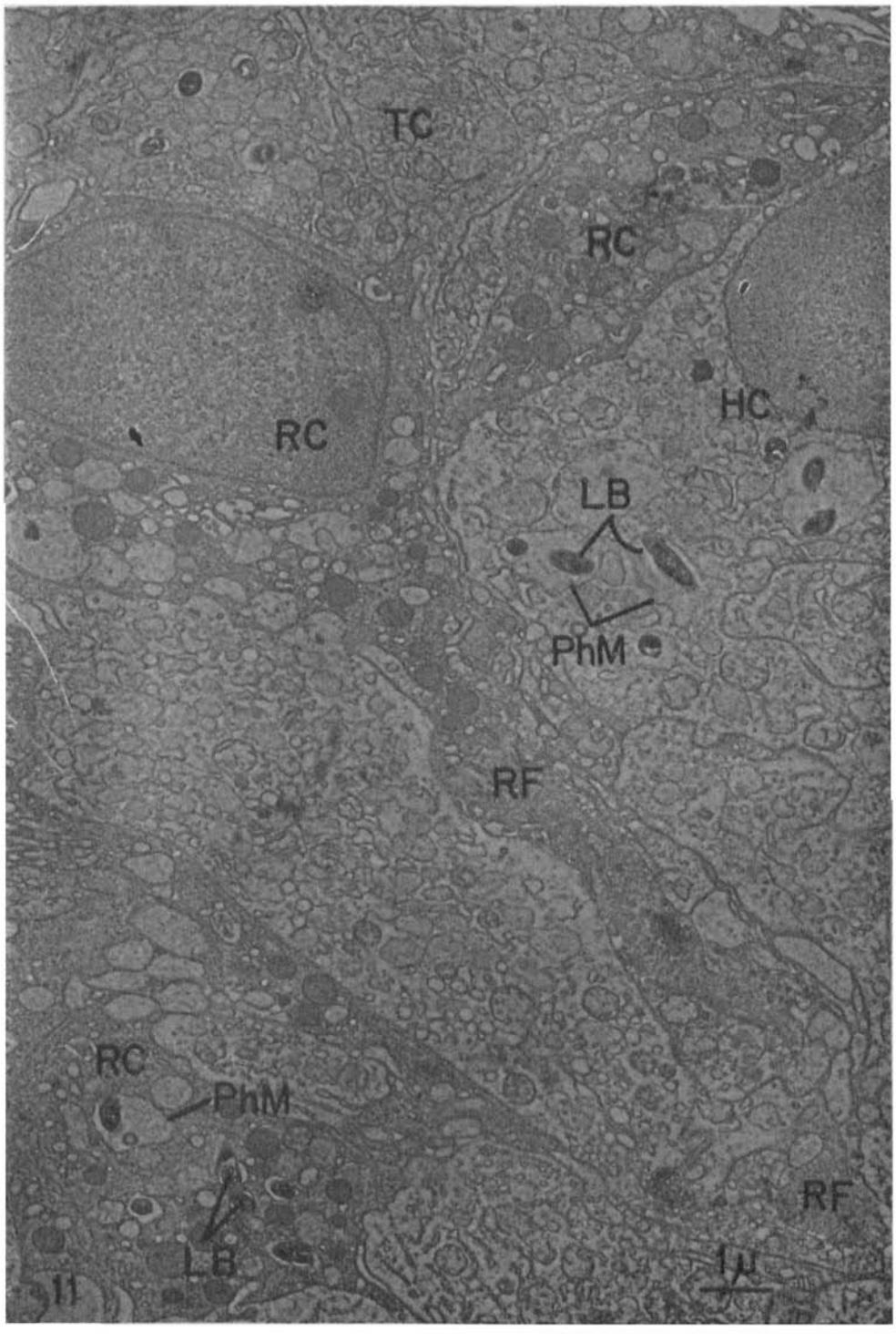
FIG. 10. A treated borderline lesion. A membrane-limited vacuole **(V)** with fine granular content is seen in an epithelioid cell. In widened extracellular space, round bodies of various sizes containing fine granules, similar to intracellular vacuoles, are exhibited **(arrows)**. Reticular fibers **(RF)** are scattered in broad intercellular spaces.

cellular division soon. In other words, lengthy bacilli may be observed frequently in lesions where bacillary multiplication takes place actively. However, bacilli are not so rapidly reproduced in borderline cases as in lepromatous cases, because of the reactivity of host cells, as described later. Therefore long bacilli are not found frequently in borderline leprosy lesions.

Intracellular distribution of M. leprae *and its microenvironment.* —In a reactional borderline condition, bacilli are usually distributed

DESCRIPTION OF PLATE

FIG. 11. Immature reticular cells **(RC)** in an untreated reactional borderline lesion. These cells show the intricate cytoplasmic prolongations and have the condensed cytoplasmic organelles together with many vesicles; thus they appear to be dense. Their mitochondrial matrix is dense, in comparison with that of other cells. Reticular fibers **(RF)** embedded in the reticulum show the close relationship with these cells. Well developed histiocytic cells **(HC)** with less cytoplasmic condensation are adjacent to the immature reticular cells. Both types of cells contain intact bacilli **(LB)** which are enclosed by phagocyte membranes **(PhM)**. In the upper part, a transitional cell **(TC)** lies between two types of cells, one dense and the other histiocytic.


TC
RC
HC
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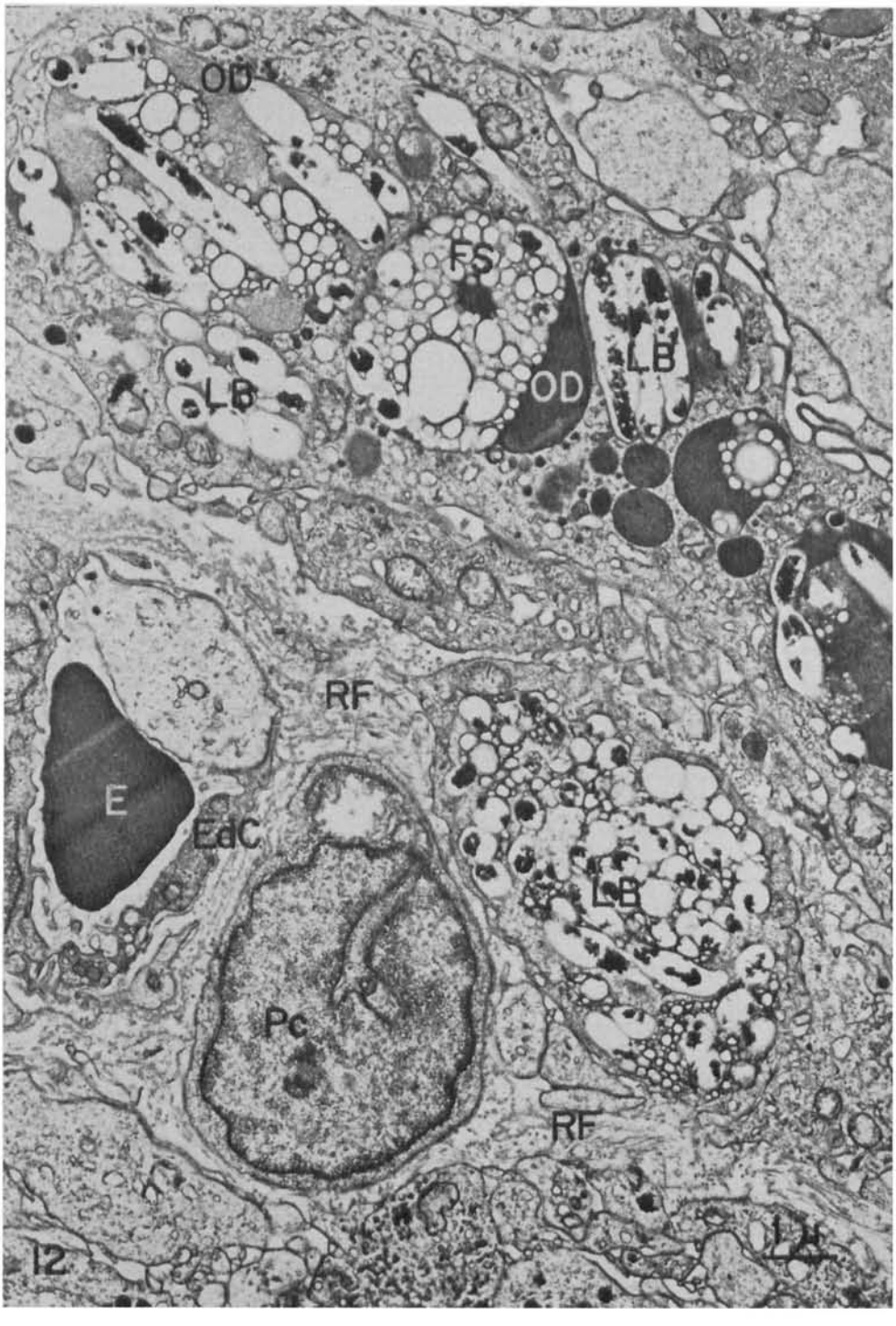
OD
FS
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12

as single individuals or small groups of a few bacilli, and never form clumps (Figs. 5, 6 and 11). In addition, they are always surrounded by membranes, presumably corresponding to phagocytic membranes, and separated from host cell cytoplasm. Usually bacilli in host cells are enclosed by a membrane a short time after phagocytosis. This phagocytic membrane disappears for some time, releasing phagocytosed bacilli into the cytoplasm.

In the reactional phase, cellular destruction may occasionally form intradermal vesicles, as shown in Figures 5 and 6, which ulcerate. Consequently, bacillated cells may not persist until bacilli multiply and accumulate in a group. In reactional borderline state, therefore, one can see only the early stage of bacillary invasion, when bacilli begin their multiplication inside the remaining phagocytic membrane.

In other cases of untreated borderline lesions bacilli occasionally accumulate in host cells and the majority of these clumped bacilli display their intact features (Figs. 3, 14 and 16). These bacilli, especially in small groups, are not wrapped with electron-transparent substance but are in direct contact with the host-cell cytoplasm. In relatively large clumps, however, the cytoplasmic matrix surrounding bacillary aggregates appears to be a low density area containing fine granules (Fig. 14), some of which are undoubtedly RNP particles. At this stage, this low density area is not delimited by a membrane but fuses into apparently intact host cell cytoplasm. In some cases, this bacterial microenvironment decreases the granular substance containing very low density substance spotted with dense material (Fig. 17). Furthermore, electron-transparent substance occurs in this case, forming the limiting membrane in the region adjacent to the surrounding intact cytoplasm.

Nishiura ([42]) postulated that electron-transparent substance may be produced around *M. leprae* in a stationary growth stage, suggesting its close relationship with bacterial growth. However, as seen in Fig. 9, electron-transparent substance also appears in a single bacillus or in a small group. On the basis of our observations, we believe that electron-transparent substance may occur as a result of cytoplasmic disintegration of host cells caused by bacterial parasitism.

It should be noted that the bacilli completely surrounded by electron-transparent substance are mostly degenerated (Figs. 9 and 14). According to Nishiura ([42]), the accumulation of electron-transparent substance may interfere with bacterial metabolism, resulting in bac-

DESCRIPTION OF PLATE

FIG. 12. A pericapillary lesion of the treated borderline patient. A pericyte **(Pc)** surrounded by a basement membrane shows a tendency to develop into a histiocytic cell, freeing itself from its original site. Epithelioid-type cells include bacilli **(LB)**, all of which are degenerated, together wth opaque droplets **(OD)** and foamy structures **(FS)**. Note that these bacillated cells still contain abundant cytoplasmic organelles. Reticular fibers **(RF)** are sparsely distributed in the moderately widened extracellular space. **EdC**: Endothelial cell of capillary. **E**: Erythrocyte.

terial death. In relation to this problem, Figure 17 exhibits a very interesting feature; irregularly shaped dense structures are occasionally found in large bacterial clumps enclosed by a small amount of transparent substance. Some of them show cytoplasmic details of bacilli, but the distinct structure of cell wall is not always visible. These structures may represent some abnormal bacterial features. It is emphasized that these structures are always evident when electron-transparent substance appears around the bacilli, especially in large clumps.

According to Mitchell and Moyle ([35]), the cell wall of the exponential phase of *Staphylococcus aureus* loses its tensile strength, and the protoplasts are released from the cell wall which protects the bacterial cytoplasm. The same authors said, in addition, that the protoplasts are lysed almost instantaneously in protein denaturants such as urea and glycine ([36]). Since electron-transparent substance contains denaturants of protein-rich cytoplasmic organelles of host cells, bacilli are influenced by the protein denaturants, although these substances are previously formed by bacilli themselves. Furthermore, it is probable that the irregularly shaped bacillary structures represent protoplasts of *M. leprae* that have been formed during a relatively active growth stage and subsequently have undergone degeneration caused by electron-transparent substance.

Similar consideration will be applied to furnish one of the reasons for the completely negative result in animal transmission using the bacilli from lepromatous cases ([9]); the inoculum contains bacilli together with electron-transparent substance which may interfere in bacterial growth in animal tissues.

Bacterial aggregation in comparison with so-called "globi."—In light microscopic studies of borderline leprosy, some reports noted that the bacilli usually form "globi" ([1, 46, 53, 54]), but Rath de Souza ([44]) noted no tendency to form "globi" in this condition. Before discussing this problem, we feel that there is some confusion concerning the terminology of "globi" ([58]). It may be worth while to note the description of "globi" by Cowdry ([10]), Gay and Steinbach ([15]), and Neisser ([40, 41]). According to the first two articles, the term "globi" was applied to the bacillary clump with the clear halo (gloea or Schleim). Neisser used the term "globi" to explain bacillary agglomerates embedded in a waxlike substance called "gloea."

Comparing these observations with electron micrographs, it is understandable that the term "globi" may be applied to bacterial aggregations encased in abundant electron-transparent substance corresponding to gloea in light microscopy. Figure 18 exhibits one of the true "globi" in a lepromatous lesion.

It should be mentioned that all our cases of untreated borderline leprosy, some of which were examined by biopsy more than one year

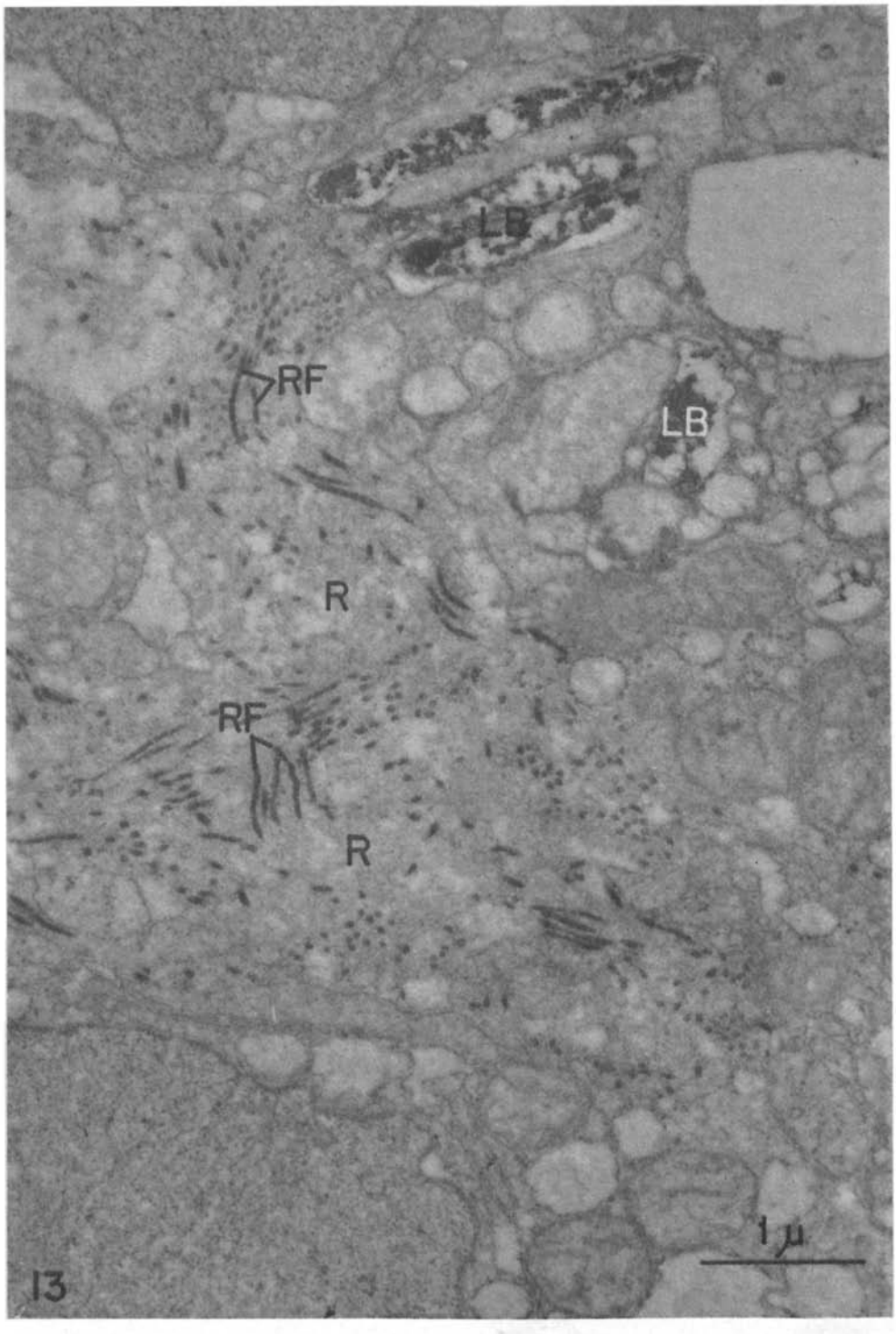


FIG. 13. This picture exhibits densely stained reticular fibers embedded in the reticulum **(R)** of moderately dense fibrillar matrix. Reticular fibers show irregular arrangement. These fiber elements are located between bacillated cells. An untreated lesion. Phosphotungstic acid stain. **LB**: Degenerated bacilli.

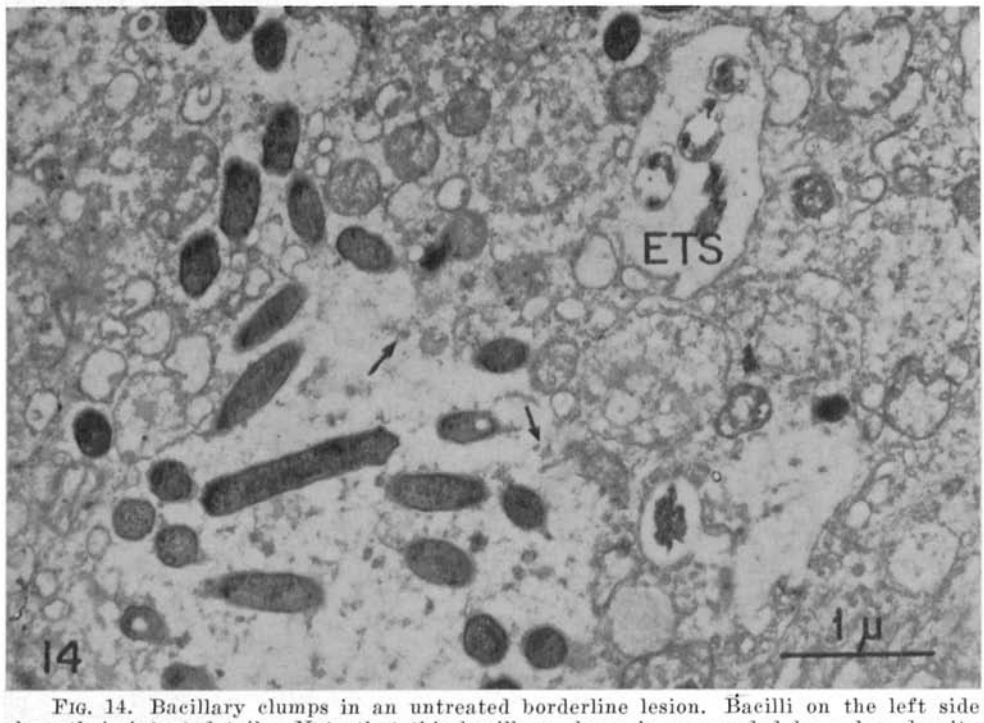


FIG. 14. Bacillary clumps in an untreated borderline lesion. Bacilli on the left side show their intact details. Note that this bacillary clump is surrounded by a low density substance containing many particles, some of which are RNP particles **(arrows)**. Also note that there is no limiting membrane between this bacterial environment and host cell cytoplasm. On the upper right side, four bacilli showing contracted cytoplasms are encased in electron-transparent substance **(ETS)**, which is delimited by a membrane from host cell cytoplasm.

after the formation of skin lesions, demonstrate a bacillary clump composed of intact bacilli, although it occasionally accompanies small amounts of electron-transparent substances. This implies that in the borderline state, true "globi," which should be accompanied by gloeal masses are not revealed in a short period, especially in untreated cases. This is different from lepromatous cases in which "globi" are formed very soon.

Since we could not observe the further evolution of untreated patients' lesions, it is somewhat speculative to discuss whether or not bacterial aggregations become "globi" without treatment. However, the occurrence of electron-transparent substance in bacillary clumps may cause bacterial degeneration in time, as described in the foregoing. Thus, "globi" are possibly formed in untreated cases, but after a long period of time.

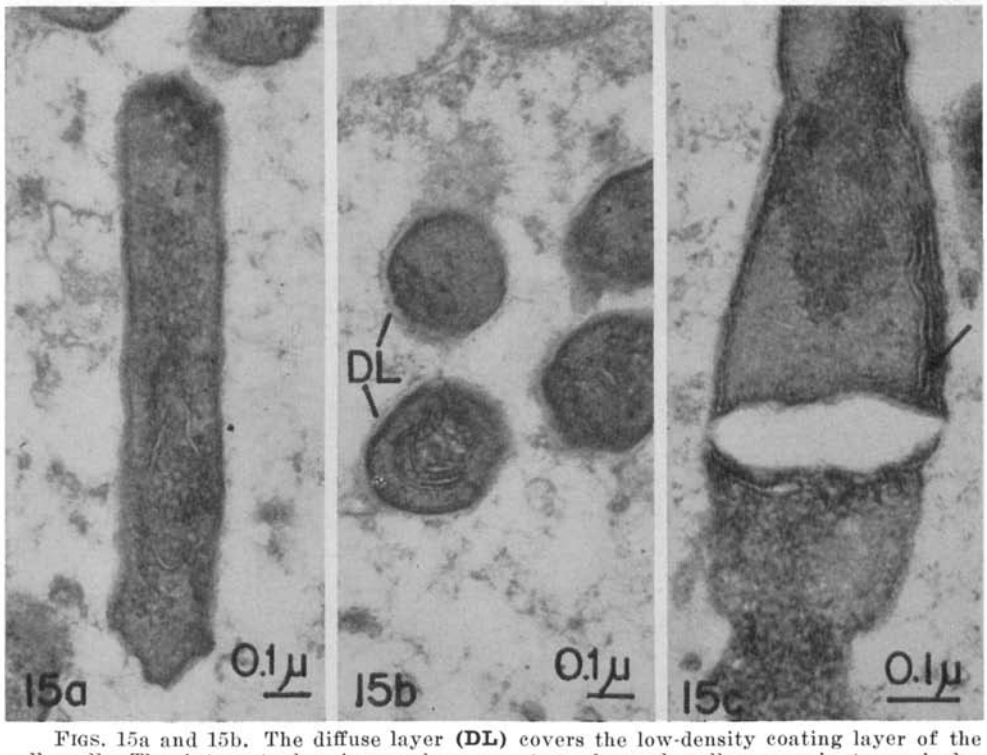
On the other hand, degenerated bacillary clumps with electron-transparent substance, as exhibited in Figures 8 and 12, occur in treated patients, possibly as a result of chemotherapy. The term "globi" should be applied to these clumps.

Wheate ([60]) recommended that "all borderline cases should be

admitted to a leprosarium, not only because of the danger of crippling deformities, but also because of *their infectivity.*'' His opinion is supported by our electron-microscopic observations, which brought out the fact that nearly all bacilli, both in clumps and in single individuals, are intact, implying their infectivity.

Bacterial growth and cellular reaction.—Borderline leprosy is believed to be an intermediate immunologic state between lepromatous and tuberculoid types, i.e., between allergic and anergic conditions. This consideration is based on clinical study of the transformation of tuberculoid to lepromatous leprosy ([5, 52, 53, 55, 56]). Although Cochrane ([3, 4]) doubted the role of an allergic process in the transformation of tuberculoid cases into the anergic process of lepromatous cases, it is conceivable that various stages of tissue response occur between the two extremes ([3, 6, 11, 39, 44]), aptly named ''pseudoallergy'' by Cochrane ([3]). Clinical and histopathologic pictures correlate with this tissue response.

In electron micrographs of borderline lesions, infiltrating cells, except for leucocytes, are always more abundant in cytoplasmic organelles than lepra cells in lepromatous cases. Rather, the cytologic



FIGS. 15a and 15b. The diffuse layer **(DL)** covers the low-density coating layer of the cell wall. The intracytoplasmic membrane system shows lamellar or minute vesicular structure, depending on sectioning angle.

FIG. 15c. The intracytoplasmic membrane system contains a moderately dense substance sandwiched between two inner layers **(arrow)**. Note the close relationship between the system and the dividing surface.

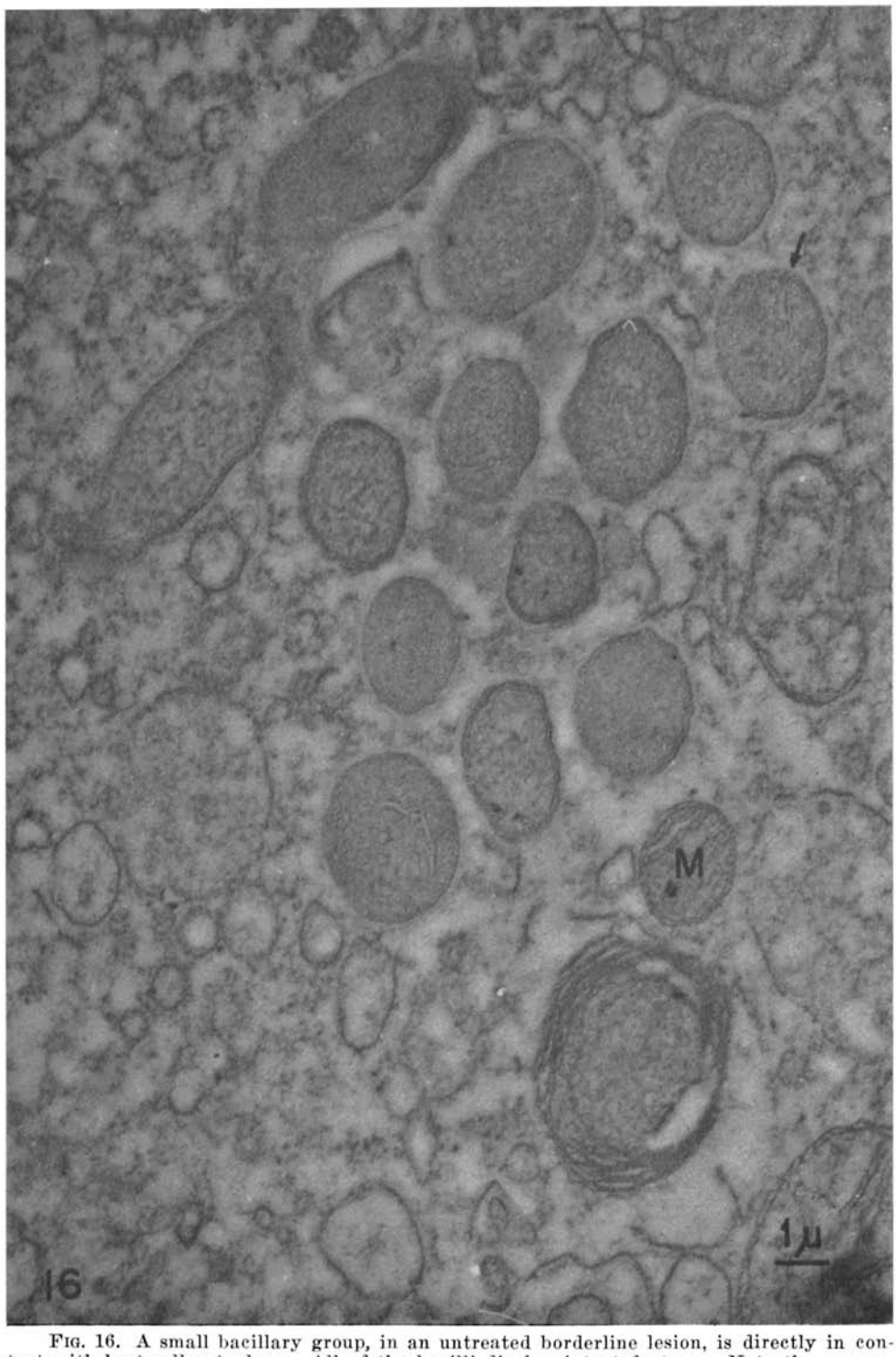


FIG. 16. A small bacillary group, in an untreated borderline lesion, is directly in contact with host cell cytoplasm. All of the bacilli display intact features. Note the connection between the intracytoplasmic membrane system and the plasma membrane **(arrow)**. **M**: Mitochondria of host cell.

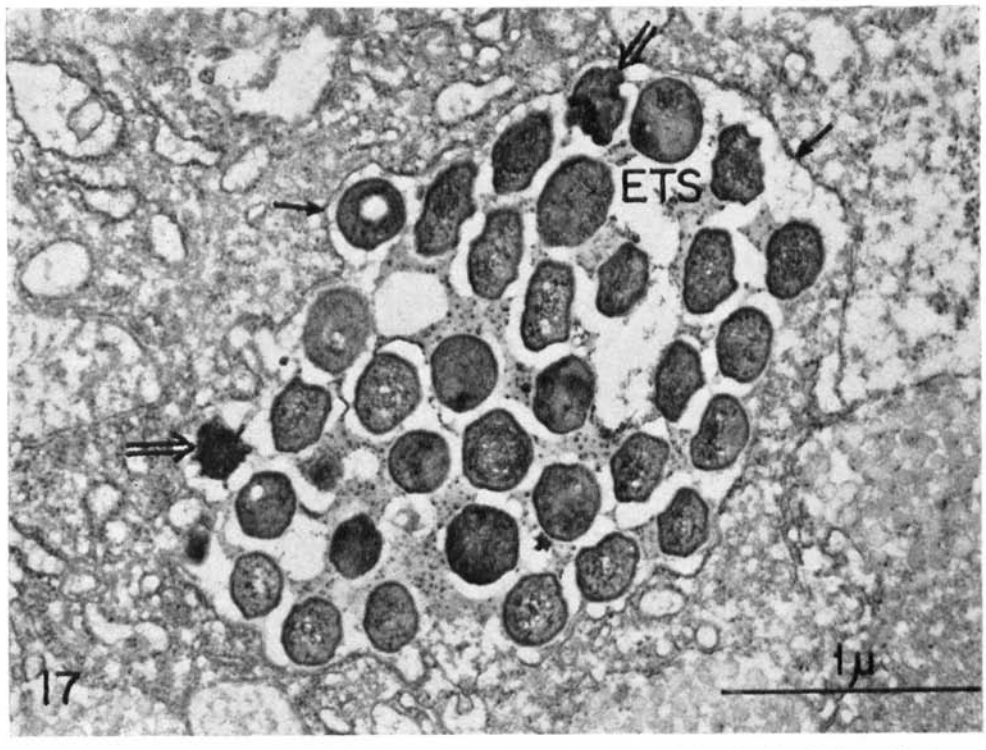


FIG. 17. A later stage of a bacillary clump in an untreated borderline lesion. Electron-transparent substance **(ETS)** occurs around the bacilli. Amorphous substance of low density, spotted with dense granules, is still observed. The limiting membrane is partially formed between electron-transparent substance, and host cell cytoplasm **(arrow)**. However, this substance is not visible in the border where low density substance fuses into host cell cytoplasm. Note that all bacilli exhibit their intact features and also that some bacilli show an intricate contour **(double arrow)**, which is not surrounded by a distinct cell wall.

aspect is almost identical with that of epithelioid cells in tuberculoid lesions. It is considered that epithelioid cells represent tissue response to *M. leprae* and that these cells destroy bacilli rapidly ([11, 33, 39, 42]). Stavitsky ([49]) postulated that the microsome fraction (the term "microsome" used in biochemistry includes endoplasmic reticulum and RNP particles) is the site of antibody formation. Hanks ([19]) explained "cellular allergy" as "antibody precursor" or "intracellular antibody." These opinions indicate that the relatively abundant distribution of cytoplasmic organelles in epithelioid-type-bacillated cells of borderline lesions may explain the fact that intracelluar antibody is still produced in this type of cell.

According to Urbach ([51]), the presence of antibody inhibits the multiplication of bacilli. On the other hand, Davey ([11]), referring to tuberculosis, emphasized that multiplying bacilli may later inhibit even partial response, when the grade of allergy is below the threshold level of effectiveness. These two opinions, apparently contradictory, best

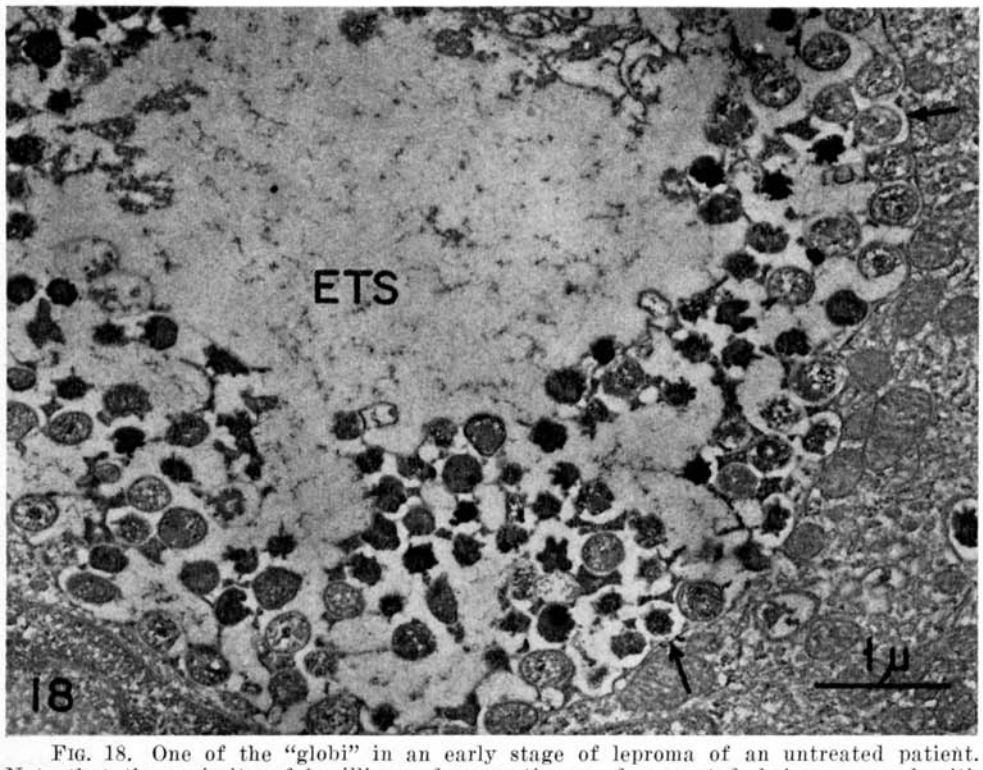


FIG. 18. One of the "globi" in an early stage of leproma of an untreated patient. Note that the majority of bacilli are degenerating or degenerated, being wrapped with abundant electron-transparent substance **(ETS)**, which is clearly delimited by a membrane from host cell cytoplasm **(arrow)**. Irregularly shaped bacilli are also noted among degenerating bacilli.

explain the host-parasite interaction in borderline leprosy. Phagocytosed bacilli multiply very slowly in histiocytic cells under moderately or slightly inhibited conditions because of the presence of intracellular antibody. These multiplying bacilli may interfere in the inductive phase of antibody formation in host cells. Therefore, cellular reactivity and bacterial activity may be balanced in borderline lesions. As biologic phenomena in general cannot be fixed in a conditional stage, the balance may be shifted according to the predominant activity of either bacilli or host cells. Once bacterial activity overcomes cellular reactivity, the cytoplasmic appearance of host cells, particularly bacterial microenvironment, becomes very similar to that of lepra cells; opaque droplets and foamy structures, which are believed to be a characteristic structure of lepra cells ([22]), are seen (Figs. 8 and 12). We believe that this deviation from a balanced condition may occur in each individual cell and may not influence other infiltrating cells, because the multiplying bacilli may only inhibit the response of their own host. Contrarily, if cellular reactivity is predominant, bacilli are destroyed before they multiply and therefore bacillated cells become epithelioid cells identical with those in tuberculoid lesions.

Response to chemotherapy.—Clinical experience has indicated that borderline leprosy responds relatively well to chemotherapy ([16, 60]). In the present study, we could not find any intact bacillus in borderline lesions after a few months of treatment (Figs. 7, 8 and 12). This dramatic effect of chemotherapy does not appear in lepromatous cases.

Wade and Perrin ([57]) suggested that some potentiality of resistance retained in borderline cases makes treatment more effective than in a lepromatous case. As discussed in the foregoing, the bacilli in borderline leprosy subside under the slightly inhibited condition. It is understandable that this cellular reactivity may play a role as an additional factor in chemotherapy.

On the other hand, it should not be overlooked that bacterial microenvironment is related to the penetration of drugs into bacilli. In borderline lesions the majority of bacilli, both in single individuals and in groups, *without* either opaque droplets or electron-transparent substance, are attached directly to the host cell cytoplasm. Therefore, the drugs may easily penetrate into bacilli. In contrast with the bacterial microenvironment of borderline cases, bacilli in lepromas are usually enclosed by opaque droplets and electron-transparent substance, which are believed to be lipid in nature ([22, 23]). These substances may prevent the drugs from reaching the bacilli.

SUMMARY

1. Seventeen cases of borderline leprosy, diagnosed clinically and histopathologically, were examined by electron microscopy.

2. All biopsied lesions were composed mainly of epithelioid-type cells, usually bacillated, together with leucocytes. The lesions consist not only of a mixture of lepra cells and epithelioid cells but also of cells that may have elements of both lepra and epithelioid cells in the same individual.

3. It is noteworthy that the majority of bacilli disclose their intact character in untreated patients. Usually clumps of intact bacilli are not surrounded by electron-transparent substance. This is in contrast to the appearance of "globi" in lepromatous lesions.

4. In lesions of patients after a few months of chemotherapy, intact bacilli no longer appear. Most of the bacilli are degenerated, and encased in electron-transparent substance. These bacillary clumps correspond to "globi."

5. From the morphologic point of view, we believe that bacterial activity and cellular reactivity are balanced in borderline leprosy. Thus, the intact bacterial characteristics persist for a relatively long time, if chemotherapy is not applied. This finding may be valuable in identifying borderline leprosy.

SUMARIO

1. Diez y siete casos de lepra límite (borderline) diagnosticados clínicamente e histopatologicamente fueron examinados por microscopía electrónica.

2. Todas las lesiones biopsiadas estaban compuestas principalmente por células de tipo epitelioide, usualmente bacileadas, junto con leucocitos. Las lesiones consisten no solamente de una mezcla de células leprosas y epitelioides, sino tambien de células que pueden tener elementos de ambas células leprosas y epitelioides en el mismo individuo.

3. Es de notar que la mayoria de los bacilos mostraban su caracter intacto en pacientes no tratados. Usualmente grupos de bacilos intactos no estan rodeados por substancia electrón-transparentes. Esto es en contraste con la apariencia de los "globos" en lesiones lepromatosas.

4. En las lesiones de los pacientes tratados varios meses con quimioterapia, no aparecen mas los bacilos intactos. La mayoria de los bacilos estan degenerados e incluidos en una substancia electrón-transparente. Estos acumulos bacilares corresponden a los globos.

5. Desde el punto de vista morfologico, nosotros creemos que la actividad bacteriana y la reactividad celular estan balanceadas en la lepra límite (borderline). De este modo, las caracteristicas bacterianas intactas persisten por un tiempo relativamente largo si la quimioterapia no es aplicada. Este hallazgo puede ser valioso en la identificación de la lepra límite (borderline).

RESUMÉ

1. Dix-sept cas de lèpre border-line, diagnostiquée par la clinique et l'histopathologie, ont été étudiées par le microscope électronique.

2. Toutes les lésions biopsées étaient constituées principalement de cellules de type épithélioïde, généralement avec bacilles, et de leucocytes. Les lésions ne consistaient pas seulement d'un mélange de cellules lépreuses et épithélioïdes mais aussi de cellules qui pouvaient réunir à la fois dans la même unité individuelle des éléments de cellules lépreuses et de cellules épithélioïdes.

3. Digne d'être noté est le fait que, chez les malades non traités, la majorité des bacilles présentent les caractéristiques de bacilles intacts. Généralement, des amas de bacilles intacts sont entourés d'une substance perméable aux électrons. Ceci contraste avec l'apparence des globi dans les lésions lépromateuses.

4. On ne voit plus de bacilles intacts dans les lésions des malades après quelques mois de chimiothérapie. La plupart des bacilles sont dégénérés, et inclus dans une substance transparente au microscope électronique. Ces amas bacillaires correspondent aux globi.

5. Du point de vue morphologique, nous croyons que dans la lèpre borderline l'activité bactérienne et la réactivité cellulaire sont en équilibre. Dès lors, les caractéristiques d'intégrité des bacilles persistent pour un temps relativement long, si aucune chimiothérapie n'est administrée. Cette observation peut être précieuse pour l'identification de la lèpre border-line.

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