

Electron Microscopy. Approach to Leprosy Research¹

Tamotsu Imaeda, M.D.²

Since 1948, when Bishop *et al.* (3) first applied electron microscopy to study the morphology of *M. leprae*, this new technic, with the advantage of ultrathin sectioning, has been utilized widely in leprosy research. In 1956 Brieger and Glauert (7) reported on the cytoplasmic reaction to the leprosy bacillus in lepra cells. Later Brieger's group (6-8) clarified more details on both cytopathology and bacterial morphology. In the field of histopathology of leprosy a Kyoto University group (14-22, 32, 33) described the ultrastructure of various types of leprosy lesions. Weddell *et al.* (33) also elucidated the process of nerve involvement of leprosy infection; their studies were further supplemented by those of Imaeda and Convit (15). Thus, the description of the bacteriology and histopathology of leprosy at the electron microscopic level has been outlined by these authors. Many basic problems, e.g., morphologic evidence on the pathogenesis of leprosy infection, and demonstration of the immunopathologic differences among various types of leprosy lesions, still remain unsolved.

In this conference I would like to discuss bacterial growth in various leprosy lesions and also the macromolecular structure of the surfaces of various mycobacteria. The latter may be related closely to both the cytopathology and the immunology of mycobacterial diseases. The submicroscopic histopathology of leprosy will not be included, because it has been thoroughly reviewed by Nishiura (22).

MYCOBACTERIAL GROWTH AND MICROENVIRONMENT IN HOST CELLS OF HUMAN LEPROSY

It is a well known fact that phagocytosed bacteria are always separated by the mem-

brane from the host cytoplasm during various periods of time (5-20, 21, 34). The ultimate fate of this membrane may depend on (1) the mechanical balance between the inner vacuolar and the outer cytoplasmic pressures across the membrane, (2) the chemical and/or immunologic interaction between the host cytoplasm and the parasite on the membrane matrix, and (3) the biologic reaction of the host cytoplasmic components on the parasite across the membrane.

The first mechanism is a function of the growth rate of the bacteria in the vacuoles. The second includes (a) the digestion of the membrane by intracytoplasmic enzymes of the host (i.e., the limiting vacuolar membrane is regarded as a foreign substance, although it is derived from its own outer cell membrane), (b) the dissolution of the membrane by some substances released from the bacteria, and (c) the immunologic reaction on the vacuolar membrane, resulting in the disintegration of this membrane. The fate of bacteria in the host cell may be influenced by these factors.

In leprosy, as far as we know, *M. leprae* is phagocytosed mainly by mesenchymal cell types, in which potential growth is possible (Fig. 1). This stage, just after phagocytosis, is directly related to the immunologic condition of cells. Hanks (13) has suggested that the phagocytic membrane may disappear or be modified more rapidly in the hypersensitive cells of tuberculoid leprosy than in the anergic cells (lepra cells) in lepromatous cases. Nishiura (22), however, showed in electron micrographs of epithelioid cells from tuberculoid lesions that the dead bacilli were still enclosed by the phagocytic membrane. This implies that the antimicrobial factors in hypersensitive cells may be located on the phagocytic membrane, which is derived from the outer cell membrane, or that the antimicrobial factors—in the immunologic sense—in the cytoplasm may pass into the vacuole without deforming the vacuolar membrane. Ac-

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²Investigator, Dept. of Experimental Pathology, Instituto Venezolano de Investigaciones Cientificas (IVIC), Apartado 1827, Caracas, Venezuela.

ording to our experience with various types of leprosy lesions, the development of the enclosing membrane appears to be more closely related to the growth of the bacilli in vacuoles than to the immunologic state of the host.

Rupture of the membrane may occur mechanically as a result of increase of bacterial cells in restricted space. Accumulated metabolites of bacilli also may play a chemical role in dissolving the membrane. Self-digestion of the membrane by the host cytoplasm was not found in our study. Arai (2) reported that the limiting membrane of phagocytic vacuoles containing India ink disappeared after a long period of time. His evidence suggests that self-digestion of the membrane may take place in vacuoles containing very slowly multiplying bacilli such as *M. leprae*. However, there is a lack of experimental evidence to clarify the relationship between the phagocytic membrane and the host-mycobacteria interaction.

In lepromatous and borderline leprosy lesions, the bacilli that become free from the vacuole are directly in contact with the host cytoplasm (Fig. 2). They proceed to grow, forming large clumps (Fig. 3). At this stage a very low density substance, almost electron-transparent, accumulates around the bacilli. Usually this substance fuses into the host cytoplasmic ground substance. Once the bacillary clump becomes very large, together with the increase in low density substance, a physicochemical membranous structure is formed at the interface between the bacillary clump and the host cytoplasm (16). Morphologically it is very difficult to distinguish this structure from the phagocytic membrane. However, the release of bacilli from the phagocytic vacuole, and the absence of a membranous structure around small groups of bacilli, without the low density substance, furnishes enough evidence to support the opinion that the membranous structure around the large clump embedded in the low density substance may be different from the original phagocytic membrane. These facts suggest also that the low density substance is composed of bacterial metabolites, possibly mixed with denatured host cytoplasmic

components caused by bacterial metabolism (16), thus forming the membranous structure at the interface of the host cytoplasmic ground substance.

In the bacillary clump, bacilli without distinct cell walls are sometimes noted (16). Noteworthy also is the fact that these abnormal bacillary forms are always evident when the large clump is associated with the low density substance. This implies that the bacillary metabolites associated with the low density substance may suppress cell wall synthesis or may dissolve the cell wall.

The other type of bacterial environment is shown in Figure 4. The bacilli are embedded in a fine granular substance, which is further separated by a membrane from the cytoplasm. This embedding substance was designated as "opaque droplets" by Yamamoto *et al.* (33), and also as "cytosome-like substance" by Brieger and Allen (6). It should be emphasized that this substance is found not only in leprosy infections but also in other bacterial infections caused by *E. coli* (4, 20, 21), *Staphylococcus* (5), *M. tuberculosis* (18, 25) and the agent causing Whipple's disease (9).

Occasionally this droplet contains a lamellar structure and mitochondria. These findings of droplets are also shown in the peculiar cytoplasmic components in other types of cells, variously termed as "cytosome," "microbody," "dense body," and "lysosome." Cytochemical techniques applied to leprosy biopsies clearly demonstrate the existence of acid-phosphatase activity in this droplet (Fig. 5). When the bacillus lies exposed in the cytoplasm, the acid-phosphatase activity is not found around it. On the basis of this evidence, it seems likely that the fine granular substance (opaque droplet) around the bacilli corresponds to the lysosome, as far as acid-phosphatase activity is concerned (10). Therefore, we propose that the opaque droplet be called "lysosomal substance."

The occurrence of the "lysosomal substance" around the bacilli is a common phenomenon in various types of leprosy lesions. On the other hand, we have noted, after examining 90 biopsy specimens, that the lesions taken from patients undergoing

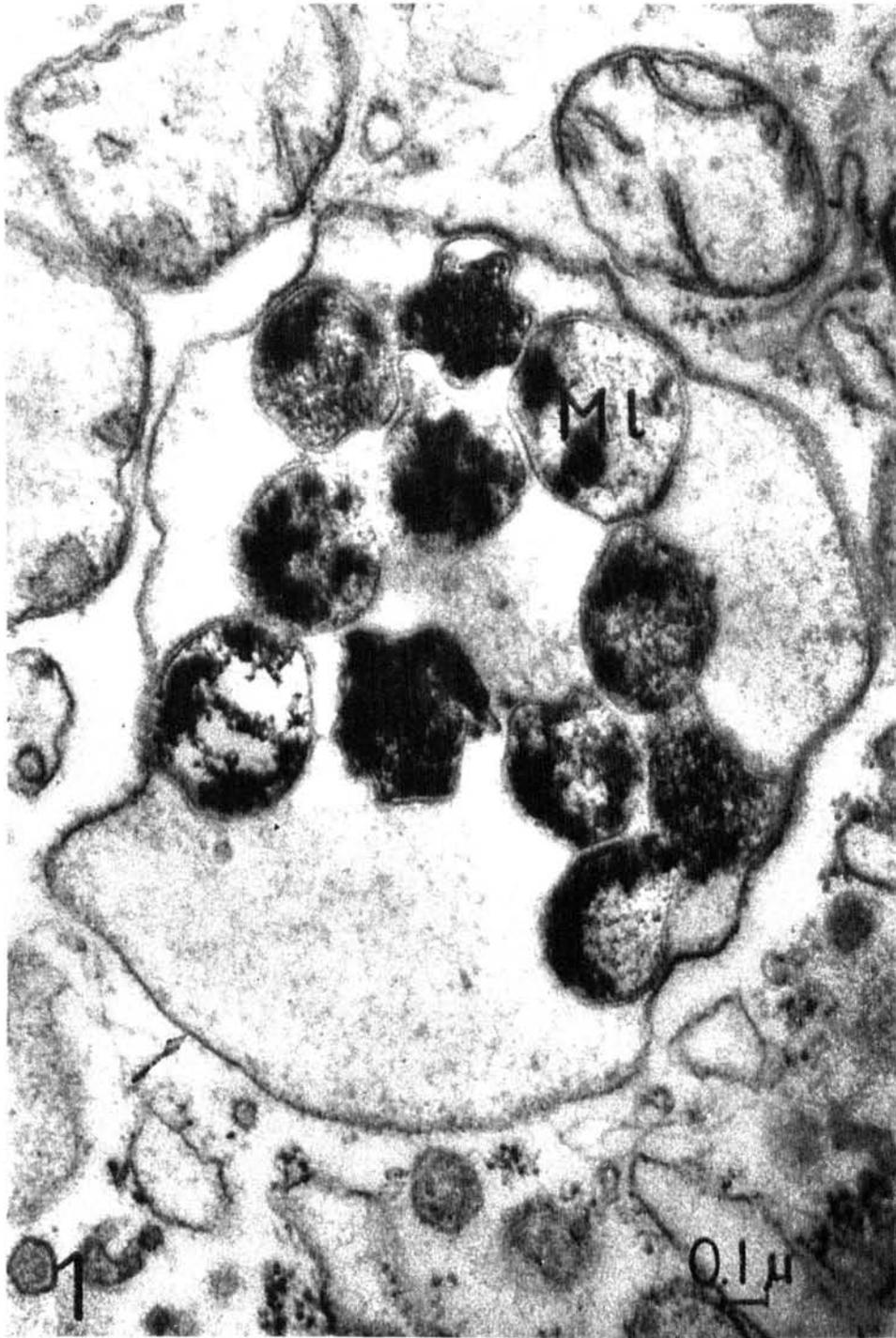


FIG. 1. Leprosy bacilli (ML) are separated by a phagocytic membrane (arrow) from a lepra cell cytoplasm.

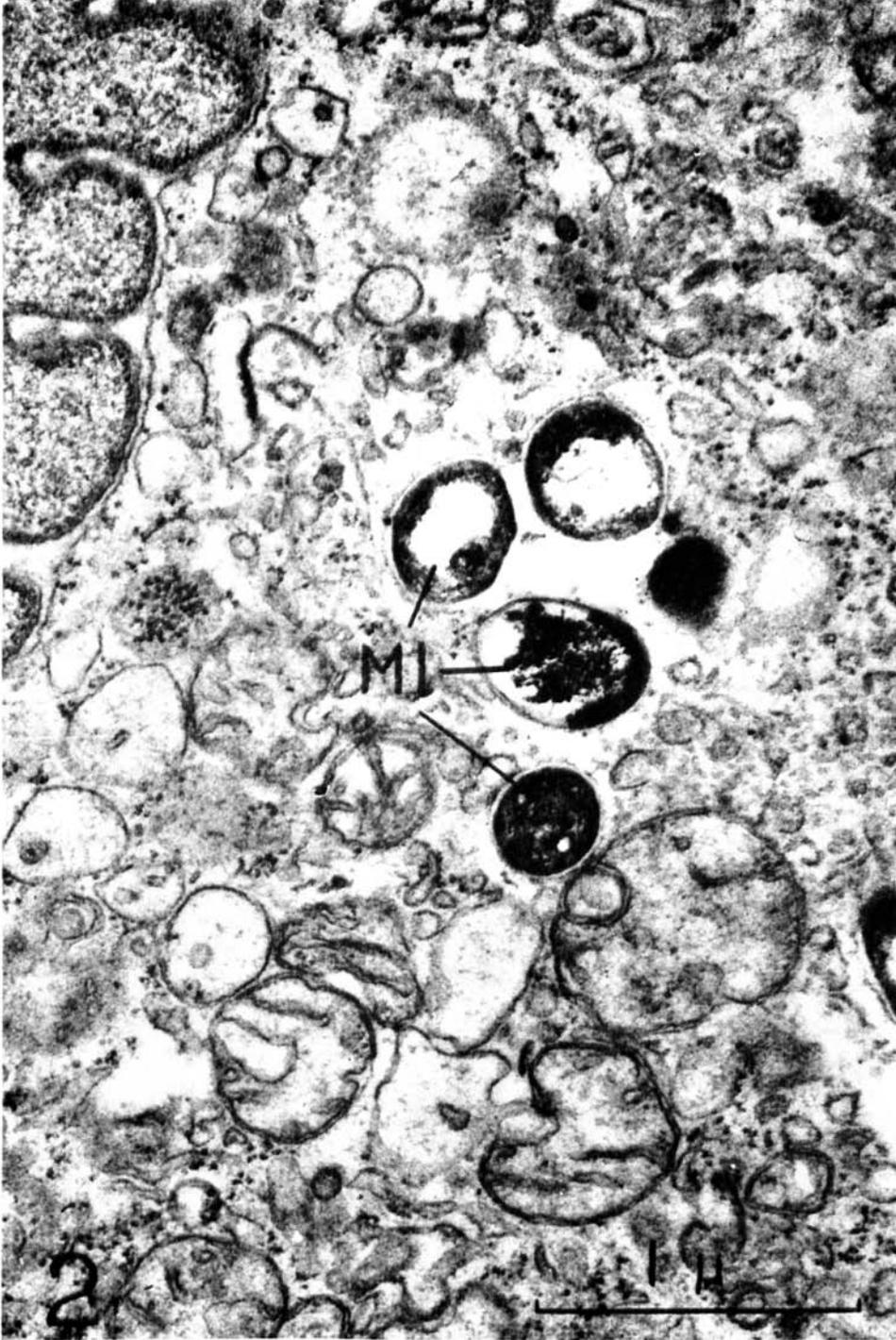


FIG. 2. Leprosy bacilli (ML) are exposed to host cytoplasm after rupture of the phagocytic membrane.

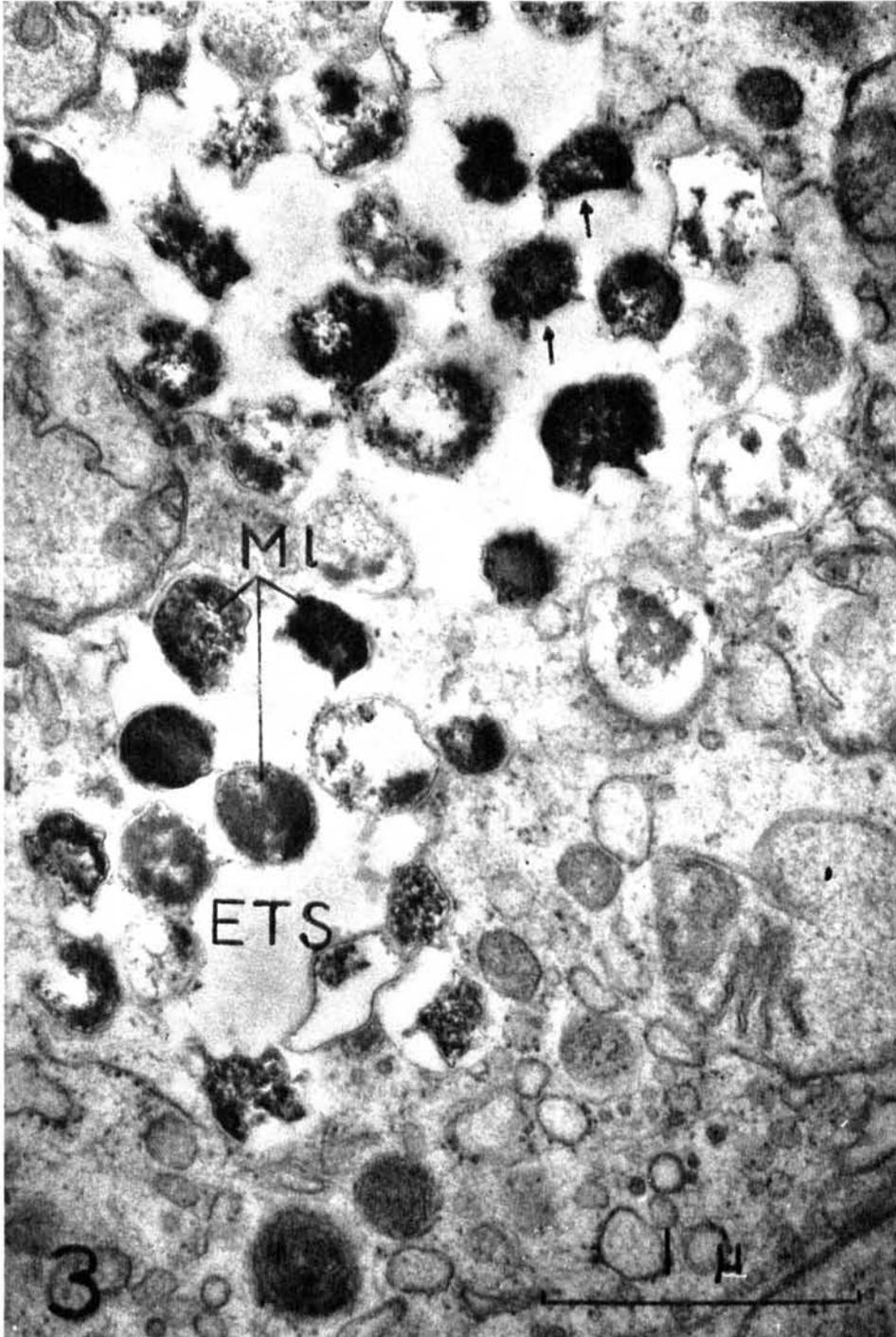


FIG. 3. Bacillary clumps of *M. leprae* (ML) in a lepra cell. A very low density substance (ETS) occurs in these clumps. Note the irregularly shaped bacilli devoid of a distinct cell wall (arrow).

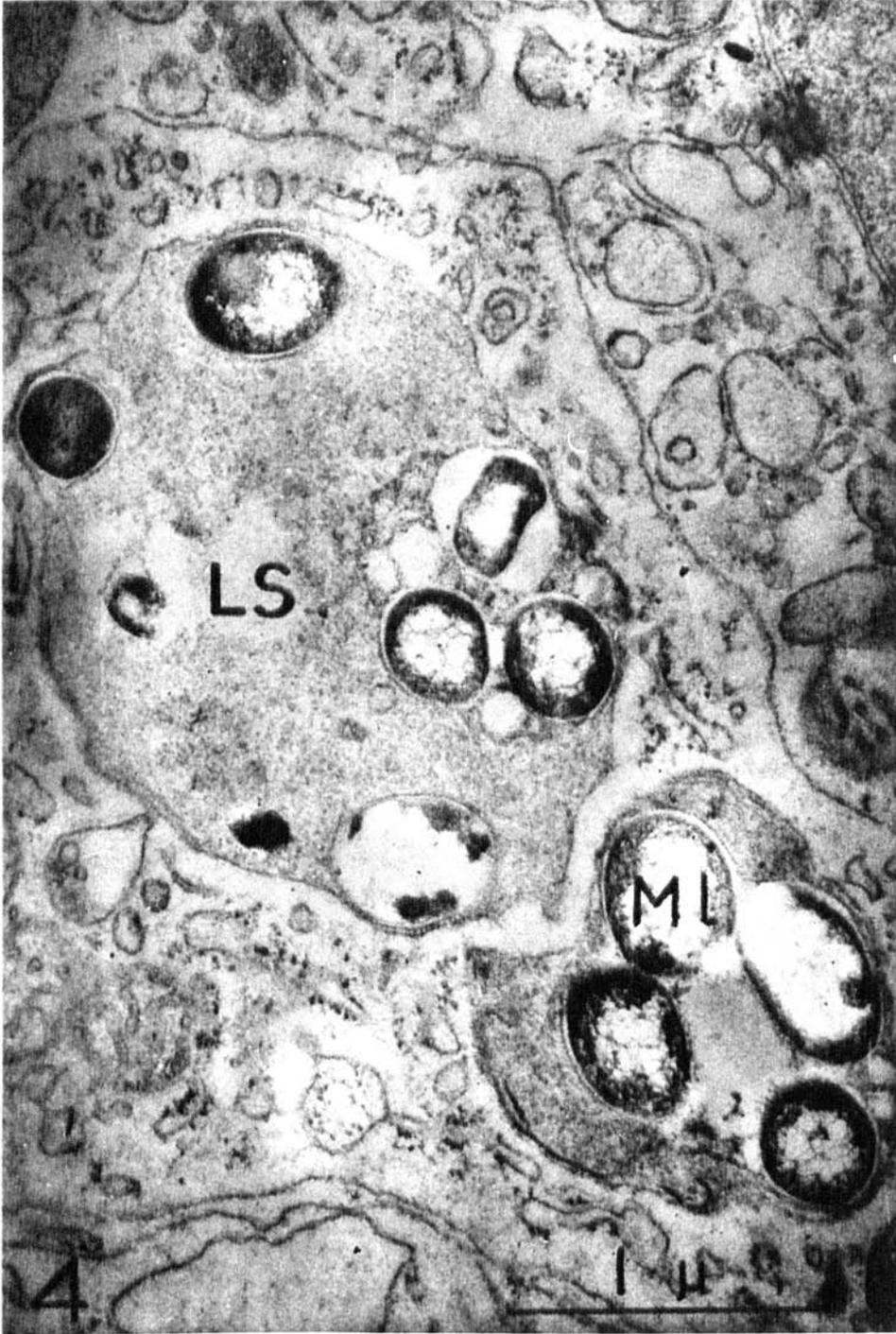


FIG. 4. Leprosy bacilli (ML) are embedded in a fine granular substance (LS).



FIG. 5. This picture exhibits the results of cytochemical electron-microscopic examination of a lepra cell. The fine granular substance (LS) surrounded by the membrane (arrow) contains dense particles which represent acid-phosphatase activity. ML = leprosy bacilli.

chemotherapy with DDS or other related compounds appear to contain much more abundant "lysosomal substance" than those from nontreated patients. In order to ascertain the effect of chemotherapy on the microenvironment of bacilli, mice infected with *M. lepraemurium* were injected with Promin. Before treatment the majority of bacilli lie in the membrane-bound vacuolar space. Four weeks after a total injection of 1.0 ml. of Promin solution, the "lysosomal substance" occurs around the bacilli. Schulz⁽²⁵⁾ also described an increase of "lysosomal substance" in alveolar macrophages after the injection of sulfonamide. From these results, it is believed that the production of "lysosomal substance" is a resultant effect of chemotherapy.

Concerning the occurrence of "lysosomal substance" around the bacilli, Imaeda⁽¹⁴⁾ suggested that the contents of the pinocytotic granules accumulate around the ingested bacilli following degranulation. The fusion of granules into the vacuole containing various bacteria has been observed by other authors^(20, 21, 34) also. They suggested that the enzymes in the granules may be discharged into the phagocytic vacuole through the limiting membrane. In a study of pinocytosis in amoebae, Schumaker⁽²⁶⁾ stated that the excess of protein bound on the cell membrane surface caused an infolding of the membrane into the cytoplasm. Because of this observation, it is possible that chemotherapy may modify the serum proteins, resulting in an increase of pinocytosis, which further promotes the formation of "lysosomal substances."

The disintegration of bacillary bodies in the "lysosomal substance" was noted in tuberculosis lesions⁽²⁵⁾ and in *E. coli* infection⁽²¹⁾, as well as in leprosy lesions. It is an obvious conclusion that the bacteria undergo degeneration more readily in the "lysosomal substance" than in an exposed state in the cytoplasm of host cells, because the "lysosomal substance" is abundant in hydrolytic enzymes that may interfere with bacterial metabolism.

With bacterial disruption, the foamy structure is formed in the "lysosomal substance." This may result from the decomposition of the lysosomal lipoprotein-like material⁽¹⁴⁾, by bacterial enzymes, as well

as inclusion of bacterial debris. It should be emphasized that the foamy structure is not characteristic of the environment of the leprosy bacillus, but appears around several species of disrupted mycobacteria in a lipophilic phase, as Galindo will note after this presentation.

In summary of the first part of this report, it may be said that the microenvironment of *M. leprae* in human host cells is represented in four forms: (1) bacilli enclosed by the phagocytic membrane, (2) bacilli in direct contact with the host cytoplasm after rupture of the enclosing membrane, (3) formation of a large clump accompanied by low density substance, and (4) bacilli in the "lysosomal substance." It is believed that the large clump with the low density substance may be the only characteristic bacterial structure contributed to the environment of host cells.

MYCOBACTERIAL SURFACE

The surface structure of mycobacteria was studied by Takeya *et al*^(27, 28, 29). They noted that the paired fibrous structure was distributed on the cell envelope of the ghost cell after treatment with antibiotics. However, neither their chemical composition nor biologic activity has been described.

On the other hand, chemical analysis of the whole mycobacterial cell has been carried out extensively, especially in Anderson's laboratory⁽¹⁾ and later by Lederer's group⁽¹⁷⁾.³ Immunologic functions of these chemically purified substances have been studied in many laboratories. Reports in this respect, however, have not been correlated with any fine structure studies.

When the mycobacteria are ingested in animal cells, the macromolecules on the bacterial surface finally come into contact with the host cytoplasm. Consequently, morphologic change on the bacillary surface at various stages of growth, both *in vitro* and *in vivo*, is connected closely with host cell reaction.

Ultrathin sections, usually fixed with osmium tetroxide, are not satisfactory for study of the bacterial surface, because the osmium fixation technic does not reveal any

³The groups of Anderson and Lederer have published many works. Only representative reports are cited here.



FIG. 6. *M. leprae* shadowed with chromium. The bacterial surface is covered with fine fibrils.



FIG 7. Sonically disrupted cells of an atypical mycobacterium. Thick fibers in an inner surface of cell wall show the spiral conjugation of fine fibrils (arrow). The latter are usually found in the outer cell surface and also show the spiral-arrangement (double arrow). Chromium shadowing.

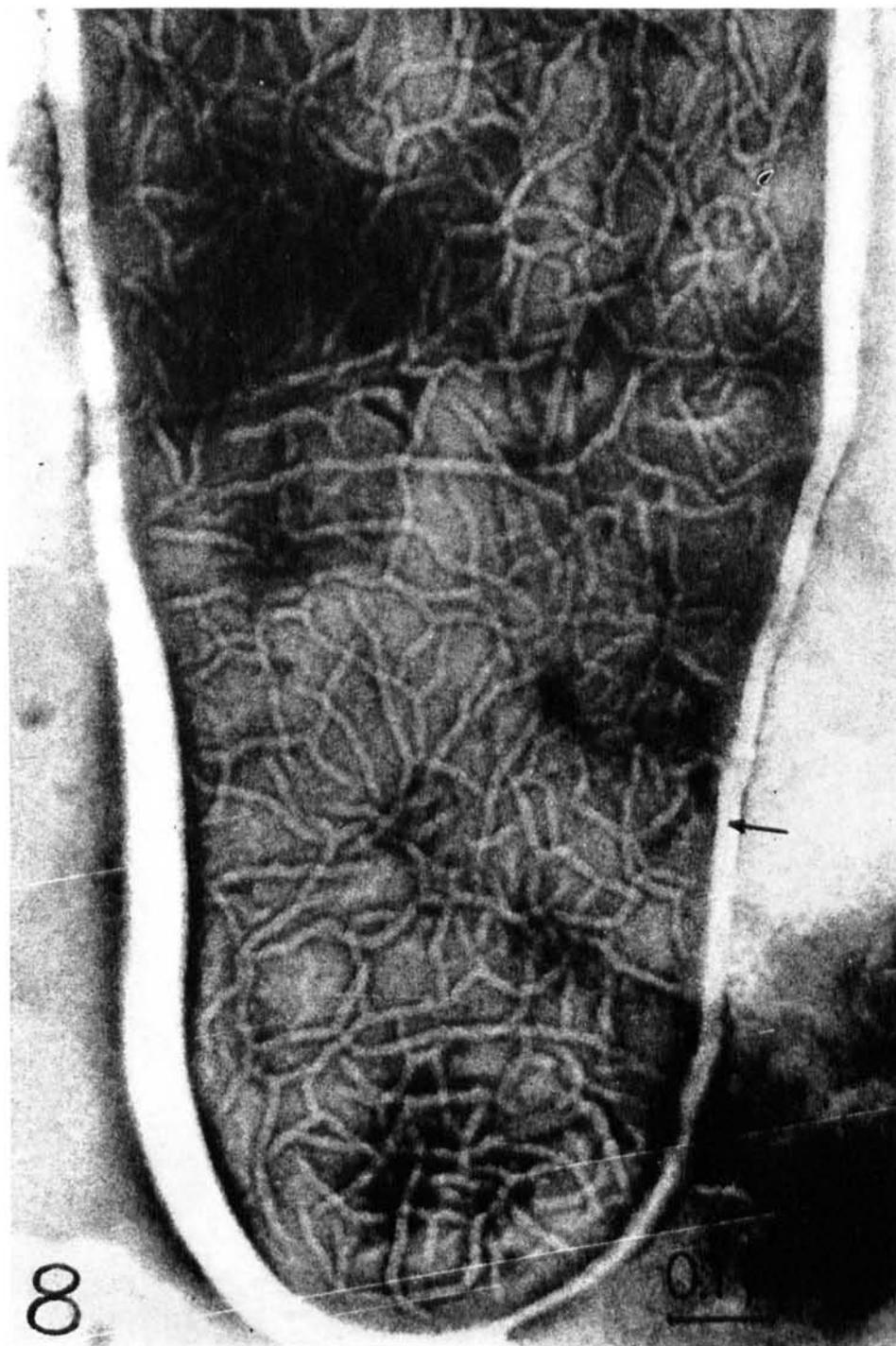


FIG 8. *M. tuberculosis* (H37Ra), negatively stained with silico-tungstic acid. Fine fibrils are seen in the cell wall. An arrow indicates the microcapsule.

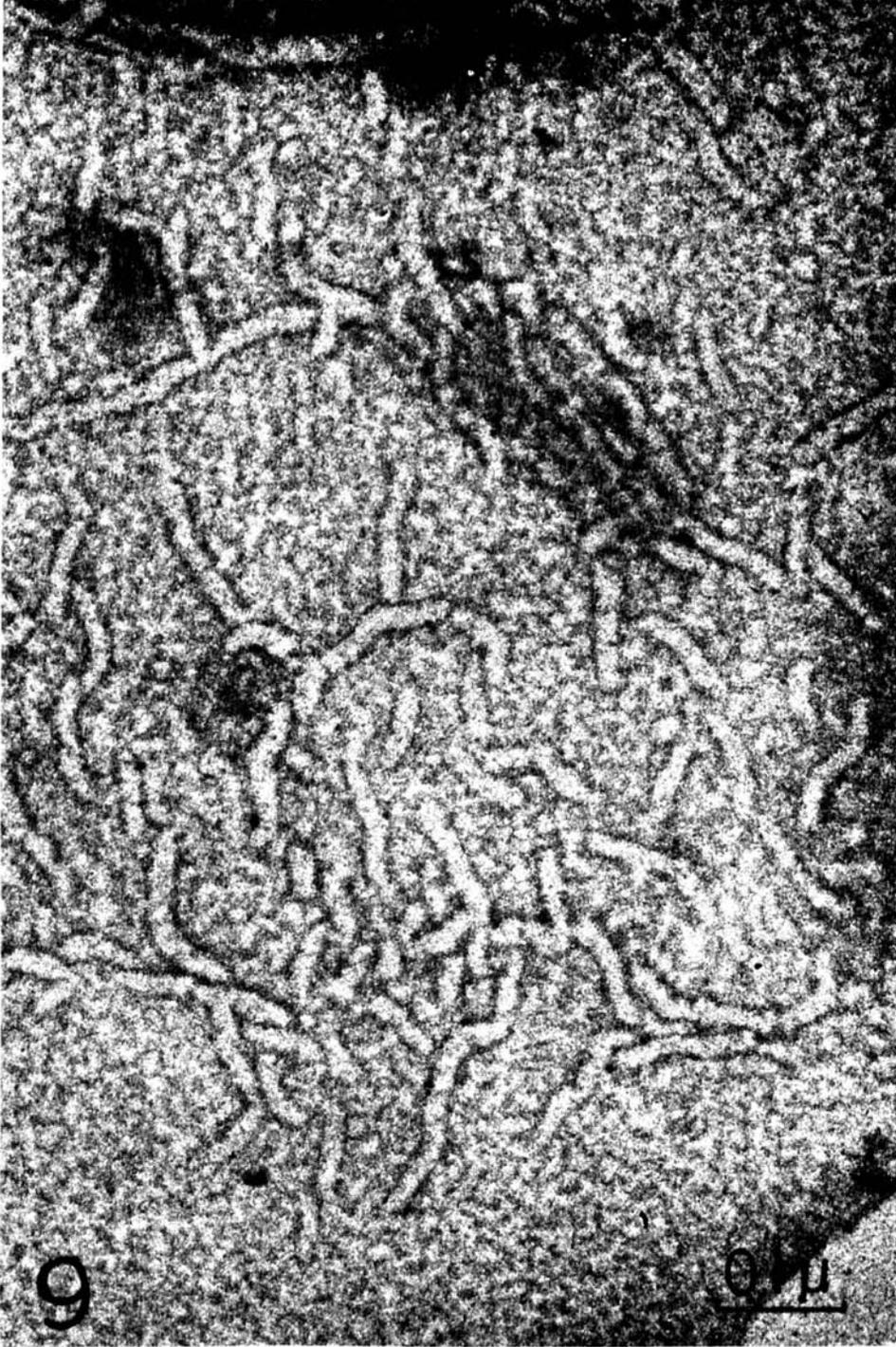


FIG. 9. Sonically disrupted cell wall of *M. tuberculosis* (H37Ra). Fibrils show the spiral arrangement. Negative stain with silico-tungstic acid.

details of substance, such as polysaccharides or saturated lipids, which are possibly located on the bacterial surface. On the other hand, simple techniques, such as shadow-casting and negative staining, are known to be useful in examining surface structure, and these methods are easily combined also with chemical fractionation.

Figure 6 shows an intact cell of *M. leprae* after shadowing with chromium. Many fibrils cover the cell surface. Their diameters vary from 3 to 30 $m\mu$.

Figure 7 shows a sonically disrupted cell of atypical mycobacteria isolated from a lepromatous patient. It should be noted that the fibrils are seen not only at the outer surface of the cell wall but also on the inner surface. In addition, thick fibers, which are frequently observed in the fine granular matrix of the inner surface, are composed of spirally arranged, thinner fibrils. Moreover, this fibril also shows a spiral configuration. Thus, the unit fibril, about 3 μ in diameter, makes the spiral and these two fibrils are conjugated in the further spiral form.

A strain of *M. tuberculosis* (H37Ra), negatively stained with silicotungstic acid, also exhibits the fibrillar structures at the cell surface (Fig. 8). A sonically disrupted and negatively stained cell of *M. tuberculosis* (H37Ra), displayed the spiral arrangement of these fibrils (Fig. 9).

Once the bacillus dies, the cell wall matrix becomes less dense and the fibrils are detached from the cell surface. At this stage, the shadowed picture reveals the fibrils in a parallel arrangement, as observed by Takeya *et al* (27, 28, 29).

These fibrillar elements, observed in the negatively stained and shadowed cells, are common structures among 14 species of mycobacteria examined in this study. Furthermore, they cannot be found in the ultrathin section fixed with osmium tetroxide.

In 1927 Mudd and Mudd (19) suggested that carbohydrates may be present on the acid-fast bacillary surface. Recently Földes (11, 12) described a possible chemical structure of the mycobacterial surface, based on phenol extraction technic. He believed that the lipoproteins may be located on the outermost layer of the tubercle bacillus cell wall. Two polysaccharides, P₁ and

P₂, with small amounts of lipids and amino acids, are described as forming the inner layer of the cell wall. Later on, Tepper (30), after referring to various chemical extraction data of mycobacteria, suggested that a peptido-muramic acid-acetyl glucosamine complex may lie at the inner layer, while a polysaccharide-lipid-amino acid complex covers the outer surface.

After phenol extraction (23) of the purified cell wall fraction, the cell wall matrix almost disappears, leaving the fibrillar component. The fibrils show the same morphologic structures and dimensions as are seen in the nontreated cell surface. This result strongly suggests that the fine granular matrix of the cell wall is composed mainly of lipoprotein, which dissolves in phenol during extraction, and that the fibrillar element may contain polysaccharides.

Chemical analysis of the fibrils obtained after phenol extraction, made by Kanetsuna, shows the following composition: chloroform soluble lipids, 29.8 per cent; reducing sugar, 18.7 per cent; hexosamine, 7.3 per cent; pentose, 15.7 per cent; nitrogen, 3.8 per cent; and total phosphorus, 1.0 per cent. The chief amino acids are glutamic acid, alanine, and diaminopimelic acid.

On the basis of this chemical analysis it is assumed that the fibrils are composed of lipopolysaccharides with peptides.

As a conclusion, I suggest that peptido-lipopolysaccharide forms the complex spiral fibrils, which support cell wall rigidity. When the bacillus loses its viability, this spiral conjugation is relaxed and yields parallel fibrils. Finally, they disappear from the cell surface. It is possible that the fibrils are released from the dead bacillus into the external environment with or without denaturation.

We believe the lipoproteins may be embedded within the framework of the fibrils, forming a moderately dense cell wall in the ultrathin section. The outer layer of the surface may be covered chiefly with these fibrillar elements of peptido-lipopolysaccharides, which are not so osmiophilic, thus displaying a low density microcapsule.

In the negatively stained "globi" from a lepromatous lesion, the majority of bacilli do not show the fibrils. This feature cor-

responds to that of the ultrathin section of "globi" in which very few bacilli are laden with cytoplasm. It is believed that the viable bacillus is solid after carbol-fuchsin staining (24). In electron microscopy, this solid, i.e., the bacillus with the complete cytoplasm, is surrounded by the dense cell wall matrix containing the fibrils. Therefore, the negative staining method may be useful in determining bacillary viability.

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DISCUSSION

Dr. Johnson. Before opening Dr. Imaeda's paper for general discussion I would like first to call on Dr. B. Galindo and then on Dr. N. E. Morrison. Dr. Galindo is also from the Venezuelan Institute for Scientific Investigation (IVIC).

Dr. Galindo. I would like to comment on Dr. Imaeda's report. The point is the occurrence of the foamy structure. Our experiment using killed intact mycobacteria, *M. tuberculosis* H37Ra and *M. butyricum* in an oil phase (Bayol and Arlacel mixture), showed that these bacilli began to disintegrate in the pulmonary capillary soon after intravenous injection into rabbits, as seen in Figure 1. This material was taken from animals 4 hours after injection. The walls of intact bacilli are in partial contact with the oil, in contrast with the disintegrated bacilli and foamy structure, which are fully surrounded by oil. The foamy structure has a vesicular configuration, which is composed of bacillary debris and fine osmiophilic granules. Figure 2 exhibits a lepra cell containing the foamy structure, which is almost identical to the former. Figures 3 and 4 show the rabbit pulmonary capillaries after injection of the same oil mixture

free from mycobacteria. Figure 3 was taken from an animal sacrificed 4 hours after injection. No foamy structure could be found. Figure 4, taken from another animal sacrificed 5 days after injection, shows, in the capillary lumen, a structure that resembles the foamy one. However, this structure is distinguishable from the foamy one, because it is devoid of the fine osmiophilic granules. In addition, the fact should be pointed out that this configuration appears only a long time after injection of pure oil mixture.

These findings strongly suggest that both lipid and disintegrated mycobacteria may play an important role in the formation of the foamy structure in our rabbit experiments.

The occurrence of the foamy structure in the lysosomal substance found in bacillated cells in human leprosy may be explained in an analogous way, since the foamy structure usually occurs around the dead bacilli enclosed by the lysosomal substance which contains lipids.

Dr. Morrison. I would like to comment on a possible relationship of the spiral structure shown in Dr. Imaeda's fine structure micrographs to chemical components de-

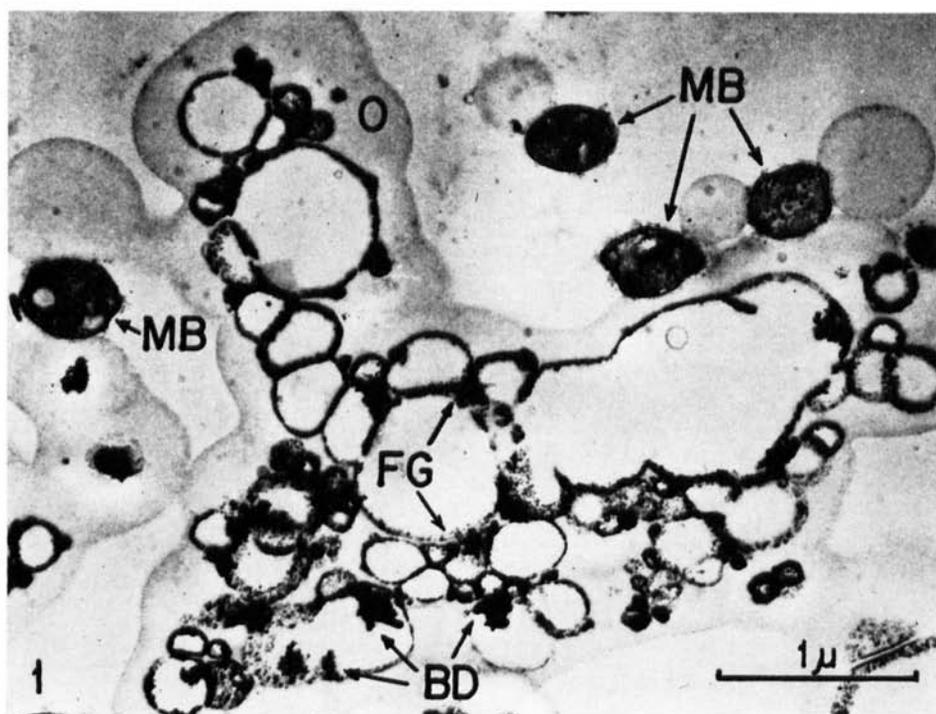


FIG. 1.—Foamy structure completely surrounded by oil (O), in a lung capillary of a rabbit 4 hours after injection. Intact *M. butyricum* (MB). Bacillary debris (BD). Fine osmiophilic granules (FG).

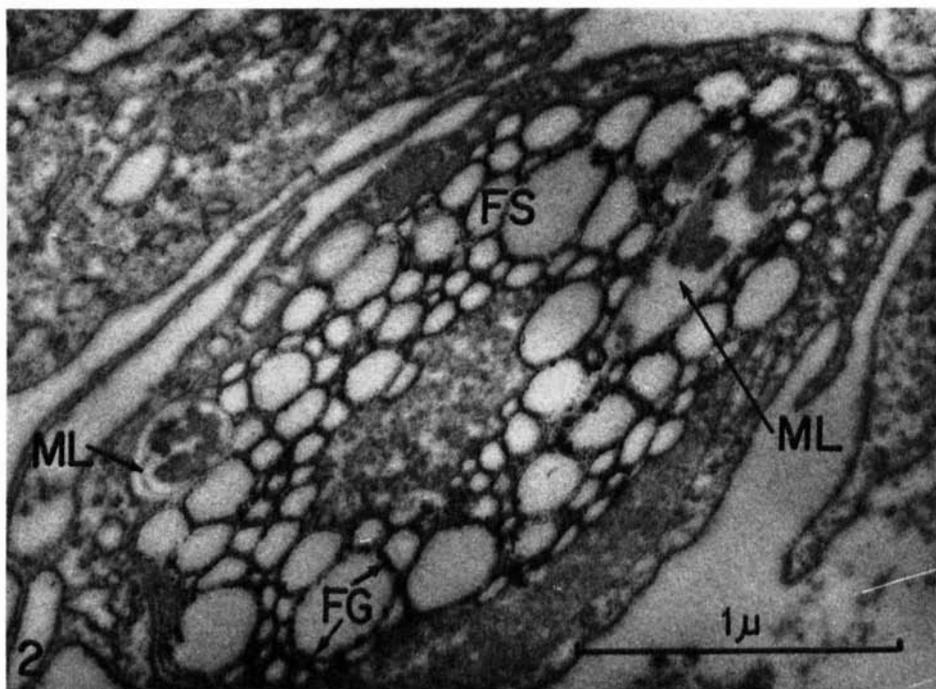


FIG. 2.—Foamy structure (FS) in a lepra cell. *M. leprae* (ML). Fine granules (FG).



FIG. 3.—Pure oil (O) in a lung capillary of a rabbit 4 hours after injection.

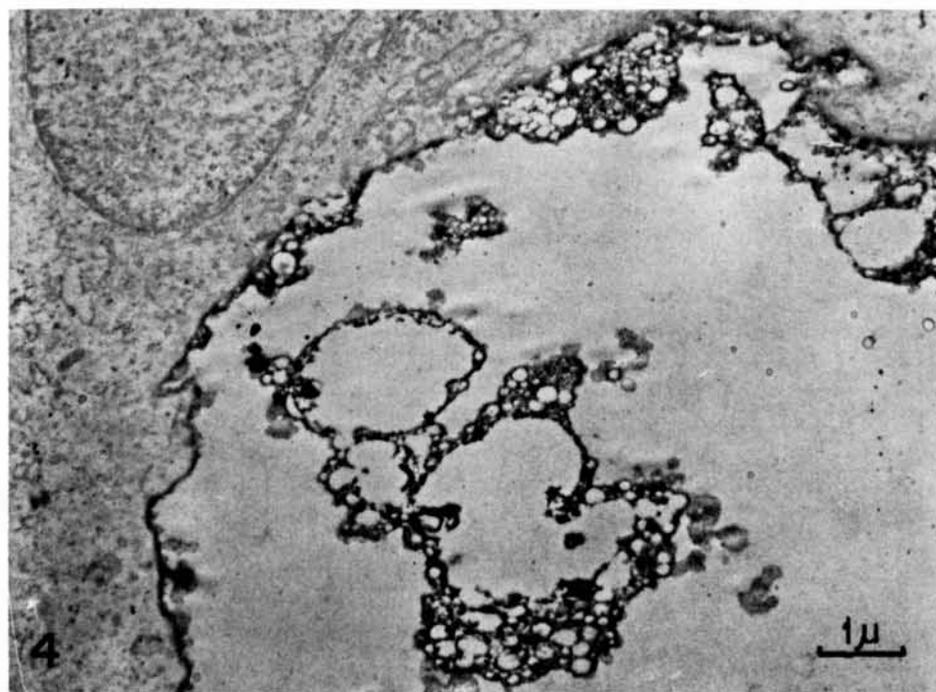


FIG. 4.—Pure oil in a lung capillary of a rabbit 5 days after injection. Note absence of fine osmiophilic granules.

rived from the cell walls of mycobacteria. Takeya and co-workers (*J. Biophys. Biochem. Cytol.* 9 (1961) 496-501) have described the presence of a "paired fibrous structure" in mycobacterial cell walls. Each fiber measured some 5-8 $m\mu$ in width. The structure was observed following growth in the presence of antibiotics. On a few occasions it was observed in cell walls from *M. lepraemurium* isolated from rat lepromas. The "paired fibrous structure" disappears upon extraction with alkaline ethanol. Extraction with neutral solvents has no effect. It thus appears that the lipid-containing "fibrous structure" is firmly bound to the cell wall.

It is possible to speculate that the "paired fibrous structure" could be related to a "bound" form of wax D. Stewart-Tull and White (*J. Gen. Microbiol.* 34 (1964) 43-49) have recently proposed a structure for wax D. The structure is considered to contain two long side-chains composed of mycolic acid residues ester-linked to polysaccharides, which in turn are linked through muramic acid to a heptapeptide containing alanine, glutamic acid, and diaminopimelic acid. The question that I would like to direct to Dr. Imaeda is if the possibility could be considered that a "bound" form of wax D, perhaps analogous in structure to the Stewart-Tull and White model, may be representative of the "paired fibrous structure" and provide a basis, as well, to explain his own observations concerning the occurrence of a fibrous spiral structure. It is conceivable that the addition of mycolic acid residues to the wax D side chains, followed by annealing of the side chains, could result in a double spiral type of structure.

Dr. Imaeda. Dr. Takeya did not describe the spiral, but he did say how the fiber appeared on the cell surface. This occurs only after bacterial degeneration or treatment by some solvents or antibiotics. We have never found such a parallel arrangement of fibers in the intact cell wall. At the same time, I must point out that these fibers, even from the intact cell, are easily discharged from the surface to an external environment. It was reported that wax D

is soluble in chloroform. However, according to our experiments, the spiral fiber isolated from the cell surface is not dissolved in chloroform but suspended in it. On the other hand, chemical components of these fibers are very similar to those of so-called wax D. From these results, I believe that the wax D may represent the detached fibers from the cell surface during the extraction procedure with chloroform. Since the dimensions of fibers are very small, the suspension in chloroform appears to be a true solution. In other words, I wonder if the so-called wax D is the substance that is really dissolved in chloroform. Regarding the question on the chemical structure of the fiber embedded in the cell surface, I cannot answer yet.

Dr. Tepper. I would like to comment on the work of Kotani *et al.* and ask Dr. Imaeda a question. Kotani released a substance from the cell walls of BCG by the action of lysozyme and his *Flavobacterium* enzyme. This material was soluble in chloroform and was similar in biologic activity to the wax D of the virulent tubercle bacillus. Electron micrographs revealed morphologic units with an apparently membranous appearance; however, there was no fine structure comparable to that just shown by Dr. Imaeda. After this "bound wax D" was dissolved in chloroform, its membranous appearance was lost. Kotani found this "bound wax D" to account for approximately 60 per cent of the weight of the delipidated cell wall. I wonder if Dr. Imaeda would comment on Kotani's observations and compare them with his own.

Dr. Imaeda. According to our experiments, spiral fibrils of peptidolipopolysaccharide are partially embedded in the lipoprotein cell wall substance even after chloroform extraction. In addition, we confirmed the fact that these fibrils still remained after lysozyme digestion, for a long period of time. Kotani's electron micrograph (Fig. 5B in their original publication) also showed the same evidence. From these results, it seems likely that the "bound wax D" obtained by Kotani *et al.*

(*Biken. J. (Osaka)* 6 (1963) 181-196) may represent the remaining peptido-lipopoly-saccharide after ethanol-ether and chloroform extraction followed by incomplete enzymatic digestion.

Dr. Johnson. Dr. Imaeda, perhaps we might want to go back to an argument that occurred yesterday regarding the relationship of *M. leprae* to *M. lepraemurium*. Can you tell us something about morphologic differences between these two organisms?

Dr. Imaeda. I expected this question. Dr. Hanks also should be interested in it. *M. lepraemurium* almost always grows inside the vacuole. With this, Dr. Rees, you would agree. On the contrary, *M. leprae*—or should I say mycobacteria in human leprosy lesions—grow after the rupture of the cell membrane. This means that the intracellular growth of the mycobacteria of human leprosy is quite different from that of *M. lepraemurium*. Another thing—I have never seen the abundant distribution of the lysosomal substance around *M. lepraemurium*. But, as I have shown, sometimes we can see the lysosomal substances surrounding the bacilli of human leprosy even without DDS injection.

Dr. Hanks. At one point, Dr. Imaeda, you pointed out in your micrographs bacilli or particles that seemed to be devoid of cell walls. Have you any thoughts on their significance?

Dr. Imaeda. Yes, there are bacteria without cell walls. I would like to say that this structure possibly represents a certain stage of formation of the L form, but I do not have any evidence to show you the growth of this form. Also sometimes we observed complete rupture of the cell walls. Other important evidence is that the bacteria without cell walls always appear in big globi. This implies that some substances or metabolites of the bacilli will cause the formation of this special structure of mycobacteria in human leprosy—especially in lepromatous leprosy.

Dr. S. C. Chang. Although the L form lost its cell wall, it still retains its cytoplasmic membrane. The bodies shown in Dr.

Imaeda's micrograph are very irregular, and probably not L forms. They could be disintegrated bacilli or any disintegrated particles.

Dr. Imaeda. Please do not forget that this is osmium-fixed material. We wonder if the original round shape of the peculiar bacterial form is maintained even after osmium fixation. We are trying to use a better fixation than osmium. So, at this time, I cannot say definitely that it is the L form. With regard to another question about the plasma membrane—of course the L forms should have the plasma membrane without cell walls. But I have never found the plasma membrane around these structures in leprosy biopsies.

Dr. Merkel. I have run electronmicrographs on *M. johnei*-infected tissue and found the same appearance in these that you find with leprosy, i.e., absence of a cell wall in many of them.

Dr. Dannenberg. Did you notice any difference between the bacteria in a vacuole and out of a vacuole, or the amount of low density substance produced in healing in lepromatous and tuberculoid leprosy? In other words, can you give any prognostic or pathogenic significance to some of these stages?

Dr. Imaeda. Do you mean the bacterial cytoplasm or the whole cell? Let us take the two extremes, tuberculoid and lepromatous leprosy. In tuberculoid leprosy it is extremely difficult to find the bacillus. In my experience during 13 years I have seen only two tuberculoid biopsies containing the mycobacteria. They were degenerated in the vacuoles. Also I have a picture of degenerated bacilli completely exposed to the cytoplasm of the host, possibly after the rupture of the vacuolar membrane. In lepromatous leprosy, on the contrary, the bacilli are enclosed by the membrane after phagocytosis, and later on this vacuole disappears. The bacilli are embedded in the lysosomal substance in the treated case; in the case with no treatment they continue their growth, without forming any membrane around the bacilli, until abundant low electron density substance occurs.

Dr. Dannenberg. You are to assume then that after therapy and healing, the lysosome membrane reforms around the bacteria and has some effect on its destruction?

Dr. Imaeda. Yes.

Dr. Rees. Would you tell us, Dr. Imaeda, why the creature there, without a cell wall and with no cytoplasmic membrane, is of bacillary origin.

Dr. Imaeda. I have no idea about the occurrence of this structure. We observed that this structure without any membrane occurs just in the globi, and speculated that it might be the bacterial cytoplasm.

Dr. Rees. So it is pure speculation?

Dr. Imaeda. Yes, it is interpreted as a certain stage of the L form.

Dr. Rees. About it being of bacillary origin?

Dr. Imaeda. I cannot say that this is the definite L form, but possibly it could correspond to the L form.

Dr. Rees. But if you cannot say it is an L form, what ground have you for saying even that it is of bacillary origin?

Dr. Imaeda. This structure clearly contains DNA and also lipid inclusions which one sees only in the intact mycobacteria.

Dr. Rees. That does not make it of bacillary origin, does it?

Dr. Imaeda. Yes, this means it is of bacillary origin.

Dr. Johnson. I fear we will have to reserve further speculations for a later time.