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An Electron Microscope Study of the Band Structure of the Leprosy Bacillus and Other Mycobacteria[1,2]

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Peculiar band structures were found on murine and human leprosy bacilli in 1958 by Okada (1). Consequently Okada postulated that there might be some process of demarcation in the bacillary body which results in replication in a manner different from ordinary cell division. Sato has also presented pictures of similar structures (2) which he termed "demarcation lines" in the leprosy bacillus.

In the course of an electron microscopic study of leprosy bacilli obtained from new lepromas of a resistant lepromatous patient, distinct band structures were found in almost all intact bacilli (Figs. 2, 3, 6, and 7). Subsequently, similar electron microscope examination of leprosy bacilli was repeated on six cases of acute leprous infection in all of which distinct band structures (Figs. 4, 5) were found. It seems probable that these band structures are a constant feature of growing leprosy bacilli.

Similar band structures have been found also in other mycobacteria, such as murine leprosy bacilli Hawaiian strain, avian tubercle bacilli Juchө strain and H37Ra strains of tubercle bacilli.[4]

MATERIALS AND METHODS

Human leprosy bacilli and murine leprosy bacilli were obtained from human and mouse lepromas respectively. They were scraped from excised tissue with a surgical knife, suspended immediately in a drop of physiologic saline and placed on Maximow slides.

Care was taken in every step of specimen preparation to minimize destruction of mycobacterial cells. The suspensions of bacilli were placed on copper grids for electron misroscopy. After the grids had dried, they were washed twice in distilled water. Later, they were shadow-casted with chromium or platinum-palladium. Electron microscopic observation at the accelerating voltage of 80,000 volts proved satisfactory for the observation of the band structures on the cell wall of mycobacterial cells. Electron microscopes used in this study were Akashi AEM-80 and Hitachi 11A and 11D.

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4 Avian tubercle bacilli and H37Ra were taken from cultures.

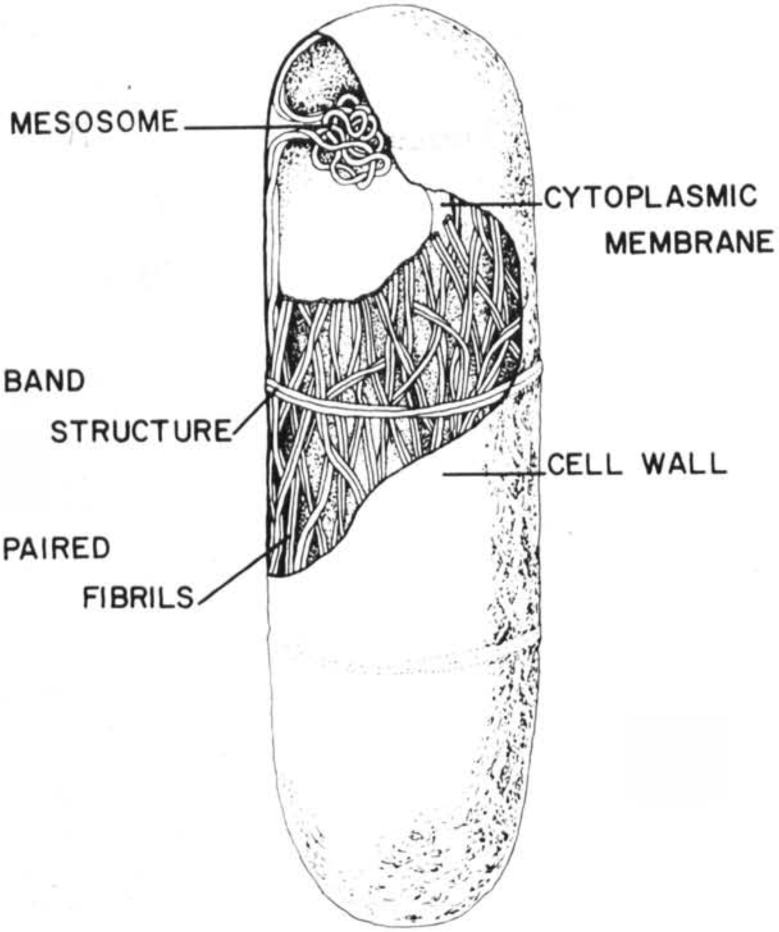


FIG. 1. Diagramatic presentation of the relationship between cell wall, band structure, paired fibrous structure, real cytoplasmic membrane and mesosome of mycobacterial cells. Band structure consists of paired fibrils arranged transversely like a bamboo-node. This is clearly shown in a negative-stained bacillus in Figure 9. Mesosome is continuous to the paired fibrils and is composed of the same fibrils. The so-called ultrastructural cytoplasmic membrane or plasma membrane is not a real cytoplasmic membrane but consists of the profiles of the paired fibrous structures beneath the cell wall. The real cytoplasmic membrane is very thin (less than 30 Å) and can be seen in negative-staining specimen as the border of the cytoplasm (Fig. 10).

Findings of the shadow-casted bacilli were compared with those of ultra-thin sections and negative stained preparations of homologous bacilli.

OBSERVATIONS AND DISCUSSION

Band structures are usually 100-300. Å thick. In chromium-shadowed specimens (Figs. 3, 4, 5), band structures appear broader than in platinum-palladium-shadowed bacilli. The real width of the band structures is most accurately measured on platinum-palladium-shadowed bacilli (160. Å) (Fig. 6). In some leprosy bacilli both band structures and paired fibrous structures ([3]) can be seen on the surface of the cell body (Figs. 5, 7), because in such cells only the thin rigid layer of the cell wall covers both structures. In such instances, band structures can be seen

FIG. 2. Human leprosy bacilli from a growing leproma. These bacilli have thick cell walls and distinct band structures. Magnification: 88,000× (Scale: 0.1 μ). Symbols: **L**, leprosy bacillus; **BD**, band structure.

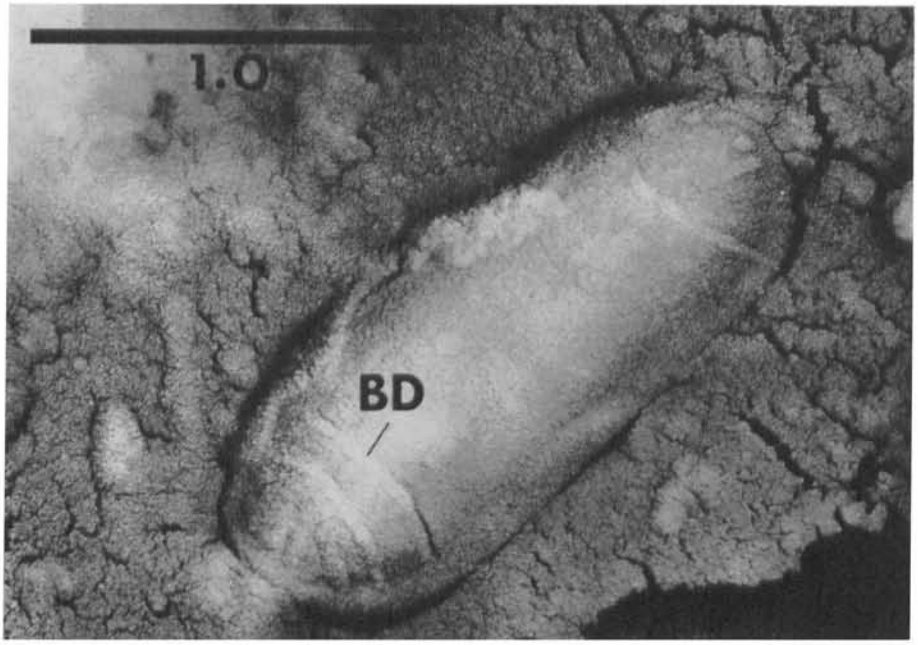


FIG. 3. A very thick human leprosy bacillus with three distinct band structures. This bacillus was obtained from a growing leproma. The cell wall is very thick and paired fibrous structures beneath the cell wall cannot be seen from outside the cell. Magnification: 58,000× (Scale: 1.0 μ). Symbol: **BD**, band structure.

interwoven with fibrous structures and seem to be lying within the same layer, i.e., beneath the rigid basal layer of the cell wall.

On the other hand, in other leprosy bacilli having a thick surface cell wall layer, no fibrous structures are visible on the surface of the cell, and only the band structures are then evident (Figs. 2, 3, 6).

In some bacilli, band structures have a paired appearance (Fig. 6). Some of the band structures are continuous with the fibrous structures (paired fibrous structures) as is seen in avian tubercle bacilli (Fig. 9). From these observations it seems most probable that the band structures and the paired fibrous structures are similar fibers, the only difference being that the band structure surrounds the bacillary body in a bamboo-joint-like arrangement, whereas the paired fibrous structures form a kind of network beneath the cell wall.

In ultra-thin sections, it is very difficult to find band structures, because they are intertwined with the paired fibrils which can be seen beneath the cell wall as the so-called cytoplasmic membrane. In point of fact, the so-called cytoplasmic membranes of mycobacteria are not real membranes, but are the profiles of paired fibrous structures (Figs. 11, 12). This is clearly shown in a picture of a negatively stained avian tubercle bacillus (Fig. 10), in which the fibrous nature of the so-called cytoplasmic membrane is clearly seen. The band structures which can be seen in negative staining specimens have the same diameters as those of the paired fibrils (Fig. 9).

The real cytoplasmic membrane of mycobacteria is a thinner membrane than the so-called cytoplasmic membrane (Fig. 10). Usually the cytoplasmic membrane of cells such as macrophages is seen as a single electron-dense line in ultra-thin sections of osmium-fixed specimens. However, the so-called cytoplasmic membrane of mycobacteria, as interpreted by electron microscopists, has a triple layered appearance even in osmium-fixed specimens (Figs. 11, 12). This suggests that the so-called cytoplasmic membrane of mycobacteria has a molecular structure different from that of the unit membrane.

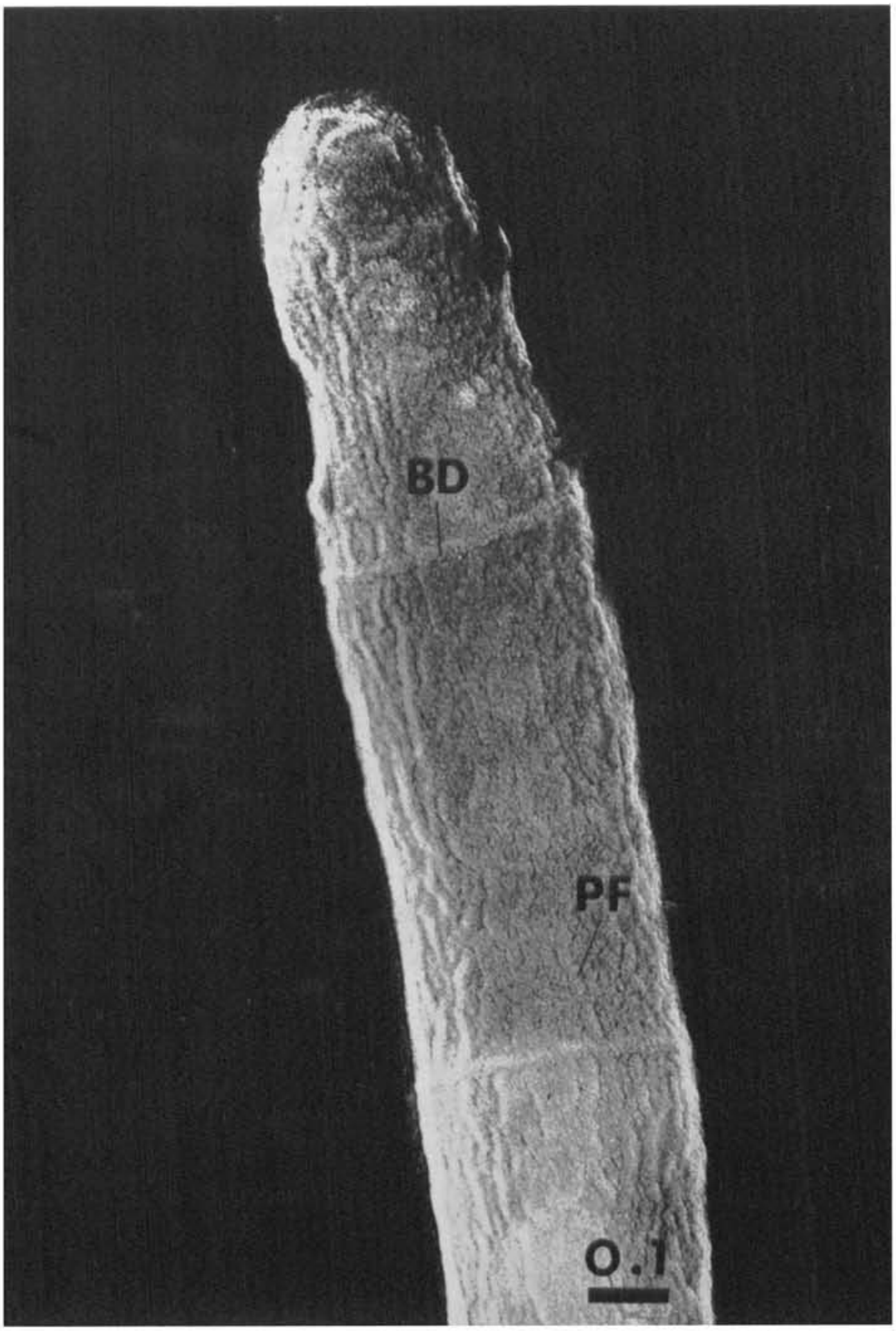


FIG. 4. Human leprosy bacillus from a case of lepromatous exacerbation. As this bacillus has a thin cell wall, fibrous structures and band structures can be seen clearly from outside the cell. Band structure and fibrous structure (paired fibrous structure) have the same thickness. Magnification: 120,000× (Scale: 0.1 μ). Symbols: **BD**, band structure; **PF**, paired fibrous structure which appears to be made of single fibers because of the chromium shadowing. However, these fibers are actually made of paired fibrils as shown in the bacillus of Figure 7.

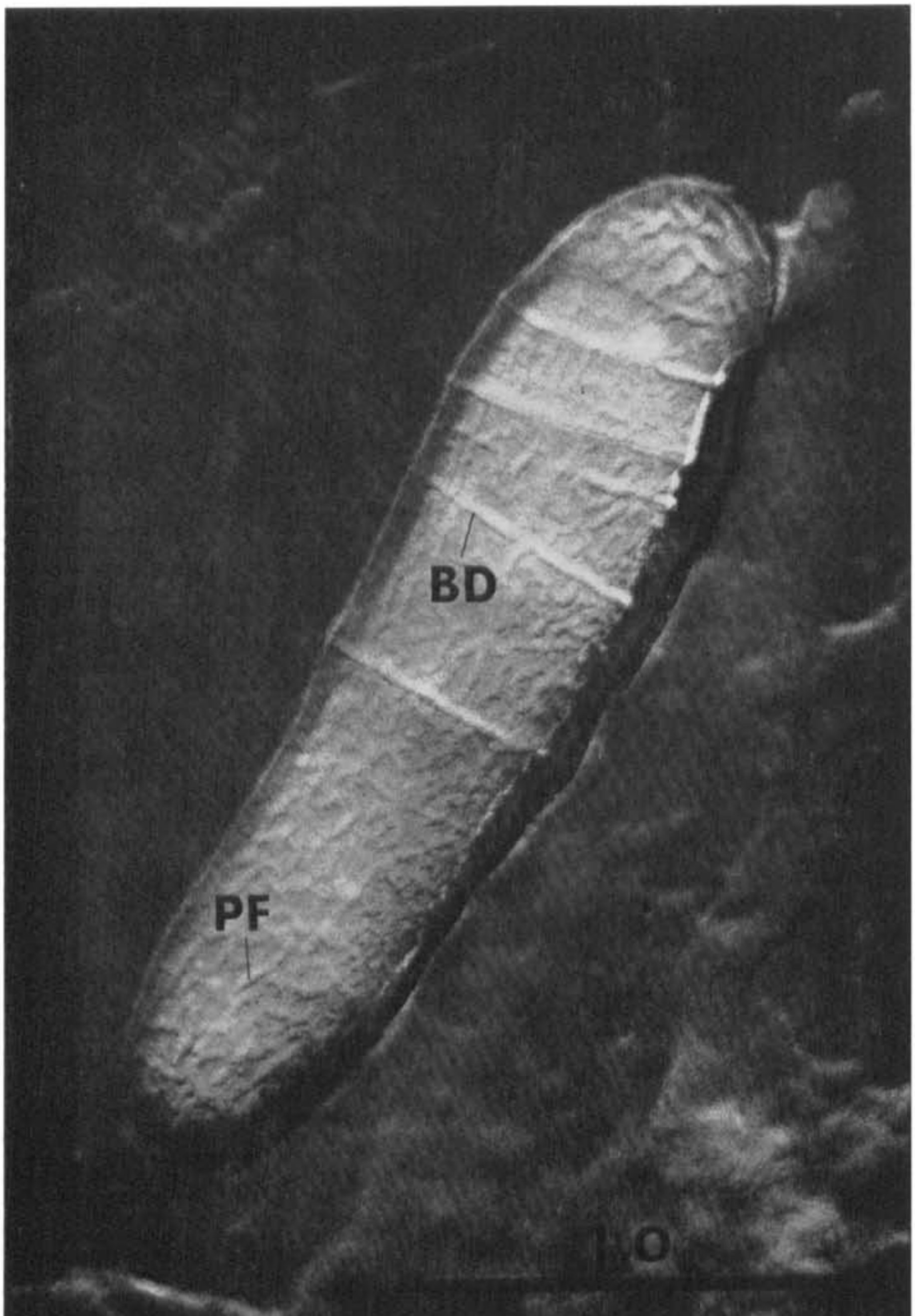


FIG. 5. Human leprosy bacillus obtained from a case of lepromatous exacerbation. Many bands are seen on this bacillus. Some of the band structures have not yet formed complete rings around the bacillary body. One of the band structures shows double fibers. Magnification: 64,000× (Scale: 1.0 μ).

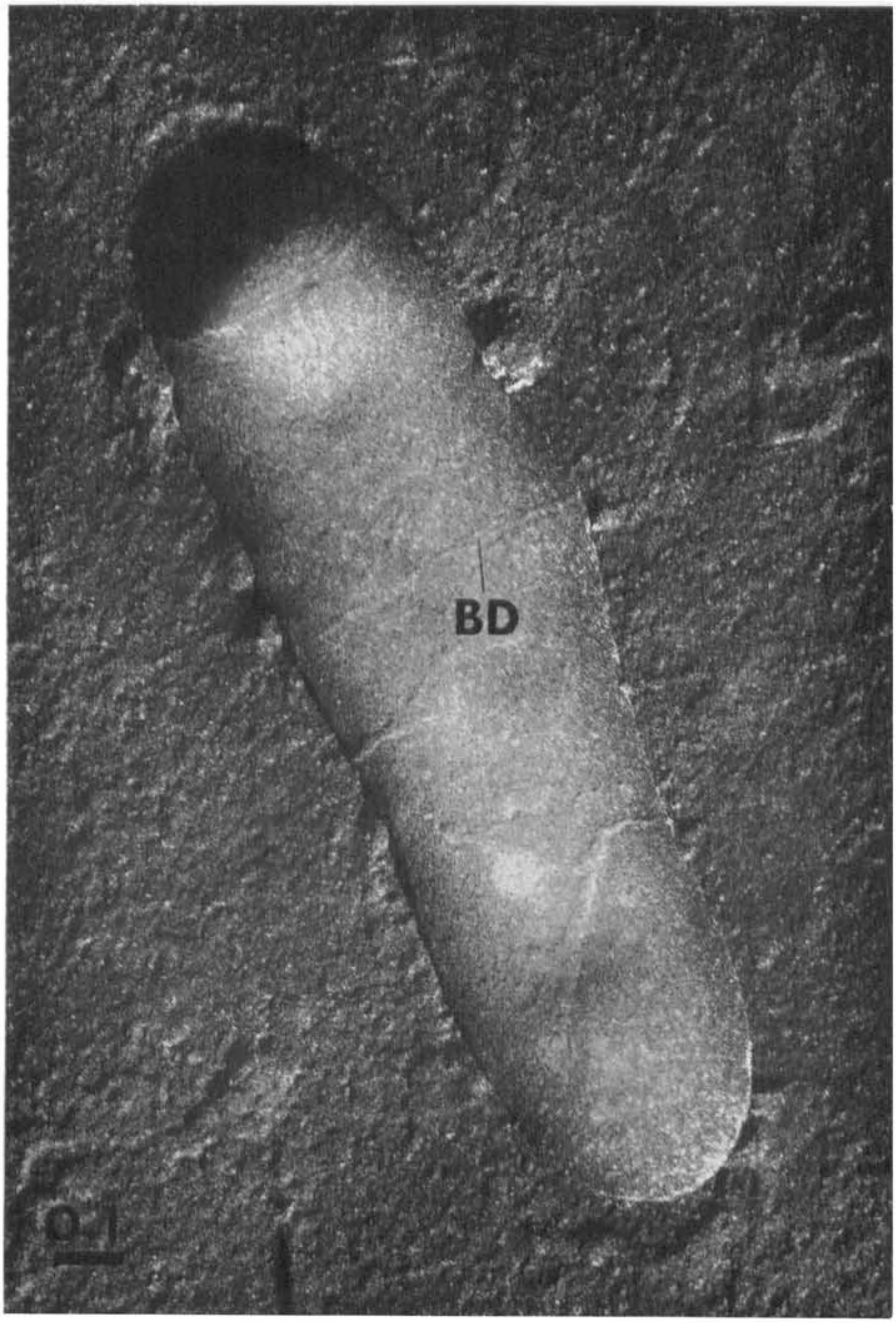


FIG. 6. Human leprosy bacillus from a growing leproma. As the cell wall of this bacillus is very thick, Takeya's paired fibrous structures beneath the cell wall cannot be seen, but two band structures are evident. These band structures show paired fibrils. Magnification: 103,000× (Scale: 0.1 μ). Symbol: **BD**, band structure which is composed of paired fibrils.

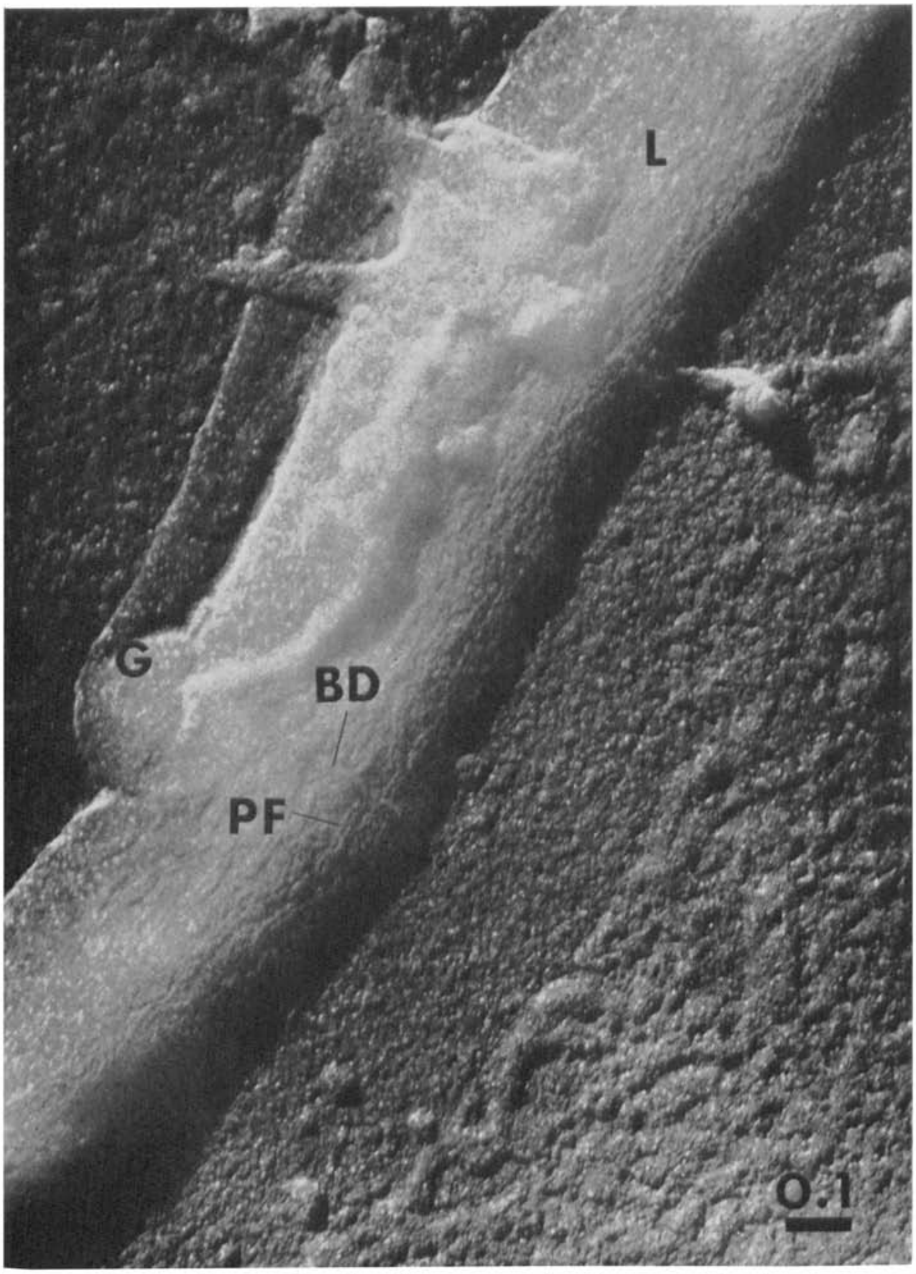


FIG. 7. Human leprosy bacillus from an active leproma. Shadow-casting with platinum-palladium. Paired fibrous structures can be seen clearly on the surface of this bacillus. These paired fibrils are actually located beneath the thin cell wall of this bacillus. Magnification: 100,000× (Scale: 0.1 μ). Symbols: **L,** leprosy bacillus; **BD,** band structure; **G,** a fragment of ghost cell wall; **PF,** paired fibrils.

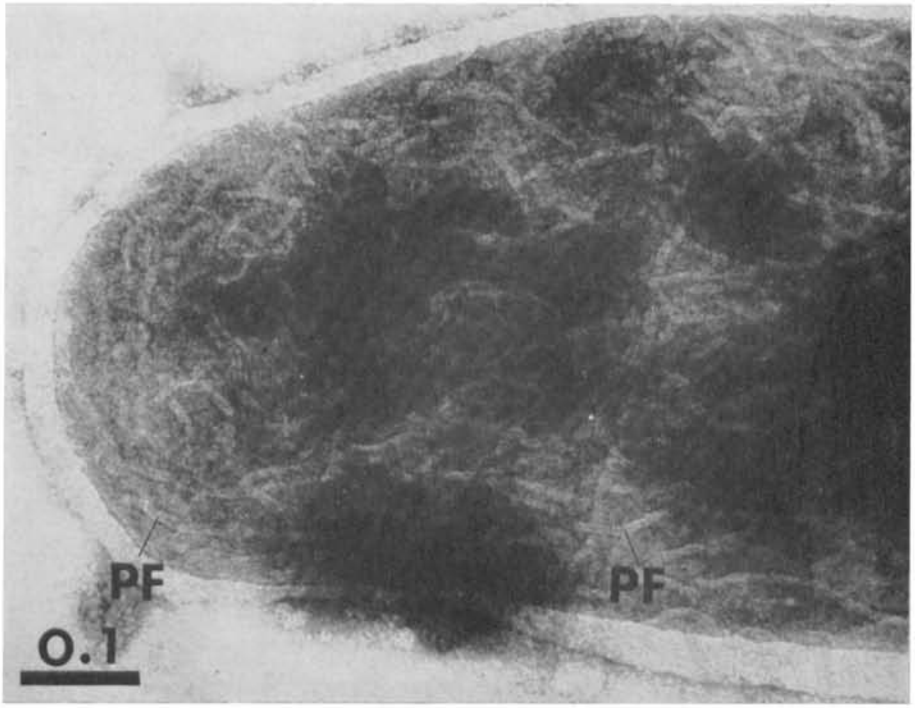


FIG. 8. Negative staining of an avian tubercule bacillus. This specimen was first fixed in OsO_4 and later negative-stained with phosphotungstic acid. Network of fibrous structures can be seen clearly. Each fiber (about 90 Å thick) is composed of two fibrils (each about 30 Å) and these paired fibrils are identical with Takeya's paired fibrils observed by shadow-casting. As these fibrils could be seen clearly only when the phosphotungstic acid entered inside the cell wall, it seems evident that this network of paired fibrils is located inside the cell wall. Magnification: 172,000× (Scale: 0.1 μ). Symbol: **PF**, a fiber composed of paired fibrils.

Some of the band structures may be related to septum formation at the site of cell division, but those band structures which can be seen near both ends of the bacillary body usually do not present underlying septum formation. At present, the function of these band structures is not evident, but as far as we have been able to determine it seems that thicker bacilli have more band structures as compared with normal-sized bacilli. Even in the same bacillus, band structures are seen more definitely on the thicker side of the bacillary body.

It seems that before the initiation of cell division, some of the paired fibrils show a bamboo-joint-like arrangement around the bacillary body. At present, the relationship between band structure formation and cell division is not clearly understood. However, judging from the fact that numerous band structures are found in bacilli taken from the lesions where bacilli are growing rapidly, this structure seems to be closely related to the cell division of leprosy bacilli. On this assumption, it may be useful to introduce the concept of the "Band Index" of mycobacterial cells as shown below, to indicate the potentiality for further cell division.

$$\text{Band Index} = \frac{\text{Total number of band structures}}{\text{Number of solid bacilli examined}}$$

Other types of band structures are also seen around leprosy bacilli. These are broader than the real band structures (Fig. 13). These broader bands are thought to be derived from the foamy structures surrounding leprosy bacilli in the cytoplasm of lepra cells. They can readily be distinguished from the real band structures.

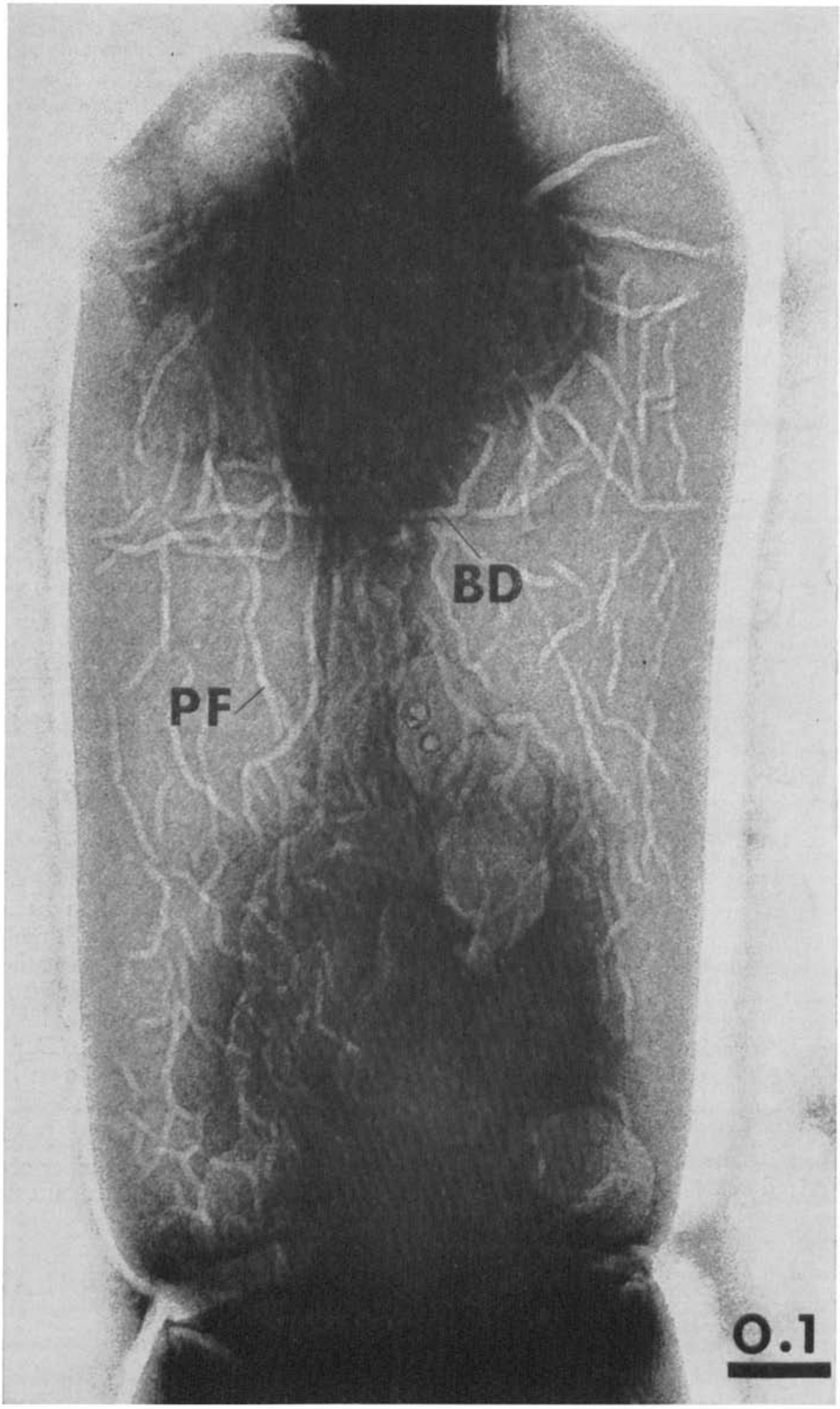


FIG. 9. Negative-stained avian tubercle bacillus. Formation of band structure by transverse arrangement of paired fibrous structures can be seen clearly in this picture. Magnification: 142,000× (Scale: 0.1 μ). Symbols: **PF**, paired fibrils; **BD**, band struc-

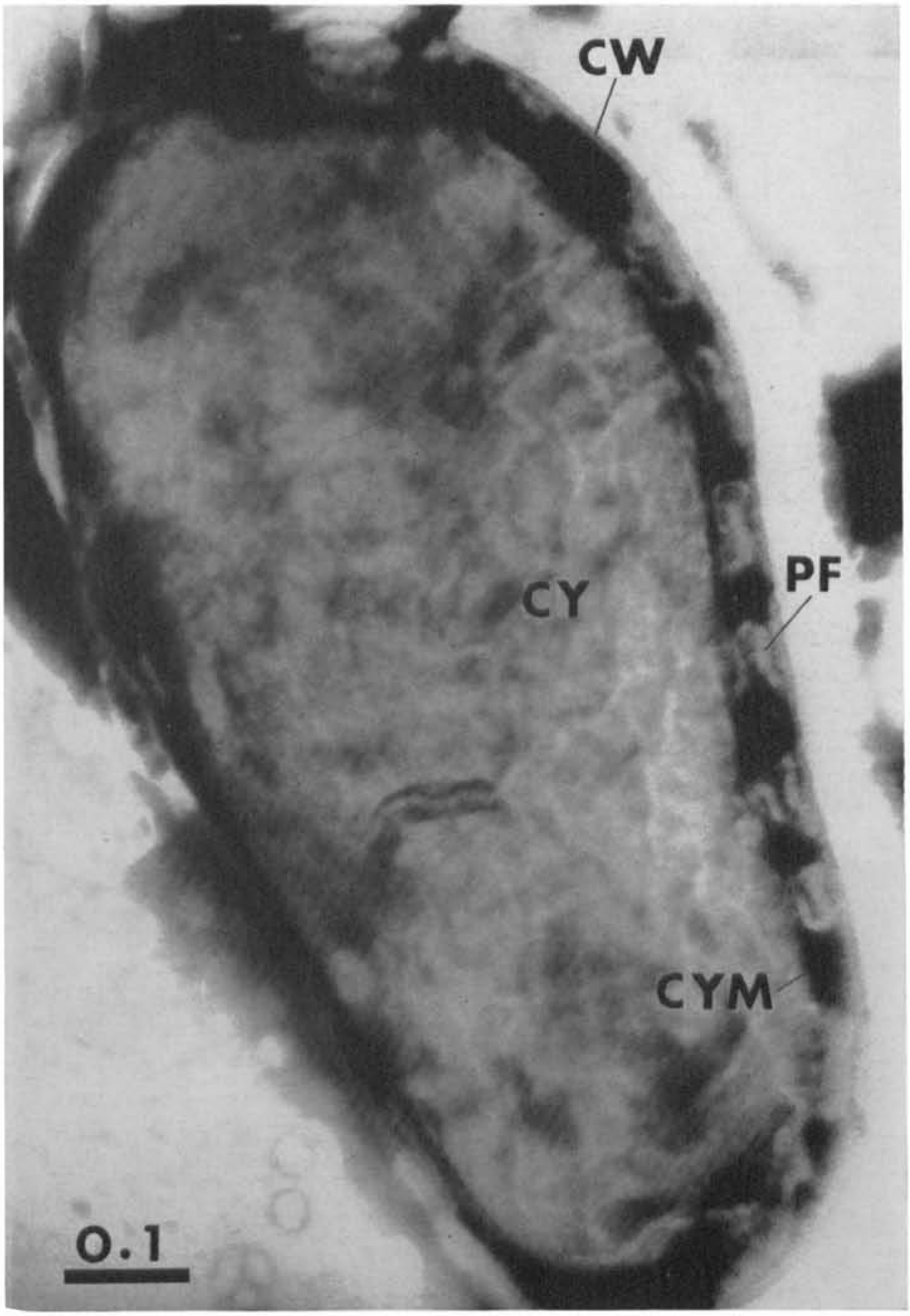
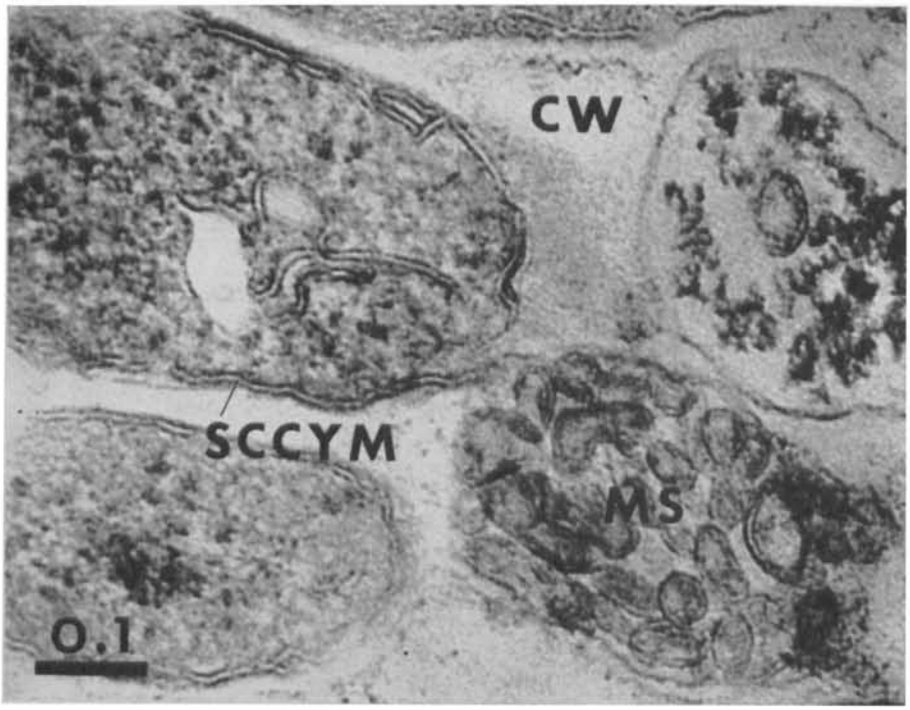


FIG. 10. Negative-staining of an avian tubercle bacillus. Phosphotungstic acid entered into the space between the cell wall and the cytoplasm. At the time of the detachment of the cytoplasm from the cell wall some of the paired fibrils were left behind in the space filled with phosphotungstic acid. The shape of these paired fibrils shows clearly that they are lying beneath the cell wall of this bacillus. At the border of the cytoplasm, there is no clear-cut electron transparent line limiting the cytoplasm. This indicated that the real cytoplasmic membrane is a very thin membrane differing from the so-called cytoplasmic membrane as observed in ultra-thin sections of mycobacteria. Magnification: 188,000× (Scale: 0.1 μ). Symbols: **CW**, cell wall; **PF**, paired fibrils; **CY**, cytoplasm; **CYM**, real cytoplasmic membrane which can not be seen as a clear-cut line because of its extreme thinness.



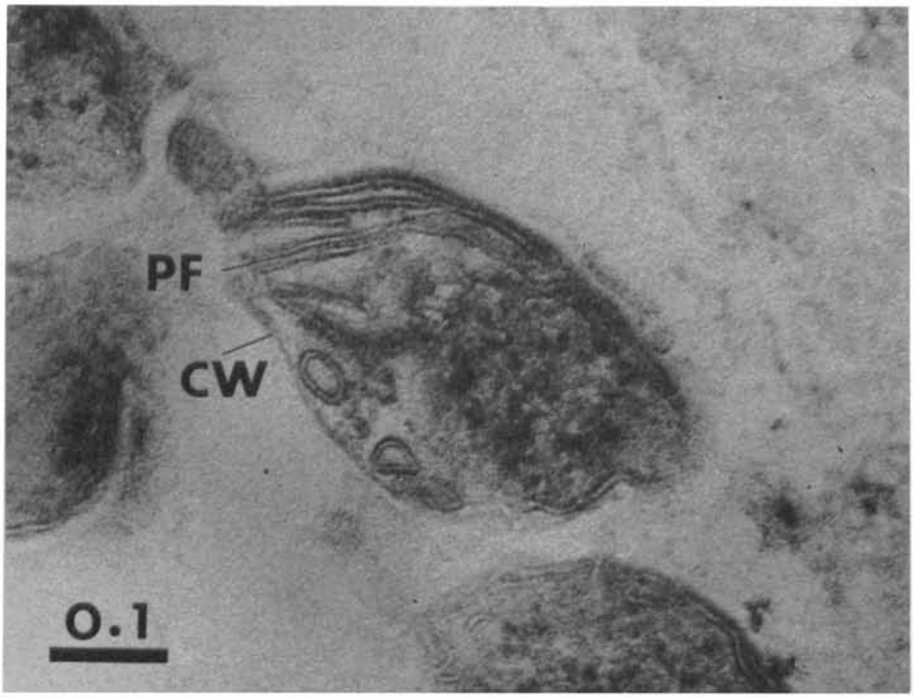


FIG. 11. Ultra-thin section of human leprosy bacilli in a lepra cell. Each fibril of the paired fibrils has a triple layered structure. The so-called cytoplasmic membrane is actually the profile of the paired fibrils. When these fibrils are detached from the cell wall, especially in mesosomes, they tend to curl up and, therefore, they show ring-like structures in ultra-thin sections. Magnification: 167,000× (Scale: 0.1 μ). Symbols: **CW**, cell wall; **MS**, mesosome with many ring structures made of the fibrils; **SCCYM**, so-called cytoplasmic membrane, but actually it is the profile of paired fibrils.

FIG. 12. Human leprosy bacillus in a lepra cell. Paired fibrils and ring-like structure made of the same fibrils can be seen in this section. Magnification: 174,000× (Scale: 0.1 μ). Symbols: **CW**, cell wall; **PF**, paired fibrils.

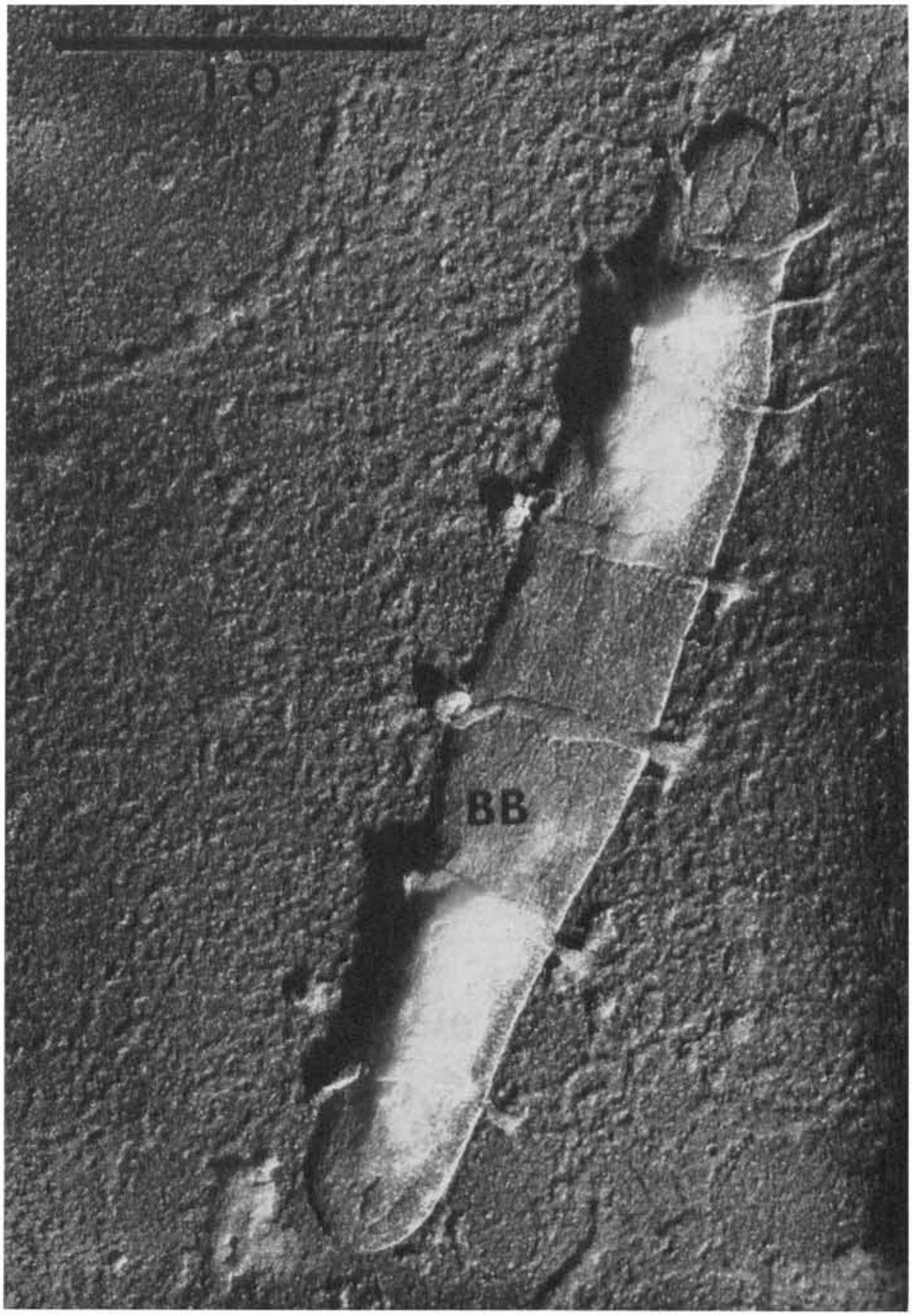


FIG. 13. Human leprosy bacillus from a lepromatous patient. There are broad bands surrounding this bacillus which are different from the real band structures. These broad bands are derived from foamy structures surrounding leprosy bacilli in the cytoplasm of the lepra cell. Magnification: 56,000× (Scale: 1.0 μ). Symbols: **BB,** broad band which is different from the real band structure of mycobacteria.

Band structures of other mycobacteria have about the same thickness and seem to be of the same nature. The pattern of the fibrous structures beneath the cell wall, however, seems to differ in different mycobacterial cells.

SUMMARY

Distinct band structures are constantly found in growing leprosy bacilli and other mycobacteria. These structures seem to be made of Takeya's paired fibrous structures and they surround the bacillary body much like bamboo-nodes. Thicker bacilli have more band structures than normal-sized bacilli.

The "Band Index" which is presented as the average number of band structures per solid bacillus seems useful as an indication of the potentiality of mycobacteria for further cell division.

RESUMEN

En el *M. leprae* y otras micobacterias se encuentran constantemente estructuras definidas semejante a bandas. Estas estructuras parecen estan hechas de las "paired-fibrous structures" de Takeya, rodeando el cuerpo del bacilo en una manera muy similar a los nodulos de bambú. Bacilos mas gruesos tienen mas de estas "band-like" estructuras que bacilos de tamaño normal.

El "band-index," es decir el numero promedio de estructuras con bandas por un bacilo sólido, parece util como indicación de la potencialidad de micobacterias por la división de celulas.

RÉSUMÉ

On observe de façon constante des structures en bande bien distincte dans les bacilles de la lèpre en croissance, ainsi que dans d'autres mycobactéries. Ces structures semblent être constituées par des structures fibreuses en paires de Takeya; elles entourent le corps bacillaire d'une manière qui évoque fortement des noeuds de bambou. Les bacilles les plus épais rèvèlent davantage de structures en bande que les bacilles de dimensions normales.

L' "Index de Bande" que l'on présente ici comme étant le nombre moyen de structures en bande par bacille solide, paraît utile en tant qu'indication de la capacité des mycobactéries à se diviser davantage.

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