The Surface Structure of *M. leprae*[1]

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Since Takeya *et al* ([6, 7]) described for the first time the so-called paired fibrous structure of mycobacteria, including *M. lepraemurium*, several reports, by several authors ([1, 2, 4, 8]), have been presented on this subject. With particular reference to the surface structure of *M. leprae*, one of us ([5]) observed that the pattern varied in chromium-shadowed and negatively stained specimens respectively; in each case the appearances were not always so delicate as in the case of other mycobacteria, including *M. lepraemurium*. Recently the authors demonstrated an elaborately formed network pattern in *M. leprae* by incubating these mycobacteria in Lockemann's liquid medium for a few days. The details are given in the following pages.

MATERIALS AND METHODS

More than ten lepromas, removed from patients with active leprosy, some with and others without regular treatment, were examined.

In the preparation of specimens, two methods were employed as follows, in order to obtain cell envelopes and ghost cells of *M. leprae*.

1. Destruction of cell bodies by grinding a leproma in a mortar. Small pieces of tissue were ground vigorously in a mortar with a little silica powder and salt. A small quantity of distilled water was added. The bacillary suspensions were left standing in a test tube for 5-10 minutes. The lower part of the supernate was then smeared on a copper grid for electron microscope observation.

2. Incubation of leproma suspension in a liquid medium. A suspension of ground leproma was incubated in Lockemann's liquid medium at 37° C for 72 hours. Parallel with this, media to which Kanacilin ("Meiji," i.e., Kanamycin + pencillin (2γ/ml.), isoniazid (2γ/ml.) and Promin (10 mgm./ml.)) was added, also were employed. After the incubation a small quantity of the suspension was smeared on a copper grid with a carbon film.

The smeared specimens so stained were treated as follows for observation. Some specimens were fixed in osmium tetroxide vapor, while others were not. All were observed under the electron microscope (Hitachi HU10 and 11B), after being shadowed with chromium or platinum-palladium (Pt-P1), and negatively stained in 0.5 per cent phosphotungstic acid (PTA) for a few seconds.

RESULTS

Findings with respect to the surface of cell envelopes of *M. leprae* obtained by mechanical destruction. (a) *Shadowed specimen.* As seen in Figure 1, the chromium-shadowed specimen (Cr) showed the formation of a coarse network consisting of many tape- or thread-like elevations (apparently) on the surface of the bacillary cell. These threads, 40-60 mμ in width, wound chiefly along the longitudinal axis of the cell throughout its entire surface. The threads, 10-40 mμ in width, were much thinner and finer, however, in platinum-palladium (Pt-P1)-shadowed specimens (Fig. 2). In view of the fact that there was no variation in the shadowing technic, the difference in the findings between Cr- and Pt-P1-shadowed specimens is probably due to a difference between Cr- and Pt-P1 particles.

(b) *Negatively stained specimens (1%-phosphotungstic acid).* A fairly well-marked difference in surface pattern was seen between cell envelopes prepared by application of mechanical force and those

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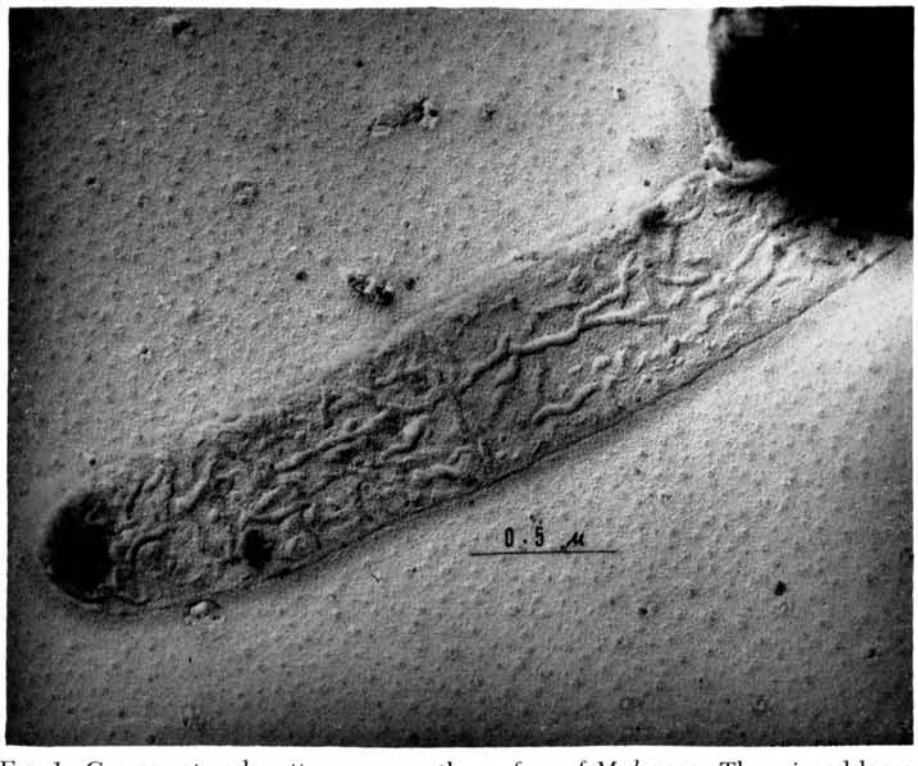

FIG. 1. Coarse network pattern seen on the surface of *M. leprae*. The minced leproma was ground vigorously in a mortar with powdered silica and salt. A small quantity of distilled water was added to the tissue gruel. The supernate of the tissue suspension was smeared on a copper grid for electron microscopic observation. Note the tape-like elevations on the bacillary surface. (Chrome-shadowing)

obtained by incubating bacilli in Lockemann's solution. The former measure was drastic and often resulted in excessive disruption of bacillary bodies, while the latter resulted in deterioration of the cytoplasm without destruction of the cell envelope. A cell envelope obtained by the first method is shown in Figure 3, and one obtained by the latter method is shown in Figure 4. The difference between the two is conspicuous; a coarse fibrous network consisting of fragments of threads is apparent in the former, while a fine, delicately formed network, consisting of closely arranged fibers, is apparent in the latter.

In each case the threads (fibers) were roughly uniform in their width, running in a complicated tangle in many directions. It was interesting to observe that where two threads crossed, lying one upon the other, there was a little greater transparence in density. The thread was 15-20 mμ in width and consisted of an inner and wider transparent tape-like part, and two outer, fine, dense lines. The latter dense lines were comparable to the so-called "paired fibrous structure" in their appearance.

DISCUSSION

As noted above, Takeya ([6, 7]) described, for the first time, with the designation "paired-fibrous structure," the surface ultramicroscopic structure of mycobacteria, including *M. lepraemurium*, as shown by chromium shadowing. The structure was demonstrable in mycobacteria lysed by phage and in their ghost cells prepared either mechanically or chemically. One of the present authors ([4]) once presented a picture of a cell envelope of *M. leprae*, obtained by grinding with silica powder, showing, by means of chromium-shadowing (Fig. 1), that a coarse and irregular network, consisting of comparatively thick fibers, formed in the surface. Later he obtained a figure showing a net-like pattern

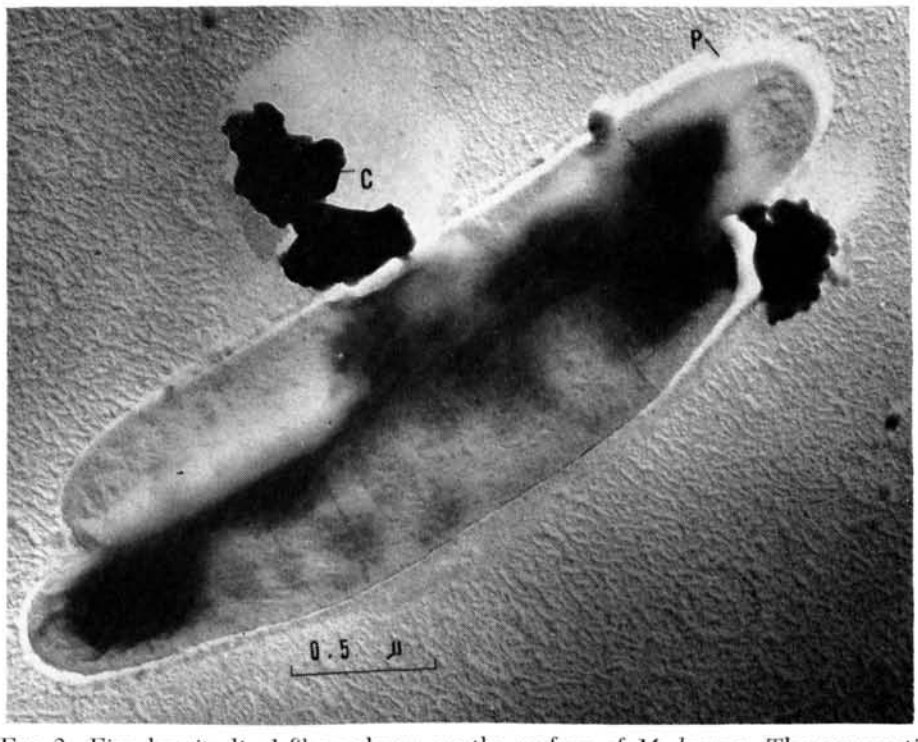

FIG. 2. Fine longitudinal fibers shown on the surface of *M. leprae*. The preparation of the specimen was made in the same way except that it was being shadowed with platinum-palladium instead of chromium. (C=cytoplasmal mass; P=peripheral transparent zone)

consisting of fragmented fibers in a negatively stained cell envelope (Fig. 3). Imaeda ([2]) discussed the problem, but his picture of *M. leprae* showing bacterial surface with fine fibrils (Fig. 8 in his paper) is not so good for the demonstration of the surface structure of the germ. According to our experience it was much more difficult to obtain as good results with *M. leprae* as with other mycobacteria, including *M. tuberculosis* and *M. lepraemurium*, if the preparation of bacterial envelopes was carried out by grinding them with sea-sand in a mortor or by means of French pressure (disintegrator) cells.[3] It proved not difficult to show a fine and elaborate network pattern in many mycobacteria other than *M. leprae* by mechanical preparation, while it was not easy in the case of *M. leprae*, the pattern of which is too irregular and simple. The structure seemed to be on the surface of a bacterial sack composed of the cell wall and cytoplasmic membrane. In an intact cell, however, scarcely any distinct surface pattern could be seen, because of the masking effect of underlying electron-dense cytoplasmic elements. The structure becomes evident if the underlying cytoplasm is removed by mechanical, chemical, or other measures. Naturally the structure is shown most conspicuously in such cells after change into ghost cells in a deteriorating environment or in cells being lysed by phage. Under these conditions the bacterial sack nearly retains its proper shape, forming cell envelopes. In contrast, cell envelopes mechanically prepared are subjected almost always to some damage, varying in degree. If the damage is very severe, the structure is completely lost, leaving a transparent sack or its fragmented remains. *M. leprae* is probably

[3] French pressure cells are described in the following articles: Graham, P. R. Preparation of chloroplasts and disintegrated chloroplast. *From* Methods in Enzymology by Colowick, S. P. and Kaplan, N. O. New York, Academic Press, 1955. Chap. 1, pp. 22-25; French, S. C. and Milner, H. V. Disintegration of bacteria and small particles by high pressure extrusion. *Ibid*, 64-67.

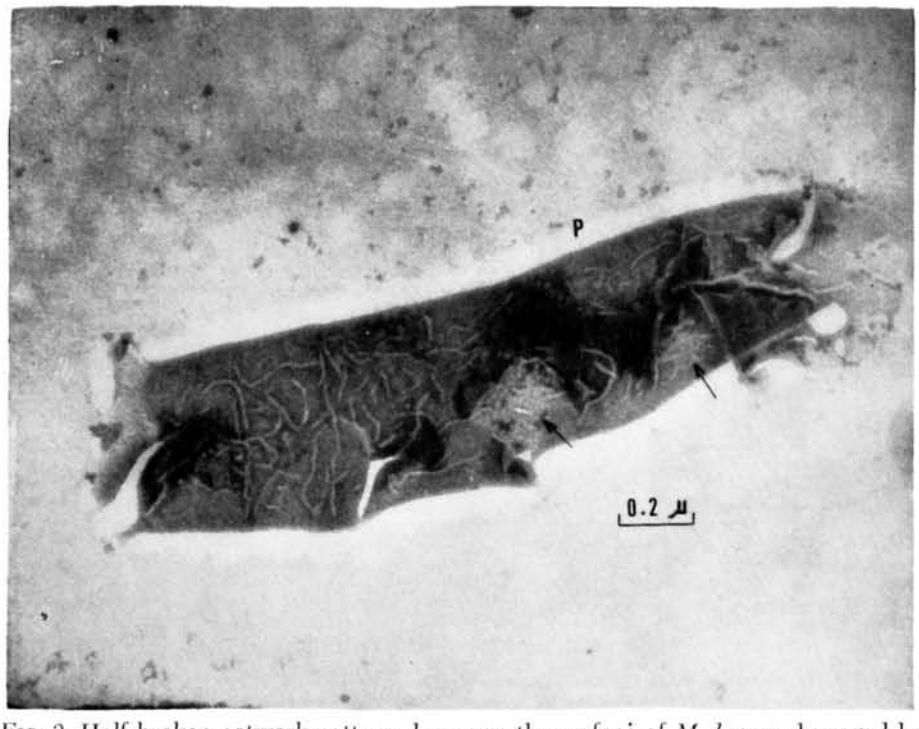

FIG. 3. Half-broken network pattern shown on the surface of *M. leprae* damaged by a grinding procedure with silica and salt in a mortar. Note the presence of fibers on the backside of a cell hull (arrow). (P=peripheral transparent zone. Negatively stained with 0.5 per cent phosphotungstic acid (PTA))

more fragile than all other mycobacteria and liable to be disrupted by the mechanical procedure of grinding. Fine fibrous threads on the cell surface are easily broken into fragments. Better specimens, however, showing a delicate and elaborate network pattern, could be observed in *M. leprae* that had been incubated in a 10 per cent serum-Lockemann medium, with or without certain antimycobacterial drugs (isoniazid, Promin, and kanacilin (Kanamycin + penicillin) for a few days. It is emphasized, however, that no difference was seen in population of ghost cells showing the network pattern among cultures, regardless of incorporated drugs.

It was also revealed that the same pattern could be found in the inner surface of the cell envelopes, although Yamaguchi ([8]) once reported this not to be true in the case of *M. tuberculosis* (Fig. 3). On the other hand, some doubt remains as to whether or not all of the cells have the same surface structure, for some, from the same batch, showed no fibrous feature among the envelopes examined. This possibly indicates that excessive decomposition may induce a complete loss of the structure. As was stated above, there were fairly marked differences in the fibrous pattern between shadowed and negatively stained specimens; i.e., a far more simple pattern, consisting of thicker and raised thread-like fibers of fine dense paired lines was observed in the former (Figs. 1, 2,), while a complicated network pattern, consisting of wider and transparent tape-like threads, each of which had a constant width, with fringes of fine dense lines, was seen in the latter (Figs. 3, 4). Consequently it seemed more reasonble to designate this surface structure as a "fibrous network" pattern rather than a "paired" fibrous structure.

Meanwhile, it is worth noting that a thin transparent zone was often seen surrounding the bacterial envelope (Figs. 2, 3). This zone is comparable to the so-called "peripheral halo" described by Malfatti ([3]). In particular, it is emphasized that the halo was often shown clearly pressed out

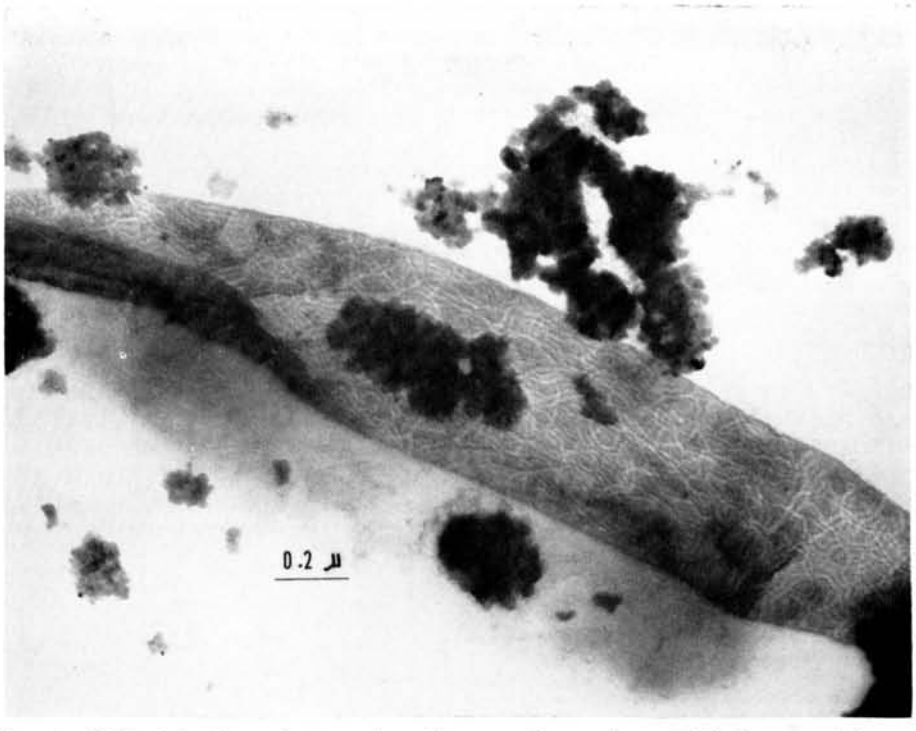

FIG. 4. Delicately formed network pattern on the surface of *M. leprae*. A leproma suspension was mounted on a copper grid for observation after incubation at 37°C for 72 hours. (Negatively stained with PTA)

by the grinding procedure (Fig. 2). This strongly suggests the presence of some substance surrounding the bacterial cell. The problem will be discussed in another paper.[4]

SUMMARY

Mycobacterium leprae as well as other mycobacteria, including *M. lepraemurium*, has a network pattern, consisting of tape-like fibers, in the cell surface.

The surface structure of *M. leprae* is fragile. Lysis of the cells by grinding destroys the structural pattern, with fragmentation of the fibrous elements of the network.

Incubation of *M. leprae* in Lockemann's solution for a few days seemed to favor demonstration of the surface structure.

It appeared more appropriate to designate the structure as a "fibrous network" pattern rather than a "paired fibrous" structure.

RESUMEN

Mycobacterium leprae, como también otras mycobacterias, incluyendo el *M. lepraemurium*, tiene un tipo de estructura consistente en fibras semejantes a cintas en la superficie celular.

La estructura superficial del *M. leprae* es frágil. La lisis de las células por la molienda destruye el modelo estructural, con fragmentación de los elementos fibrosos de la malla.

La incubación del *M. leprae* en solución de Lockemann, por unos pocos días, pareció apoyar la demostración sobre la estructura de la superficie.

[4] Very recently Prof. Nishiura of Kyoto University, presented a picture showing the presence of elevated ring-formed bands, presumably surrounding a bacillary body of *M. leprae* in its transverse axis (unpublished). Sato also once observed the formation of a similar elevated band (Human and Murine Leprosy in *Mykobakterien und Mykobakterielle Krankheiten*, Vol. 4, Part IX, Jena, 1967. Figure 4, point marked with cross, on page 8). However, he could not decide whether the band was of a demarcation line at the site of fission or of a sort of surface structure.

Se consideró más apropiado designar la estructura como una "malla fibrosa" más bien que una estructura "fibrosa pareada."

RÉSUMÉ

Mycobacterium leprae, comme d'autres mycobactéries, y compris *M. lepraemurium*, présente à la surface cellulaire un aspect en réseau, consistant en fibres ayant l'apparence de rubans (tape-like fibers).

La structure superficielle de *M. leprae* est fragile. La lyse des cellules, lorsqu'elles sont broyées, détruit leur aspect structural, et entraîne la fragmentation des éléments fibreux du réseau.

L'incubation de *M. leprae* dans la solution de Lockemann pendant quelques jours, semble faciliter la mise en évidence de la structure superficielle.

Il semble plus adéquat de désigner cette structure comme un aspect "en réseau fibreux," plutôt que comme une structure "en fibres couplées."

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