FURTHER STUDIES ON THF PAIRED FIBROUS STRUCTURE OF MYCOBACTERIAL CELL WALL

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A "paired fibrous structure" was found in "ghost cells" of mycobacteria by Takeya *et al.* (1) and was supposed to be related to the cell envelope. The present paper will describe further observations on this structure.

Cells of *Mycobacterium* Jucho strain[1] grown on a thin collodion membrane deposited on 4 per cent glycerol agar were replaced onto the same medium containing isoniazid or streptomycin 1 mg. per ml., or mitomycin C 0.1 to 100 μg per ml. After 1 to 3 days of incubation at 37°C., the collodion film was removed from the medium on copper mesh screen and "ghost cells" thus obtained were examined by the electron microscope. For further treatment of the "ghost cells," the collodion film with the "ghost cells" was again floated off on the surface of the solution containing various chemicals and incubated for 1 to 24 hours. A droplet of each solution was put on the cells on the collodion film before incubation. After treatment, the collodion film was taken on distilled water for "washing" and removed on copper mesh screen to be examined by the electron microscope. Repeated freezing and thawing, ultrasonic vibration of cell suspensions, and grinding of cells with glass beads were also used to prepare "ghost cells" of *Mycobacterium* Jucho strain and *Mycobacterium tuberculosis hominis* (H37Rv). Leproma of rats which had been infected with *Mycobacterium lepraemurium* was cut into small pieces and ground with sea sand. After removing sea sand and host cell debris by low speed centrifugation, the bacterial cells were washed and collected by high speed centrifugation. About 5 to 10 per cent of the cells collected were found to be "ghost cells" by electron microscopy.

[1] This strain was originally isolated from fowl tuberculosis and designated as *Mycobacterium avium*, Jucho strain. However, the detailed studies of the biological properties of this strain have recently disclosed that it has lost most of the properties characteristic of *Mycobacterium avium* and resembles saprophytic mycobacteria in many respects (2–4). Therefore, it is preferable to designate this strain merely as *Mycobacterium* Jucho strain henceforth.

With treatment by streptomycin, isoniazid, or mitomycin C, "ghost cells" were often found among the cells located at the margin of the microcolonies on the collodion film. Almost all the ghost cells thus obtained showed "paired fibrous structure" all over the entire cell envelope (Figs. 1, 6, and 7). The width of each fiber measures approximately 5 to 8 mμ. The more precise observations of this structure, especially at the branching sites of the "paired fibers," give the impression that this structure represents a kind of fine "canal system." Treatment with N/10 HCl, N/10 NaOH, or trypsin failed to alter the appearance of this fibrous structure (Figs. 6 and 7). However, treatment with 0.5 per cent KOH solution in alcohol removed the structure entirely leaving an extremely thin layer of the cell wall (Fig. 3). Although the same kind of fibrous structure could occasionally be obtained in the "ghost cells" prepared by freezing and thawing, by ultrasonic vibration, or by grinding with glass beads, in many cases the structure was observable only in a limited area of the cell envelope. Every "ghost cell" of *M. lepraemurium* obtained from leproma showed distinctly the same kind of fibrous structure in the entire area of the cell envelope (Fig. 2). Examinations of cell walls of *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* prepared by means of ultrasonic vibration methods failed to reveal the "paired fibrous structure."

Macromolecular structure of isolated cell wall in shadowed preparations was first reported by Houwink in a *Spirillum* species (5), and later in *Rhodospirillum rubrum* (6), a gram-positive bacterium (7), and *Bacillus aneurinolyticus* (8). The "paired fibrous structure" was found in *M.* Jucho strain, *M. phlei*, *M. smegmatis* in previous observations (1), and in *M.* Jucho strain, *M. tuberculosis hominis*, and *M. lepraemurium* in this study, but in none of the other bacteria so far examined and reported. The fact that this structure is characteristic of *Mycobacterium* species suggests that this structure may be related to the strong resistance of mycobacteria to physical and chemical treatment. This structure could be found usually in a

limited portion of the "ghost cells" prepared by mechanical means used in this experiment, whereas the structure could easily be observed in the entire area of the "ghost cells" obtained by the lysis due to mycobacteriophage (1, 9) and also by the treatment with bactericidal drugs. This seems to indicate that this structure is invisible in the intact cell wall but becomes visible by removing a cementing material. Some enzymatic reactions are supposed to account for the lysis of the cell and formation of "ghost cells" by bacteriophage infection. On the other hand, the mechanism involved in the formation of "ghost cells" by the treatment with drugs is obscure. However, it seems probable that "ghost cells" were formed by the activation of autolytic enzyme system in the cell due to the metabolic disturbance induced by the drugs. The structure was seen in the entire area of the "ghost cells" of *M. lepraemurium* found among the cells obtained by simple mechanical grinding of leproma. In this case some proportion of the cells contained in leproma might have degenerated and have been lysed by autolytic or tissue enzyme system. If these considerations are reasonable, the "paired fibrous structure" in the normal cell wall would be masked with a cementing material easily susceptible to an autolytic or other enzyme system and rather hard to remove by mechanical means. The fibrous structure was accidentally observed in "non-ghost cells" still filled with cytoplasm (Fig. 4). These cells might represent the initial stage of cell lysis by an enzymic process. This structure was found resistant to treatment with acid, alkali, and tryptic digestion, but susceptible to KOH-alcohol treatment. The elucidation of the chemical nature of the structure is now under investigation.

This structure was found inside the thin layer of the cell wall in a shadowed preparation (1). In ultrathin sections, however, the image corresponding to the structure could not be observed in any layer of the three-layered cell wall (10, 11). This may be because the "paired fibers" are so fine and/or have similar electron density to the cementing material in ultrathin sections.

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In each figure, the magnification mark indicates 1 micron.

FIGURE 1

A "ghost cell" of *Mycobacterium* Jucho strain obtained by the treatment with streptomycin (1 mg./ml.). "Paired fibrous structure" is observable all over the cell envelope. Shadowed with chromium. × 66,000.

FIGURE 2

A ghost cell of *Mycobacterium lepraemurium* obtained from leproma of rat. The same kind of paired fibrous structure as seen in Fig. 1 is observable all over the cell envelope. Shadowed with chromium. × 39,000.

FIGURE 3

An extremely thin layer of cell wall of *Mycobacterium* Jucho strain obtained by KOH-alcohol treatment. Paired fibrous structure is entirely removed. Shadowed with chromium. × 39,000.

FIGURE 4

Cells of *Mycobacterium lepraemurium* obtained from leproma of rat. Paired fibrous structure is seen in the cells still filled with cytoplasm and small electron dense granules. Shadowed with chromium. × 36,000.

Figs. 1 and 3 are republished from pictures which appeared in the preliminary report written in Japanese. (K. Takeya, Fine structures of bacterial cell, *in* Symposium on recent studies in submicroscopic structures of the cell and their function, *Proc. 15th Gen. Assembly Japan Med. Congr.*, 1959, **1,** 100.)

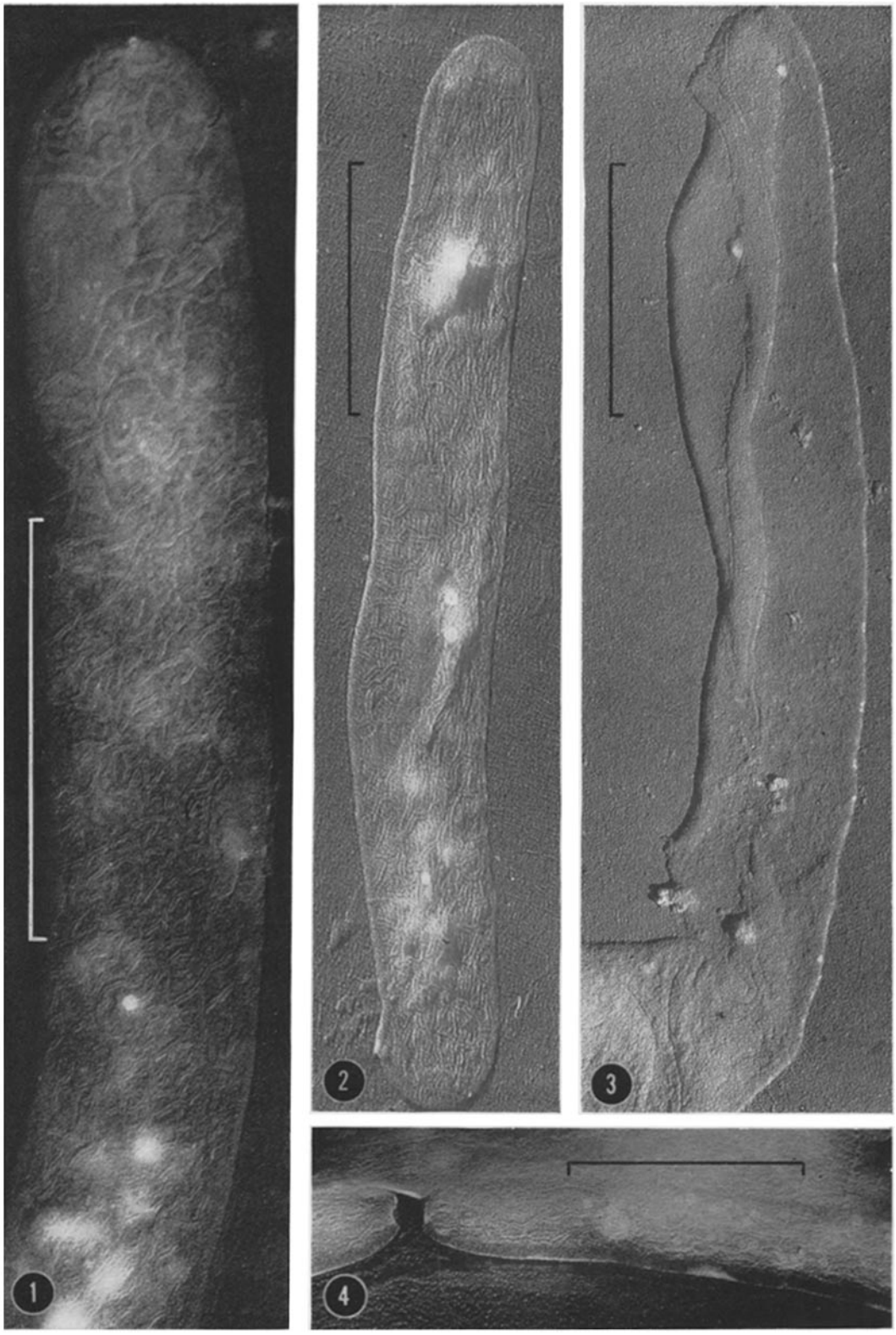
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FIGURE 5

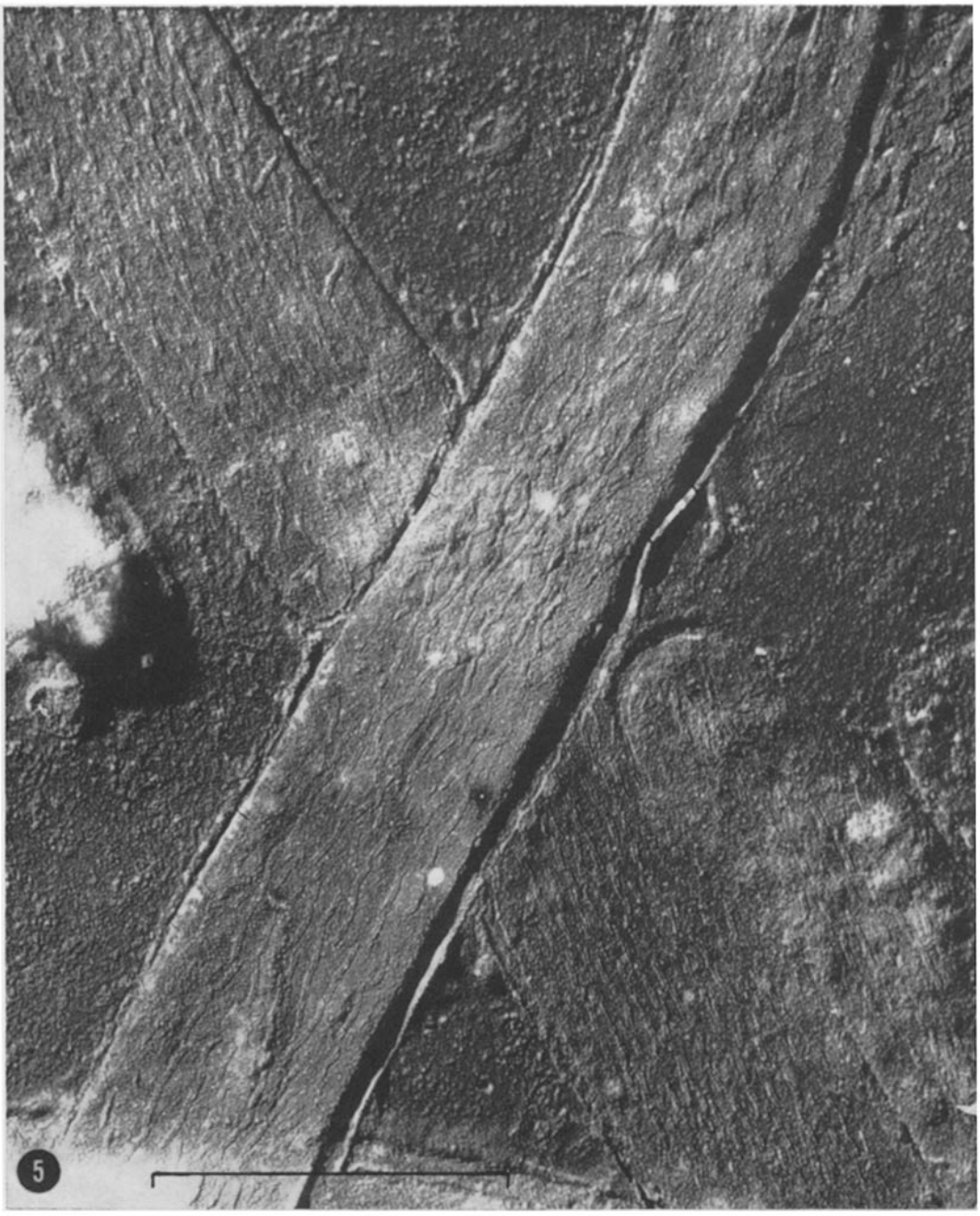
Ghost cells of *Mycobacterium* Jucho strain obtained by the treatment with mitomycin C (10 μg./ml.). Paired fibrous structure is observable all over the cell envelope of the ghost cells. Shadowed with chromium. × 52,000.

FIGURE 6

A ghost cell of *Mycobacterium* Jucho strain obtained by the treatment with streptomycin and, thereafter, treated with N/10 NaOH solution for 10 hours. Paired fibrous structure is still clearly visible. × 40,000.

FIGURE 7

A ghost cell of *Mycobacterium* Jucho strain obtained by the treatment with streptomycin and, thereafter, digested with trypsin for 3 hours. The treatment failed to alter the appearance of paired fibrous structure. × 45,000.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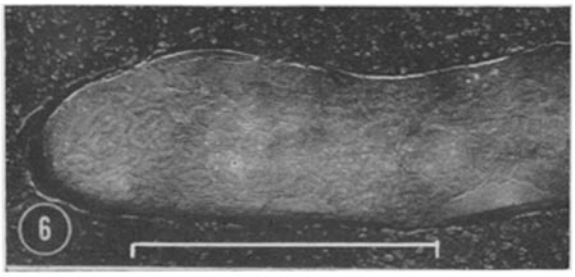
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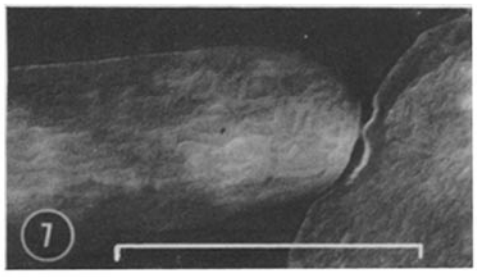
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