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Electron Microscopic Observations of Cell Division in *Mycobacterium leprae* by Means of Serial Ultrathin Sectioning [1]

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In recent studies ([1-8]) on the fine structures of leprosy bacilli in their human host, it has become evident that this bacillary form has much the same type of intracytoplasmic organelles as other bacterial cells. Even in tissues from leprous patients with untreated lepromatous leprosy, having a large number of the bacilli, dividing organisms are seldom seen. However, the morphology of *Mycobacterium leprae* from tissues of mice and their morphologic changes found at various stages in the cycle of division have been observed by electron microscopy by Edwards ([3]).

The observations reported here are concerned with the cytomorphologic studies on the cell division of *Mycobacterium leprae* in the human host cell at the electron microscopic level by means of serial ultrathin sectioning.

MATERIALS AND METHODS

Tissue specimens from leprosy patients with severe lepromatous leprosy were examined by skin biopsies. The materials were fixed by immersion in osmium tetroxide buffered to pH 6.4-6.6, as used by Kellenberger *et al* ([9]), dehydrated in graded alcohols, embedded in methacrylate resins and sectioned. The ultrathin sections were processed serially on a LKB-ultratome. The material was examined at 75 kV on a Hitachi-500 electron microscope.

RESULTS

In an attempt to elucidate the structure of the cell division of leprosy bacilli, a careful electron microscopic observation of these materials was carried out. A few dividing bacilli were observed. When the division was complete and an electron transparent zone separated the newly formed cells, the new electron-dense cell wall layer was found between the zone and the original cell wall (Figs. 1, 2a, 2b, 3a). This structure seems to show the final stage of septum formation.

Outside of the cell wall there was a mist-like layer similar to the halo (a kind of capsule) described by Terada ([10]) (Figs. 1, 2a, 2b, 3a, 3b, 4). The layer was preserved in the final stage of septum formation, surrounding the whole leprosy bacillary cell. The cytoplasmic membrane against the cell wall of newly formed cells was smoother, continuously uniformly dense and well-defined. The unit membranous structure was intimately connected with the cytoplasm (Figs. 1, 2a, 2b, 3a). An intracellular membranous organelle (mesosome) was seen in the cytoplasm of bacilli and associated with the newly formed cytoplasmic membrane, with which it was in contact (Figs. 3a, 3b).

Intracellular inclusion-granules were not observed in bacillary cytoplasm. Both the cytoplasmic membrane and mesosome appear to form a diaphragm-like membrane, that is a cytoplasmic bridge, coming into contact with the new cell wall (Figs. 1, 2a, 2b, 3a, 3b).

DISCUSSION

The fine structure of *Mycobacterium leprae* has been reported by some workers ([1-5, 10]), and Imaeda *et al* ([6-8]), who studied the structure as compared with that of other mycobacteria. Membranous systems, including the cytoplasmic membrane, and other intracellular organelles of *Mycobacterium leprae* do not differ much from those of *Mycobacterium lepraemurium* ([4, 5]).

In human material the cell division of *Mycobacterium leprae* is not frequently observed. Edwards ([3]) reported the sequential changes occurring in bacillary division of *Mycobacterium leprae* from infected foot

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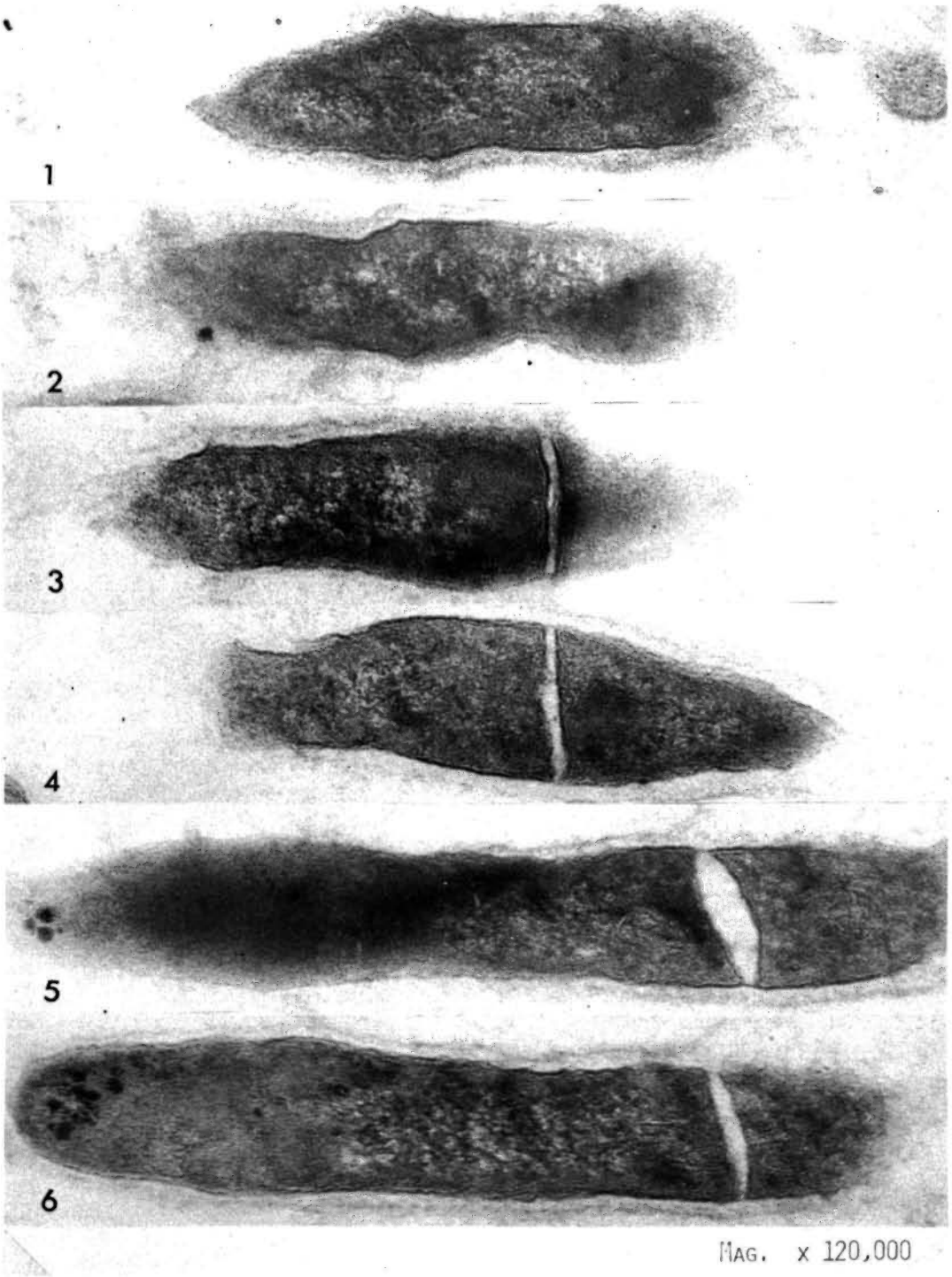

FIG. 1. Longitudinal serial thin sections. Photos 2-3 show the cytoplasmic bridge, and Photo 5 the electron transparent zone.

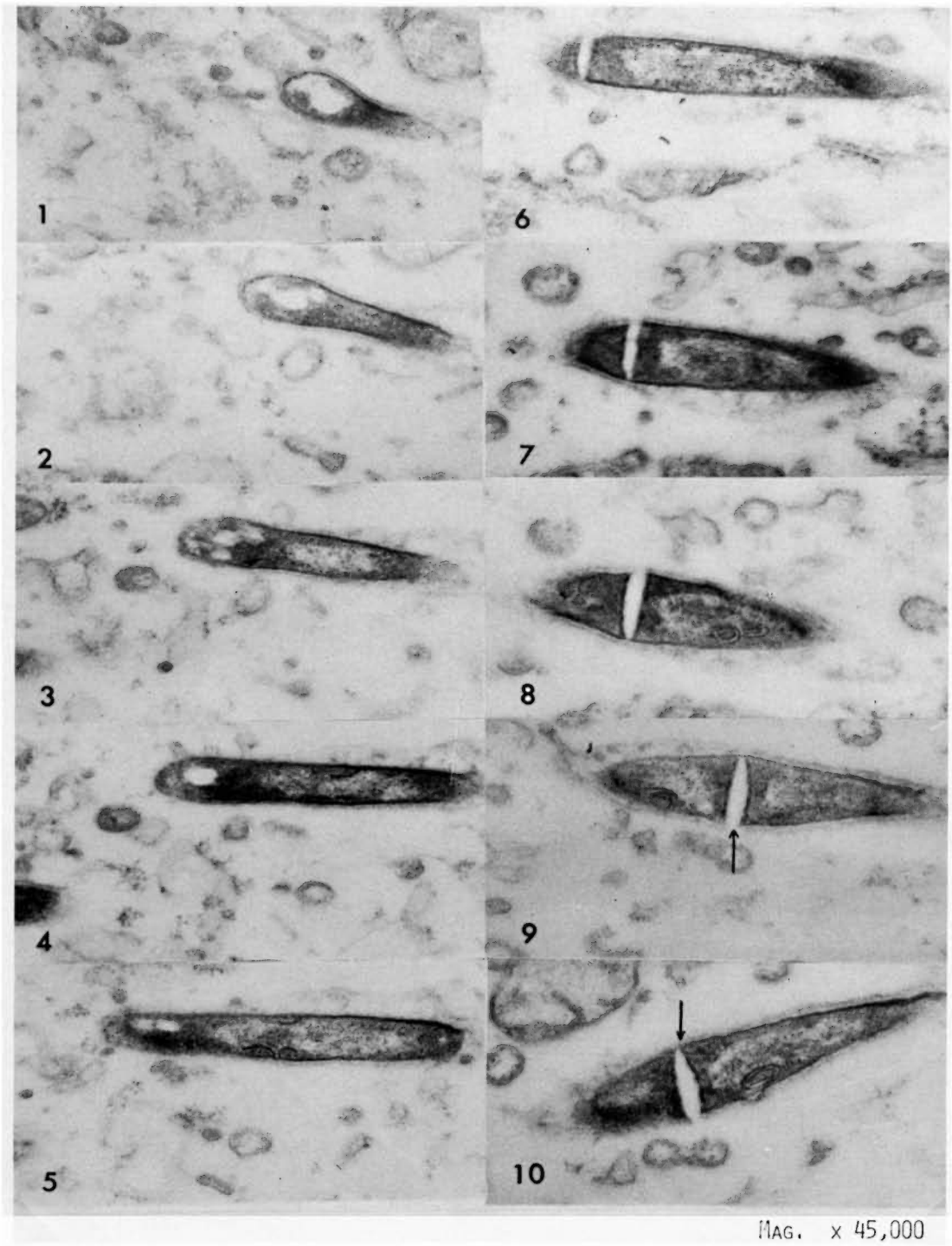

FIG. 2a. Longitudinal serial thin sections. Arrow in Photo 9 shows that the mist-like layer and the old cell wall have been broken, and Photo 10 shows that the mist-like layer has been continuous.

pads of mice. The division of *Mycobacterium leprae* seems to be completed by the growth of both the cell wall and the cytoplasmic membrane inward into the cytoplasm of the bacillary cell so as to form a septum. The septum formation was clearly observed (Figs. 1, 2a, 2b, 3a).

The intracellular membranous organelle (mesosome) was observed at or near the septum (Figs. 3a, 3b). This seems to imply that the organelle is formed as an invagination of the cytoplasmic membrane and plays a role in the process of cell division. Some membranous structures may be the site of synthesis of cell wall materials. This is implied because, as Edwards [3] suggested, the structures in some instances are found apparently in close proximity to the site of initiation of division and the cell wall of newly formed cells, although real biological functions of the membranous system in leprosy bacilli remain obscure.

The interrelation between the cell wall, the cytoplasmic membrane and the intracellular membranous organelle (mesosome) seems to be shown definitely in serial sections (Figs. 1, 2a, 2b, 3a). Many different structures of leprosy bacilli are observed, especially in lepromatous leprosy cases, and many kinds of inclusion-granules in the cytoplasm of the bacilli are found as being generally known. These granules are also found in leprosy bacilli in the different phases of multiplication [4-6]. There are, however, no ideas as to how to answer the question of whether or not these granules have biological activities in leprosy bacilli because no cultures have been obtained. However few inclusion-granules were observed in dividing bacillary cells.

SUMMARY

The division of *Mycobacterium leprae* in human skin was studied in the ultrathin sections at the electron microscopic level. A few dividing bacilli were observed. The division seemed to be accomplished by inward

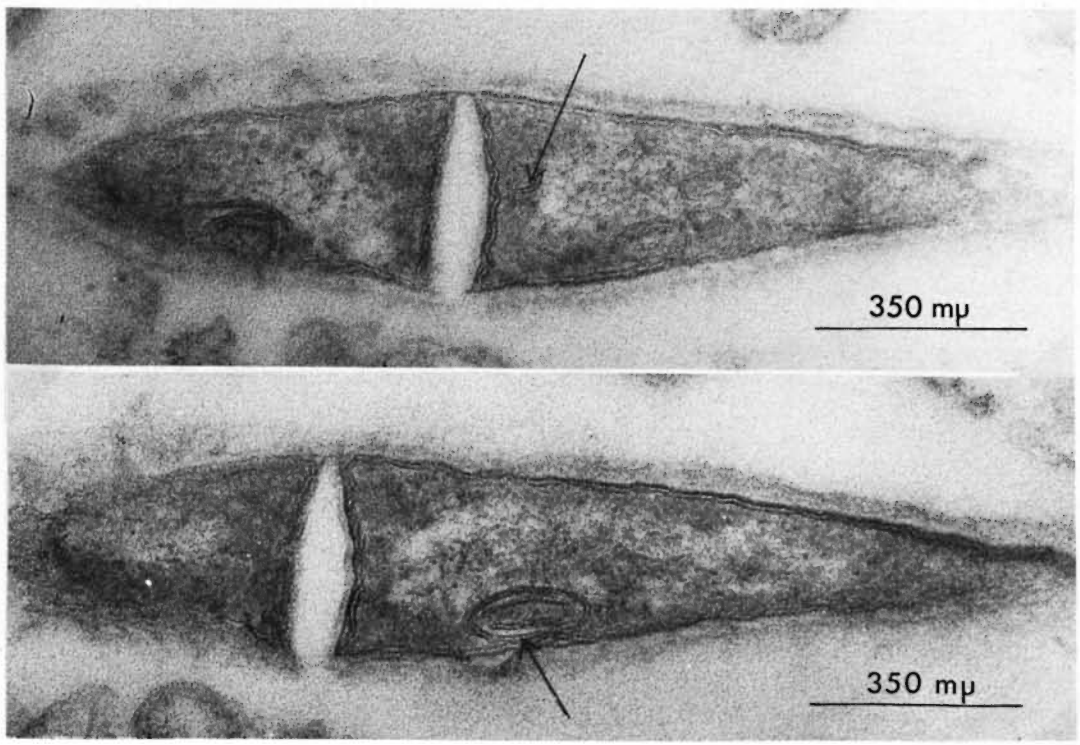

FIG. 2b. High magnification of Figure 2a, Photos 9 and 10. The mist-like layer outside of the cell wall is apparent. The arrow shows the intracytoplasmic membranous organelle.

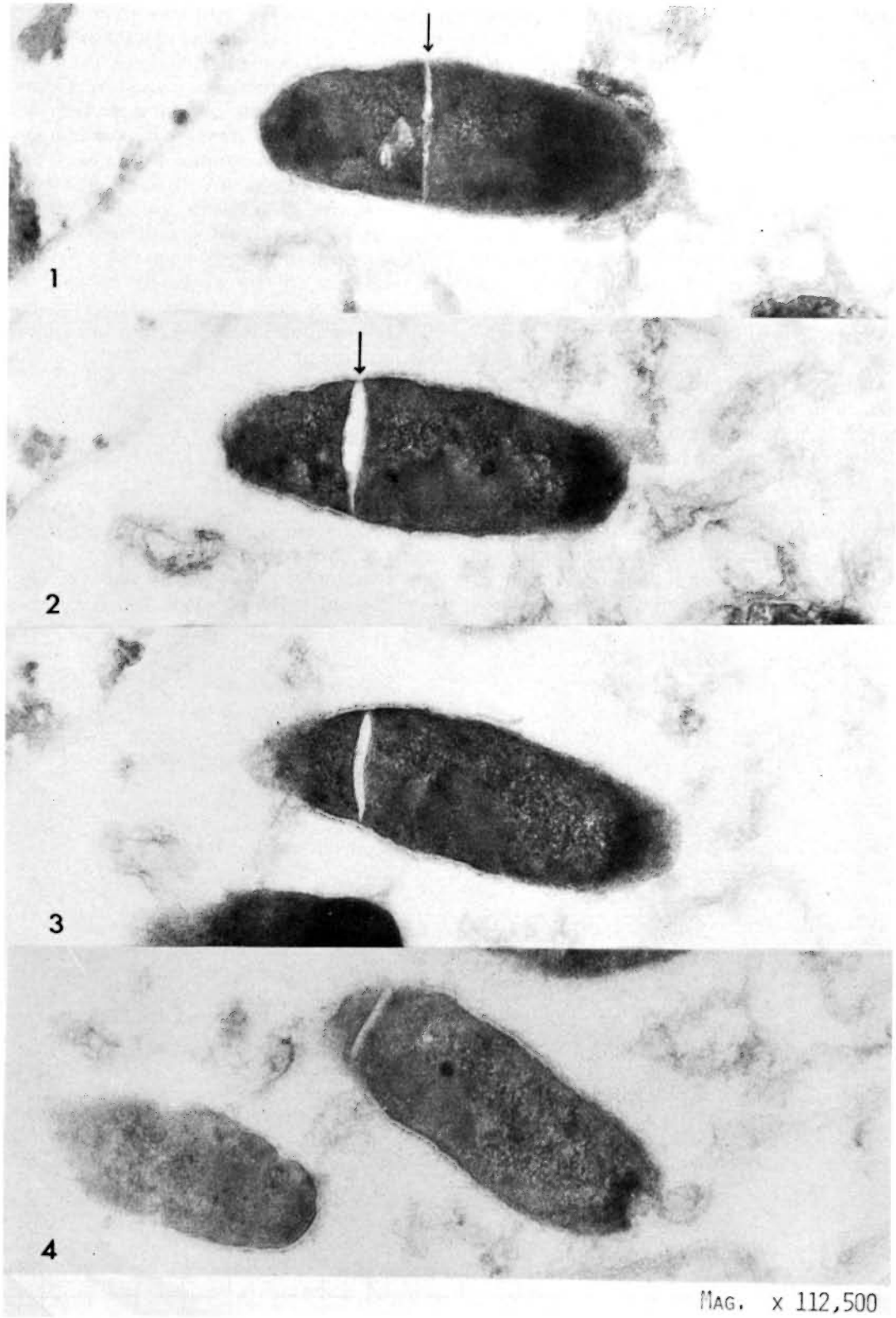

FIG. 3a. Longitudinal serial thin sections. The arrows in Photos 1-2 show partial cell division.

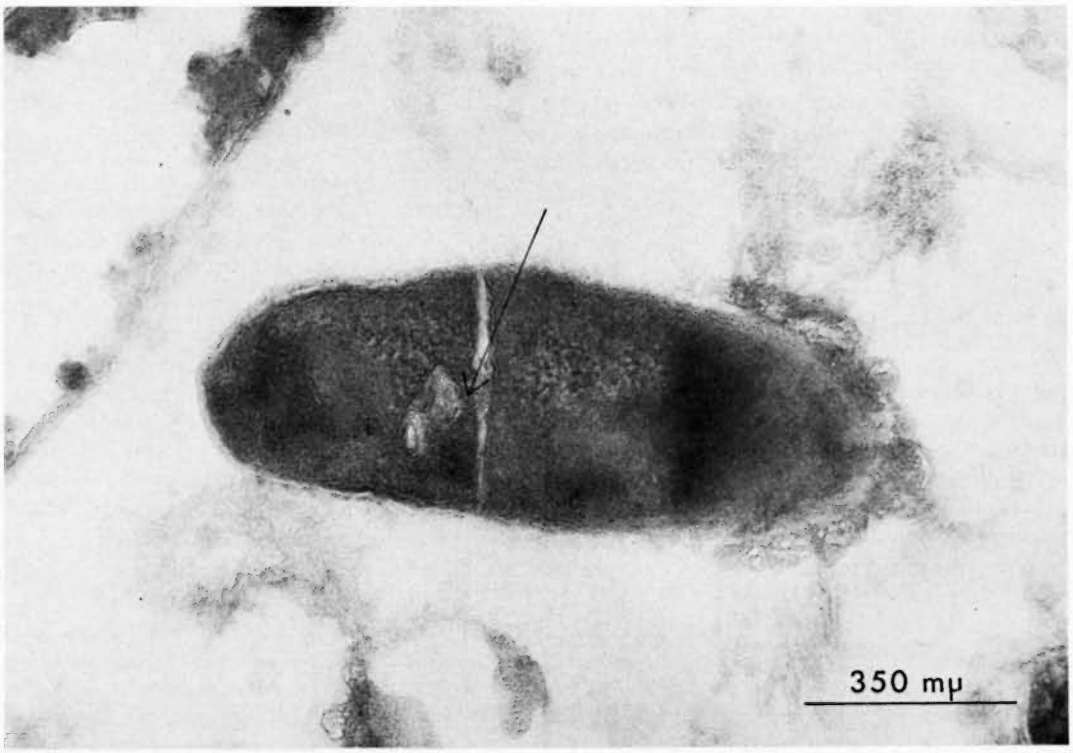

FIG. 3b. High magnification of Figure 3a, Photo 1. The intracellular membranous organelle associated with the new cytoplasmic membrane.

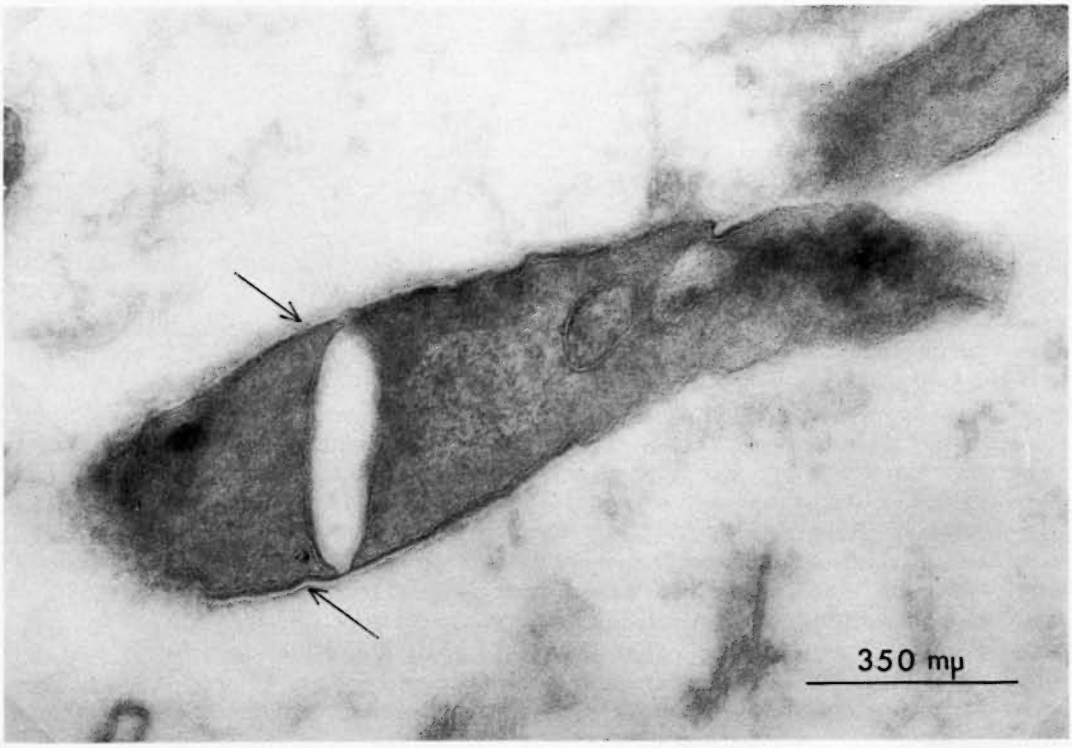

FIG. 4. The mist-like layer outside of the cell wall was preserved in the final stage of septum formation (arrow), surrounding the whole leprosy bacillary cell.

extension of both the cell wall and the cytoplasmic membrane into the cytoplasm of the bacillary cell to form a septum. The intracellular membranous organelle (mesosome) is assumed to play a role in division.

RESUMEN

Se estudió el proceso de división del *Mycobacterium leprae* en la piel humana por medio del microscopio electrónico en cortes ultradelgados. Se observaron unos pocos bacilos en división. La división pareció ocurrir por extensión, tanto de la paréd celular como de la membrana citoplásmica, hacia el interior del citoplasma hasta formar un septo. Suponemos que el organelo membranoso intracelular (mesosoma) participa en el proceso de división.

RÉSUMÉ

La division de *Mycobacterium leprae* dans la peau humaine a été étudiée dans des coupes ultra minces, à la microscopie électronique. Quelques bacilles en division ont été observés. Il a semblé que la division s'effectuait par extension à l'intérieur à la fois de la paroi cellulaire et de la membrane cytoplasmique, dans le cytoplasme de la cellule bacillaire, sous forme de septum. On présume que le mésosome, un organelle intracellulaire membraneux, joue un rôle dans la division.

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