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Electron Microscopic Observations of Intracytoplasmic Membrane Systems and Cell Division in *Mycobacterium lepraemurium*[1]

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Involutions of bacterial cytoplasmic membranes are commonly referred to as mesosomes ([18]). Fitz-James ([7]) described mesosomes as unique membranous structures in Gram-positive bacilli. The cell division process of many bacterial cells has been studied with the electron microscope. In most Gram-positive bacteria, a ring of cell wall material forms and grows inward to form a complete cross wall before division occurs ([9]). Perhaps the intracellular membranous organelles play a role in this process. Electron microscopy and thin sectioning techniques have been applied in the study of mycobacteria, especially in human ([3, 11, 12, 13, 14, 16]) and murine leprosy ([1, 5, 10, 19]). However, many aspects remain unknown regarding the interrelationships between mesosomes and cell division in *Mycobacterium lepraemurium*.

The morphology of *M. lepraemurium* from tissues of mice and their fine structures have been observed in the electron microscope. These findings are reported here together with a few observations on intracellular membranous organelles and cell division in the bacteria.

MATERIALS AND METHODS

Infection of mice. *Mycobacterium lepraemurium*, Hawaiian strain, have been maintained in mice for more than 15 years in our institute by serial transmission at 5 to 6 month intervals. Female mice (18–20 gm) of the ddY strain were given intraperitoneal injections of 0.25 ml (containing approximately 10^6 acid-fast bacilli) of a partially purified suspension of *M. lepraemurium* prepared from the homogenized liver of a mouse infected 4–6 months previously. In the present studies, the mice were sacrificed 6 months after infection.

Preparation of tissues for electron microscopy. Mice were killed with ether and pieces of liver were immediately cut into 1 mm cubes and fixed by immersion in osmium tetraoxide buffered to pH 6.4–6.6, according to the technique of Kellenberger, *et al.* ([15]). The tissues were dehydrated in graded alcohols and embedded in methacrylate resins. The ultrathin sections were processed serially on a LKB-ultratome and picked up on Formvar-covered grids. The material was examined on a JEOL-100C and/or a Hitachi-500 electron microscope operated at 50–75 kV.

RESULTS

The cytoplasm of murine leprosy bacilli contained complicated intracytoplasmic membrane systems, so-called mesosomes, as unique membranous structures which appeared as a result of invagination and a subsequent bifurcation of the cytoplasmic membrane. The mesosomes were positioned at or near the poles of the bacillary cell and seemed to protrude into the cytoplasm. In Figs. 1a, 1b, 2, 3, 4a, 4b, 4c, 4d, 4e, 4f, and 5, the appearances of the mesosomes are illustrated. They were observed in longitudinally sectioned cells as vesicular, tubular, and/or lamellar elements within a membrane envelope, which was connected to the cytoplasmic membrane. The expansion of the mesosome-neck from the cytoplasmic membrane is demonstrated in a series of seven serial sections (Fig. 2). The point of invagination can be clearly followed in these sections.

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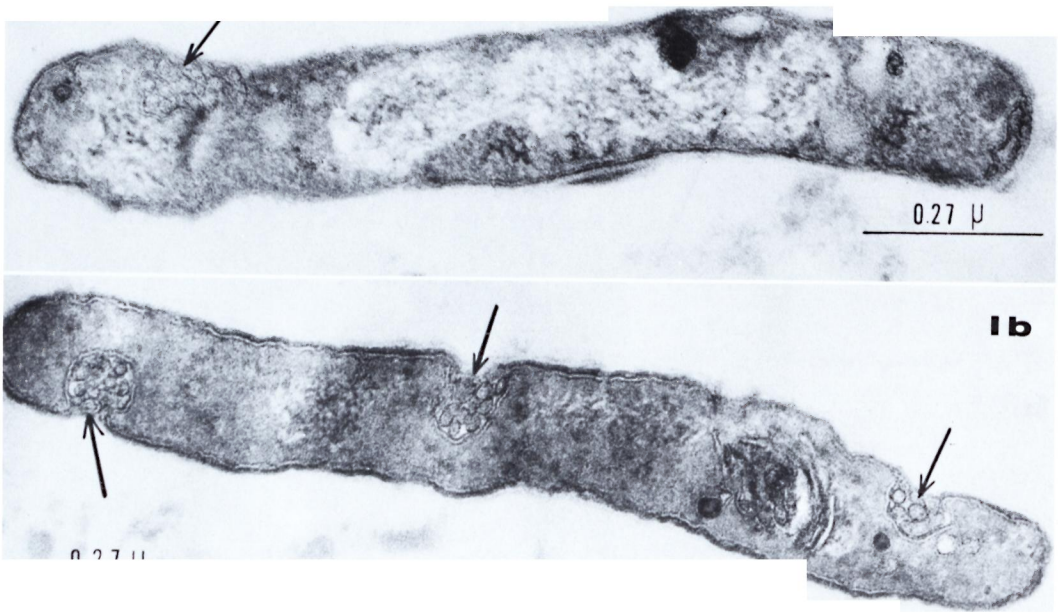


FIGS. 1a and 1b. Longitudinal thin sections. Arrows show the vesiculated type of mesosomes. Continuity of the cytoplasmic membrane with the mesosomes is apparent.

The mesosome depicted in Fig. 5 seems to be a simple form, occuring in a stage prior to the formation of the septum involved in cell division. The cytoplasmic membrane is pointing to form a slight concavity, and the septal wall, which is half completed, shows continuity with the cytoplasmic membrane. The initiation of septal wall formation, the construction of small vesicles from the pericytoplasmic space, is seen at both sides of the cell, and these take up a position facing cytoplasmic bridge formation. Just before the bridge reaches a subequatorial position, the development of a mesosome is clearly observed in serial sections of the organism (Figs. 6 and 7). The vertical position of this mesosome appears to be the tip of the new septal wall. This septal mesosome shows the clustered structure of laminar membranes and is in a characteristic location on the transverse septum. Figure 7 is the high magnification of Fig. 6, photos 3 and 4, and shows the ingrowth of the septal mesosome.

When the division was complete and an electron transparent zone separated two newly formed cells which were still connected to the mother cell wall, the new electron-dense cell wall layer was formed between the electron transparent zone and the original cell wall (Figs. 8, 9, 10a, 10b, 10c, and 10d).

DISCUSSION

Interconnections between mesosomes and cell division of *Mycobacterium lepraemurium* are described from the viewpoint of electron microscopy. The lateral extension and the centripetal growth of the septal wall seems to result from the original development of cytoplasmic membrane occurring at or near the leading edge of the nascent septum. Additionally, the association of a mesosome with the septum formation is evident morphologically (Figs. 2, 6, and 7).

Reavely and Burge [17] have recently refined the meaning of the term "mesosome" when discussing Gram-positive bacterial cells as "sac-like invaginations of the cytoplasmic membrane together with the vesicular, tubular, and lamellar membranous contents of these sacs." A recent review of the literature on mesosomes leaves no doubt

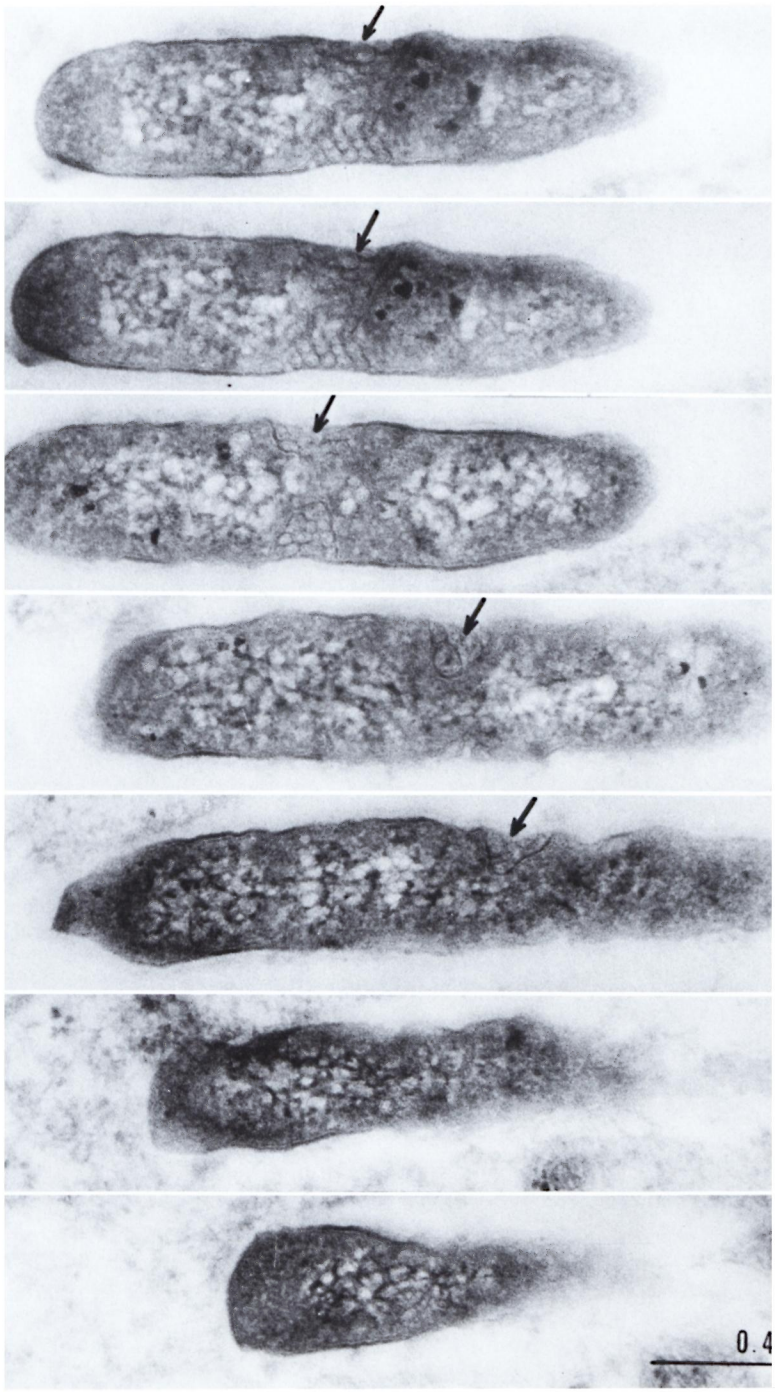


FIG. 2. Longitudinal serial thin sections. The expansion of the mesosome-neck from the cytoplasmic membrane is clearly demonstrated (arrows).

that these apparent organelles are in fact manifestations of the cytoplasmic membrane systems ([9]).

The fine structures of the mesosomes and septum in murine leprosy bacilli seem to be the same as those seen in human leprosy bacilli reported in earlier communications ([11, 12]). The mesosomal membrane systems

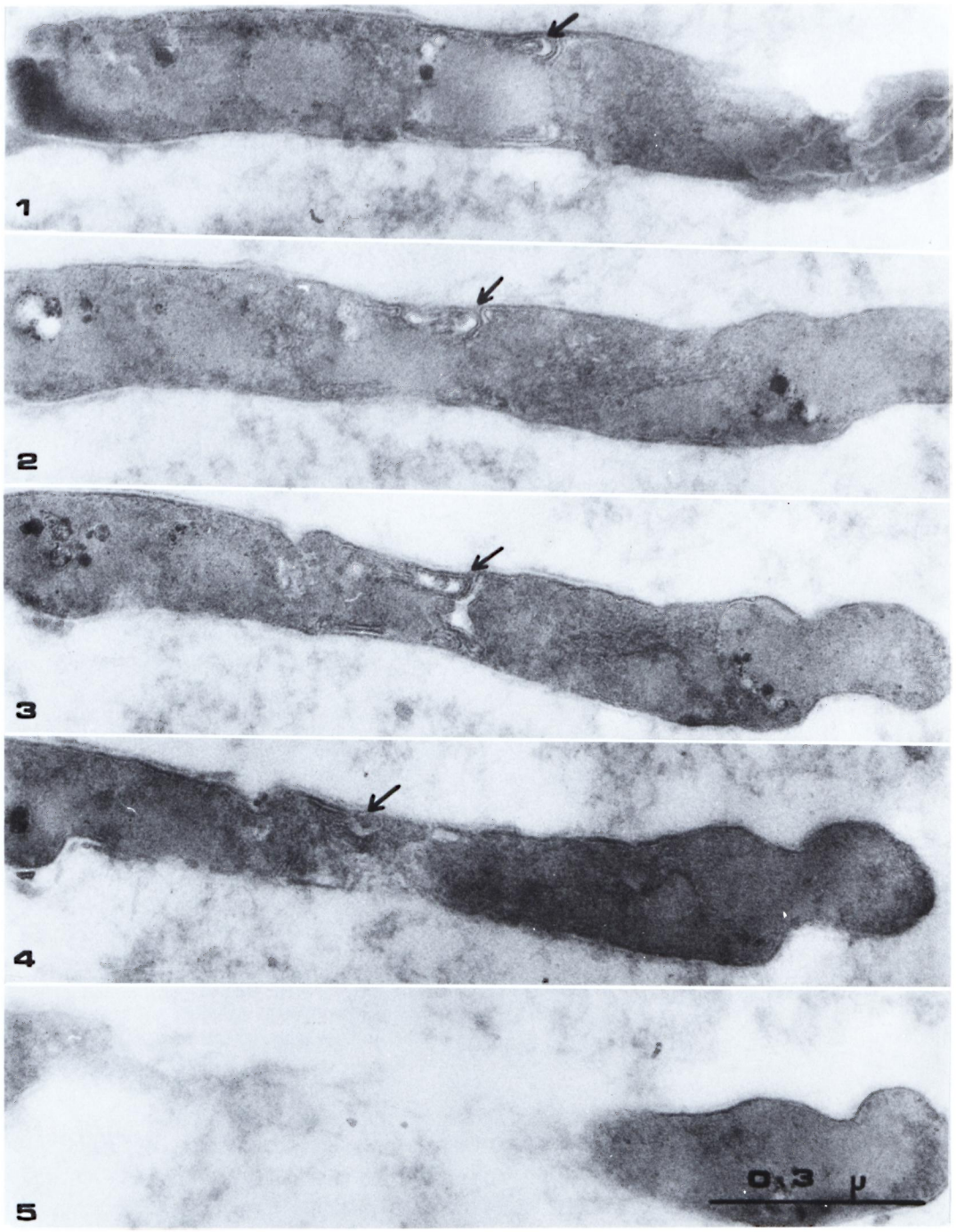
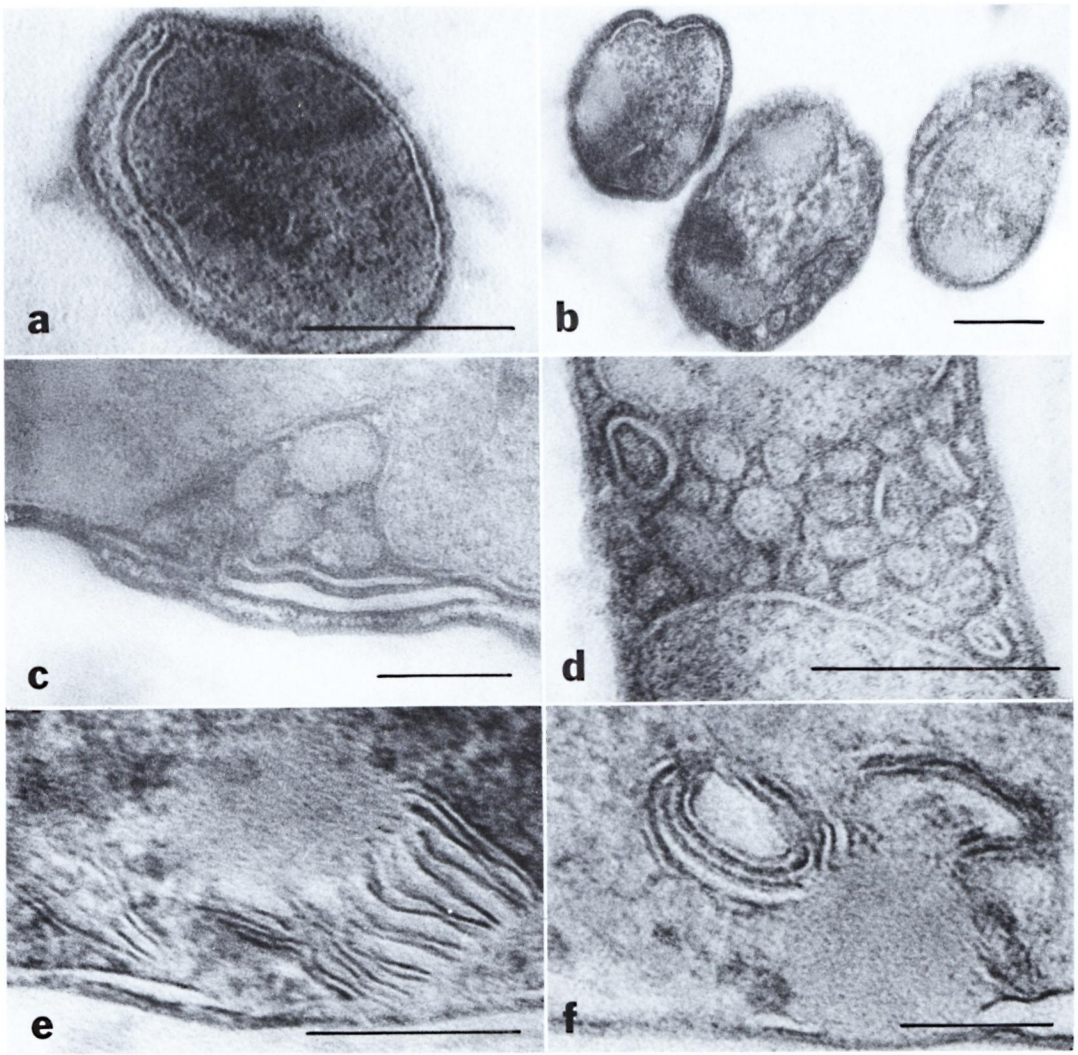


FIG. 3. Longitudinal serial thin sections. Arrows show the cytoplasmic membrane invagination.

are trilaminar, consisting of two electron-dense layers separated by an electron-transparent zone and having vesicular, tubular, and/or lamellar structures.

The type and perhaps even the location of the mesosome within the cell may vary depending upon the physiological state of the cell (Figs. 1a, 1b, 4a, 4b, 4c, 4d, 4e, 4f, and 6) [9]. Furthermore, tubulovesicular [8], lamellar-vesicular, and lamellar-tubular



FIGS. 4a, 4b, 4c, 4d, 4e, and 4f. A variety of intracytoplasmic membrane systems. Figs. 4a and 4b show thin cross sections. Figs. 4c, 4d, 4e, and 4f show longitudinal thin sections. Continuity of the cytoplasmic membrane with the intracytoplasmic membranous organelles is clearly distinguishable. Bars represent 0.1 μ in these photomicrographs.

types of mesosomes have been described ([4]). Mesosomes have also been differentiated according to their cellular location; thus septum mesosomes, peripheral or cytoplasmic membrane mesosomes, and even nuclear mesosomes have been reported ([9]). Mesosomes with diverse structures have been observed in mycobacteria, e.g., mesosomes within nucleoid in *M. tuberculosis* H_{37}Rv 102, mesosome-like structures in *M. smegmatis* 607, and others ([2, 19]).

In some of the photomicrographs (Figs. 1a, 1b, 2, 4a, 4b, 4c, and 4d), mesosomal tubes of murine leprosy bacilli can be seen to contact the cytoplasmic membrane. These probably are the sites where future septa will develop for the first cell division. In human material, bacteria that had reached the stage of completion of the new cell wall, but had not separated, were seen, but other stages were rare ([6, 11]). It may be possible to explain this finding as suggested by Edwards ([6]); i.e., although bacteria may divide as seldom as once in 2–3 weeks, the actual process of formation of the cell wall probably occurs relatively rapidly. The

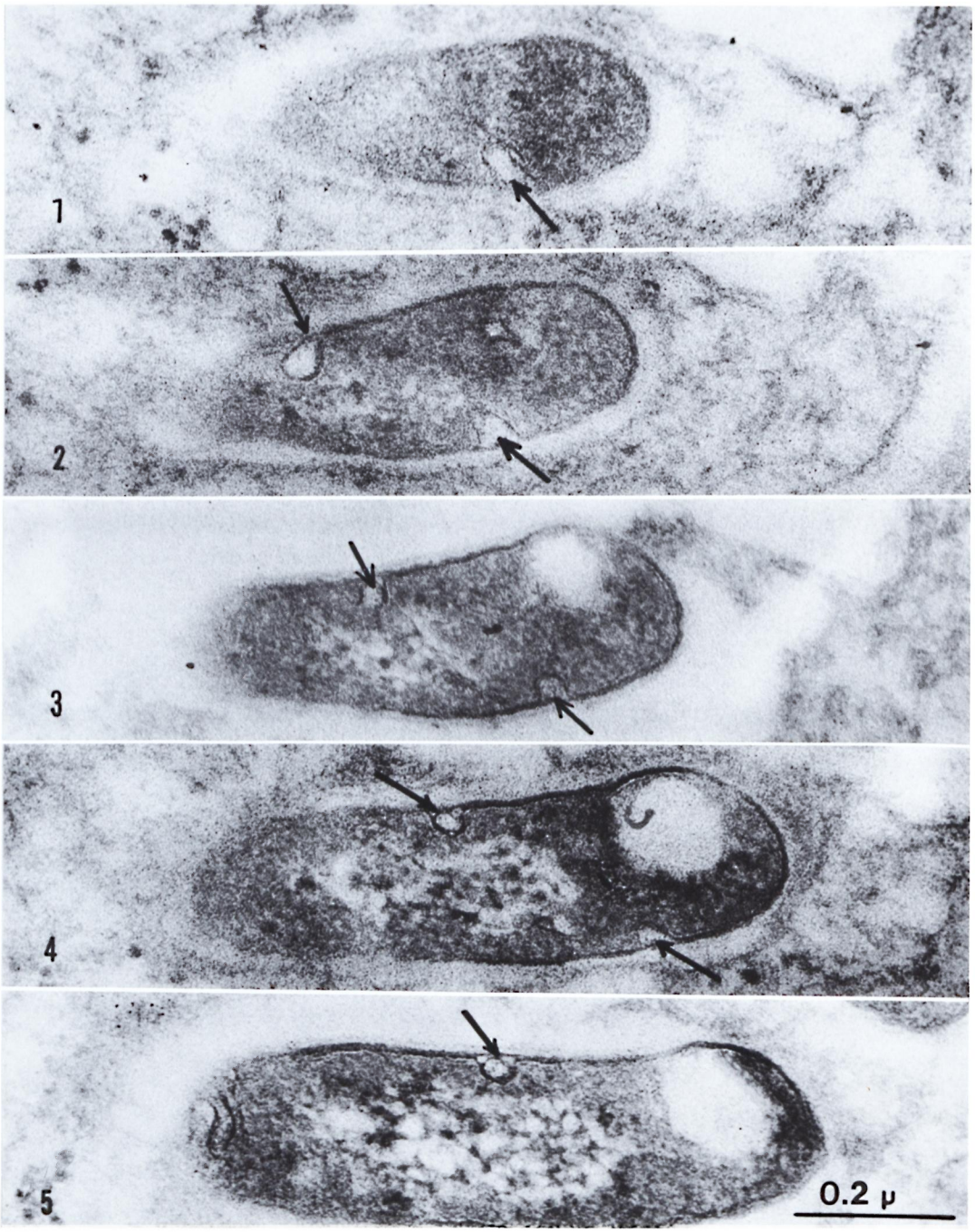


FIG. 5. Longitudinal serial thin sections. Arrows show the initial invagination of the cytoplasmic membrane.

morphological features of *M. lepraemurium* seem to be the same as those seen in *M. leprae* (Figs. 8, 9, 10a, 10b, 10c, and 10d).

Binary fission is a general and fundamental rule in the process of cell multiplication. However, the observable frequency of cell division in leprosy bacilli under the electron microscope is not yet sufficient to discuss the structure and function of the dividing process in any detail. Although any specific

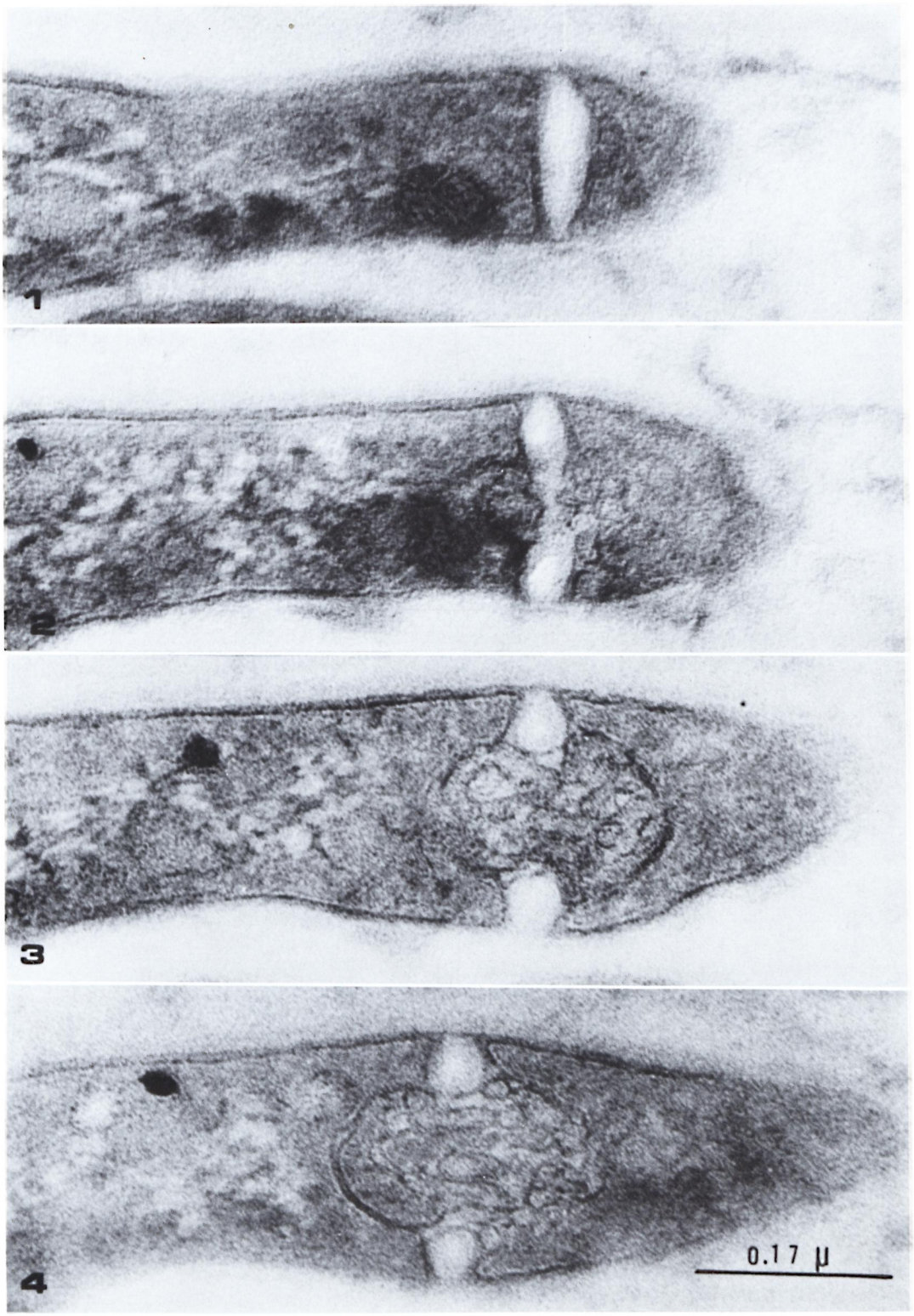


FIG. 6. Longitudinal serial thin sections. A crosswall halfway completed showing continuity with the cytoplasmic membrane and a large vesicular and/or tubular mesosome.

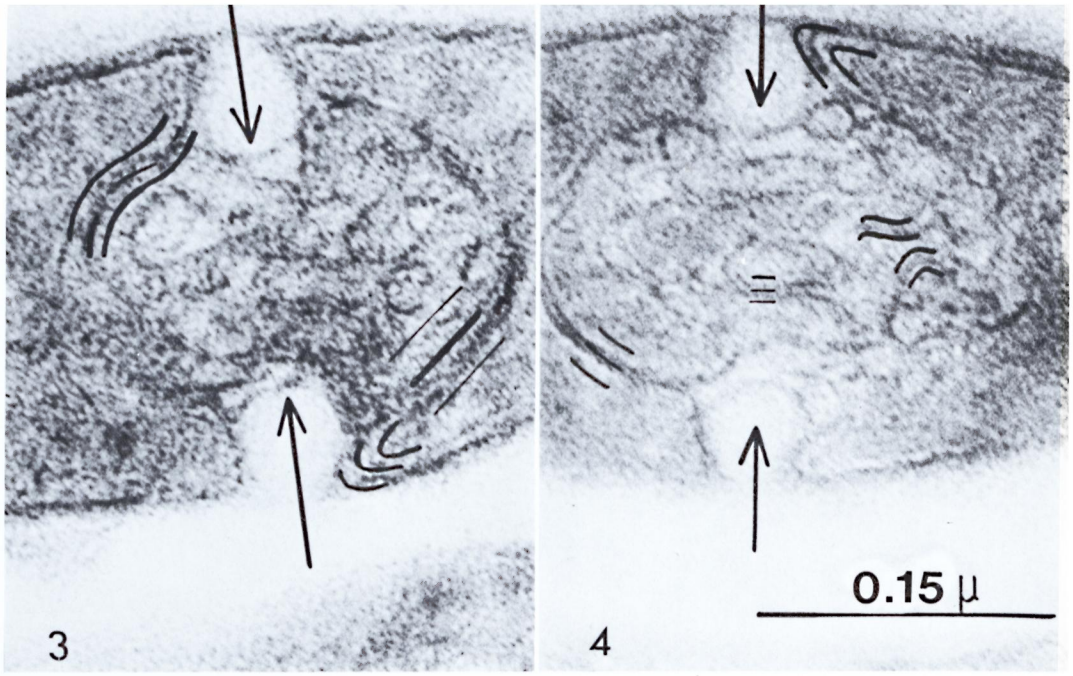


FIG. 7. High magnification of Fig. 6, Photos 3 and 4. The black lines show the continuous membranous structures.

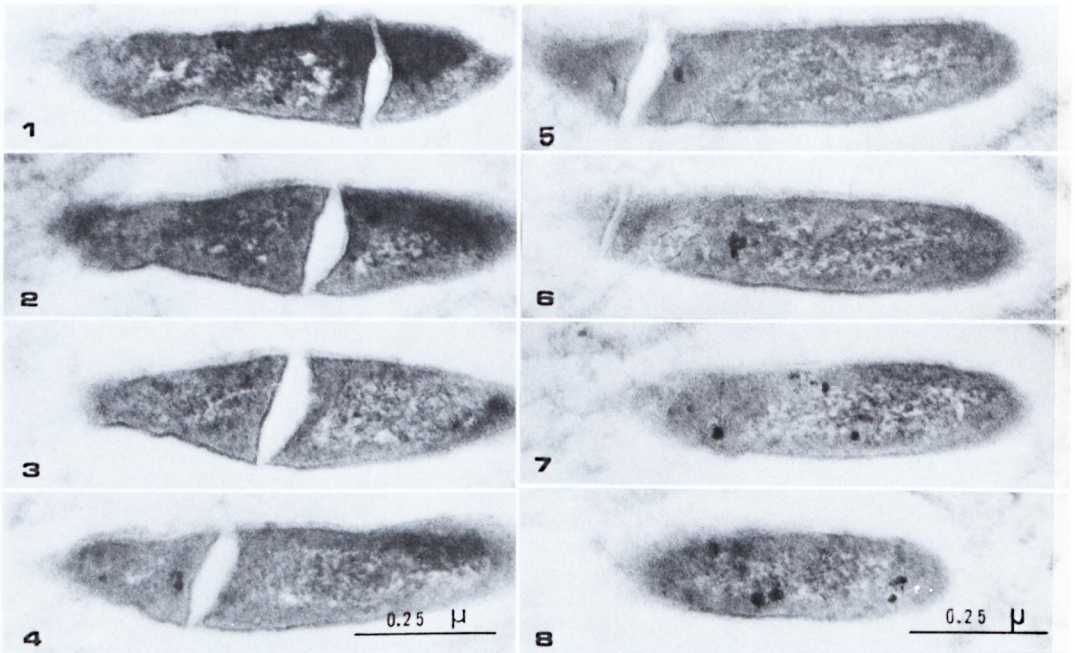


FIG. 8. Longitudinal serial thin sections. Septum formation is clearly observed.

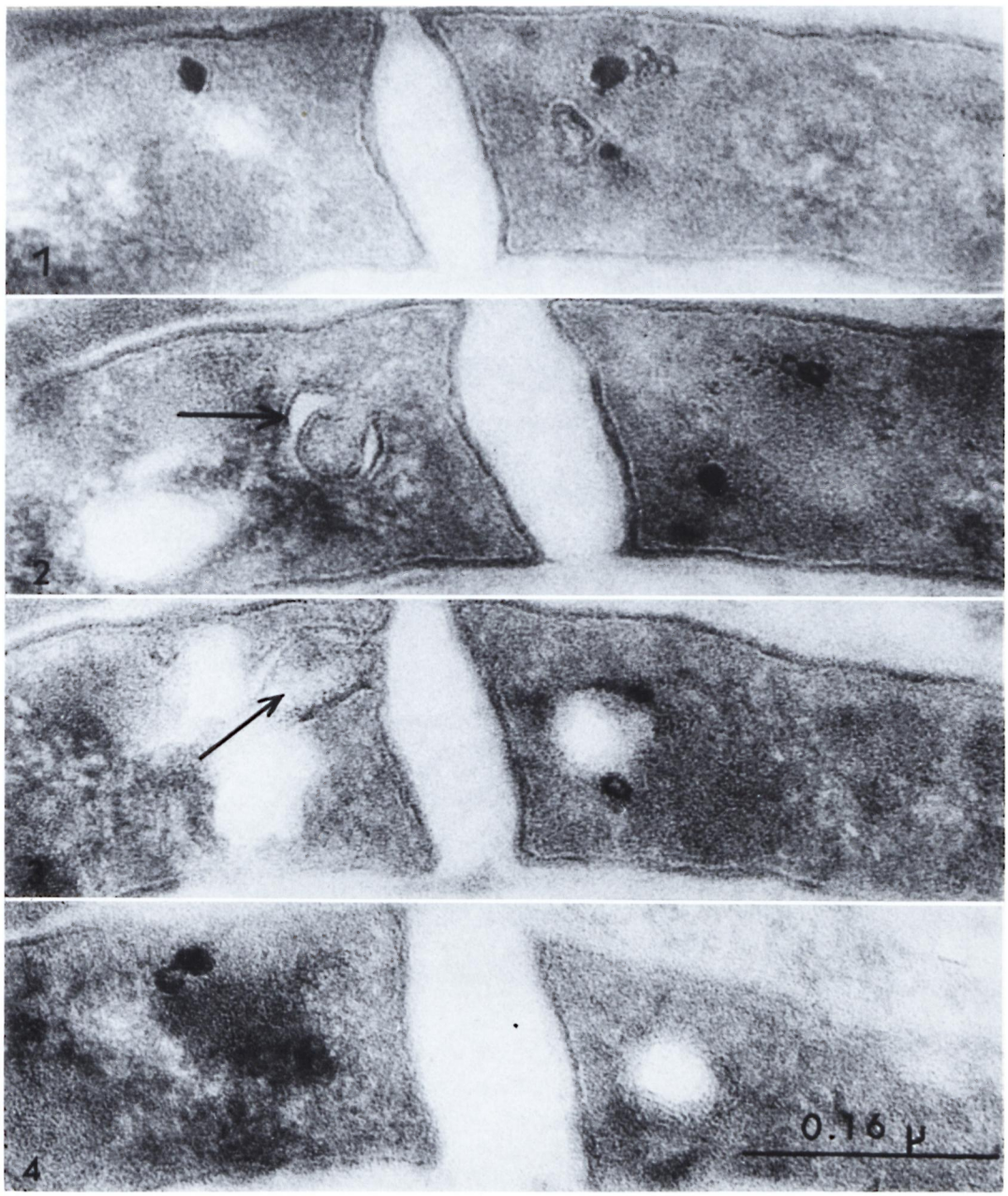


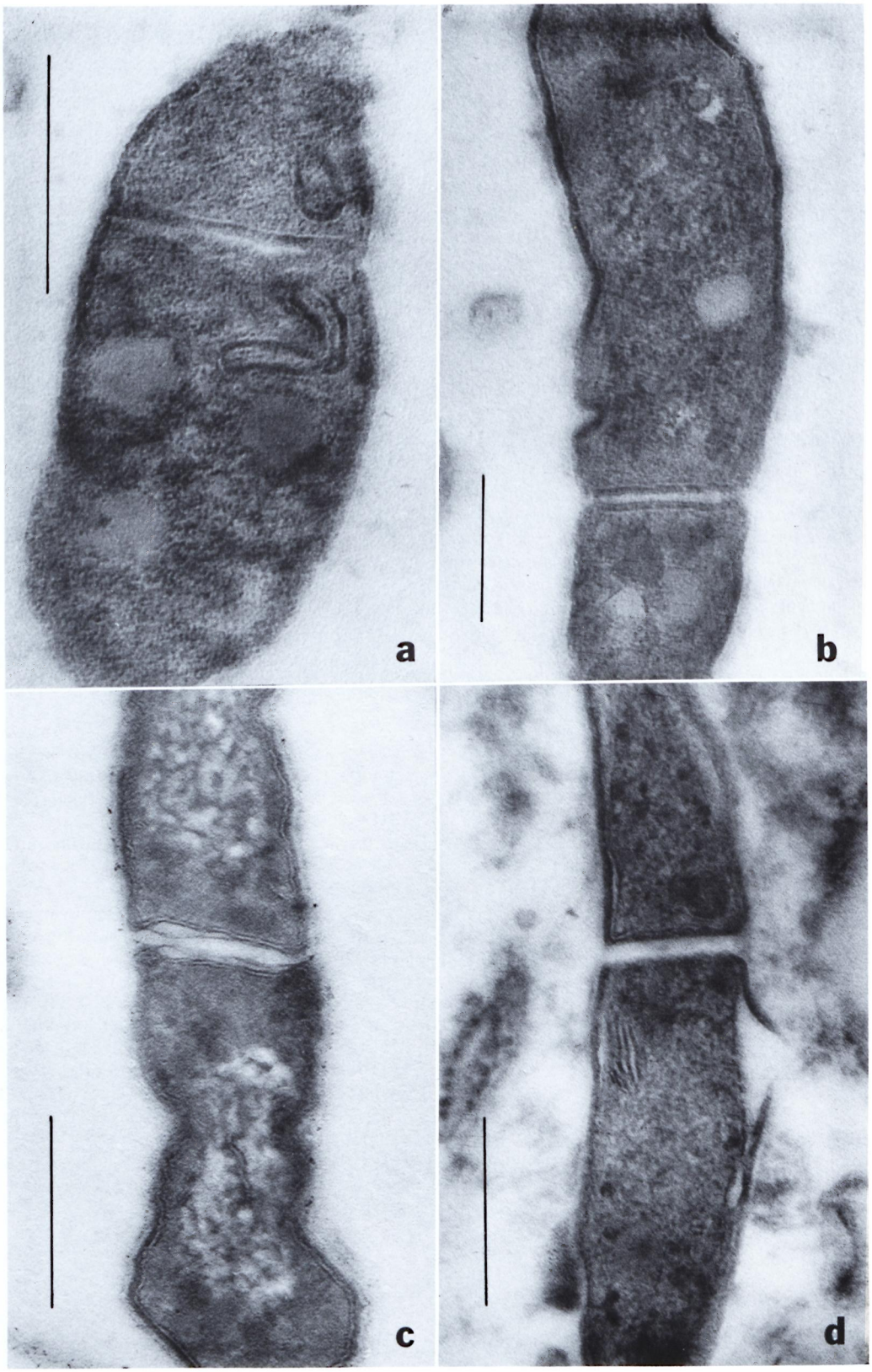
FIG. 9. Longitudinal serial thin sections. Arrows show the intracytoplasmic organelle.

mesosome function is yet to be ascertained, it should be noted that there are likely to be some functional correlations between mesosomes and cell division in murine leprosy bacilli.

SUMMARY

The fine structures and the interconnections between the intracytoplasmic membrane systems and cell division of murine leprosy bacilli in liver tissue from mice infected intraperitoneally with *Mycobacterium lepraemurium*, Hawaiian strain, were studied in ultrathin serial sections at the electron microscopic level.

Intracellular membranous organelles (mesosomes) were seen as vesicular, tubular and/or lamellar structures. The for-



FIGS. 10a, 10b, 10c, and 10d. Longitudinal thin sections. Completed stages of septal wall formation are observed. Bars represent 0.25 μ in these photomicrographs.

mation of mesosomes appeared to be initiated by invagination and/or folding of the cytoplasmic membrane.

A few dividing bacilli were observed. The lateral extension and the centripetal growth of the septal wall seemed to result from the original development of cytoplasmic membrane occurring at or near the leading edge of the nascent septum. After the septum formation was completely accomplished, the separation of two new daughter cells is assumed to occur. The mesosome was associated with the newly formed cytoplasmic membrane (septal wall).

It was shown that both the cytoplasmic membrane and the mesosome played an important part in septum formation.

RESUMEN

Se estudiaron, al nivel de la microscopía electrónica en cortes seriados ultradelgados, las estructuras finas y las interconexiones entre los diferentes sistemas de membranas intracitoplásmicos, y la relación de tales sistemas con el proceso de división celular del *Mycobacterium lepraemurium* (cepa Hawaii) encontrado en el tejido hepático de ratones inoculados intraperitonealmente con el bacilo de la lepra murina.

Los organelos membranosos intracelulares (mesosomas) se observaron como estructuras vesiculares, tubulares o laminares. La formación de los mesosomas pareció ser iniciada por invaginación o plegamiento de la membrana citoplásmica.

Se observaron algunos bacilos en división. En éstos, la extensión lateral y el crecimiento centrípeto de la paréd septal pareció derivar del desarrollo de la membrana citoplásmica ocurriendo en o cerca del borde anterior del septo naciente. Después que se completa la formación del septo, se asume que ocurre la separación de dos nuevas células hijas. El mesosoma estuvo asociado con la membrana citoplásmica recién formada (paréd septal).

Fue evidente que tanto la membrana citoplásmica como el mesosoma jugaron un papel muy importante en la formación del septo.

RÉSUMÉ

On a eu recours à des coupes, en série, ultraminces, de la qualité nécessaire pour la microscopie électronique pour étudier les structures fines et les interconnexions entre les systèmes de la membrane cytoplasmique, de même que la division cellulaire, chez des bacilles de lèpre murine séjournant dans les tissus hépatiques de souris infectées de manière intra-péritonéale par *Mycobacterium lepraemurium* de type hawaien.

Des constituants membranaires intra-cellulaires (mésosomes) ont été observés sous forme de structures vésiculaires et/ou lamellaires. La formation de mésosomes semble être déclenchée par l'invagination et/ou le plissement de la membrane cytoplasmique.

Quelques bacilles en division ont été mis en évidence. L'extension latérale, de même que la croissance centripéde de la paroi du septum, semblent être le résultat du développement original de la membrane cytoplasmique, qui survient au niveau de la tranche frontale du septum en formation, ou à proximité. Lorsque la formation du septum est entièrement achevée, la séparation des deux cellules-filles est supposée survenir. Le mésosome est associé avec la membrane cytoplasmique nouvellement formée (paroi du septum).

Il est clair que la membrane cytoplasmique, de même que le mésosome, jouent tous deux un rôle fort important dans la formation du septum.

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