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# Electron Microscopic Observations of Intracytoplasmic Membranous Structures in *Mycobacterium leprae* by Means of Serial Ultrathin Sectioning [1]

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Through studies on fine structures of the microorganisms, it has become evident that yeast or yeast-like fungal cells are eucaryotic, having differentiated intracellular organs similar to those present in higher organisms, and that bacterial cells belong to procaryotic cells because they are constructed with undifferentiated intracellular organs.

However, many aspects remain unknown regarding the characteristics of the intracellular membranous organelles of *Mycobacterium leprae,* though the intractyoplasmic fine structures are found in the bacilli from tissue samples of leprous patients ([1, 3, 4]).

The purpose of the study reported below is to describe the ultrastructural interrelations of intracytoplasmic membrane systems, that is, the cytoplasmic membrane and the intracellular membranous organelle (mesosome) in the cytoplasm of *Mycobacterium leprae.*

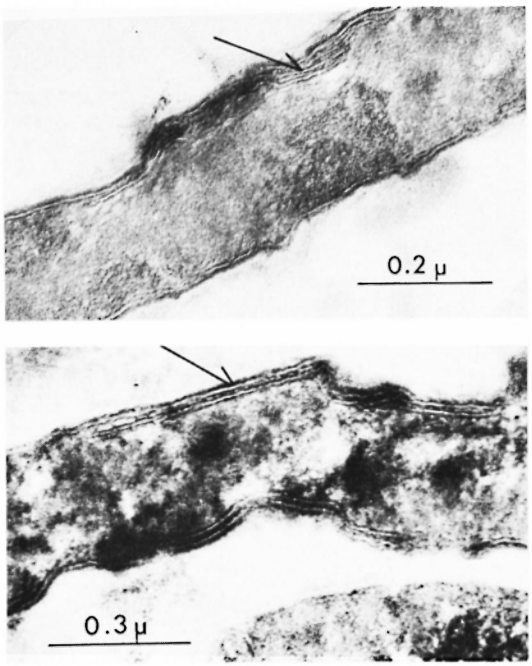
## MATERIALS AND METHODS

Tissue specimens from leprosy patients with lepromatous leprosy were examined in skin biopsies. The materials were fixed by immersion in osmium tetroxide buffered to pH 6.4-6.6, as used by Kellenberger *et al* ([5]) dehydrated in graded alcohol, embedded in methacrylate and/or styrene resins and sectioned. The ultrathin sections were processed serially on a LKB-Ultratome. The material was examined at 100 kV on a JEOL-100C and/or a Hitachi-500 electron microscope.

## RESULTS

Intracellular membranous organelles that conform to the definition of mesosomes in leprosy bacilli were seen as laminated structures and clusters of vesicles connected to the cytoplasmic membrane adjacent to the cell wall (Figs. 1a, 1b, 2a, 3, 5b, 6).

These membrane systems were trilaminar, consisting of two electrondense layers separated by an electron-transparent zone (Figs. 1a, 1b, 5b, 6). The trilaminar mesosomal membrane having laminated structures was closely associated with the trilaminar cytoplasmic membrane (Figs. 1a, 1b, 3, 6) and lay parallel to the bacillary cell envelope (Figs. 1a, 1b). The clusters of vesiculated membrane were positioned at or near the poles of the bacillary cells and were surrounded by the unit membrane; in cross section these appeared as vesicles of various sizes (Figs. 2a, 2b).




FIGS. 1a, 1b. Longitudinal thin sections. Arrows show the trilaminar membranous structures parallel to the bacillary cell envelope.

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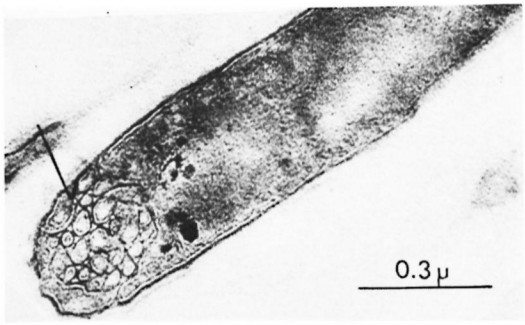


FIG. 2a. Longitudinal thin section. Arrow shows clusters of vesiculated membrane.

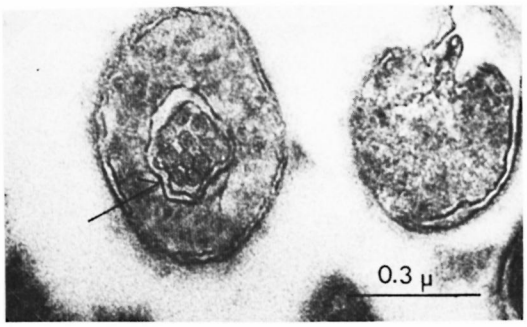


FIG. 2b. Thin cross sections. The clusters of vesiculated membrane are positioned at or near the pole of the bacillary cell and are surrounded by the unit membrane.

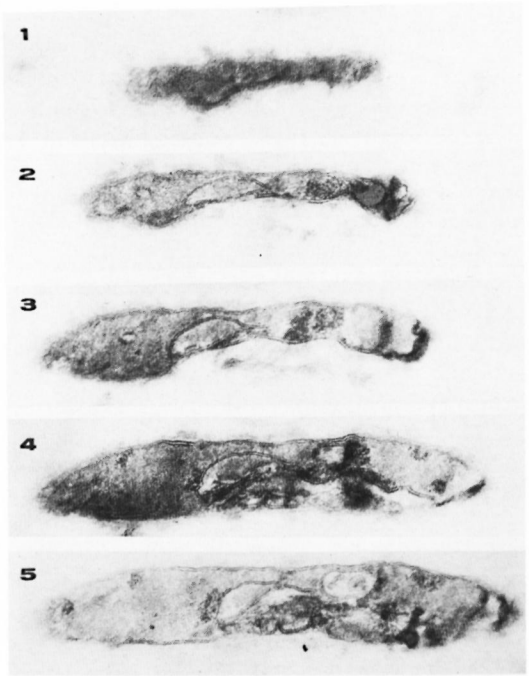


FIG. 3. Longitudinal serial thin sections. The trilaminar membrane structures are clearly visible.

The formation of mesosomes seemed to be initiated by invagination and/or folding of the cytoplasmic membrane (Fig. 6). The point of invagination and/or folding was clearly followed in serial sections (Figs. 4, 6), and seemed to become more prominent and to be adjacent to the cell wall. After that, the mesosome depicted in Figures 4, 5a and 5b appeared to show bizarre disruption of processes in a stage prior to septum formation as concerned with cell division.

The diverse morphology of the membrane sac and its internal tubular components, as well as the very limited area of attachment with the cytoplasmic membrane, was clear in serial series of micrographs. The origin of the tubules from the mesosomal sac was suggested in Figures 3, 4, 5b and 6. This was clearly shown in Figures 4 and 5b in which there are evident branching tubules as in-


FIG. 4. Longitudinal serial thin sections. The point of invagination and/or folding is clearly visible through these serial sections (see arrow in Photo 4).

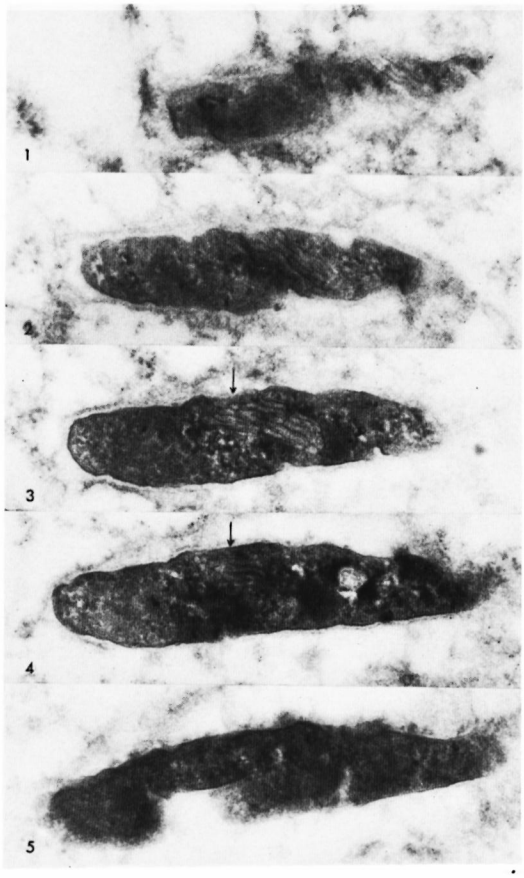

FIG. 5a. Longitudinal serial thin sections. The mesosome depicted in these serial sections shows bizarre structures.

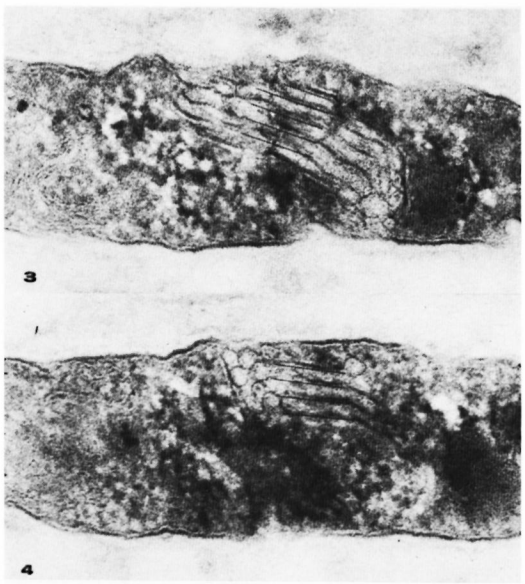

FIG. 5b. High magnification of Figure 5*a* Photos 3-4. The black lines show the laminat membranous structures.

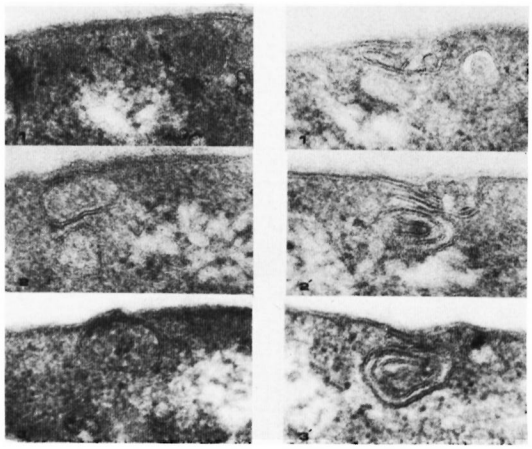

FIG. 6. A series of three sections demonstrating the expansion and distention of the mesosome.

vaginations of the mesosome sacs. Figure 6 is a series of three sections demonstrating the expansion and distention of the mesosome.

## DISCUSSION

In sections of *Mycobacterium leprae,* it sometimes seems difficult to say in what way the cell wall is different from the cytoplasmic membrane in thickness and sometimes a thin differentiated layer can be detected immediately within the cell wall. Edwards (1) suggested that the actual process of formation of the cell wall probably occurred relatively rapidly during division of *Mycobacterium leprae* and in some instances mesosomes were found apparently in close proximity to the site of initiation of division.

In spite of many reports dealing with bacterial electron-transport enzymes, some of which have been understood to be associated with the cytoplasmic membrane, it is uncertain whether the enzymes are really bound to the cytoplasmic membrane and the intractyoplasmic membrane in disintegrated bacterial cells.

Fuhs (2) discussed the functional interrelations between mesosome and nucleoid during the whole period of bacterial cell division. The interconnections between the cytoplasmic membrane, mesosomes and cytoplasm in leprosy bacilli seemed to be clearly defined from the serial sections (Figs. 3, 4, 5a, 5b, 6). The detailed analysis of serial sections of mesosomes has yielded informa-

tion other than that related to structure ([6, 7]). Although any specific mesosome function is yet to be ascertained, data suggest their participation in septum formation and synthesis of cell wall components during cell division of the leprosy bacilli since mesosomes are often seen at or near the point of cell division (Figs. 4, 5a, 5b). However, the ideal structure of cell division in *Mycobacterium leprae* was different, as noted in an earlier report ([3]).


## SUMMARY

The fine structures and interconnections between the cytoplasmic membrane and mesosomes of *Mycobacterium leprae* in human skin were studied in ultrathin sections. These intracellular membranous organelles were seen as laminated structures and as clusters of vesicles, which were trilaminar consisting of two electron-dense layers separated by an electron-transparent zone. The formation of mesosomes seems to be initiated by invagination and/or folding of the cytoplasmic membrane.

## RESUMEN

Se estudiaron, en cortes ultradelgados, la estructura fina y las interconexiones entre la membrana citoplásmica y los mesosomas del *Mycobacterium leprae* en la piel humana. Estos organelos membranosos intracelulares se observaron como estructuras laminadas y como racimos de vesículas, las cuales fueron trilaminares y consistentes de 2 capas electrodensas separadas por una zona transparente a los electrones. La formación de mesosomas parece ser iniciada por una invaginación y/o por un plegamiento de la membrana citoplásmica.

## RÉSUMÉ

Dans des coupes ultra-fines de peau humaine on a étudié les structures fines et les interconnexions entre la membrane cytoplasmique et les mésosomes de *Mycobacterium leprae*. Ces organelles membraneuses intra-cellulaires ont été observées sous forme de structures laminaires et d'amas de vésicules, trilaminaires et consistant de deux couches opaques aux électrons séparées par une zone transparente. La formation de mésosomes semble débuter par une invagination et/ou un plissement de la membrane cytoplasmique.


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