

Semi-and Ultra-thin Section Study of *Lepra Lepromatosa*Erhard Fasske¹

The first electron microscope research on skin biopsy material from patients with lepra lepromatosa was made by Brieger and Glauert (2). Since 1958 research groups from Kyoto University, Japan, and the Instituto Venezolano de Investigaciones Científicas and Ministry of Health and Social Assistance in Caracas, Venezuela (Imaeda *et al.* (3,4,5), Nishiura (6), and Yamamoto *et al.* (11,12) have been carrying out a complete study of the ultrastructure of leprosy cells and *M. leprae*. In the German literature we find a newly written review by G. Klingmüller (7) of his electron microscopic findings.

Although these micromorphologic research methods have clarified the structure of *M. leprae* and the reactions of phagocytosing cells, they have led to other problems. The position of a lepra cell in relation to surrounding tissues in an electron-microscopic investigation depends on the preparation. Examination of the fine tissue structure of fresh nerve lesions is very difficult, since preparations of the fine branches of cutaneous nerves for investigation of their ultrastructure are very hard to obtain.

It seems, purposeful, therefore, to combine the histologic investigations of light microscopy with the cytologic examinations of electron microscopy. This would allow a choice of individual cells from the same biopsy material. A method which has proved successful begins with a light microscope survey of stained semi-thin sections which were embedded in Epon or Maraglas. Sections worthy of further study can then be investigated by electron-microscopic examination of consecutive homomorphic ultra-thin sections from the same biopsy material.

MATERIAL AND METHODS

A combination of light-and electron-microscopic study of numerous skin biop-

sies from patients with lepra lepromatosa, borderline leprosy, and tuberculoid leprosy, with and without DDS treatment, was used. The biopsy material was provided through Prof. Dr. Bönicke from the Relief Center Bisidimo in Ethiopia. A more complete study of the findings of our electron-microscopic investigation will be reported later together with Dr. v. Bassewitz of Münster.

Immediately after cutting, the small tissue sections were fixed for 90 minutes in 3% buffered glutaraldehyde (phosphate buffer, pH 7.2), washed, and sent to us in buffer. After receipt of the preparations, they were fixed a second time in 2% osmium tetroxide solution for 2 hours, drained, and embedded in Maraglas 644 or Epon 812. For electron-microscopic investigation, the ultra-thin sections were contrasted with phosphotungstic acid and lead acetate. Semi-thin sections, of 1500-2000 Å thickness were stained for light-microscopic investigation in a mixture of 2% aqueous methylene blue solution and 1 N NaOH in a ratio of 2:1 at 20-30° C (Maraglas sections, 2-3 minutes; Epon sections 6 minutes). After rinsing, the sections were counterstained in saturated basic fuchsin solution at 80° C for 10-15 seconds, dried and covered. The color contrast for light-microscopic study is so distinct that cell and tissue structure, as well as mycobacteria, can be distinguished. After light-microscopic study of tissue structure, e.g., proof of leprosy cells, giant cells or little nerve trunks, the structures can be analyzed further through electron-microscopic study of consecutive ultra-thin sections.

RESULTS

The method will be demonstrated with two examples, viz., a study of a leprosy cell and of a Schwann cell. The selected tissue was taken from a skin biopsy of the right knee of a 22 year old Ethiopian with leprosy after one week of DDS treatment. Fig. 1a shows a light-microscopic photograph

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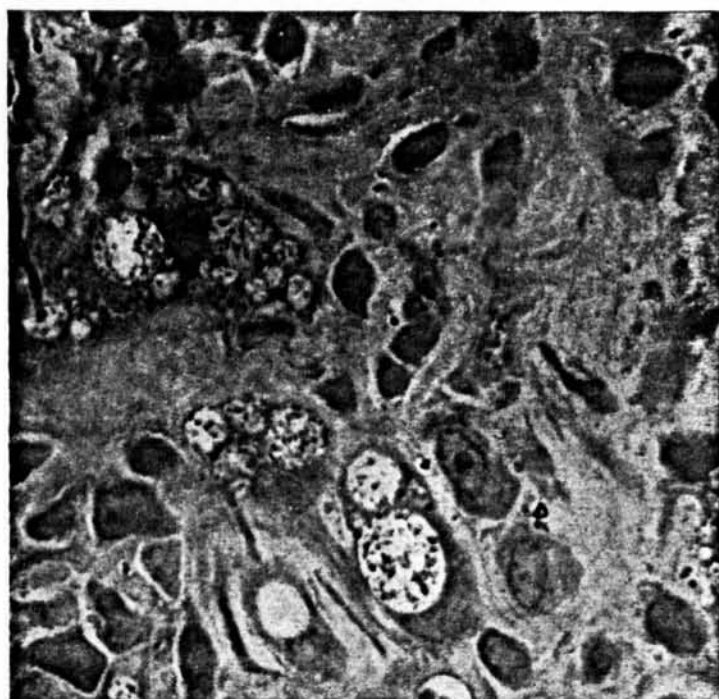


FIG. 1a. *Lepra lepromatosa*, skin biopsy. Semi-thin section of Epon-embedded material. Methylene blue-fuchsin staining. Oil immersion, x 1000. Leprosy cells with large globs. Mycobacteria rather shrivelled.

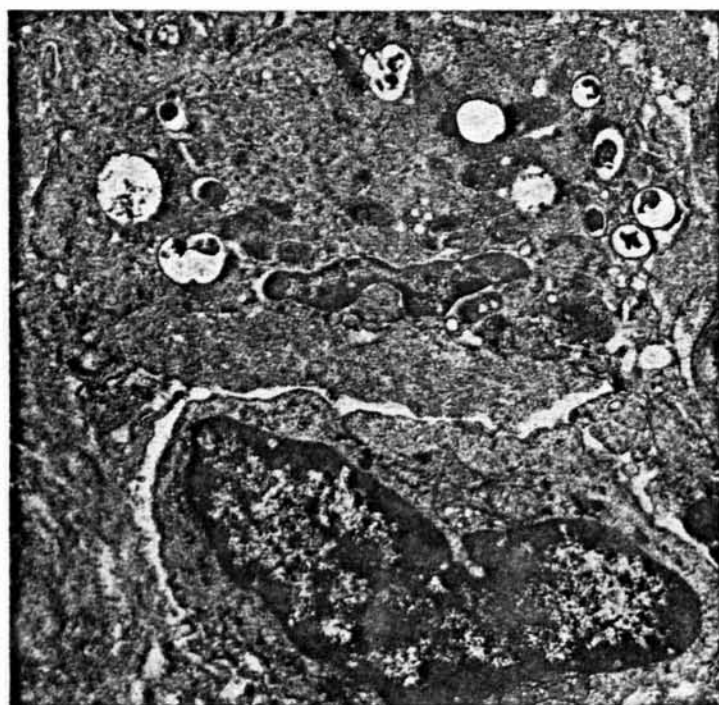


FIG. 1b. Low electron-microscopic magnification of an ultra-thin section from the same Epon block, x 8000. Above is a leprosy cell with mycobacteria inside an electron-transparent zone. Between the bacteria are large lysosomes.

(oil immersion, x 1000) of an Epon semi-thin section after methylene blue-fuchsin staining. The large and partially branched leprosy cells contain vacuoles or drops, in which numerous shrivelled and evidently degenerated mycobacteria can be recognized. These vacuole-like forms correspond to the globi. The bacteria lie in a transparent, non-stainable substance, which was earlier labelled as "Gloea" by Neisser. A low electron-microscopic magnification of a consecutive ultra-thin section (Fig. 1b) shows in the cytoplasm of a leprosy cell dense or desintegrating mycobacteria, which either lie in membrane-bound vacuoles or are surrounded by an electron-transparent zone [Nishiura (9)]. This zone probably consists of a substance formed from a metabolic product of the bacteria and a denatured cytoplasmic component of the host cell [Imaeda *et al.* (5)]. In untreated dimorphous leprosy (borderline leprosy) and in tuberculoid leprosy, such globi in the epithelioid cells do not occur [Imaeda (3)]. In lepra lepromato-

sa, evident relationships exist between the width of the electron-transparent zone and the extent of bacterial disintegration. Chemotherapy probably also influences this zone through its effect on the bacteria. In macrophages of leprosy, the number of mitochondria is smaller than in the metabolically active cells of tuberculoid leprosy [Yamamoto *et al.* (11)]. On the other hand, the cytoplasm of the leprosy cells in lepra lepromatosa contains numerous electron-dense forms of varying shape and size. These were termed "opaque drops" by Nishiura, and as "lysosomelike substance" by Brieger and Allen (1).

A higher electron microscopic magnification of this body (Fig. 1c) shows irregularly formed, often crescent-shaped small bodies, which lie adjacent to or fully enclose the mycobacteria. The bacteria are shrivelled or flake-like. In the small bodies symmetrically round, clear spots occur, which indicate either a material of less electron density—probably lipoids—or areas of dissolved material. The bodies show acid

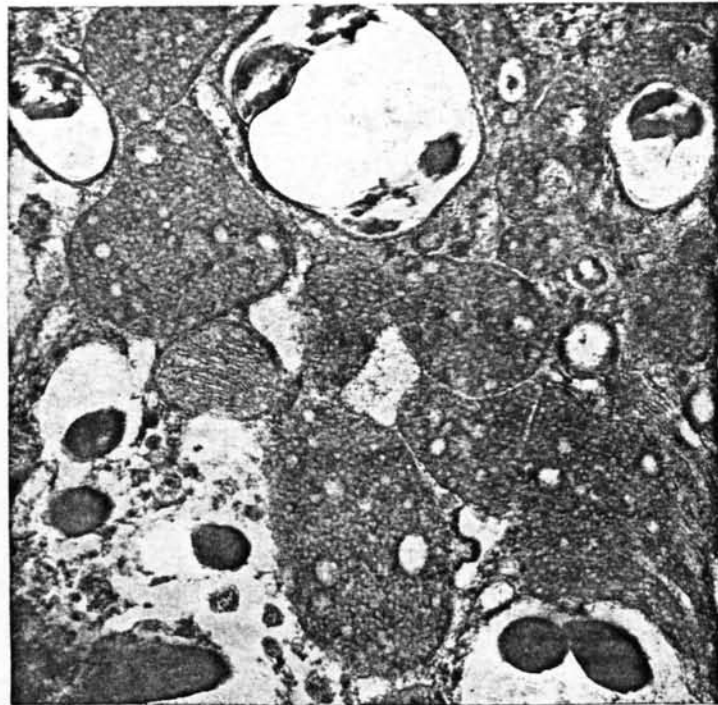


FIG. 1c. High electron-microscopic magnification of the same preparation, x 40,000. Between the mycobacteria is a large lysosome with foamy inner structure. Bacteria partially destroyed.

phosphate activity [Imaeda (³), G. Klingmüller (⁷)] and therefore represent lysosomes. Evidently the lysosome with its acid hydrolase partakes in the destruction of mycobacteria. Imaeda is of the opinion that this lysosome is found in increased and enlarged form under DDS therapy. The endproducts of lysosomal digestion are myelin bodies in the sense of the so-called "residual bodies." In these, mycobacteria are no longer recognizable through electron microscopy.

The structural relationship between host cells and mycobacteria can be divided electron-optically into five steps, according to the degree of cell reactions as follows:

1. Structurally intact mycobacteria in membrane-bound phagocytosis vesicles.
2. Mycobacteria lying free in the cytoplasm, evidently after membrane rupture.
3. Formation of globi. Inconspicuous or already degenerated mycobacteria inside an electron-transparent substance.
4. Degenerate mycobacteria inside lysosomes.
5. Residual bodies without bacterial structure.

The previous light-microscopic examination of semi-thin sections shows in addition special features, which can then be examined electron-microscopically. We were, therefore, able to demonstrate mycobacteria relatively often in the endothelial cells of the small cutaneous vessels of lepra lepromatosa. The combination method is especially useful in the location of the small cutaneous nerves. Fig. 2a is a stained semi-thin section showing branched, small marrow-containing nerve trunks of the skin of the knee region. In the Schwann cells and in the adjacent macrophages, the mycobacteria (globi) lie inside vacuoles. The electron-microscopic magnification (Fig. 2b) gives further information about the relationship between the cytoplasm of the Schwann cells and these bacteria. It can be seen that evidently intact bacteria are surrounded by an electron-transparent zone. The large window-like lysosome is lacking.

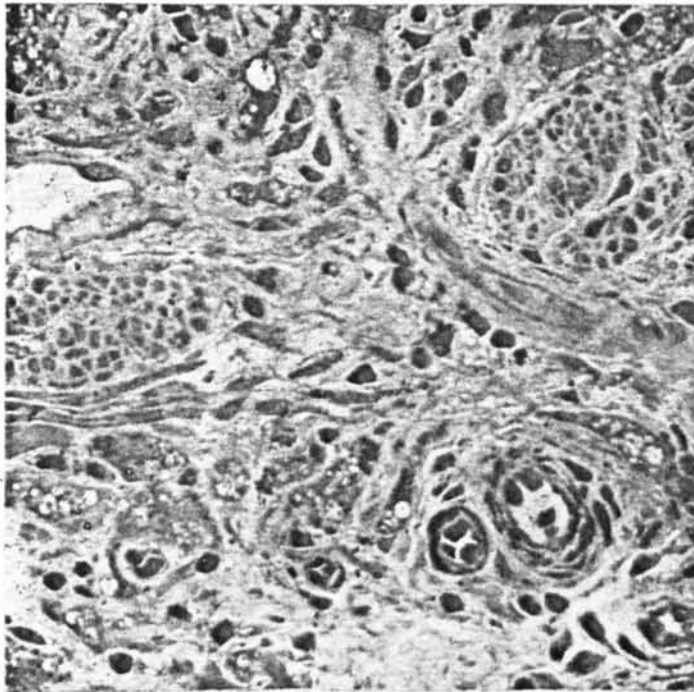


FIG. 2a. *Lepra lepromatosa*, skin biopsy. Semi-thin section of Epon-embedded material. Methylene blue-fuchsin staining, x 650. In subcutaneous tissue lie capillaries and marrow-containing nerve trunks. In Schwann cells and histiocytic macrophages lie globi with shrivelled mycobacteria.



FIG. 2b. Electron-microscopic photograph of ultra-thin section from the same Epon block. $\times 36,000$. Marrow-containing nerve trunk. In Schwann cells lie intact mycobacteria inside an electron-transparent zone.

Since, in the same biopsy piece, numerous lysosomes in the leprosy cells were demonstrated, it must be assumed that the Schwann cells have fewer or non-existent defense possibilities and therefore can be considered as a reservoir for leprosy bacteria [Kawamura and Shirasaki (⁶), Lumsden (⁸)]. Furthermore, the frequent insufficient therapeutic effect of sulfone can therefore be explained on the basis that these drugs do not reach the Schwann cells [Rees *et al.* (¹⁰)].

The two examples given here point to new problems. Foremost is the question of whether a therapeutic effect can be attained alone through a bacteriostatic influence on mycobacteria or whether the enzymatic lysosomal defense performance of the macrophages and Schwann cells can also be furthered through increased synthesis of acid hydrolases. The combination of light-microscopic, histochemical, and electron-microscopic research methods on the same excision material can contribute to the solution of such problems.

SUMMARY

A combination of light- and electron-microscopic study was used on numerous skin biopsies from patients with lepra lepromatosa, borderline leprosy, and tuberculoid leprosy with and without DDS treatment. The combination method has proved successful for light microscopic survey of stained semi-thin sections embedded in Epon and Maraglas, and for investigation also by electron-microscopic examinations of consecutive homomorphic ultra-thin sections from the same biopsy material. The method described is especially useful in the location of the small cutaneous nerves. This type of investigation is demonstrated with two examples, viz., a leprosy cell and a Schwann cell.

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