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# Myelinated and Unmyelinated Fibers in Sural Nerve Biopsy of a Case with Lepromatous Leprosy—a Quantitative Approach[1]

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In lepromatous leprosy combined qualitative and quantitative studies of myelinated fibers (MF) in semithin and ultrathin sections of biopsied peripheral nerves have rarely been published ([3, 4, 12]). Those that have been published mostly concern the index branch of the radial cutaneous nerve. As far as we can see, quantification of the involvement of unmyelinated fibers (UF) does not appear in the literature. The advantage of combining quantitative studies of both nerve fiber populations for sufficient assessment of the underlying neuropathological process may be demonstrated by the following observation. More detailed studies of MF (including teased fiber preparation), UF, and their associated Schwann cells in sural nerve biopsies of this case and three further cases of the lepromatous spectrum will be published separately.

**Case history.** A 64-year-old Cambodian merchant presented with a history of at least 18 months of an initial circumscribed numbness in the left upper arm and left foot which was followed by the appearance of nodular lesions over the face and extremities. Previous inadequate treatment could not be excluded definitively. There was no evidence for alcoholism or involvement of other toxic factors. Examination revealed a slight facies leontina, thickened earlobes, and multiple, bluish to reddish-brown, smooth nodular swellings (6–30 mm in diameter) distributed almost symmetrically over the face and whole body of this well-nourished man. Slightly thickened radial nerves were palpable in both lateral brachial sulci. There was a symmetrical loss of pain and thermal sensibility, moderate in the dermal lesions but most marked—and here not restricted to the skin lesions—over the knees, elbows, lateral feet, and (questionable) in the left paravertebral lumbar region. Muscle stretch reflexes were well preserved. Electromyography of several muscles as well as electroneurography of motor and sensory nerves, including the right sural nerve, were within normal limits. Extensive laboratory tests revealed no abnormalities except for lues latens seropositiva. A lepromin-A test was negative. A nasal mucosal swab showed acid-fast bacteria. In a skin biopsy of a lesion on the right cheek, a fully developed foam-cell granuloma with numerous acid-fast intracellular rods but only few lymphocytes was seen under the free "Grenzstreifen." The granuloma fraction (GF) was 0.6; bacterial index of the granuloma (BIG), 4. Accordingly, the histopathological index (HI) was 3.8 ([10]). The patient's leprosy was classified as lepromatous leprosy. Specific treatment with 600 mg rifampin and 50 mg dapsone daily and 100 mg clofazimine three times a week was begun 2 weeks prior to biopsy.

## MATERIALS AND METHODS

After informed consent, a biopsy of the right sural nerve was performed at the ankle level. A small part of the specimen was fixed in Formalin, dehydrated, embedded in paraplast, and stained by hematoxylin and eosin and according to Ziehl-Neelsen modified by Fite. For semithin and ultrathin sections a larger part of the tissue was treated as described elsewhere ([6]). Morphometrical evaluation was performed as indicated pre-

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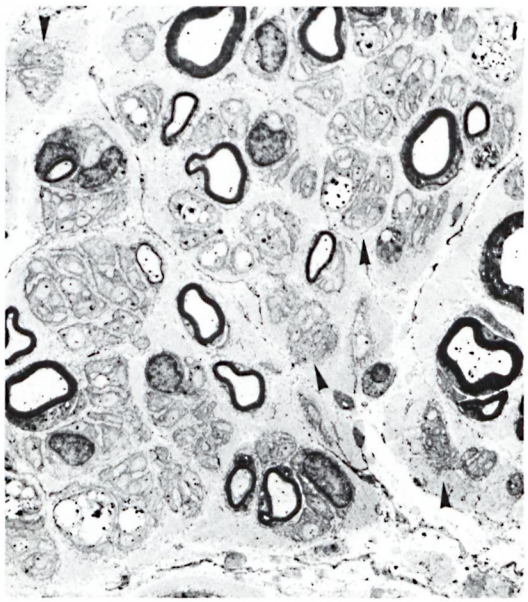

FIG. 1. Low-power electronmicrograph of cross section of sural nerve. Note incompletely remyelinated axons, clusters of Schwann cell complexes of the myelinated type without myelinated fibers (some of them shown by ▲), and Schwann cells with phagosomes containing osmiophilic bacterial remnants ( ×2400).

viously ([6]). The histograms are derived from measurements of 3203 MF on micrographs ( ×1500) and of 1666 UF on electronmicrographs ( ×21,000).

## RESULTS

**Light microscopy.** In paraplast sections, many intracellular acid-fast rods and granules were seen in groups of foam cells preferentially located in the endoneurium. Here, obviously Schwann cells contained identical particles. In semithin sections the endoneurial structure was well preserved. Many thinly myelinated fibers, rarely appearing in clusters, and some actual degeneration of MF were visible. Numerous foam cells occurred in patchy or scattered distribution, containing osmiophilic particles or filamentous network. In one fascicle a small necrotic area was detectable subperineurially. The perineurium was inconsistently thickened, in some segments invaded by foam cells and scattered mononuclear cells.

**Electron microscopy.** The state of MF was confirmed (see above). There were numerous Schwann cells of the formerly myelinated type ([9]), now containing exclusively unmyelinated axons (Fig. 1). Many granular or fragmented bacteria occurred in macrophages and/or mostly UF-associated Schwann cells, here preferentially in membrane-bound phagosomes. In most fascicles marked swelling of Schwann cell cytoplasm was obvious. Occasionally an uncharacteristic degeneration of Schwann cell cytoplasm and/or UF could be observed.

**Quantitative evaluations.** There was a normal density of 7076 MF per $mm^2$ of endoneurial area ([5, 9]), but the histogram demonstrates a moderate reduction of larger ($\geq 7$ $\mu$m) and a distinct increase of smaller ($<7$ $\mu$m) diameter fibers (Fig. 2). The density of UF was 49,025/$mm^2$, reflecting an increase if compared with respective age-related values in the literature ([5, 9]). The histogram of UF indicated an impressive shift to smaller calibers and a widening of the fiber spectrum beyond 2.4 $\mu$m diameter axons (Fig. 2).

## DISCUSSION

In the sural nerve of this fully developed and briefly treated case of lepromatous leprosy, we found a combination between a normal density of MF and a pathological histogram with reduction of larger and an increase of smaller diameter fibers. In lepromatous leprosy this combination has been reported as yet only once by Dastur and Porwal ([3]) in the unifascicular index branch of the radial cutaneous nerve. It suggests an early stage of nerve involvement with prevailing segmental demyelination and remyelination as generally acknowledged in lepromatous leprosy in man and in experimentally infected animals ([1, 2, 7, 8, 12, 13]). But since we found numerous UF in Schwann cell complexes of the formerly myelinated type by electron microscopy, most probably representing not yet myelinated regenerating sprouts of MF, a considerable additional Wallerian degeneration of MF has to be taken into account. The preservation of a sufficient quantity of large MF in our case suggests early previous degeneration of predominantly smaller MF as concluded from the animal model ([13]). This finding is in agreement with the normal results of electroneurography of the sural nerve as well.

An increase of UF, as confirmed by quantitative electron microscopy in our case, has

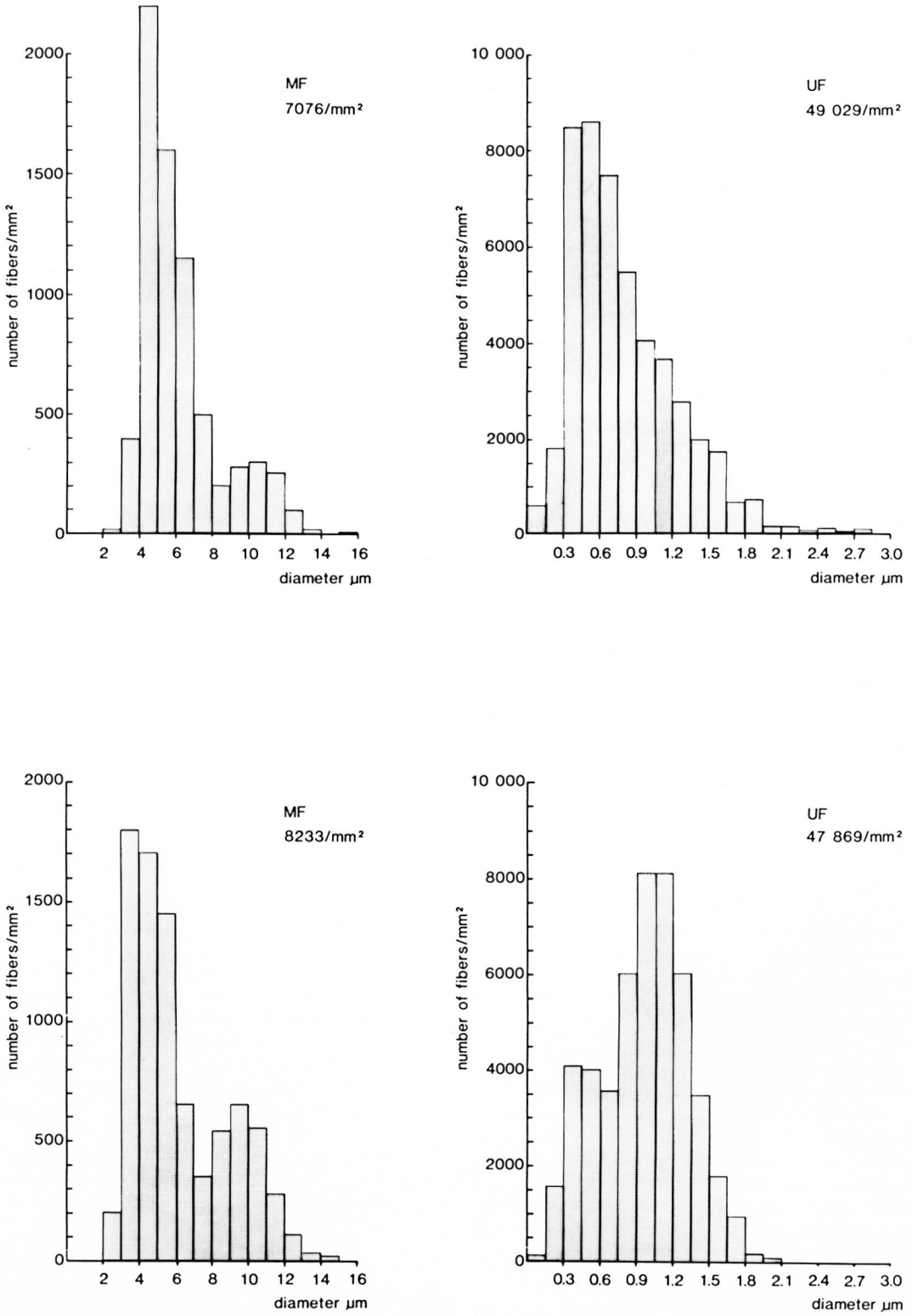


FIG. 2. Histograms and densities/mm² of myelinated and unmyelinated fibers in sural nerve biopsy of the leprosy case (top) and a 41-year-old control (bottom).

been as yet only suspected qualitatively in early lepromatous leprosy ([4]). The respective highly abnormal histogram indicates not only the presence of some large demyelinated fibers consistent with segmental demyelination of MF, but especially of a large amount of small regenerating unmyelinated sprouts presumably of formerly myelinated as well as of genuine unmyelinated axons ([9]). Accordingly, there is strong evidence for a previous loss of genuine UF, compatible with a) the predominant bacillation of UF-associated Schwann cells, b) the type of sensory deficit, and c) the current concept of pathogenesis in lepromatous leprosy ([1, 3, 11–14]).


## SUMMARY

A 64-year-old Cambodian male with lepromatous leprosy of several months' duration, with only brief previous treatment, had symmetrical patchy loss of pain and thermal sensibility including the lateral foot regions. Quantitative assessment by light and electron microscopy revealed normal counts of myelinated fibers (MF) and an increased number of unmyelinated fibers (UF). The respective abnormal histograms and the qualitative differentiation of UF-associated Schwann cells suggested a combination of segmental demyelination with Wallerian degeneration of MF, and a loss of gènuine UF, concomitant with considerable regenerative activity of both fiber populations.

## RESUMEN

Un hombre de Cambodia de 64 años con lepra lepromatosa de varios meses de duración, con solo un breve periodo de tratamiento previo, tuvo zonas simétricas con pérdida de sensibilidad térmica y al dolor, incluyendo las regiones laterales de los pies. El estudio al microscopio de luz y electrónico reveló que había cuentas normales de fibras mielinizadas (FM) y un número incrementado de fibras desmielinizadas (FD). Los histogramas anormales respectivos y la diferenciación cualitativa de FD-asociadas a células de Schwann, sugirieron una combinación de desmielinización segmental con degeneración Walleriana de las FM y una pérdida de FD genuinas, asi como una concomitante y considerable actividad regenerativa de ambos tipos de fibras.

## RÉSUMÉ

On a observé qu'un sujet cambodgien âgé de 64 ans, de sexe masculin, atteint de lèpre lépromateuse depuis plusieurs mois, et qui n'avait été traité que de manière transitoire, présentait des plages symétriques où la sensibilité douloureuse, de même que la sensibilité thermique, étaient perdues; ces zones atteignaient les régions latérales des pieds. Une évaluation quantitative à la microscopie optique et à la microscopie électronique, ont révélé un nombre normal de fibres myélinisées (MF) ainsi qu'une augmentation du nombre de fibres non myélinisées (UF). Les histogrammes correspondant étaient anormaux; la différentiation qualitative des cellules de Schwann associée aux fibres non myélinisées a fait penser à une combinaison de démyélinisation segmentaire avec dégénérescence Wallérienne des fibres myélinisées, et de perte des fibres non myélinisées, accompagnée d'une activité considérable de régénération dans les deux populations de fibres.


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