

Electronmicroscopic Demonstration of *Myco leprae* in Axons

C. K. JOB

*Schieffelin Leprosy Research Sanatorium-632106,
Karigiri, Via. Katpadi, Tamil Nadu, S. India*
and

*Department of Pathology, Christian Medical College and Hospital,
Vellore-632004, Tamil Nadu, S. India*

and

R. VERGHESE

*Schieffelin Leprosy Research Sanatorium-632106,
Karigiri, Via. Katpadi, Tamil Nadu, S. India*

Biopsies from 14 lepromatous nerves were examined. Unequivocal evidence for the presence of *Myco. leprae* in axonal cytoplasm is found in four biopsies. In the nerves the organisms are present much more frequently and in large numbers in Schwann cells, macrophages and perineurial cells and therefore the intra axonal bacilli may not have much part to play in the destructive process of the nerve. However it is suggested that axons are very likely sites for bacilli to remain protected from the bodily defence mechanisms and drugs resulting in relapse of the disease.

The affinity of *Myco. leprae* to peripheral nerves and especially to Schwann cells is quite well known and has been well established through several studies using the light microscope (Khanolkar, 1955; Weddell *et al.*, 1963; Lumsden, 1964). Nishiura *et al.* (1957, 1958, 1960) and later Imaeda and Convit (1963) in their electromicroscopic studies have reported the finding of *Myco leprae* in axons and have suggested that Schwann cell infiltration by *Myco. leprae* is a sequelae to axonal invasion. However, Job (1970) and later Dastur *et al.* (1973) studying lepromatous nerve biopsies using the electronmicroscope have clearly demonstrated and confirmed the observations of Weddell *et al.* (1963) and Lumsden (1964) that *Myco. leprae* primarily affect the Schwann cells. Although we are convinced that the target cell of *Myco. leprae* in the peripheral nerve is the Schwann cell, we would like to record here the finding of *Myco. leprae* in axons in four of the lepromatous nerve biopsies studied.

Material and Methods

In a period of three years 14 lepromatous nerve biopsies have been studied using the electronmicroscope. Thirteen of them were from the radial cutaneous nerves and one from the greater auricular nerve. They were cut into small pieces

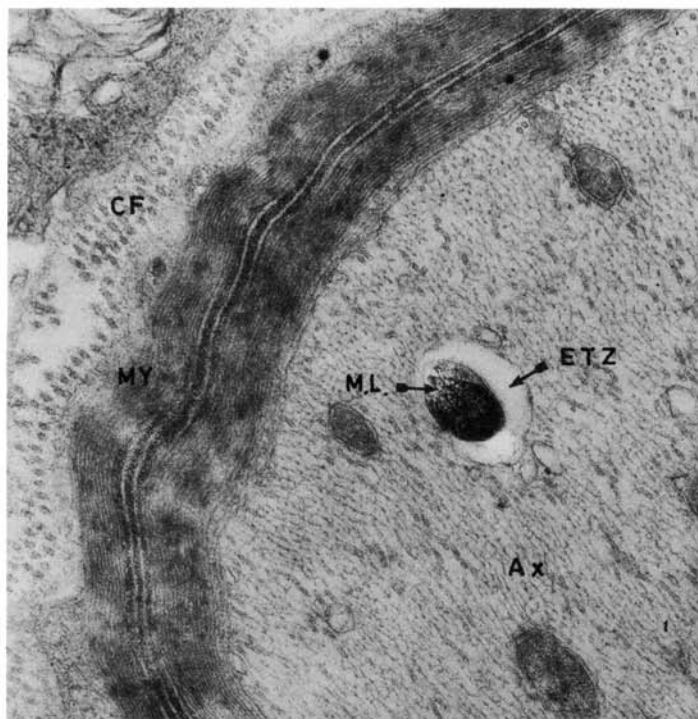


Fig. 1. Electronmicrograph to show a myelinated nerve fibre containing the cross section of a bacillus (ML) inside an axon (AX). Note electron transparent zone (ETZ) surrounding it. MY, Myelin; CF, Collagen fibres.

of about 3 mm long and 1 mm thick and were fixed in 5% glutaraldehyde solution in phosphate buffer at pH 7.2 for 4 h at 4°C. Later they were washed in buffer solution and treated with 2% osmium tetroxide, processed in grade alcohol followed by propylene oxide and embedded in araldyte. In a few biopsies part of the tissue was fixed in Dalton's solution instead of glutaraldehyde. Ultra-thin sections were prepared, stained with lead citrate and uranyl acetate and were examined under an electronmicroscope.

Findings

The results of the study of most of the lepromatous nerve biopsies have been reported earlier in two communications (Job, 1970, 1971). The nerve parenchyma in most instances was partly, and in some largely, replaced by collagen fibrils, macrophages, plasma cells, lymphocytes and fibroblasts. The number of Schwann cells with axons were much reduced. Perineurium was thickened with infiltrating collagen fibrils and proliferating perineurial cells. *Myc. leprae* were found in large numbers in Schwann cells, macrophages, perineurial cells and endothelial cells.

On searching through many ultra-thin sections we were able to find in four



Fig. 2. Electronmicrograph showing a myelinated axon containing two *Myco. leprae* (ML) inside the axon (AX). A double membrane bound vacuole (MV) contain the organisms. SC, Schwann cell; CF, Collagen fibril; LB, a lamellar body.

biopsies very occasional bacilli inside axons (Figs 1 and 2). The cytoplasm of all the intraneurally situated organisms was markedly electrondense as found in solidly staining organisms. The thin electrondense double layers of the plasma membrane were well seen. In some instances the organisms were found in the ground substance of the axon with an electron transparent zone around it (Fig. 1). In other sections there were membrane bound vacuoles which contained the organisms (Fig. 2). The axons which contained the bacilli appeared normal with no evidence of any degenerative changes. The Schwann cell in one of them

contained a large vacuole with bacillary debris and in another there is a lamellar body (Fig. 2) which normally occurs in Schwann cells.

Discussion

In this paper unequivocal evidence for the intra-axonal presence of *Myco. leprae* is presented in four different lepromatous patients. In some the bacilli were present in the ground substance of the axonal cytoplasm and in other sections the bacilli were present in membrane-bound vacuoles. There were also electron transparent zones surrounding the organisms as seen in Fig. 1.

Khanolkar (1955) had suggested earlier that bacilli enter the nerve through the naked axons in the skin, proliferate in the axonal tissue and are taken up by the Schwann cells only later. Nishiura *et al.* (1957, 1958, 1960) in their electronmicroscopic studies had sought to confirm these findings. However, Dastur *et al.* (1973) were unable to see organisms in axons. Job (1970) reported findings of organisms inside axons in two patients but suggested that they could be situated in intra-axonal Schwann cell processes. The electronmicroscopic evidence in this study leaves no doubt as to the intra-axonal presence of the bacilli. The interaction between the bacilli and the axonal cytoplasm had produced the electron transparent material around it and this is very similar to what was described in the cytoplasm of macrophages and Schwann cells (Imaeda, Convit and Lapenta, 1963; Job, 1974).

It is interesting to note that in all the four patients the bacilli were seen in myelinated fibres. Bacilli could enter the myelinated axon at the internodal sites. Intra-axonal proliferation of the bacilli is quite possible but we are not able to demonstrate it. The significance of the presence of bacilli inside axons is yet to be determined. Compared with the number present in other components of the nerve, the bacilli in axons are very uncommon and therefore it is reasonable to infer that it is only in a very rare instance bacilli enter the axoplasm. Once they enter the axons they are very much protected. No lysosomal granules are present in axons and therefore intra-axonal degradation and dissolution of bacilli may not be possible as in other cells. No fragmented bacilli were seen in the axons and all those seen were solidly staining and electron dense and therefore they could be considered viable. Axons may be sites where organisms can be protected from enzymic and other protective action of the body's defence mechanism and drugs and can remain there long enough to be responsible for relapses. If they do multiply in axons, which is also reasonable to expect, disintegration of axons directly by the organism is a possibility (Imaeda and Convit, 1963). However, this process would indeed be a rare occurrence.

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Addendum

Since submitting this paper for publication we have come across two papers on intra-axonal *Myco. leprae*, one by Yoshizumi *et al.* and another by J. Boddington, and their references are given below:

- Yoshizumi, M. O. and Asbury, A. K. (1974). Intra-axonal bacilli in lepromatous leprosy—a light and electron microscopic study. *Acta Neuropath. (Berl.)* **27**, 1-10.
- Boddington, J. (1974). The occurrence of *Mycobacterium leprae* within axons of peripheral nerves. *Acta Neuropath. (Berl.)* **27**, 257-270.

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