Diagnostic Efficacy of Cutaneous Nerve Biopsy in Primary Neuritic Leprosy

TO THE EDITOR:

I was interested to read the above-titled JOURNAL article by Drs. Mary Jacob and Rachel Mathai [Int. J. Lepr. 56 (1988) 56–60]. Nerve biopsy is certainly a valuable and revealing procedure, in the right hands, and I agree that it might be particularly helpful in primary neuritic leprosy, which appears to be relatively common in India. I am, however, very far from convinced that one can safely regard it as ". . . a simple office procedure . . ." and I would like to emphasize that in our publication on sural nerve biopsy [Haimanot, *et al.,* Int. J. Lepr. 52 (1984) 163–170], quoted by Jacob and Mathai (their reference 9), we carefully emphasized that nerve biopsy should be attempted ". . . only by experienced observers, including an operator who is trained in nerve biopsy." (One of our authors was a qualified neurologist/neuropathologist.) I also note with some concern (in Materials and Methods) that a ". . . thin sliver of the main peripheral nerve trunk, such as the ulnar, was taken in a few cases." Such trunks contain mixed fibers, and there is some risk that motor elements may be damaged. Finally (again in Materials and Methods), does the statement ". . . a 1-cm piece of the nerve was sliced with a scalpel . . ." mean that a full 1-cm length (segment) of the nerve was removed? Would this not result in permanent loss of sensation in the area supplied?

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Drs. Jacob and Mathai Reply

TO THE EDITOR:

We are writing in response to the letter from Dr. A. Colin McDougall on our article entitled "Diagnostic Efficacy of Cutaneous Nerve Biopsy in Primary Neuritic Leprosy."

We agree that nerve biopsy should be performed by experienced persons. The technique of biopsy of a cutaneous nerve is simple because the course of these nerves can be traced under the skin at specific sites. It may be noted that the cutaneous nerves chosen for biopsy were the radial cutaneous nerve at the wrist, the cutaneous branch of the common peroneal nerve above and medial to the medial malleolus, and the sural nerve at the ankle as it winds behind the lateral malleolus. Any other cutaneous nerve which was biopsied was palpable in close proximity to an area of sensory loss. All cutaneous nerve biopsies in our series were performed by dermatologists or dermatology trainees with not less than 3 months of training in minor surgical procedures.

Concern has been expressed that permanent loss of sensation will result when a full 1-cm segment of a cutaneous nerve is removed. The cutaneous nerves chosen for diagnostic purposes were from the sites of established neurological deficits. This was mentioned in the section on Materials and Methods. Thus, there is no need to fear worsening of sensory function after biopsy.

The possible danger of performing a sliver biopsy from the trunk of a peripheral nerve was also pointed out by Dr. McDougall. Sliver biopsy of the ulnar nerve above the elbow was performed by competent surgeons on two patients who presented with total (sensory-motor) neurological deficit indicative of damage to the nerve at that site. The size of the nerves in both patients was normal. It may be argued that even though there was existing neurological

deficit, if the nerve pathology was due to a reversible condition, the removal of a segment of nerve could prevent restoration of function. Each time a biopsy on a nerve trunk was done, it was done as a calculated risk. We feel it is justifiable to do this and that the clinician must take this risk so that early diagnosis can be made and treatment started on patients who have treatable disease.

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Investigations into Cultivation of *M. leprae* Under Low Oxygen Tension

TO THE EDITOR:

A critical review of the literature strongly suggests that *Mycobacterium leprae* is a microaerophilic organism. Growth of this mycobacterium in the foot pads of mice ([3]) may not necessarily be due to lower temperature but most likely because of low oxygen tension. The concentration of oxygen in the host tissues where *M. leprae* multiplies abundantly has been reported to be 2.5% ([2]). It was thus postulated that low oxygen tension of the culture medium might facilitate the multiplication of *M. leprae*. Attempts were thus made to cultivate *M. leprae in vitro* under low oxygen tension.

M. leprae cells were isolated from non-irradiated livers removed aseptically from armadillos previously infected with *M. leprae*. Both liquid and solid media were used: The liquid medium contained $(NH_4)_2SO_4$ 0.2 g; KH_2PO_4 2.0 g; glycerol 2.5 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; Na thioglycolate 0.8 g; hemin 0.002 g and water 100 ml. The solid medium, in addition, contained 200 ml of egg yolk.

Several tubes containing 9 ml of liquid medium were inoculated with 1 ml of a bacillary suspension which contained 1×10^9 bacilli. To inoculate the solid medium, 2.0 g of armadillo liver was homogenized in 4.0 ml of 2% NaOH and then neutralized. Several tubes containing solid medium were inoculated with 0.2 ml of bacillary paste. The inoculated tubes were placed in a jar. The jar was closed and excess air was removed from the jar by vacuum. The jar was then flushed with a gas mixture containing 2.5% O_2, 5% CO_2, and 92.5% N_2. Air served as the control. The cultures were incubated at 34°C. At various time intervals aliquots were taken from the liquid medium for microscopic counts. To assess the *in vitro* growth on solid medium, the growth from the surface of the solid medium was removed and a homogenous suspension was made in 100 ml of 0.05 M phosphate buffered saline solution. Appropriate aliquots were then used from microscopic counts.

It was observed that no multiplication of acid-alcohol fast bacilli (AAFB) occurred when incubated under air. However, two-fold and fourfold increases in AAFB were observed, respectively, when incubated in liquid and solid medium under a gas mixture of 2.5% O_2 and 5% CO_2 between 16–24 weeks. The morphology of the bacilli was well maintained. The bacilli did not show any growth on Löwenstein-Jensen or Dubos medium. The number of AAFB started to decline rapidly after about 26 weeks of incubation under a gas mixture, and no bacilli could be detected after about 36 weeks of incubation.

Further studies are in progress under different gas mixtures. During these studies, in addition to microscopic counts, determinations of ATP and DNA will be done and identification of the cultivated AAFB will be carried out.