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.

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