Effect of Purification Procedures on the Viability of *Mycobacterium leprae*

TO THE EDITOR:

Suspensions of *Mycobacterium leprae* prepared from infected tissues are usually contaminated with host-tissue elements. When such suspensions are used for metabolic studies, spurious results might be obtained. The enzyme activities detected could be of host-tissue origin, or the contaminant substances would inhibit the bacterial enzymes.

THE TABLE. *Effect of purification procedures on viability and o-diphenoloxidase of* Mycobacterium leprae.

Treatment	Viability: No. of bacilli harvested per foot pad[a]	*o*-Diphenol-oxidase absorbance 480 nm ($\times 10^{-3}$)
None	2.4×10^6	33
NaOH	1.9×10^6	36
Acetone-ether	1.3×10^{4}[b]	37
Trypsin	1.6×10^6	72

[a] Mean value per foot pad determined from two pools of five mouse foot pads each.

[b] One pool of five mouse foot pads had no bacilli in 90 microscopic fields; the other pool had 15 bacilli in 90 fields (2.6×10^4/foot pad).

We have reported earlier (4) that *M. leprae* separated from infected armadillo tissues do not lose their *o*-diphenoloxidase activity on treatment with dilute alkali, proteolytic enzymes, or acetone and ether. We have also shown (2) that alkali-treatment completely inactivates a host-tissue enzyme adsorbed on the bacterial surface. It was not known whether the purification procedures would impair the viability of the *M. leprae* suspensions.

Suspensions of *M. leprae* were prepared from the spleen tissue of experimentally infected armadillos, as described before (3). The bacilli were treated with the different reagents to remove adsorbed host-tissue elements, as reported earlier (4). Viability of the bacterial preparations was tested by inoculating them into the left hind foot pads of Swiss NIH mice (female). The number of bacilli inoculated in each mouse was 1×10^4. The preparations were inoculated into 20 mice each. Six months later, the bacilli in the mouse foot pads were enumerated by the method of Hanks, *et al.* (1). *o*-Diphenoloxidase of the treated suspensions was determined as described before (4).

The results are given in the Table. *M. leprae* separated from lepromatous tissue retained their viability. Alkali and trypsin did not alter the viability of the organism; however, the bacilli treated with acetone and ether failed to multiply in the mouse foot pad. None of the reagents diminished the *o*-diphenoloxidase of *M. leprae*; in fact, trypsin-treatment enhanced the enzyme activity, confirming our previous report (4). It is likely that acetone-ether treatment disrupts the bacterial cell membrane, resulting in the loss of soluble cytoplasmic contents. This might explain the failure of the organism to grow in the mouse foot pad. *o*-Diphenoloxidase is a particulate enzyme (5) firmly attached to the bacterial membranes; as such, the activity is not removed by the acetone-ether treatment. It is evident that *M. leprae* suspensions can be purified with NaOH and trypsin without impairing the viability of the bacilli.

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