other hand, all other dietary levels of minocycline tested (0.01%, 0.04%, 0.06%, and 0.1%) were bactericidal for *M. leprae* ($p \leq 0.02$).

Unfortunately, in this study the control inoculum itself had low viability. This may account for why the percentage of *M. leprae* killed was less than had been found in previous studies ([2, 4]). Nonetheless, it would appear that concentrations of minocycline required to inhibit and kill *M. leprae* are similar ([2, 3]) and easily attainable in man.

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Improved Method for Purification of *Mycobacterium leprae* from Armadillo Tissues

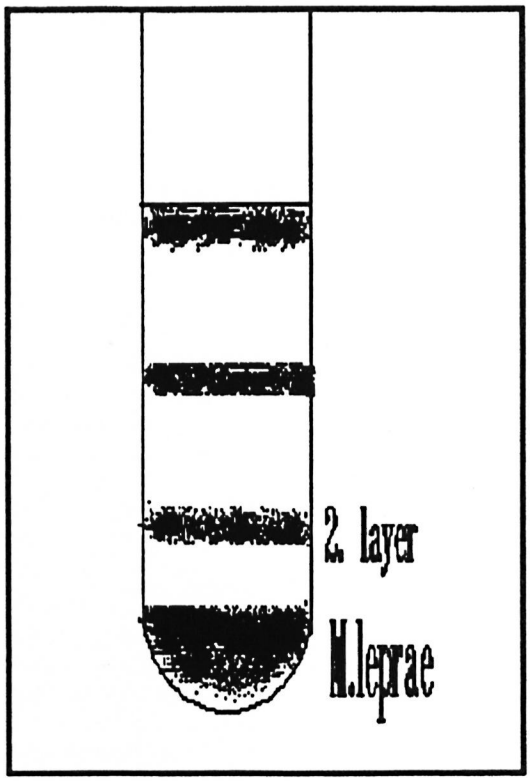
To the Editor:

An efficient, rapid, and cheap method for the preparation of pure *Mycobacterium leprae* from armadillo tissues is described. It is especially recommended for application in immunology and molecular genetics.

Because the cultivation of *M. leprae* has not yet been possible, the only source for specified living *M. leprae* are animal models, especially the nine-banded armadillo (*Dasypus novemcintus*). Difficulty in the separation and purification of the organism from armadillo tissues is one of the main problems workers face in this field, and it limits the effective use of *M. leprae* in immunology, vaccine preparation, biochemical studies, etc.

We have developed a rapid, efficient, cheap, and easy method for the preparation of *M. leprae* from armadillo tissues which provides bacteria of high quantity and high quality. The method depends on low speed/high speed centrifugation with the use of Percoll as a gradient separation medium.

Armadillo liver tissue (6 grams) from an armadillo inoculated with *M. leprae* 24 months earlier was homogenized with ultraturax at high speed in 10 ml phosphate buffered saline (PBS), pH 7.0, in a Beckman screw-cap centrifuge tube (Cat. no. 355670), and the homogenate was then centrifuged at 55 × *g* for 6 min at 5°C. The supernatant was carefully aliquoted to two or three 5-ml screw-cap tubes (Greiner, Cat. no. 124261)



THE FIGURE. Separations of *M. leprae* suspensions after centrifugation.

and centrifuged at 15,000 × *g* for 10 min at 5°C. The supernatant was discarded, and the sediment was resuspended in 6 ml Tween buffer [1 ml 10% Tween 80/100 ml distilled water/0.2 ml of 2-morpholino-ethane-sulfonic acid (MES) (1.0661 g/10 ml distilled water, pH 6.8, adjusted by NaOH)]. The suspensions were pooled in a sterile Beckman screw-cap centrifuge tube (as above), and 24 ml of 30% Percoll [30 ml Percoll/70 ml Tween buffer (as described above)] was added. The tube was centrifuged at 70,000 × *g* for 1 hr at 5°C. The bottom layer (The Figure) was taken and washed twice with Tween buffer, twice with saline solution, and twice with PBS at 13,000 × *g* for 10 min at 5°C. The sediment was resuspended in 2 ml PBS and kept for further use.

The present method takes about 2½ hr and provides *M. leprae* free of any armadillo tissue contaminations. The number of viable organisms is very high. The second layer (The Figure) can be used for methods with lesser needs for purity of the bacteria. The count in this fraction lies two powers below the bottom layer, and it is contaminated with armadillo tissue cells.

The standard protocol for purification of *M. leprae* described by Draper [1] is time-consuming compared to this new method. The efficiency of our method and the viability of the bacteria prepared with it are at least as high as those with the Draper protocol. The bacteria prepared by the present protocol were white, i.e., devoid of a ferric iron-protein complex which makes the use of the bacteria unacceptable in immunological studies in man [2, 3].

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