mals, namely, rabbits, nutrias, raccoons, and opossums. Although thorns were present in some of the animals, they were not so frequent as reported in the armadillo ([6]).

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Psychrophilic Mycobacteria in *M. leprae*-infected Tissues

TO THE EDITOR:

A multifactorial medium (MFM) was proposed for the *in vitro* cultivation of *Mycobacterium leprae* ([2, 3]). In the MFM, Na-thioglycolate served as a source of energy and mycobactin with exochelin for iron acquisition ([1–4]). Slow growth of leprosy-derived mycobacteria (LDM) occurred on the semisolid medium at pH 5.8 and incubation temperature of 32°C.

I am now able to report that a considerably higher yield and more rapid growth can be achieved in a liquid medium at an incubation temperature of 16°C to 18°C if Na-thioglycolate is replaced by ammonium thioglycolate and β-cyclodextrin replaces mycobactin-exochelin.

In a closed Erlenmeyer flask, 0.05 g of thioctic acid (Fluka Chemical Corporation, Hauppauge, New York, U.S.A.) and 5 g of β-cyclodextrin (Chinoin, Budapest, Hungary) were dissolved in 10 ml of hot ammonium thioglycolate (Fluka) (60% v/w in water).

A poor nutrient, multifactorial liquid medium was used. This contained in 1 liter of distilled water: KH_2PO_4, 2.5 g; Na_2HPO_4, 4.0 g; $(NH_4)_2SO_4$, 2 g; $MgSO_4$, 0.2 g; ferric ammonium citrate, 0.05 g; and 10 ml of the above thioctic acid-β-cyclodextrin-ammonium thioglycolate solution. The pH was adjusted to 7.0, using the PO_4 buffers. The solution, distributed 10 ml/25 ml screw-cap tubes, was autoclaved for 25 min. Optimal growth of the primary cultures and subcultures was registered at 16°C to 18°C. These results indicate that the physicochemical properties of the β-cyclodextrin might replace the iron acquisition growth factors.

No visible growth was observed at 4°C and very slow growth was seen at 32°C. At 16–18°C the inoculum increased in size into a visible growth within 2 to 8 weeks, depending on the size and quality of the inoculum. This growth consisted of strongly acid-fast cells with characteristics as previously described ([3]).

Twenty-four such cultures are now maintained, being transferred into subcultures at 6- to 10-week intervals and grown at 16°C incubation temperature.

These leprosy-derived cultures, ranging from the 2nd to the 17th subcultures respectively, are tentatively designated as "*M. psychrophilum* L.," indicating that further characterization and identification are nec-

essary. However, the designation "*M. psychrophilum* L." has been selected because the mycobacterium grows under psychrophilic conditions and cultures are obtained from *M. leprae*-infected tissues. Results indicate that LDM might have a role in the pathology of leprosy, as advocated earlier by this author.

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Multiplication of Armadillo-derived *M. leprae* in Murine Dissociated Schwann Cell Cultures

To the Editor:

For as long as viability of *Mycobacterium leprae* is thought to be responsible for its pathogenesis, its *in vitro* cultivation holds an edge over the production of structural epitopes by recombinant technology. In 1985, Mukherjee and Antia reported a sustained multiplication of human-derived *M. leprae* within Schwann cells of an organized nerve culture derived from dorsal root ganglia (DRG) of newborn mice ([5]). Since the DRG culture technique is a relatively fastidious one and requires almost 3 weeks for cellular organization, it was decided to attempt pathogen growth in dissociated Schwann cell cultures whose characteristics are likely to be different from their organized counterparts. Besides achieving ease in maintenance, reducing the culture period, and acquiring homogenous cell populations, it was also anticipated that such trials might yield leads to the Schwann cell properties responsible for supporting the intracellular growth of *M. leprae*.

This communication describes the culture conditions found to be optimal for obtaining *M. leprae* growth in dissociated Schwann cells (DSC) and to emphasize the conditions that differ from those we reported for DRG cultures in 1985 ([5]).

Since a semi-quantitative count of intracellular bacilli on culture coverslips could be subjective and therefore erroneous, a quantitative petri plate culture method was used to evaluate growth. Dissociated Schwann cells were obtained from the sciatic and brachial nerve plexuses of 1-day-old, Swiss white mice by the method of Brockes, *et al.* ([2]) and cultured in Falcon petri plates. An equal number of petri dishes with the equivalent numbers of Schwann cells in each set was terminated at time periods of 2 weeks and 4 weeks. All of the cultures were used at a maturation period of 7–12 days after initiation. Since initial viability of the inoculum is a prime factor in the outcome, *M. leprae* derived by homogenization from infected armadillo spleen and liver tissues were used in all of the experiments. *M. leprae*-infected armadillo tissues were supplied by Dr. E. Storrs, Melbourne, Florida, U.S.A. The tissues were shipped frozen to Bombay where they were aliquoted and stored at −90°C until use. For use, the aliquoted tissues were thawed and homogenized in Hanks' balanced salt solution. Tissue contamination was reduced by differential centrifugation and the bacilli quantitated. Before use the suspensions were inoculated into sterile Dubos broth for detection of contam-