Investigations into Cultivation of *M. leprae* in a Nasal Mucus Medium: A Preliminary Report

TO THE EDITOR:

The nostrils of lepromatous leprosy patients harbor a multitude of *Mycobacterium leprae* cells. These organisms are mainly responsible for spreading the disease to close contacts. A quantitative evaluation between the *M. leprae* of the skin and the nose showed that *M. leprae* from the nose had a significantly higher morphological index and greater length when compared with those in the skin of the same patients. Further, the number of globi was considerably more in nasal samples than in skin smears (Prabhakar, M. C., submitted for publication). Based on these data, it was postulated that the nasal cavity might provide some biochemical(s) which might nourish the *M. leprae* therein and facilitate their active multiplication ([3], and Prabhakar, M. C., submitted for publication). Efforts were made to cultivate *M. leprae in vitro* under conditions simulating those of the nostrils.

M. leprae cells were obtained from the nasal flushings of lepromatous patients according to the method described by Prabhakar ([1, 3]). In the author's opinion, for the cultivation of *M. leprae in vitro,* the *M. leprae* extracted from the nose of untreated lepromatous patients would be more suitable than those from any other source. In a few experiments, these organisms were used after purification according to the method described by Shepard ([4]).

Nasal mucus collected from healthy individuals was desiccated and, when completely dry, was powdered and preserved in

a tightly closed container at 4°C. Five, 10, and 15 mg per ml solutions were made in 0.1% w/v bovine serum albumin (BSA). A 0.1% solution of BSA served as control. The media were filter-sterilized, and the pH was adjusted to 6.2 (which corresponds to that of the nasal mucosa). The media were inoculated with freshly extracted *M. leprae* from the nose of lepromatous patients. Benzyl penicillin (100 U/ml) was added to each sample tube to prevent the growth of other microorganisms. Immediately after inoculation and after mixing thoroughly, smears were made with a standard loop (loop size was maintained constant), and spread in a circle 8 mm in diameter. After drying and fixing, staining was done with 3% carbol fuchsin, decolorized with 1% HCl in 70% ethanol, and counter-stained with 0.3% methylene blue. The number of organisms was counted across the diameter of the spot using a binocular Olympus microscope (100 ×). The samples were incubated at 31 ± 0.5°C (which corresponds to the temperature of the nasal passage). Smears were prepared on alternate days, dried, stained, and the acid-alcohol-fast bacilli (AAFB) were counted as described above. Control samples were also stained in the same manner, and the number of organisms counted.

It was observed that the above-mentioned medium (optimum 5 mg/ml), under the conditions stated above, encouraged the growth of AAFB. The morphology of the organisms was beautifully maintained.

Further studies are in progress to improve upon the cultivation and identification of the obtained cultures. Efforts are also being made to fractionate the nasal mucus, and cultivation is being tried in different fractions as well as with other substances and growth factors from cultivable mycobacteria ([2]) added. Further experiments are necessary before claiming the successful cultivation of *M. leprae.*

—Dr. M. C. Prabhakar

Reader in Pharmacology
University College of
Pharmaceutical Sciences
Kakatiya University
Warangal 506009, India

REFERENCES

1. Appa Rao, A. V. N., Krishna, D. R., Ramanakar, T. V. and Prabhakar, M. C. "Jala Neti," a yoga technique for nasal comfort and hygiene in leprosy patients. Lepr. India **54** (1982) 691–694.
2. Kato, L. A multifactorial culture medium with growth factors from leprosy-derived mycobacteria proposed for cultivation trials for *Mycobacterium leprae.* Int. J. Lepr. **54** (1986) 310–311.
3. Prabhakar, M. C., Appa Rao, A. V. N., Krishna, D. R. and Ramanakar, T. V. How much non-infectious are the "non-infectious" lepromatous leprosy patients? Lepr. India **55** (1983) 576–583.
4. Shepard, C. C. Acid-fast bacilli in nasal excretions in leprosy, and results of inoculation of mice. Am. J. Hyg. **71** (1960) 147–152.