CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters.

A Rapid Identification Test for *Mycobacterium leprae*

TO THE EDITOR:

We proposed oxidation of 3,-4-dihydroxyphenyalanine (dopa) as an identification test for *Mycobacterium leprae* (K. Prabhakaran and W. F. Kirchheimer, J. Cact. **92** [1966] 1267-1268). Several other mycobacteria including *M. lepraemurium* showed no oxidation of dopa to pigmented products, indicating that among mycobacteria the phenoloxidase enzyme might be specific for *M. leprae*. The main objection to the above test was that it cannot be carried out in laboratories not equipped for biochemical studies (J. Convit and M. E. Pinardi, Internat. J. Leprosy **40** [1972] 130-132). Moreover, the spectrophotometric procedure requires large quantities of organisms (1.5-2 mg protein).

We have developed a simple spot test which can be carried out without any specialized equipment and with much smaller amounts of material. A drop each of phosphate buffer (0.5 M, pH 6.8), bacillary suspension (about 100 μg protein) and D-dopa solution (about 2 mg/ml in water, made up fresh) is placed in a cavity slide. L-dopa may be used instead; however, this substrate is also oxidized by tyrosinase of melanocyte origin. Use of D-dopa obviates this difficulty. Controls consist of slides where the bacilli without dopa and dopa without the bacilli are added; to make up the volume, two drops each of buffer are used. To prevent desiccation, the slides are kept in a moist atmosphere. A simple way to accomplish this to to place the slides in a large Petri dish containing moist filter papers cut to size. It is better not to place the slides directly on the wet filter paper. For this purpose a layer of cellophane paper may be put over the filter paper. The Petri dish is covered and left overnight at room temperature or incubated at 37°C. The slide containing *M. leprae* and dopa develops a deep purplish color which gradually turns black. The slide containing dopa may develop a faint pink color due to autooxidation of dopa. The organisms by themselves do not show any change in color. The speed with which the dopa oxidation occurs varies. If the bacilli are not degenerate and more bacilli are used, the oxidation becomes apparent within 60 or 120 minutes, depending on the amount of material added. Even when smaller quantities of *M. leprae* were used, we have observed dopa oxidase activity of the organisms when the preparations were left overnight at room temperature (25°C).

Employing this procedure, conversion of dopa to melanin pigment has been demonstrated with *M. leprae* obtained from leprous human tissues, armadillo tissues and with bacilli recovered from mouse foot pads. It is hoped that this simplified technic will serve as a rapid indentification test of the leprosy bacteria. [This study was supported by the U.S-Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (Grant AI-07890).]

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