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EDITORIALS

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Problems in Identifying *M. leprae*

Essentially there are eight mooted technics and a presumed growth characteristic which may be applied toward establishing the identity of *M. leprae*. These have been developed in studies of bacilli directly derived from human tissues and little consideration has been given to possible problems attendant on attempting to utilize them as means of establishing an *in vivo* cultured strain as being *M. leprae*. A brief summary of these technics may help to put the problem in perspective.

Absence of growth on the standard media utilized in culturing acid-fast bacilli. This is essentially a negative determination in that if growth is obtained it is regarded as not being *M. leprae*. However, with cultured specimens there is the theoretical possibility of carry-over of essential nutrient material with the bacilli or of material incorporated on or in the bacilli, particularly from liquid media. The presence of bacillary growth on first culture might thus be a "false" growth contaminant, i.e., it might be a growth of the bacillus that is sought. A second subculture from such possible growth may theoretically be needed to confirm true disparate culture before disallowing the test subculture as being *M. leprae*.

Mouse foot pad inoculation. This requires six or more months and, while useful as additional final evidence, is therefore impractical as a check on growing *in vitro* cultures during the developmental phase of a culture technic. Of course, some contaminants will produce "fat foot pads" more rapidly than the *M. leprae* infection and may lead to the earlier identification of a contaminant as such.

Armadillo inoculation. Similar problems exist as with the mouse foot pad and are additionally complicated by the costs of obtaining and maintaining adequate numbers of animals. Added to these is the fact that armadillos are not all susceptible to this infection and that among those that are susceptible the time required to develop an infection is quite variable.

DOPA oxidase test. This test is proposed, and widely accepted, as a virtually positive identity test for *M. leprae*. Most of the work done on this determination as related to *M. leprae* emanates from a single laboratory. The published list of acid-fast mycobacterial strains tested[1] were apparently derived from cultures whereas *M. leprae* were derived from human tissue lesions. Beaman and

[1] Prabhakaran, K. Oxidation of 3, 4-dihydroxyphenylalanine (DOPA) by *Mycobacterium leprae*. Int. J. Lepr. 35 (1967) 42-51.

Barksdale, on the basis of their study[2] suggested that the production of the presumed enzyme might be due to "little understood conditions of growth." Hyaluronic acid alone or in combination with yeast extract, for example, gives a positive reaction by Prabhakaran's[3] spot test method. The method as used, particularly the simple rapid method,[4] has perhaps inadequately recognized possibilities for false reactions. Thus, auto-oxidation occurs and pigment is formed with DOPA and 0.5 M phosphate buffer alone at pH 6.8.

Pyridine extraction for two hours at room temperature of acid-fast quality has been reported by Convit and Pinardi[5] as having identity significance for *M. leprae*. The claim was initially based on a sample of five tested mycobacteria. We have extended this testing method to an additional seven strains as well as three acid-fast mycobacteria reported by these authors: *M. tuberculosis, var hominis, M. avium, M. phlei, M. smegmatis, M. kansasii*, a scotochromagen (Group II), *M. intracellularis* (Group III, non-chromagen), and *M. fortuitum*. Of these *M. fortuitum, M. smegmatis* and, to a slightly lesser degree, *M. phlei*, all lost their acid-fastness after pyridine extraction for two hours at room temperature (cā. 25°C). All these bacilli, as contrasted with *M. leprae* and *M. lepraemurium*, showed poor initial staining with Baker's phospholipid stain which was comparable to that of both leprosy bacilli after pyridine extraction. It should be noted, however, that in the original work of Convit and Pinardi, the human and murine leprosy bacilli were suspensions obtained from animal tissues while the other mycobacteria tested were from actively growing *in vitro* cultures. It was not stated whether these were plate or liquid media. Presumably some were from plates. If so, problems of acid-fast enhancement by oxidation of bacillary lipids should be considered. In other words, the test conditions lend no assurance to the validity of the method for determination of identity of possibly cultivated *M. leprae*. This method is of negative value in that a given strain which does not lose acid-fastness after pyridine extraction may not be *M. leprae*. There is also the possibility that *in vitro* cultivation, depending perhaps on the nature of the successful medium, may alter the quality of *M. leprae* acid-fastness, and that actively growing bacilli may have different acid-fast properties from possibly slower growing or nonviable bacilli in human tissue. Whether or not this would hold true for bacilli grown *in vitro* is, of course, not yet reported.

Lepromin test. The use of Dharmendra type antigen with a reading of the 24-48 hour Fernandez response is of value but must be interpreted in the light of possible false cross reactions reported. The Mitsuda type preparation with a reading at three to four weeks has similar but probably more specific value. Both require enough bacilli to make multiple lepromin tests and are therefore difficult to use unless an already substantially successful means of growth is available. These determinations are therefore difficult to utilize in monitoring the development of a culture technic. They should be used in ultimate determination of alleged cultivation success.

Antigen immunodiffusion analysis. Stanford and associates[6] have recently developed the possibility of identification of *M. leprae* by immunodiffusion analysis of four and possibly six specific antigens of this pathogen.

Immunologic identity determination. This type of identification is likely to be the most reliable positive method of determination and is most likely to be free from any interference due to growth alteration occasioned by transfer to *in vivo* cultivation. To date, the most feasible specific procedure is that of Abe[7] involving specific fluorescent antibody staining of bacilli by antibody to a protein leprosy nodule extract following tuberculosis bacilli polysaccharide and other

[2] Beaman, L. and Barksdale, L. Phenoloxidase activity in organisms isolated from lepromatous and tuberculoid leprosy. J. Bacteriol. **104** (1970) 1406-1408.

[3] Prabhakaran, K. A rapid identification test for *Mycobacterium leprae*. Int. J. Lepr. **41** (1973) 121.

[4] Prabhakaran, K. Rapid identification tests for *Mycobacterium leprae*: a clarification. Lepr. Rev. **45** (1974) 342-344.

[5] Convit, J. and Pinardi, M. E. A simple method for the differentiation of *Mycobacterium leprae* from other mycobacteria through routine staining techniques. Int. J. Lepr. **40** (1972) 130-132.

[6] Stanford, J. L. and Rook, G. A. W. Taxonomic studies on the leprosy bacilli. Tenth Joint Conference on Leprosy, U.S.-Japan Cooperative Medical Science Program, Bethesda, Maryland, U.S.A., Oct. 27-29, 1975.

[7] Abe, Masahide. A new method of indirect fluorescent antibody test for leprosy. Leprosy Scientific Memoranda L-367 2, 1973.

broad adsorptions of the antibody containing serum. There is obvious need for the development of specific reference antigens or antibodies.

Growth characteristics. It is generally assumed, on the basis of apparent bacillary performance in the human host and mouse foot pad, that *M. leprae* will present slow growth on *in vitro* cultivation. Though this is apparently a logical assumption, it cannot be accepted as a rigid condition of identification since the bacilli may be immunologically and/or nutritionally suppressed in living hosts and may perform quite differently *in vitro*.

It is evident that positive identification of a cultivated strain of bacilli as *M. leprae* still poses a formidable problem and that the very cultivation technic may enhance these problems and raise questions of false positive reactions not previously envisaged.

—OLAF K. SKINSNES