COMMITTEE 2: WORKSHOP ON MICROBIOLOGY

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MORPHOLOGY

Mycobacterium leprae, when stained by the Ziehl-Neelson procedure, is an acid-alcohol-fast, weakly curved bacillus, 0.3 to 0.4 μm × 2 to 7 μm, sometimes showing a metachromatic granule of unknown nature. In all mycobacteria-rich tissues it is possible to find some non-acid-fast organisms. The significance of these, and particularly the possibility that they may be young forms, should be investigated.

In human lepromatous tissues, leprosy bacilli frequently occur in round to oval clumps called globi, the organisms being situated in cigar-shaped bundles.

Commonly, the majority of bacteria stain irregularly. Provided that staining technics are carefully standardized, there is a strong correlation between regular (solid) staining in smears and viability measured by the mouse foot pad model ([10, 17, 18]). Harada, *et al* ([9]), have shown that batches of fuchsin having particular light absorbance characteristics give the most satisfactory staining.

Fisher and Barksdale ([7, 8]), and Convit and Pinardi ([4]) reported that the acid-fastness of *M. leprae* was extractable with pyridine and that this was specific for the species. However, Skinsnes *et al* ([19]), have shown that pyridine extractability of acid-fastness is a characteristic of aging, nonviable bacilli and not unique to the leprosy bacillus. Slosarek *et al* ([22]), also found that the reaction was not specific.

In the electron microscope, shadowed preparations of *M. leprae* are seen to contain both uniformly dense and beaded forms of the bacilli. After shadowing or negative staining of *M. leprae* suspensions, intertwining paired fibrous structures 10 to 30 nm in width, forming a network in the walls, can be seen. Nishiura *et al* ([14]), have described "band-like structures" parallel to the short axis of the cell.

In ultrathin sections, *M. leprae* has a cell wall 15 to 20 nm thick, apparently consisting of an inner electron-dense and an outer electron-transparent layer, surrounding the cytoplasmic membrane. Large mesosome-like structures are frequently observed, continuous with the cytoplasmic membrane. In tissues, at least, bacterial division is by transverse fission. There have been numerous reports of spheroplasts and other aberrant forms in leproma-derived material. It is important to establish the nature of these and their relation to leprosy.

BIOCHEMISTRY, METABOLISM AND GROWTH

The presence of mycolic acids in *M. leprae* was first demonstrated by Etemadi and Convit ([6]). The large amounts of *M. leprae* obtainable from armadillo tissue allowed a chemical study of the cell walls ([5]). Like other actinomycetales, *M. leprae* has in its wall mycolic acids, arabinogalactan and peptidogycan. The mycolic acids resemble two of the three found in *M. tuberculosis*, and differ from those found in *M. avium, M. lepraemurium, M. vaccae*, and *M. smegmatis*. The peptidoglycan contains diaminopimelic acid, D-alanine, glutamic acid, glucosamine, muramic acid and substantial amounts of glycine. The

simultaneous occurrence of glycine and meso-diaminopimelic acid in bacteria is rare and does not occur in other mycobacteria analyzed so far. At the present time this is an important differential characteristic of *M. leprae*.

Knowledge of metabolic processes in *M. leprae* remains limited. Among enzyme activities reported to occur are dopa oxidase ([15]), glutamate decarboxylase ([16]), β-glucuronidase and N-acetyl-β-glucosaminidase ([11]). In each case controversy persists about the origin of the activity, whether it is bacterial or host-derived. More investigation is needed.

The organism is reported to take up, and to incorporate into insoluble products, radiolabeled thymidine and DOPA ([3]). Uptake is apparently inhibited by dapsone and rifampicin, and by diethyldithiocarbamate in the case of DOPA.

The drug dapsone is bacteriostatic for *M. leprae* in very low concentrations: MIC = 0.02 μg per ml. This high sensitivity is an unusual property.

M. leprae can be readily transmitted to animals, normal mice, rats and hamsters (limited infections); armadillos, immunosuppressed mice and rats and congenitally athymic (nu/nu) mice (disseminated infections). The evolution of the infection, particularly in the mouse foot pad, is characteristic for *M. leprae* when compared with other mycobacteria. The generation time during the exponential phase of multiplication in the mouse foot pad is 11 to 13 days. Isolates of *M. leprae* in mouse foot pads vary slightly in only two properties: the average rate of growth between inoculation and harvest, and the number of bacilli in the harvest.

ANTIGENIC STRUCTURE

Abe *et al* ([1]), found two antigens in human lepromatous nodules: a protein antigen thought to be specific for *M. leprae* and a polysaccharide antigen common to other mycobacteria. Abe *et al* ([2]), have also demonstrated an insoluble antigen specific for *M. leprae* by indirect immunofluorescence. This has been useful in identifying organisms as *M. leprae*. Navalkar ([12]), and Navalkar *et al* ([13]) found four antigens common to other mycobacteria and one protein antigen specific for *M. leprae*.

Stanford and Rook ([21]) were able to detect 12 antigens in disrupted *M. leprae* from infected armadillo tissue. Six of these were common to all mycobacteria. Four were apparently species specific and two of uncertain specificity.

CULTURE

Criteria that should be applied to claims of *in vitro* culture of *M. leprae* are:

a. The isolation procedure should ideally be successful in a percentage of attempts when bacilli-rich material, derived from untreated humans or experimental animals, is used.

b. Multiplication should be regular and significant (taking into account experimental errors and possible artifacts of the technics used).

c. Ideally it should be shown that the bacilli can be passaged indefinitely.

Attempts to grow *M. leprae* in tissue culture have sometimes indicated limited multiplication, but results were irregular. Recently, Talwar's group ([23]) have shown limited but continuous incorporation of thymidine into bacteria derived from human biopsies, cultured in macrophages.

There have been several recent reports on organisms cultivable from human and armadillo leprosy tissues, notably that of Skinsnes *et al* ([20]). Proof acceptable to all workers that the organisms are identical with *M. leprae* has not yet been obtained. Among criteria that should be used to demonstrate identity are:

a. All *in vitro*-grown strains obtained should be identical in most if not all characters, including drug-sensitivity patterns. Strains may differ in a small number of tests (biotypes).

b. Strains should be identical with *M. leprae* in antigen components known to contain *M. leprae*-specific determinants.

c. The possibility of contamination from the environment should be considered; isolates should differ from currently known mycobacterial species.

d. The strains obtained should behave in experimental animals in a similar way to *M. leprae* derived from human tissue, particularly with respect to nerve invasion.

e. Standardized suspensions of killed organisms prepared from the cultures should give negative Mitsuda reactions in lepromatous forms of the disease and positive reactions in tuberculoid cases.

f. The cell-wall skeleton of the cultivated

strains would be expected to resemble that of tissue-derived *M. leprae* in its chemical composition.

g. 3,4-Dihydroxyphenylalanine (DOPA) oxidase has been reported to be active in *M. leprae* and has been proposed as a specific test. Several workers have attempted to use this test, but with contradictory results. It is important that procedures for preparing bacterial suspensions and for performing the test are standardized, so that its significance may be established.

The greater the number of characteristics in which the proposed isolate differs from *M. leprae*, the greater the possibility that it is, in fact, a contaminant. Up to the present no cultures have been shown to satisfy all the above criteria, though not all have been fully tested.

RECOMMENDATIONS

a. In order to accelerate progress towards *in vitro* cultivation of *M. leprae*, the setting up of multidisciplinary teams of investigators is recommended, so that the most recent progress in such areas as cell physiology and molecular biology is applied to the problem. Studies on electron-microscope histochemistry, incorporation of radioisotopes, DNA homology and other genetic investigations and characterization of cell components should be stimulated.

b. Detailed antigenic characterization of *M. leprae* should be pursued to determine its taxonomic relation with other mycobacteria.

c. Difficulties have arisen over the use of technics for determining viability based on morphology in different laboratories, in spite of attempts to insure that standard methods are used. Additional objective technics for identifying viable organisms should be sought.

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