Working Parties

Workshop on Cultivation of *M. leprae*[1]

Chairman: Dr. Esmond R. Long
Co-Chairman: Dr. Byron S. Tepper

Thirty-one invited participants[2] discussed mutual problems and methodology in cell-free and tissue-culture approaches to the cultivation of *Mycobacterium leprae*. It was generally agreed that lepromas from untreated patients provide the most reliable source of inocula for cultivation trials. The availability problems of contamination, and transport of material for inocula were discussed. It was suggested that, when feasible, infected thymectomized-irradiated mice could provide a more readily available source of inocula. The importance of inhibitors of microbial growth and factors toxic for tissue-culture cells that may be present in the host tissue were emphasized. Although the ultimate goal of the *in vitro* studies is the unquestionable multiplication of *M. leprae*, it was suggested that microscopic counts of inocula and incubated cultures could provide leads toward favorable nutritional and environmental conditions for growth. The results of cultivation trials that were negative or yield only limited multiplication were discussed. There was a similarity in problems of inocula and methodology for the tissue culture and cell-free approaches to cultivation. In the tissue-culture approach additional problems were the choice of cell lines and the maintenance of original properties during prolonged propagation periods. The morphologic and physiologic changes that occur during cell subculture were emphasized, and it was suggested that original cell lines be preserved by freezing, to be used in serial transplants or as reference standards.

Criteria were suggested for characterizing acid-fast organisms arising in culture as the leprosy bacillus. The criteria are (1) the organisms do not multiply on or in ordinary bacteriologic media; (2) the organism multiplies in typical fashion in the mouse foot pad under defined conditions; (3) it invades peripheral nerves; (4) skin test lepromins prepared from the organism duplicate the results of standard lepromins, particularly in lepromatous patients, (5) the organism oxidizes certain phenolic compounds such as 3,-4-dihydroxyphenylalanine, and (6) it does not elongate in Hart-Valentine media. It was suggested that some of these characteristics may be lost during growth *in vitro*, but that it did not seem likely that all would be lost at the same time. An exception would be the possibility that the etiologic agent in leprosy may first be cultured as non-acid-fast or as L forms of the organisms. Methods for the isolation, cultivation, morphologic study and identification of "soft-forms" were discussed.

The open discussion and scientific sincerity of the participants of this workshop made this meeting highly successful. This workshop illustrated the value of small group gatherings for free discussion of specific problems that could not be covered in any such detail in the general scientific program. In this sense it seemed to offer a precedent for future general meetings.—B. S. TEPPER

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[2] W. L. Barksdale, L. M. Bechelli, C. H. Binford, C. Brown, Y. T. Chang, T. K. Choi, J. Convit, P. Draper, A. H. Fieldsteel, J. H. Hanks, P. D'A. Hart, G. R. F. Hilson, L. Kato, W. F. Kirchheimer, J. Lew, E. R. Long, R. S. Merkal, T. Mori, N. E. Morrison, T. Murohashi, T. Nakayama, S. R. Pattyn, R. J. W. Rees, C. V. Reich, W. A. Rightsel, G. Munoz Rivas, C. C. Shepard, O. K. Skinsnes, B. S. Tepper, F. F. Wilkinson, Y. Yoshie.