In Vitro Grown *Mycobacterium leprae* Probably a Member of the *Mycobacterium scrofulaceum* Species

To The Editor:

In a hyaluronic acid based medium, Skinsnes and collaborators (Int. J. Lepr. **43** [1975] 193-203) grow regularly a pigmented strain of mycobacteria from human and mice leprotic nodules. These authors claimed the successful cultivation of *M. leprae*. One of the strains was submitted for investigations in several laboratories where reputable and highly trained microbiologists are familiar with the identification of mycobacteria. Pattyn (Leprosy Scientific Memorandum [LSM], 1976 Memo L-790) was the first to report that the strain isolated by the Honolulu group from leprous tissue does grow perfectly well on Lowenstein-Jensen medium, and it can be easily identified as belonging to a well-known mycobacterial species. Pattyn identified the strain as *M. scrofulaceum* and concluded: "That the organism isolated by O. Skinsnes is entirely different from the etiologic agent of leprosy."

In cooperation with Dr. Edith Mankiewitz, we submitted the strain isolated by the Skinsnes group to an identification test battery. We are now able to report that the culture is a scoto-chromogenic mycobacteriur belonging to the *M. scrofulaceum* species. In this respect, our findings are in full agreement with the report of Pattyn, however, by careful considerations, we came to a different conclusion.

Little or nothing is known about the biological, biochemical and enzymatic characteristics of mycobacteria grown in the host. No information whatsoever is available concerning the substrates which pathogenic mycobacteria utilize in the living or necrotic host tissues as sources of energy, carbon and nitrogen for growth, multiplication and virulence. These bacilli grow and multiply *in vitro* on substrates like asparagine, glycerol, coagulated egg albumin, bovine serum albumin and oleic acid. These substrates are certainly not available to them in the host tissue. In the test tubes, however, they are rapidly adapted to such ingredients which are supplied to them in the culture media. They soon recognize these strange substrates as food stuff. They then multiply fast or slowly, then eventually produce pigments in the presence or absence of the nuclear magnetic energy of light. They produce enzymes to metabolize the new substrates and retain certainly many of the metabolic characteristics which they carry over from their host-adapted existence. According to the substrates supplied to them, they present a metabolic, morphologic and growth profile. Accordingly, *homo sapiens microbiologicus* identifies and classifies them by convenient systems and a pragmatic philosophy.

Little or nothing is known about the biology of *M. leprae* in the host cell. Not a single biological entity has been recognized which *M. leprae* oxidizes in the host to derive energy. For a while, we believed that it oxidizes DOPA but lately even this attractive theory has become more and more demystified (LSM, 1976 Memo 788). We have the faintest idea what characteristics *M. leprae* will possess once grown on artificial culture media. We even don't know whether it will be an acid-alcohol resistant microorganism. Probably it will be slow-growing, fast-adapted to the Lowenstein-Jensen medium on which it might form pigments like a scoto-chromogenic, and its biochemical and other characteristics will force the trained microbiologist to classify and identify it as one of the known or a hitherto unrecognized scrofulaceum.

According to the source of isolation, *M. scrofulaceum* shows quite considerable heterogeneity; the multiple human isolates, sporadic human isolates and soil isolates. Subgroups can be differentiated according to the susceptibility to ethambutanol, tolerance to hydroxylamine and urease activity. Some are more virulent for mice than the others (M. Tsukamura; Tubercle **50** [1966] (51-59). The scoto-chromogenes implicated in human disease failed to hydrolize Tween while those isolated from tap water hydrolize it (Wayne, L. G.; Am. Rev. Resp. Dis. **93** [1966] 919-927). The scrofula type and the aqua type scoto-chromogenes can be easily

differentiated but they certainly belong to the same species. It is now clear that among the scrofulacea, there are several varieties of subspecies. It is therefore conceivable that, while the Honolulu strain still awaits final identification and classification, the real *in vitro* grown *M. leprae* might fit into the *M. scrofulaceum* species as a new variety or new subspecies.

These were the thoughts which came to my mind when we fully confirmed the results of Pattyn concerning the *in vitro* characteristics of the strain isolated by the Skinsnes group from human leprous tissue. In agreement with Pattyn, we recognize the strain as *M. scrofulaceum*. I am, however, unable to subscribe the same conclusion. Since nothing is known about the characteristics of *in vitro* grown *M. leprae,* I cannot conclude that the Honolulu strain is not identical with the etiologic agent of leprosy. While we are certainly not yet convinced that the strain is identical with *M. leprae,* I am inclined to reserve some free space for *M. leprae* on the pages of the classification for mycobacteria probably in the group of the scoto-chromogenes close to *M. scrofulaceum*.

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