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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.

M. leprae Versus *M. scrofulaceum*

TO THE EDITOR:

In response to the report from Dr. Pattyn as referred to in the letter from Dr. Kato (IJL **44** [1976] 385-386) and in elaboration of Dr. Kato's polemic, the following observations would seem to be of significance.

The determinations reported by S. R. Pattyn relative to our cultured isolate HI-75 are of significance and are supported by some observations of our own. The differentiation of *M. leprae* in culture from other mycobacteria, notably those commonly designated *M. scrofulaceum,* may, however not be as easy as implied.

Our initial isolates, in phosphate buffer, from human biopsy tissues were plated directly on Wallenstein, Ogawa egg yolk, Tarshis, MacConkey, blood agar and eosin-methylene blue media. There was no growth from the isolates reported (IJL **43** [1975] 192-203). These isolates all grew in primary and in subcultures in LA-3 medium. We, of course, also had other isolates of mycobacteria which did not have the immunofluorescent FITC characterization described (IJL **43** [1975] 204-209) and which grew on some of the standard media listed. We also had an acid-fast contaminant in one isolate, to the presence of which we were first alerted by the FITC determination.

Subsequently, successive generations of the reported isolates were periodically reinoculated directly from the LA-3 cultures to the standard media. Some of these, particularly with heavy inoculations, now grew on standard media, notably Wallenstein's. However, when subcultures were made from these growths to the standard media there were no growths. Prior to developing the LA-3 medium we had, over a period of many weeks, attempted to grow leprosy isolates on various standard mycobacterial media flooded with hyaluronic acid, suspended in hyaluronic acid, or having hyaluronic acid incorporated in them. There had been growth of some isolates under these conditions where there was no growth on control plates. The growth was limited and we had been unable to maintain and propagate them. Efforts were then made, which went through three phases, to develop a suitable medium and which resulted in the LA-3 medium. On the basis of these experiences determinations were made which led to the conclusion that the initial growth on the standard media from direct LA-3 inoculations resulted from carry-over of LA-3 medium with the inocula.

This characteristic held true for a third generation LA-3 subculture three months after first isolated. Direct inoculation with culture in LA-3 medium to Wallenstein medium yielded growth in three weeks but subculture to the same medium yielded no growth though incubated for five weeks. When HI-75 had been cultivated through 11 transfers in eight months, adaptation to cultivation was evident. There was growth on Wallenstein medium in two weeks following direct inoculation from LA-3 culture. Subsequently 4 successive Wallenstein culture to Wallenstein plate transfers at two week intervals have all presented colony growth.

HI-75 has now been intensively cultivated in LA-3 medium for 12 months. During this year HI-75 has maintained its characteristic reaction with LL serum, FITC conjugated antibody—a characteristic not shared by the strains of *"M. scrofulaceum"* studied. This has been confirmed and substantiated by a markedly improved technic and is supported by the findings of a recently developed complement fixation technic (IJL **44** [1976] 301-314). Two different pooled serum preparations have given the same results. Pattyn ignores, and does not report attempting, the FITC determinations. Since bacilli directly isolated from LL patients, and since bacilli in frozen sections of LL skin biopsies have

shown the same immunofluorescent response, it is not valid to assume that, "the organism isolated . . . is entirely different from the etiologic agent of leprosy."

In a small series of five TT-BT leprosy patients recently available to us, we found that a Mitsuda type antigen, prepared from an eight week old culture of HI-75 according to WHO lepromin standards, elicited gross, raised, erythematous and indurated reactions paralleling, at 21 and 28 days, those called forth by a concomitantly given Mitsuda type lepromin prepared by the Instituto de Leprologia, Rio de Janeiro, Brazil (Partida 85). Nine bacillary positive LL patients presented no visible response to either antigen. There was, however, in each LL subject a deep-lying small induration at the site of HI-75 injection only. These determinations were observed and concurred in by an experienced leprologist not a member of our laboratory. We judged these to be in the range usually regarded as a negative lepromin reaction and tentatively concluded that this difference in response to the two antigens might indicate stronger antigenicity on the part of the HI-75 antigen. There are obvious differences in problems of standardization which will have to be worked out between the usual lepromin and lepromin prepared from actively growing cultures where bacillary size differences and possible degenerative effect of host cells on biopsied bacilli will have to be considered. Counting bacilli will not suffice. There was no visible or palpable reaction in any of these 14 Chinese patients to 0.1 ml full strength LA-3 medium similarly inoculated. While we do not claim that this is an adequate or definitive series, we are not aware that antigen preparations from mycobacteria commonly called *M. scrofulaceum* have this characteristic even for small groups of patients.

Pattyn's concluding sentence may be misleading if not carefully read since it implies, on the basis of analogous reasoning, that HI-75 presents a mouse foot pad inoculation response incompatible with that of *M. leprae,* and this is adduced as an argument against its identity with *M. leprae*. It would seem that in the 26 days elapsing between receipt of our HI-75 culture and the appearance of Pattyn's memo in the LSM office there was not time for this determination to have been performed. No mouse foot pad studies with this culture have been reported.

Mouse foot pad studies are in process. At up to eight months, washed HI-75 inocula have not presented the pattern of response described by Pattyn for *M. scrofulaceum* (Ann. Inst. Pasteur **109** [1965] 309-313), but have given every appearance of similarity with that described for *M. leprae* isolated directly from human biopsies save, as might be expected, that there seems to be earlier and more rapid proliferation of bacilli. It is noteworthy that mouse foot pad inoculations should be with washed bacilli and not in LA-3 medium. In the latter instance, though there is no so-called "fat" foot pad, there may be earlier dissemination to peritoneum and viscera. This is compatible with our earlier report of the stimulative effect of hyaluronic acid on the growth of *M. leprae,* isolated directly from the human host, in mice (IJL **43** [1975] 1-13) and as subsequently found to be the case in a more extended, but as yet unreported study.

We have been pursuing a series of comparative studies on the patterns of mycobacterial growth, including those of *"M. scrofulaceum"* and HI-75, in LA-3 medium. These include varied and extensive transmission and scanning EM observations. While not ready for publication, indications are that these are helpful in mycobacterial differentiation and we tentatively think that we can also in this manner differentiate HI-75 from isolates commonly called *M. scrofulaceum*. The problem is that mycobacteria designated as *M. scrofulaceum* show considerable heterogeneity, as nicely illustrated by Jenkins, Marks and Schaefer (Tubercle **53** [1972] 118-127), and differentiation from one or two strains by such cultural studies alone may not be valid for strains isolated from variant sources. This is the line of reasoning which led us over a year ago to opt for the development of an immunologic identification technic for use in identifying *M. leprae* culture and in monitoring such cultures regularly.

Pattyn's determinations are valuable, are supportive of those made by Laszlo Kato and Edith Mankiewitz, and strengthen their suggestion that HI-75 (perhaps as *M. leprae*) and *M. leprae* have a taxonomic relationship with *M. scrofulaceum*. Within this concept HI-75 (apparently *M. leprae*) and *M. leprae* may be *M. scrofulaceum,* but *M. scrofulaceum* in the sense conveyed by the conclusions in Pattyn's report—we think not.

Runyon (Tubercle **55** [1974] 235-240), rather than specifying *M. scrofulaceum,* used the terms *"scrofulaceum complex"* or *"M. avium-scrofulaceum* complex" and suggested that a fluorescent antibody "may be useful for diagnosis of leprosy in the future."

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