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 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.

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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 deep­lying small induration at the site of HI-75 injection only. These determinations were ob­erved 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 [1963] 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 "flat" 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 (J.I. 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 *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 Lauzo Kato and Edith Mankiewitz, and strengthen their sug­ gestion 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 conceived 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." — Olaf K. Skinsnes

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Study of Subcutaneous Fat in Leprosy Patients

To The Editor:

A. Carayon in a recent paper (1) pointed out the high proportion of serious and contagious forms of leprosy, serious and widespread nerve damage, a very high percentage of serious and bilateral facial paralysis, and the high proportion of an intractable type of ulnar palsy at the wrist without nerve damage at the elbow in leprosy patients in Iran. He stated that the cold weather and nutrition are responsible for such epidemiologic and clinical findings.

I also examined many of these patients and fully agree with the concepts of A. Carayon who believes that, "the lipid balance seems to be an active factor to prevent the transmission of leprosy and that malnutrition interferes in the spread of leprosy." He also quoted that, "the various lipid diets in the northern and southern provinces of Iran may explain the differences in the spread of leprosy" and the "hypothesis is advanced that more than cold, variations in lipid diet in the north and south may explain the high prevalence in the north and the low in the south. Chemical studies of subcutaneous fat of the two groups are necessary to bring scientific proof and this study would be a good goal for the next year's program."

In regards to the last point, i.e., the study of subcutaneous fat, I indicated the importance of this work in many of my publications which I summarized recently (2). In this paper I discussed my concept on the relationship between pathogenesis of leprosy and autooxidation of lipids, peroxidation, antioxidants, tocopherols, unsaturated fatty acids, subcutaneous fat, as well as the biological antioxidant activity of aminodiphenylsulfone, and the growth of *M. leprae* in animals with prooxidant diets (low in vitamin E and high in unsaturated fatty acids).

In summary, I believe that the study of subcutaneous fat recognized by Carayon and myself should be done in order to complete the study of the pathogenesis of leprosy.

— Meny Bergel
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REFERENCES
1. CARAYON, A. The effects of cold weather and nutrition in the spread and the afflictions to leprosy. Seminar on Evaluation of Leprosy, 21-23 June 1976, Tehran, Iran.

Leprous Myositis

To The Editor:

This is with reference to the paper entitled "A Histopathologic Study of Striated Muscle Biopsies in Leprosy" by J. C. Gupta et al., published in the JIE 43 (1975) 348-355.

I am a little disturbed by this paper which includes unclear and incorrect muscle pathology, and fails to take note of one earlier paper on this subject which described most of the changes that occur in the muscle and the neuromuscular endings in both tuberculous and lepromatous leprosy.

While the authors have described and tabulated a lot of histopathological "changes," many of these are nonspecific and in fact frequent end-results of myopathies and denervation atrophies. Thus, in Figure 1, "Intramyosial granulomas extending along