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Letters to the Editor

Rapid Identification Tests for *Mycobacterium leprae*: A Clarification

I reported earlier a method for "A Rapid Identification Test for *Mycobacterium leprae*" (Prabhakaran, 1973). The method involved incubating a drop of the bacterial suspension with a drop of dopa (3,4-dihydroxyphenylalanine) solution on a slide. Of over two dozen species of mycobacteria tested in our earlier studies, only *Myco. leprae* was found to convert dopa to a pigmented product *in vitro*. Since the publication of the report, inquiries have been received asking for more details of the procedure, especially regarding the amount of bacilli used. In our experiments, the bacterial suspensions usually contain 1.0×10^9 organisms/ml. Sometimes, suspensions with 1.0×10^{10} bacilli are also used. It is important that, when different species of mycobacteria are tested, the number of organisms in all the suspensions is kept the same, so that photomicrographs taken will show the distinction between the enzymic and the non-enzymic oxidation of dopa. We enumerate the bacilli by the Hanks-Lechat-Chatterjee method, modified by Kirchheimer. It is essential that the spots on the slides are of approximately the same diameter and that they are not allowed to dry up. The bacterial suspensions we use are free of visible tissue debris and have occasionally been treated with alkali, ether or acetone. The success of the method depends on the condition of the bacilli as well. We have shown that bacilli separated from tissues of armadillos infected with *Myco. leprae* oxidize D-dopa to melanin. When the organisms were obtained from autopsy material, the dopa oxidase activity was rather low and inconsistent, indicating that the enzyme is labile. If the animal had been dead for a long time, the bacteria contained very low levels of the enzyme. However, the organisms from biopsy material gave good results (Fig. 1). The biopsies were fresh or frozen.

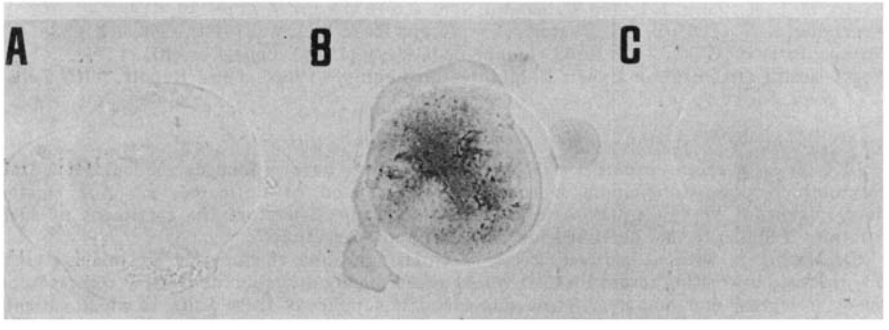

Fig. 1. A. Bacilli (Armadillo). B. Bacilli + D – Dopa. C. D – Dopa.

It has been reported (Convit and Pinardi, 1972) that when smears of *Myco. leprae* or tissue sections containing *Myco. leprae* are treated for 2 h with pyridine at room temperature, the bacilli lose their acid-fast staining property; several other mycobacteria tested retained their ability to stain with carbolfuchsin. The results were interpreted to suggest that in *Myco. leprae,* pyridine removes some component essential for Ziehl-Neelsen staining. This might well be so. It is also likely that the property is correlated with the *o*-diphenoloxidase of *Myco. leprae.* This enzyme activity has not been detected in other mycobacteria. The *o*-diphenoloxidase of the leprosy bacillus is non-specific and it oxidizes, besides dopa, a variety of other substrates. One of these substrates is mimosine. This compound has a pyridine ring instead of the benzene ring, although the alanine side chains are the same in both dopa and mimosine (Prabhakaran *et al.*, 1972). Mammalian and plant "tyrosinases" were inhibited by mimosine. Our observations suggest that *Myco. leprae* can bind pyridine, while other mycobacteria do not. The phenol in carbol fuchsin probably enables the dye to penetrate the bacterial cell. Since *Myco. leprae* can bind pyridine, pretreatment with pyridine would interfere with penetration of the dye into the leprosy organisms, and as such the bacilli fail to stain with carbol-fuchsin.

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The Terminological Question

In reference to the terminological question, I was pleased to read (*Lepr. Rev.* (1973) **44**, 94) that Dr Skinsnes considers my point about the situation in Brazil being different from Hawaii in many respects, "perhaps well taken." Unfortunately, it has never been easy for minorities to have their opinions accepted, so that I was not surprised when he added that "indeed, this is one reason that one wonders at the effort to change world-wide practice in order to achieve a social and cultural change in Brazil"—to which I must add a much needed prophylactical progress as well.

Our future would perhaps appear less gloomy if we could think of "leprosy" ("lepra", etc.) not quite as a "world-wide practice", but only as a word with ancient pejorative overtones in the English and Romance languages, which could be abandoned with a bit of goodwill. Also, when it is realised that Brazil is far from being the only victim of the complex "Leprosy, the Word, the Disease," (*Lepr. Rev.* (1972) **43**, 96) other countries might eventually join in our fight as well.

Studies conducted in the U.S. by Rolston and Chesteen (which concluded that "leprosy" is "the most negative of all medical terms") and at Carville, U.S., by Pearson, as well as by Mangiaterra in Argentina, should convince Dr Skinsnes that the movement to eradicate the term "leprosy" ought not to be brushed aside as pure "emotionalism". This is further emphasized by the fact that the new terminology has been recommended by three Brazilian congresses, accepted by the Brazilian *Nomenclatura Dermatologica* (Rabello) and *Nomina Dermatologica* (Gaspar and Gaspar), put into practice by six state public health services and about 40 medical schools in 12 states.

Denying that he was a "determined opponent" of the term "Hansen's disease" in Hawaii, Dr Skinsnes states that "Dr Rotberg clearly did not make a reasonable 'search of the literature' as he implies". Clarifying my controversial point 8, what I meant was that a determined opposition did exist *in Hawaii,* well known to all who keep up with the literature. I cited Dr Skinsnes because I thought of him as a good representative of that opposition: in three of the five articles of his series "Leprosy in Society" (*Lepr. Rev.* (1964) **35**, 175–(1968) **39**, 222–*Int. J. Lepr.* (1970) **38**, 294) the ancient movement against the appelation "leprosy" is criticized. The pamphlet he sent to me was just one example, but it did not actually provide me with any new information on this matter, as it practically repeated what I had read years ago in *Lepr. Rev.* (1964) **35**, 175. Furthermore, his antagonism to a new terminology continues even after the conclusion of the activities of the Hawaiian Citizen's Committee: Dr Skinsnes' letter to the *Far East Medical Journal* ((1971) **9**,307) of south-east Asia, opposing the highly favourable opinion of Dr Mallac, of Geneva, in regard to the Brazilian changes (*Far East Med. J.* (1971) **7**, 108), contributed to my appraisal of his position. According to Dr Skinsnes I overestimated his role, but the essence of point 8 remains: a determined opposition in Hawaii, in contrast with the present wide acceptance of the new terminology "hanseniasis" in Brazil, might have been one of the causes of the non-success of the Hawaiian experiment with "Hansen's disease".

In response to an appeal by 117 signatories from 15 countries, the Council of the International Leprosy Association decided at Bergen (1973) that each country may adopt their preferred terminology. This is not going to be of great value to us as long as the powerful English and French literature conserves the ancient word, but is at least an acknowledgment of the inconvenience of the term "leprosy" in some areas. We are hoping that this new policy of the I.L.A. will be accepted by its members, and we will be counting on Dr Skinsnes' prestige and influence to help us in this respect.

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