Lepr. Rev. (1977) **48**, 49–52

Letters to the Editor

Confirmation of the Spot Test for the Identification of *Mycobacterium leprae* and Occurrence of Tissue Inhibitors of DOPA Oxidation

We reported previously a spot test using D-dopa for the identification of *Myobacterium leprae* (Prabhakaran, 1973, 1974). In these reports, no controls using heat-inactivated *M. leprae* were given. Results obtained with both heated and unheated bacilli are presented in this communication. Some recent observations on the occurrence inhibitors in armadillo tissues which interfere with oxidation of dopa by the bacilli are also discussed.

SPOT TEST

Suspensions of *M. leprae* were prepared from the liver of an experimentally infected armadillo. The preparation contained $2.0 \pm 0.12 \times 10^{10}$ bacilli/ml. Part of the bacterial suspension was heated at 100° C for 30 min. $Na_2HPO_4 - KH_2PO_4$ buffer (0.5 M, pH 6.8) and D-dopa solution (0.01 M) were used in the reaction. The dopa solution may be also made up in the buffer.

In Fig. 1, spot 1 contained a drop (approximately 0.05 ml) of bacterial suspension and a drop each of buffer and dopa solution; spot 2 contained a drop of the bacilli and 2 drops of buffer; and spot 3 a drop of dopa and 2 drops of buffer. In the upper spots unheated bacilli were used and in the lower spots the heated bacilli. The reaction was started at 4 p.m. and left overnight at room temperature (25° C). The photographs were made the following day at about 8 a.m.

It is evident that the bacilli oxidized D-dopa giving rise to melanin pigment. Under identical experimental conditions, the heated bacilli with dopa and dopa by itself show little colour development. The results clearly distinguish between the enzymatic and the non-enzymatic conversion of dopa to pigment. Similar tests were done with a cultivable mycobacterium, *M. phlei* (grown on Proskauer-Beck medium, Youman's modification) and 2 unidentified strains of mycobacteria separated from the skin and liver tissues of 2 species of mammals. (The tissues were received from elsewhere.) *M. phlei* and the organisms separated from the infected tissues showed no reaction with dopa.

TISSUE INHIBITORS

Recently, it was observed that suspensions of *M. leprae* separated from the liver tissues of some armadillos (especially those that were infected intravenously with heavy inocula of the bacteria) contained inhibitors which interfered with dopa oxidation by the bacilli. At the same time, organisms separated from other tissues like the spleen of the animals oxidized dopa. Some bacterial preparations from fresh liver (not kept frozen) which failed to oxidize dopa had a greenish tinge, probably indicating the presence of bile pigments. It is important that tissue

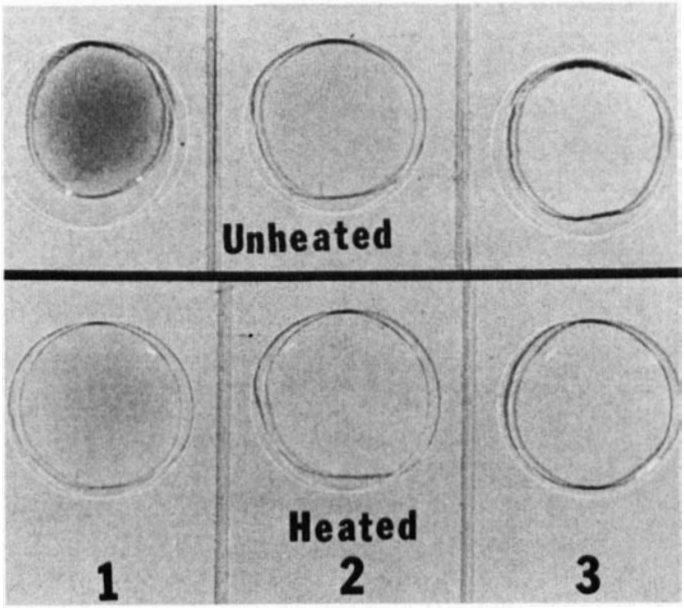

Fig. 1. Upper spots: unheated bacilli; lower spots: heated bacilli. 1, bacilli + D-dopa; 2, bacilli; 3, D-dopa.

inhibitors be excluded from *M. leprae* preparations before testing them for dopa oxidation. We obtain concentrates of *M. leprae* from infected organs by differential and density-gradient centrifugation in solutions of sucrose and KCl; further purification is achieved by treatment with trypsin, acetone and ether or dilute alkali.

USPHS Hospital,
Carville, Louisiana 70721,
U.S.A.

K. PRABHAKARAN, E. B. HARRIS
and W. F. KIRCHHEIMER

References

Prabhakaran, K. (1973). A rapid identification test for *Mycobacterium leprae. Int. J. Lepr.* **41**, 121.

Prabhakaran, K. (1974). Rapid identification test for *Mycobacterium leprae:* a clarification. *Lepr. Rev.* **45**, 342.

Note added in proof When the dopa oxidase reaction is used for the indentification of presumed *M. leprae* cultures, false positive results may be obtained, if the organisms are not thoroughly washed to remove the components of the culture media. This is best done by growing the bacilli, whenever possible, in liquid media.

Primary Sulphone Resistance

Several thousand leprosy patients live at the Agua de Dios Sanatorium, Columbia, in close contact with an even greater number of healthy people. Sulphone resistance, ascribed to irregular treatment, is believed to occur quite frequently among these patients, and is considered as such on clinical and histological grounds (secondary histoid leprosy); evolutionary grounds (lepromatous reactivation in spite of sulphone treatment); and bacilloscopic grounds (the reappearance of solid staining bacilli in patients under sulphone treatment). A case of what is believed to be primary sulphone resistance is reported here.

CLINICAL HISTORY

The patient P. B. (female), was born in this environment 33 years ago and lived here for 14 years. In August 1968 she came for consultation as a result of the appearance in the elbow area of an anaesthetic, erythematous and infiltrative plaque. Smears were made from the lesion, the nasal septum, the right elbow, ear lobe and knee. With the exception of the nasal septum all the samples were positive for AFB, with globi and solid staining bacilli. The lepromin reaction (Mitsuda) was negative.

Treatment was started with dapsone at a dose of 300 mg/week. In January 1969 the original lesion remained unchanged and a similar lesion 6 cm in diameter had appeared on the right leg. The same treatment was continued, and in June 1970 erythematous spots appeared on both thighs, with smears continuing positive both in skin and in nasal mucosa. Sulphone treatment was however persisted with until July 1971 (35 months), when severe clinical deterioration was apparent, characterized by extensive lepromatous infiltration of the face and right ear lobe, perforation of the nasal septum, and numerous large areas of iron hard purplish infiltration on the torso and limbs. Sulphone resistance was suspected and the treatment was changed to Ciba 1906 (1.5 g/day). The patient in fact took only 500 mg daily, notwithstanding which clinical improvement was noticeable 2 months later, and in 6 months had become marked, with bacilloscopy of the nose negative, and though skin smears remained positive, only granular bacilli were seen. In October 1972, i.e. 15 months after treatment with Ciba 1906 was started, only a small iron-like spot persisted at the site of the original lesion, the nasal mucus continued negative and only occasional granular bacilli were found in the skin. At that time she was mistakenly put back again on dapsone treatment on a dose of 300 mg/week and this was continued until May 1973, at which time the patient again presented extensive lepromatous infiltration of the face, infiltrated plaques in the lumbar areas, arms and legs, and the reappearance of solid bacilli in skin smears.

The patient had been continuously regular in taking treatment, and these facts strongly support the suggestion that she was suffering from lepromatous leprosy caused by a sulphone-resistance mutant strain of *Mycobacterium leprae,* acquired at the Agua de Dios Sanatorium, where there is strong evidence of many cases of sulphone resistance.

FABIO LONDOÑO

Centro Dermatológico,
Federico Lleras Acosta,
Bogota, Colombia,
S. America

Acid-fast Bacilli in the Fingers of Long-treated Lepromatous Patients

May I be permitted to offer some comments on the conclusion reached by the authors in the final paragraph of their paper entitled "Acid-fast bacilli in the fingers of long-treated lepromatous patients" (*Lepr. Rev.* (1976) **47**, 93). This paragraph reads as follows:

> "The fingers are yet another possible site of the persister bacilli that may be responsible for relapse after prolonged therapy. The public health importance of bacilli in the fingers is difficult to evaluate, but the facts that solid forms are so often present there after they have disappeared from all other skin sites and the nose, and that fingers are one of the most likely sites for skin to skin contact, are hazards that cannot be overlooked."

In my view the presence of "persister bacilli" in the fingers of such patients cannot possibly constitute a public health hazard. The concept of skin to skin transmission has been scientifically challenged and strong evidence adduced that leprosy bacilli seldom emerge, if ever, from intact lepromatous skin (Pedley, 1970*a*,*b*). In view of this, can anyone seriously believe that bacilli lurking in the finger (perhaps in Pacinian corpuscles—mentioned by the authors) would be able to work their way to the surface of the skin of the finger pulp—there to emerge in viable form, and thus constitute a public health hazard? To my mind this highly speculative suggestion is fanciful in the extreme, and, because of the stigma which attaches to the disease (Pedley, 1972), is an unfortunate conclusion to an otherwise interesting paper.

33 Aldwick Crescent,
Findon Valley,
Worthing,
Sussex BN14 0AS,
England

J. C. PEDLEY

References

Pedley, J. C. (1970*a*). Composite skin contact smears: A method of demonstrating the non-emergence of *M. leprae* from intact lepromatous skin. *Lepr. Rev.* **41**, 31.

Pedley, J. C. (1970*b*). Summary of the results of a search of the skin surface for *M. leprae*. *Lepr. Rev.* **41**, 167.

Pedley, J. C. (1972). The stigma of leprosy—in four countries. *Lepr. Rev.* **43**, 94.