Lepr Rev (1980) **51**, 359–362

Letters to the Editor

QUANTITATIVE STUDY OF THE SEPARATION OF *MYCOBACTERIUM LEPRAE* FROM ARMADILLO TISSUE

Sir,

Studies using relatively large quantities of *Mycobacterium leprae* at present depend on concentrates of the bacillus separated from lepromatous tissues of experimentally infected armadillos. Since the infected tissues are available only in limited amounts, it is important to recover as much bacilli as possible from them. For metabolic studies, *M. leprae* has to be obtained in a state involving the least damage to its enzymatic activities. For other purposes like the preparation of purified proteins or other fractions, maximum recovery of the organisms would be the main consideration.

In this report the yield of *M. leprae* from 2.0 g samples of liver tissue, obtained from an armadillo experimentally infected with *M. leprae*, was compared using two different separation techniques: (i) differential and density-gradient centrifugation of the tissue homogenate in sucrose and KCl solutions as previously reported by Prabhakaran *et al.*[1] and (ii) a modification of the chloroform extraction procedure introduced by Dharmendra.[2]

The chloroform extraction procedure was done as follows: 2.0 g of liver tissue was homogenized in 20 ml of 0.85% NaCl using a Braun Model Potter S homogenizer. The resulting homogenate was centrifuged at 24,500 × *g* for 30 minutes in a refrigerated centrifuge and the supernatant discarded. The residue fraction consisting of *M. leprae* and cell debris was transferred to a large mortar and the bacilli extracted with five, 10 ml aliquots of chloroform. All of the chloroform extracts were pooled in a large beaker kept on wet ice.

The chloroform was removed by evaporation under a stream of cool air, leaving a residue of bacilli and lipids. The lipids were removed by suspending the residue in 0.1 N NaOH and centrifuging at 24,500 × *g* for 30 minutes. The pellet consisting of acid-fast organisms was washed by resuspension in 20 ml of water and centrifugation at 24,500 × *g* for 30 minutes. The washed pellet was resuspended in 2.0 ml water and the bacilli enumerated by the method of Hanks *et al.*.[3]

Ether can be used instead of NaOH for extracting the lipid material, and may be desirable when preparing antigenic fractions; however, it was difficult to prepare a homogenous suspension from the resultant powder, for bacterial enumeration.

A comparison of the chloroform-extracted tissue residues and the residues normally discarded when using the sucrose–KCl method showed considerably fewer acid-fast rods remaining in the former. Data on the percentage of organisms recovered by the two procedures are given in Table 1. The percentage recovery is based upon bacterial counts in tissue samples taken before processing. We also observed that chloroform extraction after saline washing resulted in the removal of almost all the remaining bacilli in the residue fraction that is discarded from the sucrose–KCl procedure.

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TABLE 1. Percentage recovery of *M. leprae* from armadillo tissue

Separation method	Experiment			
	1	2	3	$\bar{X}$
Density-gradient centrifugation	26.9	32.7	36.5	32.0
Chloroform extraction	67.3	63.5	63.5	64.8

REFERENCES

1 Prabhakaran K, Harris EB, Kirchheimer WF. Binding of ^{14}C-labeled dopa by *Mycobacteriun leprae in vitro*. *Int J Lepr,* 1976, **44**, 58–64.

2 Dharmendra. Studies of the lepromin test: (5) the active principle of lepromin is a protein antigen of the bacillus. *Lepr India,* 1941, **13**, 89–103.

3 Hanks JH, Chatterjee BR, Lechat MF. A guide to the counting of mycobacteria in clinical and experimental material. *Int J Lepr,* 1964, **32**, 156–67.

ADVANCE NOTICE OF SEMINARS, INTERNATIONAL MEETINGS AND CONFERENCES ON LEPROSY

Sir,

We receive *Leprosy Review* regularly and find it a valuable way to keep in touch. Some of the excellent accounts of international meetings which have taken place prompts me to ask if it would be possible for the Editorial Board to consider the regular publication of forthcoming events in the international field, preferably very well in advance of the relevant date, together with details of application for attendance and cost, etc. I understand that plans are often made for these meetings at least one year in advance: their earliest publication in your journal would be appreciated, especially by those working in remote areas.

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DOES CLOFAZIMINE HAVE ANY VALUE IN THE MANAGEMENT OF REVERSAL REACTION?

Sir,

I refer to the letter from W F Ross, *Leprosy Review* **51** (March 1980), 92–3. On 7 May 1980 I returned a questionnaire on Clofazimine to Drs W Vischer and O de S Pinto, Geigy, Basle.

In a separate letter I summarized my comments on Clofazimine. I quote from the letter:

> As for B.663 being anti-inflammatory in borderline leprosy reactions I must say that clinical evidence has not proved this.
>
> I have tried B.663 alone in borderline reactions (cell-mediated immunity reactions), both downgrading and reversal, and could not control the reaction without giving corticosteroids as well.
>
> I have tried to increase and reduce the dosage of B.663 and found that 300 mg B.663 weekly given *with* corticosteroids controls the cell-mediated reaction, but the steroids really are effective and not the B.663. The latter is merely given then as an anti-mycobacterial drug.

I do not think B.663 has an anti-flammatory effect in cell-mediated immunity reactions.

I agree with Ross. The alleged effectiveness of clofazimine in cell-mediated reactions is a myth.

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THE SFG (SOLID, FRAGMENTED, GRANULAR) INDEX FOR BACTERIAL MORPHOLOGY

Sir,

We have recently been looking with renewed interest into the various methods which have been described for assessing the morphology of leprosy bacilli in stained smears, using classic Ziehl–Nielsen techniques. In talking to our laboratory technicians, and in the process of checking and supervising their work, we have been impressed with the fact that several of them have difficulty in consistently distinguishing between solid, and non-solid staining bacilli, as in the Morphological Index (MI), whereas they find it easier and quicker to assess a slide using the SFG, as described by Ridley in 1971 (*Leprosy Review* **42**, 96–7). In fact this article begins by saying that the MI is unnecessarily time-consuming on the part of the technician.

We have also been impressed by what seems to be a clear correlation between an increasing percentage of granular bacilli and the progress of regular treatment. Our purpose in writing this letter is to ask if other workers have adopted the SFG index? Should it perhaps be tried out more systematically in assessing the effect of treatment in the field?

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P A M SCHREUDER
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LEPROSY: SOCIO-ECONOMIC CONDITIONS AND PRIMARY HEALTH CARE

Sir,

Your reference to Horst Buchmann's publication struck an interesting chord with me. I have been watching the progress of leprosy in this part of the world with interest since 1951, when I started the 'Centre de Salud Menonita, Km 81' in Paraguay. There was an effort to raise the socio-economic level in Paraguay – the Alliance for Progress – during this time, which if it had been successful would have shown this effect that Buchmann points out. This programme did not work out successfully. Now that WHO proposes Dr Buchmann's approach, I find great hopes for improvement in the control of leprosy. As is known by all, control work of leprosy in the world today is not getting anywhere. Drs Jacobson and Hastings in their article in *The Star* (Vol. 39, No. 3) on Hansen's Disease Control state in their conclusion: 'but drastic improvements in this area are unlikely during the 1980s unless a major breakthrough in terms of vaccine development or therapy were to occur'.

It may be of interest to record that we have records of Mennonite refugees who came from Russia, from areas where there was no leprosy, but seven persons developed the disease here in Paraguay within the first 20 years, in spite of not having much contact with the Paraguayan population. None, however, have shown up with it in the last 25 years, in spite of the fact that they have had much more contact with the Paraguayans than they did

the first 20 years. The one main difference that is associated with this is the improved hygiene and standard of living. This same thing has been born out in many places in the world in the past.

For those who are afflicted with Hansen's disease, we must obviously pursue conventional drug and other treatment. For a more meaningful control programme, I contend that our best approach would be to improve socio-economic conditions for those who are at risk, and the Primary Health Care approach so ably described by Horst Buchmann.

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