An *in vitro* test using cholesterol metabolism of macrophages to determine drug sensitivity and resistance of *Mycobacterium leprae*

ISHWARI NAIR and P. R. MAHADEVAN
The Foundation for Medical Research, 84-A, R. G. Thadani Marg, Worli, Bombay 400 018, India

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**Abstract.** Macrophages that have ingested live *Mycobacterium leprae* show a preferential accumulation of cholesterol ester. Such an accumulation is not seen, on the ingestion of dead bacteria. Among the macrophages that ingest live *Mycobacterium leprae*, the presence of dapsone or rifampicin prevents largely the alteration in the anticipated increase in the cholesterol ester indicating the sensitivity of the bacteria to the drug. In the small number of relapsed patients, the presence of dapsone did not reduce the cholesterol ester increase, suggesting that the *Mycobacterium leprae* present are either resistant or escaped detection. The method provides a rapid drug screening system for anti-*Mycobacterium leprae* activity of known and unknown compounds.

**Keywords.** *Mycobacterium leprae*; cholesterol; cholesterol ester; macrophage; drug sensitivity/resistance.

**Introduction**

The screening of antimicrobials for anti leprosy activity has not been extensively undertaken. The mouse foot-pad growth technique with *Mycobacterium leprae* is claimed to be a well defined and reliable technique to test the viability and growth of *M. leprae* (Shepard, 1960). The same technique can be used to find out whether the *M. leprae* are susceptible to a drug or not by determining the growth in the foot-pad of mice, fed on a diet containing the drug (Shepard, 1967). This method has been refined and made more sensitive using thymectomised mice (Rees, 1966) or genetically bred nude mice (nu/nu) (Kohsaka *et al.*, 1978). However all these test systems need a minimum of 6-8 months even before preliminary assessment could be made. In view of this there is an urgent need for a rapid test system to determine drug sensitivity/resistance of *M. leprae*. The major limitation that has to be overcome with *M. leprae* is our inability to grow it *in vitro*, on any of the currently available bacteriological media in a reasonably short time.

We describe here a method for testing viability that could also be used for drug sensitivity testing of *M. leprae*, when the bacteria are resident in macrophages. The basic reaction monitored in this test system, is the influence that only live *M. leprae* exert on the cholesterol metabolism of the macrophages (Ishwari Kurup and

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Abbreviations used: DDS, Dapsone (di-(4-aminophenyl)-sulphone; DOPA, dihydroxyphenylalanine.
leading to the formation of cholesterol ester from cholesterol taken up, as compared with macrophages with no live *M. leprae* inside. The present paper describes the adaptation of this observation for the development of a suitable and rapid drug sensitivity test that can even be used in an anti-leprosy drug screening programme.

**Materials and methods**

*Mycobacterium leprae* were obtained from lepromatous tissue of bacillary positive, untreated patients or those under varying periods of chemotherapy. Bacillary suspensions were prepared (Ambrose *et al.*, 1974) and shown to be acid-fast and free from other contaminating bacteria. These fail to grow in normal mycobacteriological media. The bacilli were counted and $5 \times 10^6$ bacilli were added to each Leighton tube containing cultured macrophages. *M. leprae* obtained from infected armadillo tissue (supplied by Dr. E. Storrs, Florida, USA) were also used in some of the experiments.

**Macrophage cultures**

Macrophages from Swiss white mice were obtained from the peritoneal cavity following injection of 5 ml Eagle’s minimal essential medium +20% inactivated human serum (AB blood group) into the cavity, after killing the animal by cervical dislocation immediately after the injection of the medium. The peritoneal fluid was collected after agitating the cavity, and 0.7 ml of the fluid was added to each Leighton tube. The macrophages obtained from the peritoneal fluid adhered to the Leighton tubes. The medium was changed every 24 h; to remove non adherent cells. After 3 days of such culturing, esterase positive phagocytic cells, were predominantly distributed as an uniform layer at the flat bottom of Leighton tubes. There were no contaminating neutrophils, and non-adherent lymphocytes were not present in any significant numbers. Such tubes were divided into 5 sets. One set received viable *M. leprae* ($5 \times 10^6$/Leighton tube) either from human biopsies or armadillo and another heat-killed *M. leprae* ($5 \times 10^6$/Leighton tube). The control sets did not receive the *M. leprae* inoculum. The level of *M. leprae* to be added had earlier been confirmed as a good dose to elicit clear response from the macrophages (Ishwari Kurup and Mahadevan 1982). After 24 h of phagocytosis of the bacteria, the excess bacilli were removed and the macrophages were incubated with $[^3H]$-cholesterol. The experiments were also carried using $[^{14}C]$-acetate as precursor. Some of the cultured macrophages were exposed to drugs like dapsone (di(4-aminophenyl)-sulphone (DDS) (Burroughs Wellcome Co., Bombay) at a concentration of 1 µg/Leighton tube (1 µg/0.7 ml of medium) or rifampicin (Sigma Chemicals Co., St. Louis, Missouri, USA) (10 µg/ml) for 72 h before *M. leprae* were added. Some of the control macrophages were not infected with *M. leprae* and used to study the effect of the drug alone and cholesterol uptake on lipid synthesis. In the drug treated cultures after 24 h of phagocytosis excess bacilli was washed off and the macrophages were labelled with $[^3H]$-cholesterol/$[^{14}C]$-acetate and left for 4 days for studying the uptake and synthesis of the lipids in the presence of the drug.

$[^3H]$-Cholesterol was obtained as crude tritiated product from Bhabha Atomic Research Centre, Bombay and purified by repeated thin layer chromatography and