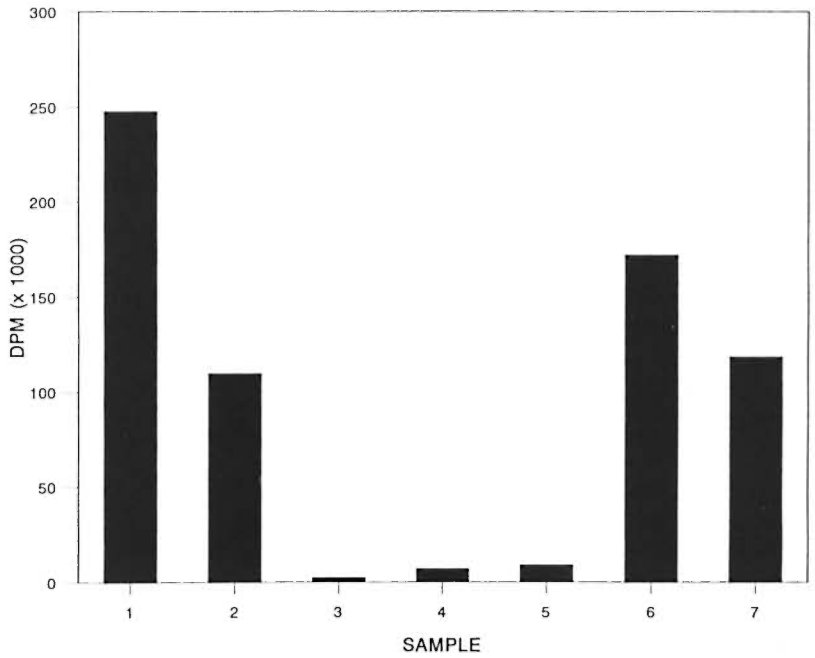
Radiometric Procedure for Detecting a Cultivable *Mycobacterium* in *Mycobacterium leprae*-Infected Armadillo Tissue

TO THE EDITOR:

For the past two decades, experimentally-infected armadillo tissues have been the principal source of *Mycobacterium leprae* for microbiological investigations. The results of earlier studies have revealed the presence of armadillo-derived mycobacteria (ADM) in some armadillos infected with *M. leprae* ([4, 5]). Contamination of purified *M. leprae* suspensions with ADM would undoubtedly interfere with any research exploiting the unique characteristics of *M. leprae*. In this communication, we describe a rapid radiometric procedure for detecting a mycobacterial species other than *M. leprae* in armadillo tissues.

Earlier *in vitro* studies have demonstrated the ability of *M. leprae* to significantly oxidize ^{14}C-palmitic acid when incubated in



THE FIGURE. Evolution of $^{14}CO_2$ from labelled substrate incubated with *M.leprae* and *M. avium* for 5 days under the following experimental conditions: 1 = 10^7 *M. avium*, in medium, + ^{14}C-acetate; 2 = 10^8 *M. leprae*, in medium, + ^{14}C-palmitate; 3 = 10^8 *M. leprae* + ^{14}C-acetate; 4 = 10^8 *M. leprae* + liver homogenate + ^{14}C-acetate; 5 = liver homogenate + ^{14}C-acetate; 6 = 10^7 *M. avium* + 10^8 *M. leprae* + liver homogenate + ^{14}C-acetate; 7 = 10^7 *M. avium* + liver homogenate + ^{14}C-acetate.

an axenic medium ([2]). However, previous data from our laboratory (unpublished observations) provided evidence that *M. leprae*, unlike most other organisms, does not utilize exogenous ^{14}C-acetate *in vitro* to any measurable degree for fatty acid synthesis or oxidation. Based on this finding, it was possible to add ^{14}C-acetate to armadillo tissue homogenates containing *M. leprae* or cultivable mycobacteria and compare acetate utilization in one particular reaction (e.g., oxidation to carbon dioxide).

Noncontaminated liver tissue was obtained from a naive armadillo and a 10% homogenate was prepared in Middlebrook 7H9 complete culture medium. *M. leprae* suspensions were prepared from experimentally infected, athymic nude mice as previously described ([3]). A strain of *M. avium*, previously isolated from armadillo tissue experimentally infected with *M. leprae*, was cultivated in medium as described above. Aliquots (1.0 ml) of liver homogenate were distributed into small screw-capped vials and inoculated with appropriate bacterial suspensions as outlined in The Figure. Control vials contained only culture medium in place of liver homogenate. Each vial was pulsed with 0.5 μCi of ^{14}C-acetate (58.2 mCi/mmole), capped loosely, and transferred to wide-mouthed scintillation vials. The viability of *M. leprae* was assessed in the control medium by incubating aliquots of the organism in the presence of 0.5 μCi ^{14}C-palmitic acid (850 mCi/mmole). The evolution of $^{14}CO_2$ was measured at suitable time intervals using a method described by Buddemeyer, *et al.* ([1]). Briefly, the $^{14}CO_2$ released was trapped on filter-paper strips previously soaked with a specially prepared liquid scintillation solution, air dried, and placed inside the counting vials. The vessels containing only *M. leprae* were incubated at 33°C; those containing *M. avium* were incubated at 37°C.

The data depicted in The Figure compare the release of $^{14}CO_2$ from radioactive substrate incubated in the presence of various combinations of armadillo homogenate, *M. leprae* and *M. avium* over a period of 5 days.

An increase in the release of $^{14}CO_2$ was observed in liver homogenate and medium incubated with *M. avium.*

A similar result was noted with homogenate incubated with a combination of *M. avium* and *M. leprae.* By contrast, the addition of *M. leprae* to the liver homogenate or *M. leprae* incubated in medium alone did not significantly alter $^{14}CO_2$ production. It was interesting to note a reduction in the amount of $^{14}CO_2$ released in the suspensions of liver homogenate containing *M. avium,* hypothetically, from substrate competition or inhibition by tissue components. Since significant amounts of $^{14}CO_2$ were released when *M. leprae* were inoculated in the presence of ^{14}C-palmitate, we may conclude that the bacilli were metabolically active.

The outcome of this study is consistent with our previous observation on the apparent inability of *M. leprae* to utilize exogenous ^{14}C-acetate. We have already emphasized the importance of obtaining armadillo-derived *M. leprae* free from contamination by other mycobacteria. By exploiting the failure of *M. leprae* to actively metabolize ^{14}C-acetate under these experimental conditions, we present a possible method for rapidly distinguishing *M. leprae* from this potential mycobacterial contaminant.

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