from *M. leprae* and *M. lepraemurium*. Based on the yields of DNA extracted by all of the methods used, both M2 and M5 are the time-saving (less than 4 hr), effective and simple methods for DNA extraction from *in vivo*-grown *M. leprae* and *M. lepraemurium*.

—Zhang-Qing Zhang, Ph.D.
Post-doctoral fellow
—Muhammad Ishaque, Ph.D.
Professor
Applied Microbiology Research Center
Institute Armand-Frappier
University of Quebec
C.P. 100
Laval, Quebec, Canada H7N 4Z3
Reprint requests to Dr. Ishaque.

Acknowledgment. This investigation was generously supported by the Military and Hospitaller Order of Saint Lazarus of Jerusalem, Canada.

REFERENCES

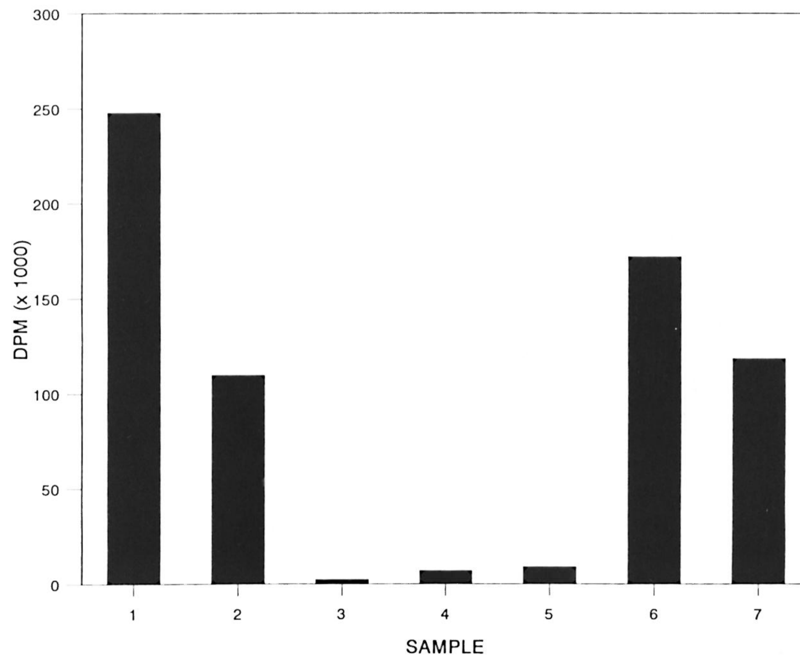
1. Athwal, R. S., Deo, S. S. and Imaeda, T. Deoxyribonucleic acid relatedness among *Mycobacterium lepraemurium, Mycobacterium leprae,* and selected bacteria by dot blot and spectrophotometric deoxyribonucleic acid hybridization. Int. J. Syst. Bacteriol. **34** (1984) 371–375.
2. Bollet, C., Gevaudan, M. J., De Lamballerie, X., Zandotti, C. and de Micco, P. A simple method for the isolation of chromosomal DNA from gram positive or acid-fast bacteria. Nucl. Acids Res. **19** (1991) 1955.
3. Clark-Curtiss, J. B., Jacobs, M. A., Docherty, W. R., Ritchie, L. R. and Curtiss, R., III. Molecular analysis of DNA and construction of genomic libraries of *Mycobacterium*. J. Bacteriol. **161** (1985) 1093–1102.
4. de Lamballerie, X., Zandotti, C., Vignoli, C., Bollet, C. and de Micco, P. A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification. Res. Microbiol. **143** (1992) 785–790.
5. Franzblau, S. G. and Hastings, R. C. *In vitro* and *in vivo* activities of macrolides against *Mycobacterium leprae*. Antimicrob. Agents Chemother. **32** (1988) 1758–1762.
6. Kato, L. and Ishaque, M. Separation of *Mycobacterium lepraemurium* from the subcutanenous tissues of the rat. Int. J. Lepr. **43** (1975) 16–20.
7. Jacobs, W. R., Jr., Kalpana, G. V., Girillo, J. D., Pascopella, L., Snapper, S. B., Udami, R. A., Jones, J., Barletta, R. G. and Bloom, B. R. Genetic system for mycobacteria. Methods Enzymol. **204** (1991) 537–555.
8. Rodde, C., Mohamed, A. A. F., Lüesse, H. G. and Kazda, J. Improved method for purification of *Mycobacterium leprae* from armadillo tissues. Int. J. Lepr. **60** (1992) 277–278.
9. Sambrook, J., Frisch, E. F. and Maniatis, T. Molecular cloning: A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1989.
10. Via, L. E. and Falkinham, J. O., III. Comparison of methods for isolation of *Mycobacterium avium* complex DNA for use in PCR and RAPD. J. Microbiol. Meth. **21** (1995) 151–161.
11. Visuvanathan, S., Moss, M. T., Stanford, J. L., Hermon-Taylor, J. and McFadden, J. J. Simple enzymic method for isolation of DNA from diverse bacteria. J. Microbiol. Meth. **10** (1989) 59–64.

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.

—Eugene B. Harris, B.S.
Rita M. Sanchez, B.S.

Laboratory Research Branch
GWL Hansen's Disease Center at
Louisiana State University
P.O. Box 25072
Baton Rouge, LA 70894, U.S.A.

REFERENCES

1. Buddemeyer, E., Hutchinson, R. and Cooper, M. Automatic quantitative radiometric assay of bacterial metabolism. Clin. Chem. **22** (1976) 1459–1464.
2. Franzblau, S. Oxidation of palmitic acid by *M. leprae* in an axenic medium. J. Clin. Microbiol. **26** (1988) 18–21.
3. Franzblau, S. and Hastings, R. Rapid *in vitro* metabolic screen for antileprosy compounds. Antimicrob. Agents Chemother. **31** (1987) 780–783.
4. Larsson, L., Draper, P. and Portaels, F. Use of gas chromatography to differentiate *Mycobacterium leprae* from cultivable armadillo-derived mycobacteria, *M. avium/intracellulare* and *M. lepraemurium* by analysis of secondary alcohols. Int. J. Lepr. **53** (1985) 441–446.
5. Portaels, F. and Pattyn, S. R. Isolation of fastidiously growing mycobacteria from armadillos infected with *Mycobacterium leprae*. Int. J. Lepr. **50** (1982) 370–374.

A Plea for Routine Use of Fine-Needle Aspiration Cytology in the Diagnosis and Follow Up of Leprosy

To the Editor:

We have previously described diagnostic fine-needle aspiration cytology in nodular lepromatous leprosy ([1]) and subsequently in leprosy lesions through the Ridley-Jopling (R-J) spectrum ([2]). The techniques we used to obtain material for cytological study were: a) fine-needle aspiration cytology using a 10-ml syringe attached to a 23-gauge needle and b) cytopuncture, performed without negative pressure ([2]). These procedures were performed by a pathologist.

Cytologic evaluation of leprosy patients is being tried on a larger scale in our institution. The procedures are now being performed by dermatologists at the time of initial examination of the patient. The simplicity and rapidity of the technique, the abundance of cytologic material obtained, the amount of information generated enabling classification in the R-J scale by reading May-Grunwald-Giemsa in conjunction with Ziehl-Neelsen-stained smears has enthused our clinical colleagues. In addition, the absence of trauma and scarring are appreciated by the patient, and reports can be issued in a few hours.

Cytology is an established tool in the diagnostic workup of a large number of in-