CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

A Mexican Armadillo (*Dasypus novemcinctus*) Colony for Leprosy Research

TO THE EDITOR:

We would like to inform the JOURNAL's readers of the establishment of an armadillo colony for leprosy research in México City, México. The project was started in 1980 with a small group of animals. Our present colony is working at relatively low cost in facilities that fulfill the basic security requirements for both the animals and the personnel in charge of them. The facilities include a crematory and two independent working areas; one designed to receive and house the animals for their adaptation to the new conditions (quarantine section) and another in which armadillos under study are maintained until completion of the experiments. In the quarantine section, there is enough room to keep up to 18 animals; in the experimental section, up to 36 animals can be housed. Cages in both sections are ceramic-lined to facilitate cleaning and they are equipped with iron doors to prevent escape. Appropriate nets protect doors and windows in both areas. Hay, which is used as the bedding, is changed once a week, and it is incinerated when discarded (Fig. 1). In the experimental section, the armadillos' urine and stools drain into a reservoir where they are mixed with boiling water and bleach before being poured into the general sewer. On arrival, each animal is immediately tattooed for permanent identification.

Initially, the armadillos (*Dasypus novemcinctus*) came from the state of Nuevo Leon (in northern Mexico), but presently most of them are captured in the high part of the state of Michoacan, a temperate region. All of the animals captured on the warm Pacific Coast near Acapulco, Guerrero, died soon after arrival (Fig. 2). So far, more than 160 animals have been received, and some of the surviving ones have been inoculated with *Mycobacterium leprae*.

The daily diet per animal includes raw, ground horse meat (200 g), an egg (one apiece), a zucchini (one apiece), a potato (one apiece), cucumber (half apiece), and fruit in season (watermelon, pineapple, cantaloupe, etc., 250 g). A teaspoonful of children's vitamins is incorporated into the meal once a week. In general, the animals soon adapt to this diet without major digestive disorders. On the average, 40% of the animals that arrive in the colony die within the first 3 months of captivity.

Some armadillos have been inoculated with *M. leprae*. Previous to inoculation, the animals are thoroughly examined for the presence of acid-fast bacilli (AFB) and for the presence of other obvious infections and injuries. AFB are looked for in smears made from nasal exudates, ear imprints, and cutaneous lymph.

For the experimental infection of armadillos, fresh lepromas from untreated leprosy patients, lepromas from previously infected armadillos, and infected armadillo tissue that has been frozen in liquid nitrogen for over 3 years, have all been successfully used. The "standard" inoculation consists of intravenous administration of 10^8 bacilli, leaving part of the inoculum deposited sub-

FIG. 1. Armadillos in a ceramic-lined cage. During the day, armadillos spend most of the time hidden within the bedding, but they show themselves when, hungry, they come out for food.

FIG. 2. Map of Mexican Republic showing areas where armadillos (*D. novemcinctus*) are usually captured, the states of Nuevo Leon, Michoacan, and Guerrero. Our experimental armadillo colony is located in Mexico City (D.F.).

cutaneously at the injection site on the femoral region. From the 30 armadillos so far inoculated with *M. leprae*, 10 animals have developed a systemic disease of variable degree ([7]). On the average, the earliest external signs of disease appear 11 months after inoculation, and include enlargement of the inguinal and cervical lymph nodes, and the appearance of small nodules in the inner aspect of the shell border and sometimes between the shell bands (Fig. 3). At this time, bacilli are easily found in smears from ear lymph. Autopsy of the sacrificed infected animals shows all of the morphological and histopathological changes already described by other groups ([6]). The collected infected material is preserved without trimming at −70°C until used. The remains are immediately incinerated.

Mycobacterium leprae-infected tissue has already been used for: a) the production of lepromin A, according to the WHO recommendations ([10]); b) bacilli purification, according to Draper's protocol 9 ([2]) for diagnostic purposes and for the immunochemical characterization of *M. leprae* ([3]); and c) for the preparation of phenolic glycolipid-I (PGL-I), according to Hunter and Brennan ([5]), for the early diagnosis of subclinical infection. These materials have been used in preliminary studies in Mexico, Sri Lanka, and the U.S.A., in cooperation with Dr. T. M. Buchanan (University of Washington, Seattle, Washington, U.S.A.[1]). In addition, diverse immunobiological, immunochemical, and biochemical studies on

FIG. 3. Evident signs of disease in an armadillo >1 yr after inoculation with *M. leprae*: enlargement of inguinal lymph nodes and the appearance of multiple, variable-sized lepromas all over the lower abdominal region. Infarction of cervical and axilar lymph nodes, and lepromas in the inner aspect of the shell border and between the shell bands (not shown) are also common signs of infection. Insert at lower right shows abundant subcutaneous lepromas in the same armadillo.

M. leprae-infected armadillos are currently in progress in our Institution ([4, 8, 9]).

So far, no evidence for natural infection has been found in over 100 armadillos examined. Examination includes macroscopic assessment of changes in external and internal structures, and bacilloscopic studies in those specimens of the animals that die within the adaptation period.

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REFERENCES

1. Buchanan, T. M., Dissanayake, S., Young, D. B., Miller, R. A., Acedo, J. R., Harnisch, J. P., Khanolkar, S. R. and Estrada, P. S. Evaluation and significance of antibodies to phenolic glycolipid of *M. leprae* in leprosy patients and their contacts. Int. J. Lepr. **51** (1983) 658–659.
2. Draper, P. Purification of *M. leprae*, protocol 1/79. Report of the Enlarged Steering Committee for Research on the Immunology of Leprosy (IMMLEP) Meeting of 7–8 February 1979. Geneva: World Health Organization, 1979, Annex 1, p. 4.
3. Estrada, G. I., Quesada, P. F., Santos, A. L., Flores, R. L., Estrada, P. S. and Buchanan, T. M. The early serodiagnosis of leprosy; the counterelectrophoresis test and the enzyme-linked immunosorbent assay. Rev. Latinoam. Microbiol. **26** (1984) 267–272.
4. Guerra, I. F., Santos, A. L., Quesada, P. F. and Estrada, P. S. Cinética de las poblaciones linfoides en armadillos inoculados experimentalmente con *M. leprae*. XVIII Congreso Nacional de Microbiología, Acapulco, Gro., Abril 27–30 (1987) 80.
5. Hunter, S. W. and Brennan, P. J. A novel phenolic glycolipid from *Mycobacterium leprae* possibly involved in immunogenicity and pathogenicity. J. Bacteriol. **147** (1981) 728–735.
6. Kirchheimer, W. F., Storrs, E. E. and Binford, C. H. Attempts to establish the armadillo (*Dasypus novemcinctus*) as a model for the study of leprosy. II. Histopathologic and bacteriologic post-mortem findings in lepromatoid leprosy in the armadillo. Int. J. Lepr. **40** (1972) 229–242.
7. Quesada, P. F., Rojas-Espinosa, O., Jiménez, A., Estrada, P. S. and Buchanan, T. M. Infección experimental de armadillos (*Dasypus novemcinctus*) con *Mycobacterium leprae*. Rev. Latinoam. Microbiol. **25** (1983) 11.
8. Rojas-Espinosa, O., Quesada, P. F., Oltra, R. A., Arce, P. P., Estrada, P. S. and Buchanan, T. M. Biochemical alterations in the serum of armadillos (*Dasypus novemcinctus*) infected with *Mycobacterium leprae*; a preliminary report. Int. J. Lepr. **53** (1985) 262–268.
9. Rojas-Espinosa, O., Méndez, A. P., Oltra, R. A. and Arce, P. P. Antimycobacterial antibodies in *Dasypus novemcinctus* infected with *Mycobacterium leprae* and their correlation with the serum levels of lactate dehydrogenase. Lepr. Rev. **57** (1986) 317–327.
10. Recommended safety requirements for the preparation of lepromin. WHO Memorandum. Bull. WHO **57** (1979) 921–923.

Serological Reactivity and Early Detection of Leprosy Among Contacts of Lepromatous Patients in Cebu, The Philippines

To the Editor:

One of the challenges in the epidemiology of leprosy is the quest for a tool to indicate the presence of infection prior to the onset of clinically recognized disease. We and others familiar with the long and somewhat indefinite incubation period have been looking for a marker which would allow us