3. JOPLING, W. H. A report on two follow-up investigations of the Malta-project. Symposium on Multidrug Therapy in Leprosy, 24–26 April 1986, Wurzburg, West Germany. Lepr. Rev. **57** Suppl. 3 (1986) 47–52.
4. JOPLING, W. H. The disease; bacterial and pathological aspects. In: *Handbook of Leprosy*. 3rd edn. London: William Heinemann Medical Books Ltd., 1984, p. 8.
5. WHO STUDY GROUP. Chemotherapy of leprosy for control programmes. Switzerland: World Health Organization, 1982, WHO Tech. Rep. Ser. No. 675.

Search for Leprous Infection in Some Small Wild Animals of Louisiana

TO THE EDITOR:

It has been reported by several workers that wild armadillos (*Dasypus novemcinctus*) have lepromatous leprosy similar to the disease produced in experimental infections with *Mycobacterium leprae* ([4–6]). Each animal with advanced disseminated disease has approximately 200 g of infected tissue and each gram contains approximately 10^{10} organisms ([3]). Upon death, the armadillo carcass is often eaten by other animals and what is left disintegrates and gets mixed in with the soil. With that much load of infected material in the environment, it is possible that other animals which share the same environment, living in burrows and even consuming dead armadillos, might contract the disease.

In our earlier study it was found that histopathological examination of both ears of those armadillos killed on the road by automobiles was an effective and simple method for surveying wild animals for the leprosy infection ([2]). In that study, 2.02% of the 494 armadillos examined had the disease.

We report here the results of a similar study of four other animals sharing the same environment with armadillos. Both ears from 51 rabbits, 56 nutria, 17 raccoons, and 311 opossums killed on the road by automobiles were collected. Of these, one pair of rabbit ears and eight pairs of opossum ears were decomposed and therefore were discarded. All of the other specimens were fixed in 10% Formalin and processed for paraffin sections; 5 μm sections were cut, stained with a modified Fite's stain ([1]), and examined microscopically under oil immersion. The distribution of animals according to the parishes in Louisiana is given in The Table.

None of the specimens studied showed acid-fast organisms. Thorns associated with foreign-body reaction and granuloma formation were detected in 2 rabbits, 3 raccoons, and 3 opossums. One specimen from an opossum showed an unidentified fungal granuloma. Granulomatous inflammation with no identifiable etiologic agent was present in the specimens of two nutria and 15 opossums. Skin ulcers were seen in 21 and acute abscesses in six specimens from opossums.

In conclusion, *M. leprae* infection was not detected in samples of four species of ani-

THE TABLE. *Distribution of animals sampled by Louisiana parishes.*

Parish	Rabbits	Nutria	Raccoons	Opossums
Ascension	32	11	5	185
Iberville	8	1	3	73
East Baton Rouge	6	1	4	34
Livingston	1			1
Tangipahoa			1	2
West Baton Rouge	1			3
Jefferson Davis			1	1
St. Martin	1		2	
St. John the Baptist	1	1	1	1
St. James	1	1		1
St. Charles		20		
Iberia		10		
Terrebonne		6		
St. Mary		5		
West Feliciana				3
East Carroll				3
St. Landry				1
Avoyelles				1
Tensas				1
Madison				1
Total	51	56	17	311

mals, namely, rabbits, nutrias, raccoons, and opossums. Although thorns were present in some of the animals, they were not so frequent as reported in the armadillo ([6]).

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REFERENCES

1. JOB, C. K. and CHACKO, C. J. G. A modification of Fite's stain for demonstration of *M. leprae* in tissue sections. Int. J. Lepr. **58** (1986) 17–18.
2. JOB, C. K., HARRIS, E. B., ALLEN, J. L. and HASTINGS, R. C. A random survey of leprosy in wild nine-banded armadillos in Louisiana. Int. J. Lepr. **54** (1986) 453–457.
3. KIRCHHEIMER, W. F. Advances in biomedical research. Jpn. J. Lepr. **49** (1980) 209–219.
4. MEYERS, W. M., WALSH, G. P., BINFORD, C. H., STORRS, E. E. and BROWN, H. L. Indigenous leprosy in nine-banded armadillos. The armadillo as an experimental animal model in biomedical research. Pan American Health Organization Scientific Publication No. 366 (1978) 67–76.
5. SMITH, J. H., FOLSE, D. S., LONG, E. G., CHRISTIE, J. D., CROUSE, D. T., TEWES, M. E., GATSON, A. M., EHRHARDT, R. L., FILE, S. K. and KELLY, M. T. Leprosy in wild armadillos (*Dasypus novemcinctus*) of the Texas Gulf Coast: epidemiology and mycobacteriology. J. Reticuloendothel. Soc. **34** (1983) 75–88.
6. WALSH, G. P., STORRS, E. E., BURCHFIELD, H. P., COTTRELL, E. H., VIDRINE, M. F. and BINFORD, C. H. Leprosy-like disease occurring naturally in armadillos. J. Reticuloendothel. Soc. **18** (1975) 347–351.

Psychrophilic Mycobacteria in *M. leprae*-infected Tissues

TO THE EDITOR:

A multifactorial medium (MFM) was proposed for the *in vitro* cultivation of *Mycobacterium leprae* ([2, 3]). In the MFM, Na-thioglycolate served as a source of energy and mycobactin with exochelin for iron acquisition ([1–4]). Slow growth of leprosy-derived mycobacteria (LDM) occurred on the semisolid medium at pH 5.8 and incubation temperature of 32°C.

I am now able to report that a considerably higher yield and more rapid growth can be achieved in a liquid medium at an incubation temperature of 16°C to 18°C if Na-thioglycolate is replaced by ammonium thioglycolate and β-cyclodextrin replaces mycobactin-exochelin.

In a closed Erlenmeyer flask, 0.05 g of thioctic acid (Fluka Chemical Corporation, Hauppauge, New York, U.S.A.) and 5 g of β-cyclodextrin (Chinoin, Budapest, Hungary) were dissolved in 10 ml of hot ammonium thioglycolate (Fluka) (60% v/w in water).

A poor nutrient, multifactorial liquid medium was used. This contained in 1 liter of distilled water: KH_2PO_4, 2.5 g; Na_2HPO_4, 4.0 g; $(NH_4)_2SO_4$, 2 g; $MgSO_4$, 0.2 g; ferric ammonium citrate, 0.05 g; and 10 ml of the above thioctic acid-β-cyclodextrin-ammonium thioglycolate solution. The pH was adjusted to 7.0, using the PO_4 buffers. The solution, distributed 10 ml/25 ml screw-cap tubes, was autoclaved for 25 min. Optimal growth of the primary cultures and subcultures was registered at 16°C to 18°C. These results indicate that the physicochemical properties of the β-cyclodextrin might replace the iron acquisition growth factors.

No visible growth was observed at 4°C and very slow growth was seen at 32°C. At 16–18°C the inoculum increased in size into a visible growth within 2 to 8 weeks, depending on the size and quality of the inoculum. This growth consisted of strongly acid-fast cells with characteristics as previously described ([3]).

Twenty-four such cultures are now maintained, being transferred into subcultures at 6- to 10-week intervals and grown at 16°C incubation temperature.

These leprosy-derived cultures, ranging from the 2nd to the 17th subcultures respectively, are tentatively designated as "*M. psychrophilum* L.," indicating that further characterization and identification are nec-