carious island setting of Venice. If the consumption in both Marghera and Venice is held constant from 1973 onward as it has been since 1969, about 3 cm of further subsidence is to be expected (curve 1). A complete cessation of the Venice extraction alone in 1974 would save about 1 cm of this (curve 2). If the Venice pumping were stopped in 1974 and the Marghera consumption reduced to 0.75 of its present value, the subsidence at Venice would be arrested at essentially its present value (curve 3). A shutdown of all wells in 1974 would arrest the subsidence at its present level and would provide a modest rebound of perhaps 2 cm in the next 25 years (curve 4). Artificially recharging the depleted aquifers would increase the rate of rebound but not the ultimate amount. Over 85 percent of the Venice subsidence is nonrecoverable.

The predictions presented in this study are based on a model calibration that was hampered by the sparseness of available data. These predictions should be viewed as first estimates that may require substantial modifications as further data become available. They are not firm enough to allow the conclusion that subsidence is no longer a problem in the Venice area. We recommend continued data acquisition and continued evaluation of the Venice subsidence situation.

GIUSEPPE GAMBOLATI
IBM Scientific Center,
S. Polo 1364, Venice, Italy
PAOLO GATTO
Consiglio Nazionale delle Ricerche
Laboratorio per lo Studio della
Dinamica delle Grandi Masse,
S. Polo 1364, Venice, Italy
R. ALLAN FREEZE
Department of Geological Sciences,
University of British Columbia,
Vancouver 8, Canada

References and Notes

1. G. Gambolati and R. A. Freeze, *Water Resour. Res.* **9**, 721 (1973).
2. G. Gambolati, P. Gatto, R. A. Freeze, *ibid.*, in press.
3. R. Serandrei-Barbero, *Cons. Naz. Ric. Lab. Stud. Din. Grandi Masse Tech. Rep. 31* (1972).
4. M. Caputo, G. Folloni, A. Gubellini, L. Pieri, M. Unguendoli, *Cons. Naz. Ric. Lab. Stud. Din. Grandi Masse Tech. Rep. 9* (1971).
5. J. F. Poland and G. H. Davis, *Rev. Eng. Geol.* **2**, 187 (1969).
6. K. Terzaghi, *Sitz. Akad. Wiss. Wien* **132**, 125 (1923); M. A. Biot, *J. Appl. Phys.* **12**, 155 (1941); A. Verruijt, in *Flow through Porous Media*, R. J. M. De Wiest, Ed. (Academic Press, New York, 1969), p. 331; P. A. Domenico and M. D. Mifflin, *Water Resour. Res.* **1**, 563 (1965).
7. This work was carried out while R.A.F. was at the IBM Thomas J. Watson Research Center in Yorktown Heights, N.Y.

12 October 1973; revised 30 November 1973

Leprosy in the Armadillo: New Model for Biomedical Research

Abstract. *Eight of twenty armadillos* (Dasypus novemcinctus *L.*) *developed severe lepromatous leprosy 3 to 3.5 years after inoculation with viable* Mycobacterium leprae. *A total of 988 grams of lepromas containing an estimated 15 to 20 grams of leprosy bacilli has been harvested from these animals. The large amounts of material now available will permit in-depth studies of the biochemistry and metabolism of the leprosy bacillus, and the animal model should make possible definitive studies on the immunology, chemotherapy, and epidemiology of the disease.*

Leprosy is one of the major public health problems in the world today. The study of leprosy has been seriously impeded by the fact that the causative agent, *Mycobacterium leprae*, cannot be grown in artificial media and that, until recently, only a mild infection with microscopic lesions could be obtained in immunologically intact animals. This has left many unanswered questions relating to the epidemiology of the disease (modes of transmission, effects of genetic and nutritional factors, and so forth) and also has resulted in slow progress in the search for drugs to halt the disease process quickly and effectively. In addition, the limited amounts of *M. leprae* available have not been sufficient for in-depth biochemical and metabolic studies of the organism, studies that are necessary for a complete understanding of the disease.

Leprosy is classified with the granulomatous diseases; because of the diverse manifestations in individuals infected with *M. leprae*, leprosy qualifies as a complete model for the study of granulomatous diseases. Resistance to leprosy appears to be primarily regulated by cell-mediated immunity—the same immunologic machinery that is thought to control neoplastic growth and the rejection of foreign tissue transplants and of other infectious agents (viruses, fungi, and so forth).

Storrs (*1*) and Kirchheimer and Storrs (*2*) reported the development of leprosy in a single nine-banded armadillo (*Dasypus novemcinctus* L.) 15 months after inoculation with leprosy bacilli obtained from a human patient. Postmortem examination and subsequent histopathological evaluations confirmed the presence of advanced, disseminated leprosy in this animal (*3*). Thirty-three other armadillos have developed lepromatous leprosy, 11 of which have been necropsied and subjected to histopathologic examinations. These have yielded a total of 1235 g of granulomatous lepromas.

The average survival time of the adult animals from inoculation until death from leprosy or its complications appears to be about 31 months. Therefore, the only significant information on the incidence of susceptibility of the armadillo to leprosy and the severity of the disease in this species can be obtained from three groups totaling 20 animals which were inoculated with viable bacilli in February and June of 1970 (Table 1).

All animals in these groups were adults captured from the wild and adapted to captivity for at least 6 months before inoculation (*4*). The inoculum was one of the following: leprosy bacilli from an untreated lepromatous leprosy patient from Surinam; *M. leprae* (supplied by the Public Health Service Hospital in Carville, Louisiana) from a patient who was not responding to drug (Dapsone) therapy; and human bacilli grown in the mouse foot pad (supplied by L. Levy of the Public Health Service Hospital in San Francisco, California).

Suspensions of these materials were inoculated by three routes: intradermal injection of 0.1 ml in the abdomen (right and left sides), ear lobe, or foot

Table 1. Data on inoculum and number of animals developing leprosy.

Group	Animals per group			Inoculum		Animals developing leprosy	
	Total	♂	♀	Source	Bacterial count (acid fast bacilli per milliliter)	No.	%
1	4	3	1	Human	8.9×10^7	2	50
2	3	2	1	Human	6.0×10^6	1	33
3	13	7	6	Mouse*	1.4×10^6	5	40
Total	20					8	40

* Mouse foot pad passage of human *M. leprae*.

pad; intravenous injection of 0.5 to 1.0 ml into the right saphenous vein; or abrasion of the ear lobes followed by treatment with inoculum.

During approximately 3 years after inoculation, eight of these animals developed severe lesions containing myriads of *M. leprae* (Table 2). All infected animals died or were killed, and the disease was diagnosed as leprosy by histopathologic examination (*3, 5*). Seven animals are still alive and negative as determined by biopsy. Five animals died from causes unrelated to leprosy and at the time of death were negative. These animals died 21 to 31 months after inoculation, so at least some of them were probably resistant to the infection and would not have developed leprosy had they survived longer.

The slowness with which the disease developed in adult armadillos captured from the wild and inoculated with leprosy bacilli is illustrated by the fact that severely infected animals died from leprosy or its complications 15, 26, 30, 31, 33, 34, 37, and 41 months after inoculation. If it is assumed that all animals that are now negative will remain negative and that none of the animals that died from other causes would have developed leprosy, 40 percent of the animals inoculated developed lepromatous leprosy.

These estimates are provisional. Factors other than immunologic competence which could influence incidence of infection include routes of inoculation, number of bacilli injected, strain of bacilli, and nutritional status of the animals.

The salient features of leprosy in the armadillo are the high incidence of susceptibility (estimated at 40 percent), the high degree of pathologic involvement, and the enormous numbers of bacilli produced. In some cases, the central nervous system and lungs became seriously infected, a finding not reported in man. Although some involvement of the bone marrow is usually observed in human lepromatous leprosy, the degree and distribution of involvement in the armadillo far exceeds that found in human disease with the exception of leprotic osteitis occasionally seen in digits. It seems likely that armadillos in the late stages of disease become depressed immunologically because of massive invasion of the bone marrow and related reticuloendothelial tissues by leprosy bacilli.

The most important finding, from the standpoint of the biochemist, is that armadillos that died of leprosy 26 to 41 months after inoculation yielded 988 g of lepromas at autopsy (Table 2). Since removal, this tissue has been stored at −70°C and is estimated to contain 15 to 20 g of *M. leprae* as judged from bacterial counts averaging about 10^{10} per gram compared to 10^7 to 10^8 per gram in advanced human cases. This material contains very little stroma, so isolation of 95 percent "pure" bacilli is easily achieved. This material and the live bacilli harvested from living animals should make possible studies on the biochemistry and the metabolism of the leprosy bacillus which were hitherto impossible.

Table 2. Data on armadillos necropsied with advanced leprosy; I.V., intravenous.

Animal	Route of inoculation	Inoculation to death (months)	Lepromatous tissue harvested (g)
		Group 1	
5	Dermal	30	116
8	Dermal	15	*
		Group 2	
9	Dermal	41	13
		Group 3	
14	Dermal	34	172
16	Dermal	33	354
17	I.V.	31	85
18	I.V.	26	127
24	Dermal	37	121

* Not determined.

We conclude that it is now possible to produce disseminated leprosy in an intact animal system and that studies related to the epidemiology, immunology, and chemotherapy of the disease can now be performed. It is felt that information obtained from these pursuits will be of value in other biomedical research areas as well as in leprosy research.

ELEANOR E. STORRS
G. P. WALSH
H. P. BURCHFIELD
Gulf South Research Institute, New Iberia, Louisiana 70560
C. H. BINFORD
Armed Forces Institute of Pathology, Washington, D.C. 20306

References and Notes

1. E. E. Storrs, *Int. J. Lepr.* **39**, 703 (1971).
2. W. F. Kirchheimer and E. E. Storrs, *ibid.*, p. 693.
3. ——— and C. H. Binford, *ibid.* **40**, 229 (1972).
4. E. E. Storrs and W. E. Greer, *Lab. Anim. Sci.* **28**, 823 (1973).
5. Papers presented at the Tenth International Leprosy Congress, Bergen, Norway, August 1973, by E. E. Storrs; E. E. Storrs, G. P. Walsh, W. E. Greer, H. P. Burchfield, and S. L. Issar; S. L. Issar and C. H. Binford; C. H. Binford and S. L. Issar; D. T. Purtillo, G. P. Walsh, E. E. Storrs, and I. S. Banks; S. C. Chang, J. D. Balentine, and S. L. Issar; and J. D. Balentine, S. C. Chang, and S. L. Issar. Abstracts will be published in *Int. J. Lepr.*, in press.
6. Supported by grant CC-00476.

16 October 1973; revised 12 November 1973 ■

Site of Biosynthesis of Galactolipids in Spinach Chloroplasts

Abstract. *The envelope of the spinach chloroplast is the site of galactolipid synthesis.*

The chloroplast envelope is a continuous boundary of two osmiophilic membranes and has an important role in thylakoid synthesis (*1*). This conclusion is based on electron micrographs showing that membranes are continuously produced by the invagination of the inner membrane of the plastid envelope (*1*). With biochemical evidence lacking it was interesting to determine whether the envelope is the site of the synthesis of some structural component of the thylakoid. I chose to investigate the site of synthesis of galactolipids because they represent more than 70 percent of the total polar lipid of the thylakoid (*2*). Their active synthesis in isolated spinach chloro-

Table 1. Specific activities (micromoles of P_i formed per hour per milligram of protein) of marker enzymes, chlorophyll content (micrograms per milligram of protein), and galactolipid synthesis (picomoles of galactose incorporated per minute per milligram of protein) in fractions obtained after disruption of intact chloroplasts by hypotonic treatment.

Fraction	Specific activity (μmole hr^{-1} mg^{-1})			Chlorophyll (μg/mg protein)	Galactolipid synthesis (pmole min^{-1} mg^{-1})
	Trypsin-activated Ca^{2+}-dependent ATPase	Fructose-1-6-diphosphatase	Mg^{2+}-dependent ATPase		
Intact chloroplast	28	7.5	0.5	65	62
Thylakoid	60	0.7	0.3	147	39
Stroma	0.1	14	0.3	0	2
Envelope	0.1	0	12.9	0.8	1200