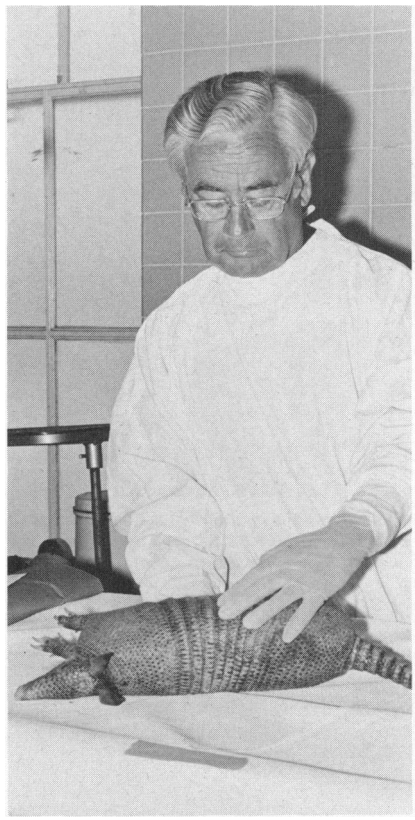
Experimental Leprosy in the Nine-Banded Armadillo

W. F. KIRCHHEIMER, MD, PhD



Kirchheimer examining anesthetized armadillo before performing a biopsy

ADVANCES IN KNOWLEDGE of leprosy have been hampered by an insufficient supply of leprosy bacilli. Unlike most bacteria, *Mycobacterium leprae* has not yet been cultured in artificial culture media. The usual scant supply from human lepromas has diminished because of widespread use of sulfones since the forties. Experiments to transmit the leprosy bacilli to animals were generally unproductive until 1960. The experiments were not conducted quantitatively, and no means existed to determine the viability of *M. leprae* in inocula and harvests. Only a few individuals of any particular animal species were inoculated, despite the observation that in man, even in areas where the disease is highly endemic and socioeconomic conditions are poor, only a minority of the people seem to be susceptible and develop disseminated (lepromatous) leprosy.

In 1960, Shepard *(1)* reported that self-limiting, localized multiplication of *M. leprae* could be induced in the footpads of normal mice. Many practical applications have been made of this discovery, such as determinations of viability of *M. leprae,* the screening of antileprosy drugs, and testing of a patient's leprosy bacilli for sensitivity to drugs.

The unaltered mouse has not, however, provided the quantities of bacilli needed for extensive study of *M. leprae.* In addition, the mouse is not suitable for studying the fundamental characteristics of leprosy as it occurs in man or for many areas of applied leprosy research *(2, 3).* Also, mice are too short lived (2 to 3 years), and the equivalent of human lepromatous leprosy does not develop in them.

In artifically immune-suppressed mice, leprosy infections resemble more closely the leprosy seen in highly susceptible human beings *(4).* Such mice, however, have not supplied the quantities of leprosy bacilli needed for research. Furthermore, in contrast to those human beings who are highly susceptible to leprosy, the cell-mediated immunity of these mice appears to be rather generally suppressed. For this reason alone, they do not qualify as suitable models for study of the naturally occurring and specific hypersusceptibility to infection by *M. leprae* that a few human beings exhibit.

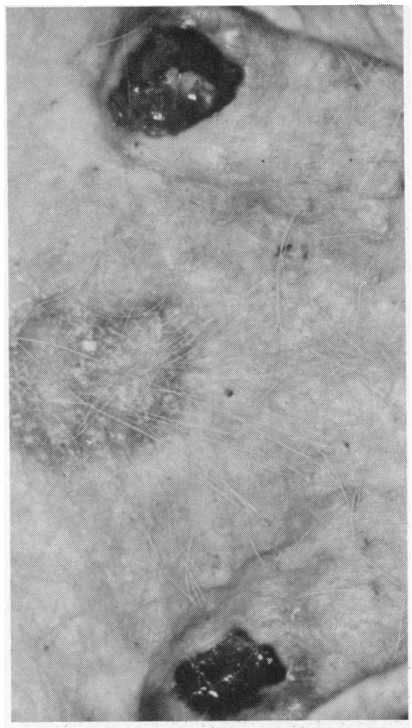
In 1971 *(2)* and 1972 *(5),* Kirchheimer and Storrs first reported disseminated leprosy in some experimentally infected nine-banded armadillos whose immunity was not artifically suppressed. Subsequently, in armadillos with disseminated leprosy that had been experimentally infected, Kirchheimer and co-workers *(6)* reported extensive tissue involvement and exceedingly high numbers of living bacilli in all organs, even in the lungs and meninges (which, in man, are not involved) and in peripheral nerves.

Recently Yoshizumi and coworkers *(7)* described the results of observations, by light and electron microscopy, of the peripheral nerves of an armadillo that had developed lepromatous leprosy following experimental infection. The close resemblance of the lesions in its nerves to the lesions observed in human lepromatous neuritis provides evidence that armadillos may be suitable for the study of leprous nerve lesions in man.

□*Dr. Kirchheimer is chief of the Laboratory Research Branch, Public Health Service Hospital, Carville, La., and clinical professor of bacteriology, medicine, tropical and medical parasitology, Louisiana State University Medical Center, New Orleans.* *The article is based on a paper of the same title that Dr. Kirchheimer gave, on invitation by the Sasakawa Memorial Health Foundation, at a Seminar on Leprosy Control Cooperation in Asia held Aug. 20–22, 1975, in Tokyo, Japan.* *Tearsheet requests to W. F. Kirchheimer, MD, PhD, Chief, Laboratory Research Branch, U.S. Public Health Service Hospital, Carville, La. 70721.*

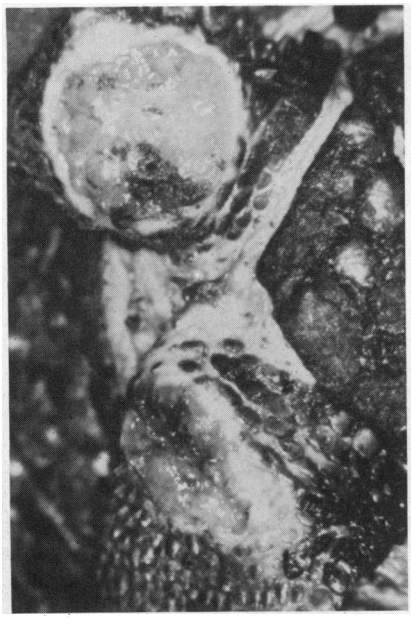
The number of leprosy bacilli per gram of lepromatous armadillo tissue is 100 to 1,000 times greater than the number found in human skin lepromas. From a single lepromatous armadillo liver weighing 180 gm, one can obtain 5 × 10^{12} (5,000 billion) leprosy bacilli. Since one can transmit leprosy from armadillo to armadillo fairly regularly by infecting them intravenously with several hundred million bacilli, an almost unlimited supply of leprosy bacilli could now become available. With the help of the armadillo, one of the major roadblocks in the way of leprosy research, the lack of bacilli, has thus been removed. Such a potentially unlimited supply of leprosy bacilli will undoubtedly have far-reaching effects on biomedical leprosy research.

The World Health Organization, for example, is sponsoring studies on the feasibility of replacing the hard-to-get lepromin derived from human tissue (lepromin H) with preparations derived from armadillo tissue (lepromin A). The results to date show good correlation between the Fernandez and Mitsuda reactions obtained with lepromin H and those obtained with Carville's lepromin A. Both (tests) reactions to lepromin are routinely used to monitor the state of resistance of leprous and of non-leprous exposed persons. Positive reactors, whether or not they have leprosy, seem more resistant to *M. leprae* than nonreactors. There is little doubt that lepromin A soon will replace lepromin H. Also, one objective of WHO's Immunology of Leprosy Project (IMMLEP) is the preparation of antigenic fractions from *M. leprae* derived from armadillo tissue. Such fractions might be of value as a diagnostic and epidemiologic tool, and also, it is hoped, as an immunological reagent for incorporation into a vaccine.

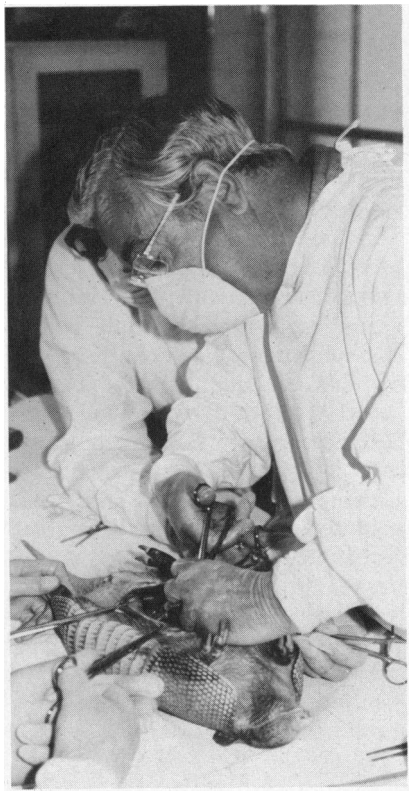


Leprous lesions in abdominal skin of an armadillo 16 months after leprosy bacilli were inoculated into these two sites

Leprous ear lesions at site of inoculation

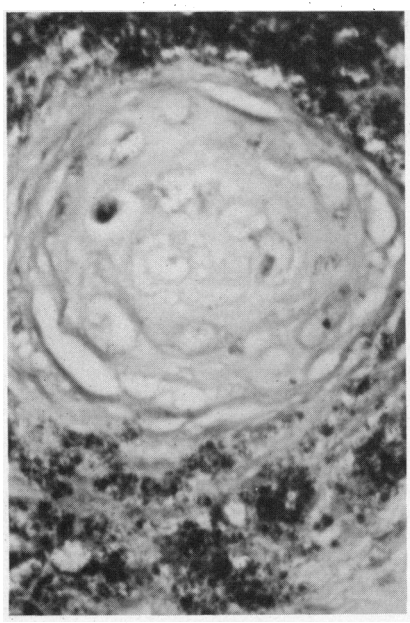


Kirchheimer obtaining leprous organs from armadillo



The Institute of Allergy and Infectious Diseases of the National Institutes of Health has awarded the Public Health Service Hospital at Carville, La., a contract for the continuous supply of leprous armadillo tissues to research laboratories. Leprosy bacilli separated from experimentally infected armadillos are being used at Carville, to study the metabolic characteristics of *M. leprae*. The final objective of these studies is to find a rational basis for *in vitro* culture methods and techniques to evaluate specific anti-*M. leprae* drugs.

Leprologists have known for many years that *M. leprae* multiply best in the cooler tissues of human beings *(8)* and of mice *(9)*. Epidemiologists and geneticists have long suspected also that excessive susceptibility to leprosy might have a genetic basis *(10,11)*. Armadillos were known to have a relatively low body temperature *(12)*, to live for 12 to 15 years, and to give birth regularly to genetically identical quadruplets. Their relatively low temperatures and long survival time would seemingly favor rich harvests of leprosy bacilli. Also, the possibility of obtaining genetically identical material has raised hope that armadillos may eventually serve as the needed model for study in fundamental and applied areas of human leprosy *(3)*.



Cross-sectioned cutaneous nerve in skin. There are an enormous number of M. leprae *in the skin of this armadillo, and there are also some in the cutaneous nerve (acid-fast stain)*

Unfortunately, armadillos have not been bred under controlled conditions—a prerequisite, of course, for genetic studies. With the objective of obtaining viable births, a comprehensive study of the reproductive physiology of male and female armadillos is being conducted by Dr. Richard Peppler, Louisiana State University Medical Center, New Orleans, in collaboration with Carville. There is also an additional requirement that is part of the objective to develop nine-banded armadillos into a model for a broad investigation of leprosy, namely, the development of a test which predicts the degree of susceptibility in the uninfected armadillo.

It is obvious that nonleprous, susceptible animals are needed to verify the different postulated modes of transmission of leprosy *(13)*. As I have stated elsewhere *(3)*, the same requirement holds if one wishes to investigate the mechanics of resistance or susceptibility at the cellular level.

Kirchheimer and Sanchez *(14)* have previously explained the rationale for using skin tests with heat-killed *M. leprae* to determine susceptibility. In their later experience, tests with heat-killed *M. leprae,* in which results were based on cell responses and the fate of these bacilli at the test site, have often seemed difficult to interpret. To circumvent these difficulties, we are seeking to determine whether in armadillos vaccinated with heat-killed *M. leprae,* a delayed type of hypersensitivity develops to the *M. leprae*-protein which we have prepared from the abundant leprosy bacilli *(15)*. On theoretical grounds, one would expect that highly susceptible armadillos would not be able to respond with delayed-type hypersensitivity reactions to skin tests with the protein or with increased blast transformation to *M. leprae* antigen.

A test to determine the susceptibility to leprosy of uninfected armadillos, as well as the successful breeding of armadillos under controlled conditions, would be significant steps toward our objectives of investigating on an animal model *(a)* the validity of the hypothesis that there is a genetic basis for susceptibility to leprosy, *(b)* the mechanism of susceptibility and resistance, *(c)* the modes of transmission, and *(d)* the efficacy of various prophylactic measures. With an abundant source of *M. leprae,* the immunochemist can systematically study the antigenic and biological activities of the various components. These are the basic prerequisites to developing *(a)* a reliable skin test antigen for field diagnosis, *(b)* a practical serologic test for diagnostic purposes and monitoring the course of the disease, and *(c)* ultimately a vaccine composed of one or more of the antigens that are most efficient in promoting protective cellular immune responses in the susceptible individual.

References

1. Shepard, C. C.: The experimental disease that follows the injection of human leprosy bacilli into the footpads of mice. J Exp Med 112: 445–454 (1960).
2. Kirchheimer, W. F., and Storrs, E. E.: Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. Int J Lepr 39: 692–701 (1971).
3. Kirchheimer, W. F.: Animal models for research in leprosy. Lepr India 45: 6–11 (1973).
4. Rees, R. J. W., Waters, M. R. F., Weddell, A. G. M., and Palmer, E.: Experimental lepromatous leprosy. Nature 215: 597–602 (1967).
5. Kirchheimer, W. F., and Storrs, E. E.: Leprosy in experimentally infected armadillos. Int J Lepr 40: 212–213 (1972).
6. Kirchheimer, W. F., Storrs, E. E., and Binford, C. H.: Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. II. Histologic and bacteriologic post-mortem findings in lepromatoid leprosy in the armadillo. Int J Lepr 40: 229–242 (1972).
7. Yoshizumi, M. O., Kirchheimer, W. F., and Asbury, A. K.: A light and electron microscopic study of peripheral nerves in an armadillo with disseminated leprosy. Int J Lepr 42: 251–259 (1974).
8. Brand, P. W.: Temperature variation with leprosy deformity. Int J Lepr 27: 1–7 (1959).
9. Shepard, C. C.: Temperature optimum of *Mycobacterium leprae* in mice. J Bacteriol 90: 1271–1275 (1965).
10. Newell, K. W.: An epidemiologist's view of leprosy. Bull WHO 34: 827–857 (1966).
11. Spickett, S. D.: Genetic mechanisms in leprosy. *In* Leprosy in theory and practice, edited by R. G. Cochrane and T. F. Davey. Williams & Wilkins Company, Baltimore, 1964, pp. 98–124.
12. Anderson, J. M., and Benirschke, K.: The armadillo, *Dasypus novemcinctus*. Exp Biol Lab Animal Care 16: 202–216 (1966).
13. Kirchheimer, W. F.: The role of arthopods in the transmission of leprosy. Lepr. India 45: 29–34 (1973).
14. Kirchheimer, W. F., and Sanchez, R. M.: Leprosy-susceptibility testing of armadillos. I. Cellular responses to intradermally inoculated heat-killed leprosy bacilli. Microbios (Cambridge) 7: 31–35 (1975).
15. Kirchheimer, W. F., et al.: Preparation of protein from *Mycobacterium leprae* and skin-test responses of vaccinated armadillos. Lepr India: 47: 142–150 (1975).