INTERNATIONAL JOURNAL OF LEPROSY

Volume 39, Number 3
Printed in the U.S.A.

INTERNATIONAL JOURNAL OF LEPROSY

And Other Mycobacterial Diseases

VOLUME 39, NUMBER 3 JULY-SEPTEMBER, 1971

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[1]

W. F. Kirchheimer and Eleanor E. Storrs[2]

In October 1965 the Leprosy Panels of the U.S.-Japan Cooperative Medical Science Program issued a "Joint Report on Research in Leprosy," in which it was noted that animal transmission was among the scientific areas deserving special attention. In this report it was stated that an experimental system for the growth of *Mycobacterium leprae* in the mouse foot-pad was currently available, and that it should be introduced into more research laboratories for the purpose of assisting the other objectives described in the report, such as "Drugs against leprosy", "Chemoprophylaxis" and "Vaccination." It was further stated that improvements in the system should be sought by increasing bacillary growth, both locally and systemically. Increased yields of bacteria, it was said would supply bacilli for other research and a systemic infection might provide a better model of human lepromatous disease. Therefore, search for suitable animal models should be continued.

Numerous attempts have been made in the past and are being continued at this time to "transfer leprosy" to different species of mammals, birds ([7]) and poikilotherms ([8, 10]). Failure to enunciate criteria of success often caused differences in interpretation of results by pathologists and bacteriologists. From our present vantage point, it seems that many early attempts were carried out in a haphazard fashion. No attention was given to the quantity and quality of the leprosy bacilli, the number of recipients required, their life span, and the optimum time for evaluating results.

Binford initiated the first sustained, comprehensive, long-range program designed to achieve reproduction of human leprosy in animals. At the First Carville Conference on Research Potentials in Leprosy, in 1956 ([3]), he stated that because in man it appeared that the disease primarily affected the cooler parts, he would select the cooler parts of animals for inoculation. Although he succeeded in obtaining mild

[1] Received for publication August 11, 1971.

[2] W. F. Kirchheimer, M.D., Ph.D., Chief, Laboratory Research Branch, U.S.P.H.S. Hospital, Carville, Louisiana, 70721; Eleanor Storrs, Ph.D., Director, Department of Biochemistry, Gulf South Research Institute, New Iberia, Louisiana.

mycobacterial infections accompanied by nerve invasion in the ears and feet of five rodents [4], he did not obtain progressive infection and dissemination. With the exception of small non-progressive lesions with tuberculoid histology that occurred occasionally in a chimpanzee at the site of inoculation, his efforts to infect monkeys, chimpanzees, hogs, dogs, and fruit bats have so far been unsuccessful.

The thesis that the cooler parts of the body were most severely involved in lepromatous leprosy was advanced by Brand [6] at the Seventh International Leprosy Congress, Tokyo, 1958, who observed that *M. leprae* grows better at temperature below body temperature, or exercises its damaging effect on the body at lower temperatures.

That temperatures of approximately 32° C are optimum for the multiplication of leprosy bacilli in the mouse foot-pad was shown by the results of Shepard's [24] careful studies. Nevertheless, the low-temperature hypothesis has been challenged by Palmer, Rees and Weddell [14]. However, recent observations at Carville by Hastings, *et al.* [11], and some of Rees' own findings in immune-suppressed mice [21] tip the scale in favor of the need for a relatively low temperature.

Gunders in Siberia, in 1958 [11], reported the results of the intravenous inoculation of a young chimpanzee with *M. leprae*. Eleven months post-inoculation, the animal exhibited numerous small nodules in the skin of the distal parts of the extremities. Histologically, the nodules resembled borderline leprosy. Small numbers of acid-fast bacilli were present. The nodules receded within three months.

Up to now, no animal species has been uncovered that possesses individuals naturally as susceptible to infection with *M. leprae* as some human beings. When we speak of a naturally susceptible animal, we mean one which has not been subjected to experimental immunosuppression, and which develops a spreading infection with the bacteriologic and histopathologic characteristics of leprosy. We believe that only an animal with a disease resembling human lepromatous leprosy can serve the major purposes for which it is needed, such as providing a model for the study of experimental chemotherapy, and for the study of the mechanism of natural resistance and susceptibility.

We must discuss now in what way the best animal models presently available fail to be satisfactory, thus necessitating continued search for a naturally susceptible animal.

Shepard in 1970 [24] showed that *M. leprae* can multiply in the mouse foot-pad under well defined conditions in a very characteristic but limited way. This has been confirmed in many laboratories in different countries [19, 20, 23]. However, Shepard's mouse foot-pad model does not resemble lepromatous leprosy either bacteriologically or pathologically. Bacterial multiplication in the mouse foot-pad is numerically limited and mainly restricted to the site of inoculation. Nevertheless, this model has been used to advantage for several purposes and studies such as:

1. Identification of *M. leprae*.
2. Screening of drugs for efficacy in suppressing multiplication of leprosy bacilli, including determination of drug-resistance.
3. Efficacy of vaccines to suppress multiplication of *M. leprae*.
4. Maintenance of the viability of leprosy bacilli under various environmental conditions, particularly refrigeration.
5. Investigation of the possible role of arthropods in the transmission of leprosy [15].

Prior to the work of Rees with thymectomized, whole body x-irradiated mice [21], none of the different methods used to suppress resistance resulted in either conversion of a localized into a disseminated infection, or in any significant increase of leprosy bacilli which can be harvested from the inoculated foot. By suppressing the immune mechanism prior to foot-pad inoculation, Rees increased the yield of leprosy bacilli per foot-pad up to 100 times that obtainable from normal animals. Following intravenous injection of leprosy bacilli into such mice, bacterial multiplication occurred in all the cooler parts of the skin. The histologic changes in the ensuing

lesions were said to be typical of lepromatous leprosy ([22]). However, no proof is at hand that skin became involved which had not been previously supplied with leprosy bacilli from the intravenous injection.

Several objections can be raised against the mouse as an animal suitable for the study of leprosy. One is the fact that the life span of even the undamaged mouse is less than the probable incubation period of leprosy in susceptible members of the human species. Another handicap is the fact that only physiologically severely altered mice develop an infection resembling lepromatous leprosy in some respects. In the regimen developed by Rees *et al.*, following x-irradiation (900r), mice must be kept alive by means of syngeneic bone marrow transfusion. Therefore, a highly inbred strain was necessary. Great losses occur from trauma and intercurrent infection.

Binford *et al* ([5]) modified the regimen of Rees *et al.* by shielding with lead a segment of the bone marrow of mice during irradiation. This method, which did not require syngenic bone marrow transfusion, provided good immunosuppressive and hematologic recovery. Although this procedure simplifies the immunosuppression procedure, it does not alter the drawback that such physiologically altered animals might not be suitable for studies into the mechanism of resistance. Apparently, they have a general loss of their antimicrobial defense mechanism. This is in sharp contrast to lepromatous leprosy where the defect seems quite specific. For various reasons, they might not be satisfactory for chemotherapeutic studies. For example, they do not live sufficiently long to study the effect of low doses on the rate of emergence of drug-resistance. These considerations strengthen our belief that search for a naturally susceptible animal should continue.

Our choice of the armadillo (*Dasypus novemcinctus*) as a possible model for the study of leprosy is supported by the following facts:

1. Armadillos have a life span of 12 to 15 years. This is far in excess of ordinary mice (2 years), or even *Mystromys albacaudatus* (5 years), presently used in Binford's laboratory ([4]).
2. Armadillos have a temperature (rectal) of 30 to 36° C and the temperature of their tissue changes in accord with the ambient temperature.
3. *Dasypus novemcinctus*, Linn. has unique potential for leprosy research because it regularly produces monozygous quadruplets, making it possible to replicate experiments with genetically identical animals. This is particularly relevant to leprosy research because of the genetic basis of the assumed mechanism of resistance of human beings to leprosy ([26]).

The purpose of the present publication is to report the development of lepromatoid infection in an armadillo experimentally infected with leprosy bacilli.

EXPERIMENTAL DESIGN

Armadillos have been reared to only a limited extent in captivity ([2, 27]). However, Storrs has kept them in the laboratory for the past eight years. Presently, she maintains a colony of approximately 300 animals at the Atchafalaya Basin Laboratories of the Gulf South Research Institute in New Iberia, Louisiana. These animals are being used as the nucleus for a breeding colony of laboratory raised animals.

Armadillos of either sex, and adapted to life in captivity have been, and will be in the future, infected with living leprosy bacilli at various ages of their postnatal life. Viability of *M. leprae* in the inoculum is determined from the ratio of solidly staining bacilli in the inoculum ([18]) and by ability to multiply in the mouse foot-pad. A careful pedigree is kept of all animals in the colony to make it possible to follow individuals of identical inheritance. Various modes of infection such as percutaneous, intracutaneous, subcutaneous, intravenous, intraconjunctival and intranasal mucosa have been or will be used. Follow-up is by gross inspection of the animals, and by bacterial enumeration of biopsied tissues as well as histopathologic evaluation of periodically conducted biopsies. Here again, survival of *M. leprae* in the tissues is determined as indicated above. Where it seems necessary, biopsies from uninoculated skin sites and search for acid-fast bacteria in the

blood and within the blood-macrophages is included in the assessment.

MATERIALS AND METHODS

Preparation of bacterial suspension. The bacterial inoculum ordinarily is prepared in the Carville Research Laboratories from biopsies of untreated lepromatous patients received from different parts of the world. In one instance, the inoculum was prepared from a biopsy from a Carville patient with sulfone-resistant leprosy bacilli. All materials received or prepared at Carville are cultured on ordinary bacteriological media and on Lowenstein-Jensen slants, incubated at 37° C and 33° C. Suspensions of the acid-fast bacilli are inoculated into mouse foot-pads. The solid ratio of the inoculum is established. Enumeration of the acid-fast bacilli is by a modification of the method described by Hanks, Chatterjee and Lechat ([12]). The inoculum for the armadillos is transported by car on wet ice to New Iberia, as soon as possible after its preparation. In several instances the inoculum consisted of suspensions of leprosy bacilli prepared by Dr. Louis Levy from infected mouse foot-pads at the U.S.P.H.S. Hospital, San Francisco, from where they were shipped on wet ice to New Iberia.

The viability of these bacilli was determined in the Carville Laboratory.

The following steps describe the method used for the preparation of leprosy bacilli suspensions at Carville:

1. Remove the epidermis from biopsy specimen.
2. Wash specimen in three changes of sterile distilled water.
3. Mince the tissue with a pair of scissors.
4. Grind thoroughly with a pestle in a mortar with addition of Hanks' Balanced Salt Solution (BSS), little by little. Make up to 5 ml.
5. Centrifuge in the cold at 220 x g for 10 minutes.
6. Remove the fatty substances floating on the top of the supernatant with a sterile swab.
7. Transfer the supernatant to a fluted flask containing a magnetic bar.
8. Grind the residue again and suspend in 5 ml of Hanks' BSS.
9. Centrifuge in the cold at 220 x g for 10 minutes
10. Pool the supernatants in a fluted flask.
11. Add 6 ml of 0.05% trypsin per 10 ml of tissue suspension.
12. Gently stir at 33° C for 30 minutes.
13. Centrifuge in the cold at 220 x g for 5 minutes.
14. Discard the residue and centrifuge the supernatant in the cold at 2700 x g for 30 minutes.
15. The residue contains the bacteria and suitable suspension can be made in tissue culture medium.

It might be desirable to immerse the biopsy specimen for 15 minutes as soon as possible after obtaining it in Hanks' BSS containing penicillin and streptomycin.

Processing of Biopsies from Armadillos. The armadillos are ordinarily injected at multiple sites, which, where possible and necessary, are then marked by tatooing. Biopsies are taken in New Iberia and immediately transported by car on wet ice to Carville. Each biopsy specimen is weighed and then divided in two parts. One is fixed in neutral formaldehyde, embedded in paraffin and sectioned. The sections are stained routinely with hematoxylin-eosin and with the Fite modification of the acid-fast stain. The remaining part of the biopsy specimen is re-weighed and used for preparation of the bacterial suspension for bacillary enumeration, inoculation into mouse foot-pads, and all bacteriologic work mentioned above. In the future, such suspensions can be used for armadillo passage of the bacteria, and preparation of lepromin.

RESULTS

Findings on Armadillo No. 8. Up to the present (July 1971) 44 armadillos have been inoculated with leprosy bacilli by various routes and with different amounts, some of them as early as 17 December 1969 and others as recently as 16 June 1971.

Armadillo Number 8 was one of four male animals which had been inoculated on 10 February 1970, with 0.1 ml of an

inoculum that contained 8.9 ± 0.4 x 10^7 bacilli per milliliter into each of two cutaneous sites at the abdomen and with an equal number of bacteria into both ear lobes.

The inoculum was prepared from a biopsy specimen of untreated lepromatous leprosy obtained by Dr. S.J. Bueno de Mesquita, Chief of Leprosy Service, Paramaribo, Surinam, on 2 February 1970, and sent to Dr. C.H. Binford, Washington, D.C., who shared a part of the specimen with us. The material was kept continuously in a thermos bottle packed with wet ice.

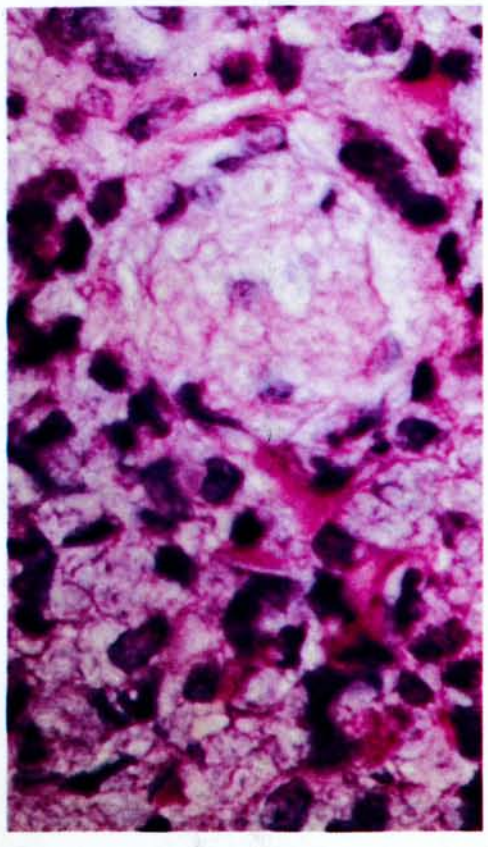
More than one year after infection, infiltrated lesions became visible at all injection sites. On 24 May 1971 biopsies were obtained from one of the abdominal lesions and from the lesion on one of the ears. The following are the numbers of acid-fast bacilli found in these respective lesions:

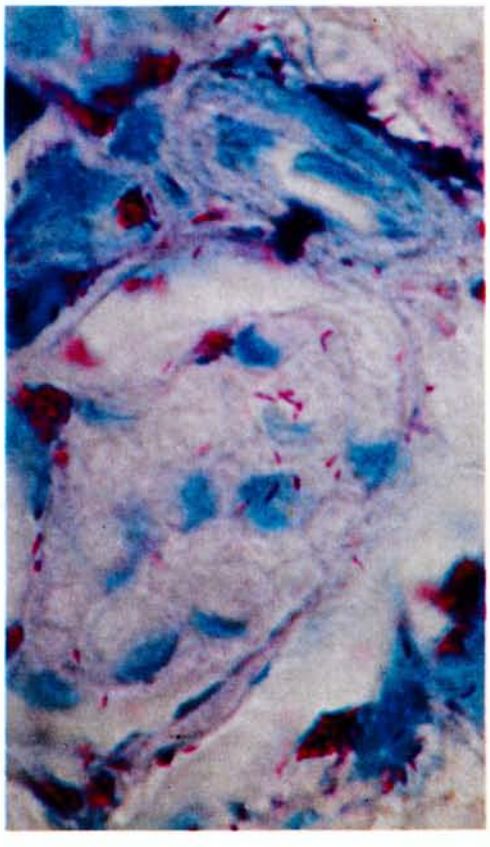
abdominal lesion: 4.5 x 10^9/200 mg of tissue;
ear lobe lesion: 1.4 x 10^9/70 mg of tissue.

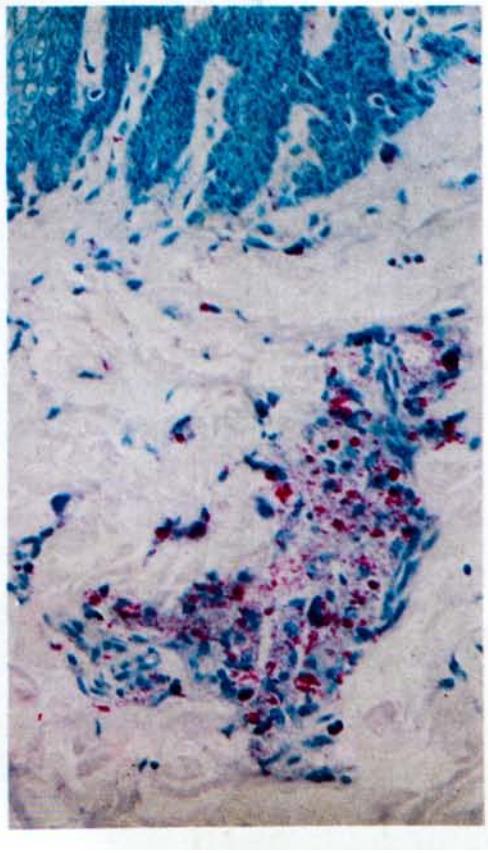
On July 1, 1971 an additional biopsy was obtained from the contralateral abdominal lesion and from normal appearing abdominal skin at some distance from the injection site. Four milliliters of heparinized blood were also obtained on this occasion. Colored photographs were taken of the four lesions prior to taking the specimens. In the abdominal lesion taken this time, 9.2 x 10^9 acid-fast bacilli were found in 1220 mg of tissue. The acid-fast bacteria from the biopsies taken at both times were planted on Lowenstein-Jensen medium and no mycobacterial growth has appeared up to now (9 July) at 37° C and 33° C. The bacteria were also injected into the foot-pads of mice for future reference. Foot-pads inoculated with the bacteria (10^4) obtained 24 May 1971 were histologically normal and bacteriologically negative one month later. The bacteria were capable of oxidizing D-dopa. This reaction is not only characteristic of the phenolase of *M. leprae*, but also excludes the possibility that the reaction might have been due to mammalian enzyme, which oxidizes only L-dopa. No other known mycobacterium oxidizes phenolic compounds ([14, 17]).

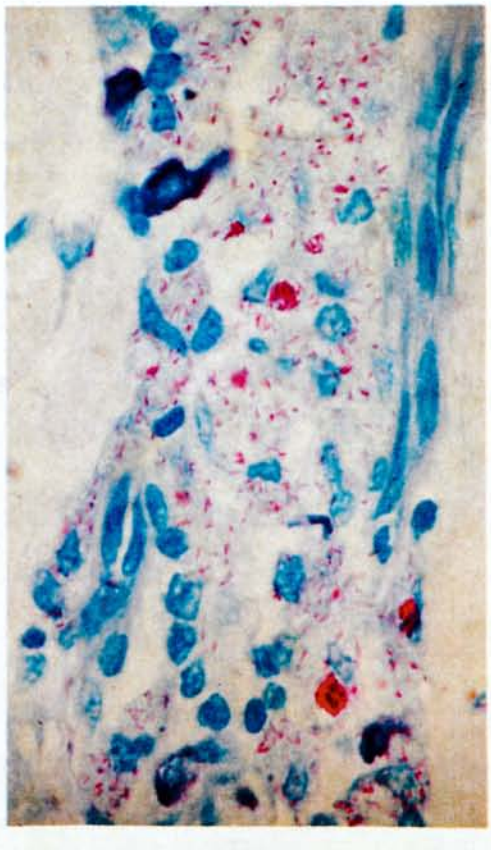
The histologic examination of all biopsy specimens showed an enormous number of acid-fast bacilli arranged in clumps within macrophages in the dermis, typical lepra cells and invasion of dermal peripheral nerves by acid-fast bacteria and cellular elements. It is necessary to stress particularly that this was also observed in the uninoculated skin. Microphotographs are shown in Fig. 1. Acid-fast bacilli were seen in films made from the buffy-coat of the blood. They were not, however, intracellular. This we believe is lepromatoid infection by *M. leprae* based on the following criteria: nonculturable acid-fast bacilli, which give a positive dopa oxidase test; enormous numbers of bacilli in typical lepra cells; dermal peripheral nerve invasion by acid-fast bacteria; spread of the infection to uninoculated sites with nerve invasion in those locations and the presence of bacteria in the blood streams. It is not known at this time, whether or not Armadillo No. 8 has the sensory defects typical for leprosy. This will be carefully explored. In several of the other animals inoculated more than one year ago, acid-fast bacteria have been seen in histologic preparations made from biopsies of the injected sites. There were modest numerical increases in some instances. Further developments must be awaited before a diagnosis of lepromatous disease can be confirmed or excluded.

FIG. 1 *Top left.* Ear-lesion at inoculation site. Observe, around a dermal nerve a compact infiltrate of foamy "lepra" cells. With the Fite-Faraco stain many bacilli were in the nerve and the "lepra" cells were densely filled with acid-fast bacilli. H and E stain X 640, AFIP Photo No. 71-6675. *Top right.* Inoculation site. Outside of the area of dense "lepromatous" infiltration was a small nerve illustrated in this picture. Observe the perineurally and intraneurally located acid-fast bacilli. Fite-Faraco stain X 1280, AFIP Photo No. 71-8414. *Bottom left.* Skin, abdomen. Non-inoculated site. This slide was made from a biopsy taken from the side of the abdomen opposite to the inoculation site. This therefore is evidence of dissemination of the infection. Observe the numerous bacilli in this small infiltrate and the similarity to lepromatous leprosy. Fite-Faraco stain X 180, AFIP Photo No. 71-6678. *Bottom right.* Skin of non-inoculated abdomen. Similar microscopic field as shown in "top left". Fite-Faraco stain X 1280.









DISCUSSION

It is generally agreed that there are great differences in innate resistance to infection with *M. leprae* among individuals of the human species. Experience with this disease shows that in the highly resistant majority, infection with the Hansen bacillus remains without perceptible consequences. The tissues of other individuals exhibit some measure of resistance and the bacteria multiply to only a very limited extent. Individuals with such characteristics develop tuberculoid leprosy if they develop leprosy at all. A minority of persons, on the other hand, appears completely lacking in resistance, apparently specifically against the leprosy bacillus. This microorganism is able to multiply in their tissues without noticeable host repression. Individuals of this genre will develop, when infected, a sustained and spreading disease known as lepromatous leprosy. It must be kept in mind that the two polar forms of leprosy, tuberculoid and lepromatous, are not interconvertible. They seem to reflect innate differences in susceptibility which are not subject to change. Intermediate degrees of resistance or susceptibility are reflected by a not quite so stable a spectrum of disease with tuberculoid and lepromatous characteristics mixed in various and variable proportions. This is called intermediate, dimorphous or borderline leprosy.

The mechanisms of resistance to infection with *M. leprae* are obscure, but it is believed that they involve mesenchymal cells. Analogy with experimental tuberculosis ([1]) might suggest enzymic differences in the macrophages of resistant and susceptible individuals. Others have stated that susceptibility is linked with a defect of the cellular immune mechanism. Both macrophages and lymphocytes are thought to participate in the cell defense mechanism of mammals. Therefore, it would seem that there is no real conflict between the proponents of the two concepts. What is clear, however, is that the mechanism of resistance or susceptibility must be explored at the cellular level, and best with material from resistant and susceptible individuals who do not themselves have leprosy. Observations by Dharmendra and Chatterjee ([9]) can lead one to believe that it might be possible to recognize susceptibility in the nonleprous person. It would be difficult, however, to arrange for a study on such individuals.

In selecting the armadillo for our attempts to reproduce leprosy in an animal, we were guided by the concept that it is unrealistic to search for a species which consists entirely of susceptible individuals. We did, however, consider that it might be possible to develop a colony of susceptible armadillos because of their propituous and unique reproductive characteristics, once we have found a male and a female animal which furnish proof of their susceptibility by having developed lepromatous leprosy. Their assumedly susceptible offspring can be used to study the resistance and susceptibility at the cellular level in leprosy. Such studies might be of general medical importance because they may also throw light on the role of similar events in the pathogenesis of other infectious diseases, especially some systemic mycoses. In addition, investigations of leprosy at the cellular level might yield information applicable to non-infectious events in which macrophages play a role. Among these are: allergy, allograft rejection, resistance to cancer, and radiation injury. Refocusing sights on leprosy, there are many other investigations possible with susceptible and lepromatous armadillos which can not be profitably investigated with the animal models now on hand, such as chemotherapy and chemoprophylaxis, and alternative drugs for treatment of emergent drug-resistant strains of leprosy bacilli. Another possible investigation would relate to the immediate-type hypersensitivity phenomena in lepromatous leprosy such as *erythema nodosum leprosum*.

In addition, lepromatous armadillos might become a plentiful source of the rare commodity *M. leprae* from untreated individuals. From not quite 1.5 gm of tissue from Armadillo No. 8 we were able to harvest 15.1×10^9 leprosy bacilli. It does not take a great deal of imagination to foresee the impact of this on interested bacteriologic and immunologic laboratories.

Finally, this type of animal model may also facilitate the study of nerve invasion and its sequelae.

It is realized that the selective breeding of armadillos may have its difficulties because of their reluctance to breed readily in captivity. It might become necessary to resort to artificial insemination and hormonal stimulation.

SUMMARY

It is reported that an armadillo (*Dasypus novemcinctus*) has developed lepromatoid infection with *M. leprae* approximately 14 months after inoculation of leprosy bacilli, from an untreated case of lepromatous leprosy, into the skin of its abdomen and ear lobes. The diagnosis of lepromatoid leprosy is supported bacteriologically by over 1000 fold increase in the inoculation sites of acid-fast bacteria, which do not grow on mycobacterial culture media and which oxidize D-dopa. In addition, these acid-fast bacteria have been found in great numbers at a skin site remote from the inoculated area. The remote skin site was of normal appearance. The inoculated skin sites were converted into massive nodular lesions. The acid-fast bacteria were intracellular, and typical lepra cells made up much of the lepromas. Bacilli were also seen in cutaneous nerves. It is too early yet to evaluate the results of the mouse foot-pad inoculations of the bacilli. So far, however, sections of the foot-pads show what one would expect of *M. leprae* after one month.

The reasons for attempting transfer of leprosy to the armadillo and the possible future significance of the armadillo in leprosy research have been discussed.

RESUMEN

Se ha reportado que un armadillo (*Dasypus novemcinctus*) ha desarrollado una infección lepromatoide con *M. leprae* aproximadamente 14 meses después de la inoculación de bacilos de lepra, de un caso de lepra lepromatosa no tratada, en la piel del abdomen y de los lóbulos de las orejas. El diagnóstico de lepra lepromatosa está basado bacteriológicamente en el aumento de más de 1000 veces de la cantidad de bacterias en el sitio de inoculación, bacterias que no han crecido en medios para micobacterias y que oxidan la D-dopa. Además, estas bacterias ácido-resistentes han sido encontradas en grandes cantidades en un sitio de la piel distante de la zona de inoculación. El sitio de piel distante tenía una apariencia normal. Los sitios de inoculación se han transformado en lesiones nodulares masivas. Les bacterias ácido-resistentes eran intracelulares y la mayor parte de los lepromas estaban formados por típicas células leprosas. También se observaban bacilos en los nervios cutáneos. Aún es muy temprano para evaluar los resultados de la inoculación de estos bacilos en la almohadilla de la pata del ratón. Hasta el momento sin embargo, los cortes de las patas de ratón muestran lo que sería de esperar con el *M. leprae* después de un mes.

Se exponen las razones por las cuales se intentó transferir lepra al armadillo y la posible significación futura del armadillo en la investigación leprológica.

RÉSUMÉ

On signale dans cet article qu'un armadillo (*Dasypus novemcinctus*) a développé une infection lépromatoïde à *M. leprae*, environ 14 mois après avoir été inoculé par des bacilles de la lèpre provenant d'un cas non traité de lèpre lépromateuse. Cette inoculation avait eu lieu dans la peau de l'abdomen et dans les lobules de l'oreille. Le diagnostic de lèpre lépromateuse a été confirmé bactériologiquement par une augmentation de l'ordre de 1.000 dans le nombre des bactéries acido-résistantes trouvées aux endroits d'inoculation; ces bactéries ne poussaient pas sur des milieux de culture mycobactériens, et entraînaient l'oxydation de la D-dopa. De plus, on a retrouvé des bactéries acido-résistantes, en grand nombre, en un endroit de la peau fort distant de la région d'inoculation. Cet endroit de la peau, distant du site d'inoculation, présentait une apparence normale. Les endroits d'inoculation cutanée se sont transformés en lésions nodulaires massives. Les bactéries acido-résistantes étaient intracellulaires, et des cellules lépreuses typiques constituaient la plus grande partie de la masse des lépromes. Les bacilles ont été également observés dans les nerfs cutanés. Il est encore trop tôt pour évaluer les résultats des inoculations pratiquées avec ces bacilles dans la sole plantaire de la souris. Jusqu'à présent, toutefois, des coupes de coussinets plantaires ont montré, après un mois, des résultats qui sont en accord avec ce que l'on pourrait attendre de *M. leprae*.

Les raisons qui ont conduit à essayer de transmettre la lèpre à l'armadillo et la signification possible pour l'avenir de l'utilisation de l'armadillo dans la recherche sur la lèpre, sont ici discutées.

Acknowledgments. The authors wish to express their gratitude to the following who have contributed substantially to the project:

Dr. S. J. Bueno de Mesquita, Paramaribo,

Surinam, who supplied the biopsy material used for the inoculum.

Dr. K. Prabhakaran, Biochemist, USPHS Hospital Carville, La., identified the mycobacteria as *Mycobacterium leprae* by use of the phenolase reaction.

Miss R. M. Sanchez, Technologist, USPHS Hospital Carville, La., assisted in the preparation of the inoculum and the enumeration and examination of the bacilli.

Dr. William E. Greer, Veterinarian, Gulf South Research Institute, New Iberia, La., assisted in the inoculations, the observation of the animal for lesions and supervised the general care of the animal.

This work was supported by research grant CC00476 from the Center for Disease Control, Atlanta, Georgia, and research grants AI-03636 and AI-07890 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health of the Department of Health, Education and Welfare, U.S.A.

Addendum: Since the preparation of this manuscript, preliminary autopsy evaluation of armadillo No. 8 confirms the presence of systemic lepromatoid acid-fast dissemination. The final autopsy report will be submitted later.

REFERENCES

1. Allison, M. J., Zappasodi, P., and Lurie, M. B. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. I. Studies on cells of normal noninfected animals. Am. Rev. Resp. Dis., **84** (1961) 364-370.
2. Anderson, J. M., and Benirschke, K. The armadillo, *Dasypus novemcinctus*. *In:* Experimental Biology Lab. Animal Care **16** (1966) 202-216.
3. Binford, C. H. Comprehensive program for the inoculation of human leprosy into laboratory animals. U.S.P.H.S. Rep. **71** (1956) 995-996.
4. Binford, C. H. The transmission of *M. leprae* to animals. Attempts to find an experimental model. Presented at Ninth International Leprosy Congress (September 1968), London, England.
5. Binford, C. H., Walsh, G. P., and Theocheung, J. L. Transmission of *M. leprae* in immuno-suppressed mice. Use of bone marrow shielding in preventing death from irradiation. Sixth Joint Leprosy Research Conference. U.S.-Japan Cooperative Medical Science Program. Bethesda, Maryland, July 26-28, 1971.
6. Brand, P. W. Temperature variation with leprosy deformity. Internat. J. Leprosy **27** (1959) 1-7.
7. Carpenter, C. M., and Miller, J. N. Experimental transmission of leprosy. *In:* Leprosy in Theory and Practice. R. G. Cochrane and T. F. Davey, eds., The Williams and Wilkins Company, Baltimore, Maryland (1964) 19-25.
8. Da Silva, N. C., E Silva, C. D. O., and De Andrade, L. C. Attempts at experimental transmission of leprosy to terrestrial Chelonia from the Amazon region, Brazil. Presented at Ninth International Leprosy Congress (September 1968), London, England.
9. Dharmendra and Chatterjee, K. R. Prognostic value of the lepromin test in contacts of leprosy cases. Leprosy in India **27** (1955) 149-152.
10. Fite, G. L., Wrinkle, C., and Sanchez, R. Inoculation of *M. leprae* into reptiles. Internat. J. Leprosy **32** (1964) 272-278.
11. Gunders, A. E. Progressive experimental infection with *Mycobacterium leprae* in a chimpanzee; a preliminary report. J. Trop. Med. & Hyg. **61** (1958) 228-230.
12. Hanks J. H., Chatterjee, B. R., and Lechat, M. F. A guide to the counting of mycobacteria in clinical and experimental materials. Internat. J. Leprosy **32** (1964) 156-167.
13. Hastings, R. C., Brand, P. W., Mansfield, R. E., and Ebner, J. D. Bacterial density in the skin in lepromatous leprosy as related to temperature. Leprosy Rev. **39** (2) (1968) 71-74.
14. Kirchheimer, W. F., and Prabhakaran, K. Metabolic and biologic tests on mycobacteria once labelled as leprosy bacilli. Internat. J. Leprosy **36** (1968) 162-165.
15. Kirchheimer, W. F., and Sharma, K. B. The role of arthropods in the transmission of leprosy. Work in progress under agreement No. BHS-DDHS-Ind-1, 1968, between U.S. and Jawahrlal Institute of Post-graduate Medical Education and Research, Pondicherry, India.
16. Palmer, E., Rees, R. G. W., and Weddell, A. G. M. Site of multiplication of human leprosy bacilli inoculated into the foot-pads of mice. Nature (London) **206** (1965) 521-522.
17. Prabhakaran, K., and Kirchheimer, W. F. Use of 3,4 dihydroxyphenyalanine oxidation in the identification of *Mycobacterium leprae*. J. Bact. **92** (1966) 1267-1268.

18. REES, R. J. W., and VALENTINE, R. C. The appearance of dead leprosy bacilli by light and electron microscopy. Internat. J. Leprosy **30** (1962) 1-9.
19. REES, R. J. W. Limited multiplication of acid-fast bacilli in foot-pads of mice inoculated with *Mycobacterium leprae*. Brit. J. Expt. Path. **45** (1964) 207-218.
20. REES, R. J. W. Recent bacteriologic, immunologic and pathologic studies of experimental human leprosy in the mouse food-pad. Internat. J. Leprosy **33** (1965) 646-655.
21. REES, R. J. W., and WATERS, M. F. R. (May 1966) Enhancement of *Mycobacterium leprae* in the mouse foot-pad by thymectomy and irradiation. Japan-U.S. Cooperative Medical Science Program Leprosy and Tuberculosis Conference, Tokyo.
22. REES, R. J. W., WATERS, M. F. R., WEDDELL, A. G. M. and PALMER, E. Experimental lepromatous leprosy. Nature **215**, No. 5101 (1967) 597-602.
23. SHARMA, K. B. Private communication. Jawaharlal Institute of Post-graduate Medical Education and Research, Pondicherry, India (1968).
24. SHEPARD, C. C. The experimental disease that follows the injection of human leprosy bacilli into the foot-pads of mice. J. Exper. Med. **112** (1960) 445-454.
25. SHEPARD, C. C. Temperature optimum of *Mycobacterium leprae* in mice. J. Bact. **90** (1965) 1271-1275.
26. SPICKETT, S. D. Genetic mechanisms in leprosy. *In:* Leprosy in Theory and Practice. R. G. Cochrane and T. F. Davey, eds., The Williams and Wilkins Company, Baltimore, Maryland (1964) 98-124.
27. STORRS, E. E. Individuality in monozygotic quadruplets of the armadillo *Dasypus novemcinctus*, Linn. Doctoral dissertation, The University of Texas, 1967.