REPORTS OF WORKSHOP COMMITTEES

COMMITTEE 1: WORKSHOP ON EXPERIMENTAL LEPROSY

Chairman: C. H. Binford

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Observers: E. Elsler
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At the X International Leprosy Congress in 1973, in Bergen, the Committee on Advances in Experimental Leprosy reported the developments in the use of the normal mouse, the thymectomized irradiated mouse, the neonatally thymectomized Lewis rat, and the nine-banded armadillo *(Dasypus novemcinctus)*. Also initial results with the Korean chipmunk were reported.

During the past five years there has been intense activity in experimental leprosy and several new models were introduced. The members of the workshop who reported on experiments in new animals brought several sets of appropriately stained histopathologic sections for study under the 12 microscopes which were generously provided by the Organizing Committee.

EUROPEAN HEDGEHOG (ERINACEUS EUROPAEUS)

In Germany, experimental inoculation with *M. leprae* was initiated in the European hedgehog *(Erinaceus europaeus)*, a small animal (less than 1 kg) with a body temperature of 35°-36°C when not in hibernation. This animal is readily adjusted to a laboratory environment and will breed in captivity. In some animals intracutaneous and subcutaneous inoculation with *M. leprae* resulted, after many months, in local lesions that histopathologically showed epithelioid cell granulomatous reactions with small numbers of AFB. Investigators in the United Kingdom, stimulated by the work in Germany, inoculated a group of hedgehogs in foot pads and another group intravenously. Two intravenously infected hedgehogs sacrificed 20 months post-inoculation showed widespread dissemination with large numbers of AFB in macrophages. The lepromatous lesions were found in lymph nodes, liver, spleen, tongue, foot pads, and ears. Intraneural involvement with *M. leprae* was observed in dermal and sciatic nerves.

The members of the workshop were impressed by the results obtained in these initial experiments with this small animal which is readily available in Europe and easily adjusted to laboratory environment, and recommended that the investigators vigorously continue their experiments.

NUDE MOUSE

The nude athymic mouse (BALB/C-nu/nu) was presented by investigators from Tokyo and Osaka as a model for experimental

lepromatous leprosy. The mice were maintained under specific pathogen-free conditions. Severe lepromatous lesions were obtained in liver, lung, spleen, nerves, bone marrow, testis, ears, tail, and the inoculated foot. Eight months after inoculation, up to 10^{10} *M. leprae* could be recovered from foot pads.

The investigator from Tokyo reported enhancement of the development of the lepromatous disease in the nude mouse by inoculating the animal with young thymic cells (1-2 weeks). The members of the workshop were convinced that with further study the nude mouse gives promise of becoming a valuable model for immunologic investigations and experimental chemotherapy.

NEONATALLY THYMECTOMIZED LEWIS RAT

An account was given from the USA of the widespread dissemination of bacilli in this model following the intravenous injection of 10^7 *M. leprae* 12 months previously. Moreover, in contrast to the mouse foot pad in which the maximum inoculum is 10^4 bacilli (more than this being immunogenic), it is possible to inoculate 10^7 bacilli into the foot pad or the NTLR and still detect bacillary growth. Heavily infected NTLR are being used in chemotherapy experiments in order to provide information on persistence of *M. leprae*. This information would be useful in the design of therapeutic regimens for patients with multibacillary leprosy. Monitoring of therapy in this experimental model and in human patients is being carried out by inoculation of large numbers of bacilli into the foot pads of NTLR.

ARMADILLOS

The use of the armadillo in leprosy research has advanced rapidly during the past five years. For the first time in the history of leprosy, sufficient quantities of *M. leprae* could be made available for basic research in microbiology, immunology and biochemistry. WHO has seized this opportunity by developing an extensive comprehensive program in immunology (IMMLEP) utilizing lepromatous tissue of armadillos captured in the southern part of the USA.

In May 1977, the Pan American Health Organization conducted at the Instituto Nacional de Dermatologia, Caracas, Venezuela a five day workshop on the Armadillo as an Experimental Model in Biomedical Research. The report of this workshop will be made available in December 1978 in the PAHO Scientific Publication No. 366 (178 pages).

Stimulated by the workshop in Caracas, programs to use armadillos in leprosy research are developing in many countries of the Americas. In addition to *D. novemcinctus*, several other species found only in South America are being investigated. Active programs utilizing nine-banded armadillos from the southern USA are being carried out in countries where armadillos are not naturally available.

Reports on progress of leprosy research with armadillos were made by several participants of this workshop.

Venezuela. Very encouraging results were reported in the *Dasypus sabanicola*, the eight-banded armadillo, native to certain regions of Venezuela and Colombia. This animal is much smaller (1.5 kilos) than *D. novemcinctus* and more easily handled in the laboratory. On inoculation with *M. leprae* a very significant number of *D. sabanicola* develop severe lepromatous leprosy, while some animals develop near-tuberculoid lesions, suggesting that this model may be useful in studying the several types of leprosy found in man.

United Kingdom. The report from the UK revealed that it is possible to successfully maintain colonies of the nine-banded armadillo *(D. novemcinctus)* in a country where this animal is not naturally available. Fifty-six percent of 16 armadillos surviving from a group of 20 which were donated by the Gulf South Research Laboratory, New Iberia, Louisiana, within two years after inoculation developed disseminated lepromatous lesions involving major tissues and organs. Dermal nerves were frequently involved. Large nerve involvement was seen in some animals. Histopathologically, the lesions were similar to those reported from Carville and New Iberia, Louisiana.

Argentina. In Argentina three species native to the country, *Zaedus pichey, Chaetophractus villosus,* and *Dasypus hybridus*, were inoculated by intradermal and intracardiac routes. Repeated inoculations were done in some animals. In *Z. pichey* and *C. villosus* granuloma developed at the sites of inoculation. Some granulomas histopathologically

showed tuberculoid changes. The lesions did not advance locally or disseminate. The results of inoculation in *D. hybridus* could not be learned because no animals survived more than six months.

Brasil. A large program for using armadillos in leprosy research is in progress. After unsuccessful results in several species, the project now is principally concerned with determining the susceptibility of the native *D. novemcinctus* to leprosy. Eight *D. novemcinctus* were found not to be infected after periods of observation up to 18 months. Much longer periods of observation will be used.

Paraguay. In Paraguay, the inoculations of *Chaetophractus vellerosus* and *Euphractus sepcticinctus* resulted in some localized tuberculoid reactions but not in disseminated lepromatous lesions. The first trials with *D. novemcinctus* were interrupted by loss of the colony from an epizootic infection. An extensive program using principally *D. novemcinctus* is now underway.

Mexico. In Mexico, facilities for use of the native *D. novemcinctus* have been provided and 18 animals have now been adapted to the laboratory environment. Basic information on the natural bacterial flora of these armadillos and hemotologic profiles have been obtained.

Carville, La. Programs previously reported have been continued and valuable information has been provided on results of massive intravenous inoculation to produce early harvests of lepromatous tissues for basic research.

An interesting report was made on the results obtained from vaccinating armadillos with heat-killed whole *M. leprae* in incomplete Freund's adjuvant eight months prior to challenge with viable *M. leprae*. The results in a small group of animals were promising and it is hopeful that further experimental vaccination of armadillos will be done.

New Iberia, La. Inoculation with *M. leprae* of two *Dasypus hybridus* obtained from Argentina resulted after 24 months in severe, disseminated lepromatous leprosy in one animal and no infection in the other. Histopathologically, the lesions were indistinguishable from those seen regularly in infected *D. novemcinctus*. The positive result in this animal should provide impetus for effectively using *D. hybridus* in programs of experimental leprosy in Argentina where this species is readily available.

Experience with the inoculation of approximately 500 *D. novemcinctus* showed that up to 60% will develop disseminated leprosy at doses of 10^7 to 10^8 *M. leprae*. Histopathologic evaluation of tissues from the experimentally-inoculated animals provided experience that later was invaluable in the study of armadillos naturally infected with mycobacteria later identified as *M. leprae*.

Studies of the tissues and organs from normal newborn and adult armadillos showed the reticuloendothelial system (thymus, spleen, lymph node, tonsils, and Peyer's patches) of *D. novemcinctus* to be well developed and morphologically intact. From these studies it was concluded that the susceptibility of the nine-banded armadillo to leprosy was not a result of malformation of organs or tissues needed to mount an effective immune response.

A shift in program emphasis from the experimental disease to the naturally acquired disease was made following the discovery in 1974 of indigenous leprosy in wild caught nine-banded armadillos.

Indigenous leprosy in wild armadillos. The most recent data on indigenous leprosy in *D. novemcinctus* captured from the wild were presented. Sixty animals from 11 locations in Louisiana and one location in Texas have been found by the Gulf South Research Institute, New Iberia, Louisiana to have lepromatous leprosy. Confirmation of indigenous leprosy in armadillos has been reported by the CDC, Atlanta, the University of Texas, Galveston and the USPHS Hospital, Carville. An armadillo captured in Texas and kept for two years in the zoo at San Diego, California was found to have disseminated lepromatous leprosy.

At New Iberia, the best method for identifying leprosy in a live animal was by examination of slit smears from the base of the ears. Lymph nodes were consistently involved but obtaining biopsy specimens of lymph nodes from live animals during a survey study is not practical.

By the criteria proposed at the London Congress (1968) and methods subsequently developed (pyridine extraction, fluorescent antibody methods) the cause of the indigenous disease is indistinguishable from *M. leprae*.

The indigenous leprosy found in *D. novemcinctus* in the USA provides an opportunity for comprehensive studies on the epizootiology of this disease, and from such studies it is hoped that some answers to problems in the epidemiology of human leprosy will be found.

Reproduction of armadillos in captivity. A scientist from the Medical Research Institute, Melbourne, Florida reported current studies in reproduction being carried out in that institute and previous studies carried out at New Iberia. Reproduction was given top priority at the PAHO workshop in Caracas in 1977, and there is general agreement that the full potential of the armadillo in biomedical research will not be obtained until breeding in captivity can be achieved and laboratory-bred animals provided in numbers sufficient for experimental work.

It was emphasized that the stress of captivity is probably responsible for interrupting the normal ovulation phases of the female. Sperm cells of males in captivity, although lower than from males when captured, appear adequate to fertilize ova of females ovulating regularly. Although artificial stimulation of ovulation is possible, it has not resulted in successful impregnation and gestation. Modification of the environment of captivity to reduce stress may prove to be the solution to reproduction.

At the Zoonosis Paramedical Center (CEPANZO) in Argentina in an area where *D. hybridus* is readily available, facilities have been developed for experimental breeding and it is hoped that successful breeding of *D. hybridus* in numbers adequate for experimental use will result.

KOREAN CHIPMUNK

Unfortunately, because of transportation difficulties, Joon Lew, M.D., Ph.D., who was scheduled to present his progress in experiments with the Korean chipmunk *(Eutamias sibiricus asiaticus)*, arrived after the conclusion of the workshop but presented his finding to some members of the workshop. In these animals inoculation of 10^7 *M. leprae* in foot pads resulted in gross nodules appearing 7-17 months post-inoculation in 10% to 20% of the animals. Histopathologically, the lesions were advanced lepromatous. Nerves were distinctly involved. Lymph nodes were massively involved and liver and spleen less involved.

These chipmunks, while readily available in nature so far, have not bred in captivity. Obviously, work with this animal which without immunosuppression develops lepromatous leprosy, should continue.

CONCLUSION

This workshop has provided information on several new models for the study of lepromatous leprosy. During the period since the last congress, significant progress has been made on the use of the nine-banded armadillo *(D. novemcinctus)* in leprosy research. At this workshop the eight-banded armadillo *(D. sabanicola)* has also been shown to be a model for lepromatous leprosy, and hopefully, for other types of leprosy.

With the opportunities now available for investigators in experimental leprosy, the use of these models in studying the pathogenesis, prevention, epidemiology and treatment of human leprosy appear unlimited.