TRANSMISSION OF *MYCOBACTERIUM LEPRAE* TO ANIMALS

Nerve Involvement in the Ears of Hamsters[1,2]

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In experiments on the transmission of *Mycobacterium leprae* to animals conducted since January 1956, several types of small animals and two species of monkeys have been used, but the largest number of experiments were in the golden hamster (Syrian hamster). Because in man leprosy affects principally the cooler parts of the body, as was stated in the plans for the experiments that were begun in 1956 (1), the inoculations have usually been made in the cooler parts of the animal.

OBJECTIVE OF TRANSMISSION EXPERIMENTS

These transmission experiments were designed for the purpose of attempting to reproduce in the animals an infection with *M. leprae* and to observe histopathologically the results of that infection during the life of the animal. The objective of these experiments, therefore, was to induce a disease in an animal and not to quantitate the multiplication of bacilli in animal cells.

Golden hamsters rarely live longer than two years, a period which is much shorter than the three to five years that Cochrane (3) gave as the generally accepted incubation or latent period in human leprosy. It is doubtful that *M. leprae*, even in a highly susceptible animal, would produce lesions any more rapidly than in man; therefore, in the post-inoculation life span of hamsters, it was not reasonable to hope that gross lesions would develop. The objective, therefore, was to obtain, in an animal, evidence of infection that histologically could be compared with the early microscopic lesions in man. Histologically the earliest lesions in human leprosy are insignificantly mild and cytologically indeterminate, but even in the mild human lesions, bacilli are frequently found in the tiny nerves of the skin (Fig. 1).

This report deals with the nerve lesions that have developed in the dermal nerves of hamster ears many months following inoculation

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with *M. leprae*. The results of 21 human to hamster experiments in 301 animals are given. With the exception of a few animals killed to provide inoculum for passage to other hamsters, all died spontaneously.

METHODS AND MATERIALS

On the basis of the geographic source of the skin specimens the experiments have been divided into four groups. All specimens were transported in thermos bottles packed in wet ice. Except as noted all patients were clinically classified as lepromatous.

GROUP I. One experiment consisting of 29 hamsters inoculated in September 1958 with an inoculum prepared from skin specimens obtained from patients in the USPHS Hospital, Carville, La. (in thermos bottle 24–36 hours).

GROUP II. Five experiments carried out in 46 hamsters inoculated in December 1958 with skin specimens from patients in the Philippines, Central Luzon Sanitarium.[4] One specimen was from a patient clinically diagnosed borderline leprosy (approximately 8 days in thermos bottle).

GROUP III. One experiment composed of 25 hamsters inoculated in February 1959 from a specimen obtained from a patient in North Carolina[5] (in thermos bottle 6 hours).

GROUP IV. Fourteen experiments started in March 1959. The specimens were from 10 patients in Surinam[6] (in thermos bottle 48 hours). One specimen was from a patient clinically diagnosed borderline leprosy.

Preparation of inoculum.—The inoculum was prepared by homogenizing the surgically removed skin specimens in an all-glass tissue grinder (Ten Broeck) and suspending the inoculum in physiologic saline. The bacilli in the inoculum were not counted, but the saline suspensions were evaluated by examining stained smears and estimating the number of bacilli per oil immersion field. The inoculum generally contained 10 to 50 bacilli per oil immersion field. The inoculations were made by one or two injections of 0.5 ml. each beneath the epidermis of the external ear (dorsal aspect) and 0.15 ml. into each testis.

Bacteriology.—Cultures on Loewenstein-Jensen medium were planted from the inoculum used in all of the human to animal experiments in this group and for the animal to animal transfer experiments made later. In none of these were mycobacteria cultivated. The culture tubes were observed for a period of 4 months.

Controls.—To furnish a basis for histopathologic comparison of results, in the majority of experiments, groups of animals were similarly infected with inoculum that had been heated in boiling water for 20 minutes.

Length of experiments.—In these experiments the greater number of the hamsters survived more than 15 months post-inoculation and a few survived 20 to 26 months.

Pathology.—Autopsies were made on all animals. The parts

[4] Specimens transported personally by Dr. Charles C. Shepard, Communicable Disease Center, Atlanta, Ga.

[5] Patient of Dr. M. E. McCrae, Greensboro, N.C.

[6] Patients from Dr. S. J. Bueno de Mesquita, Paramaribo, Surinam.

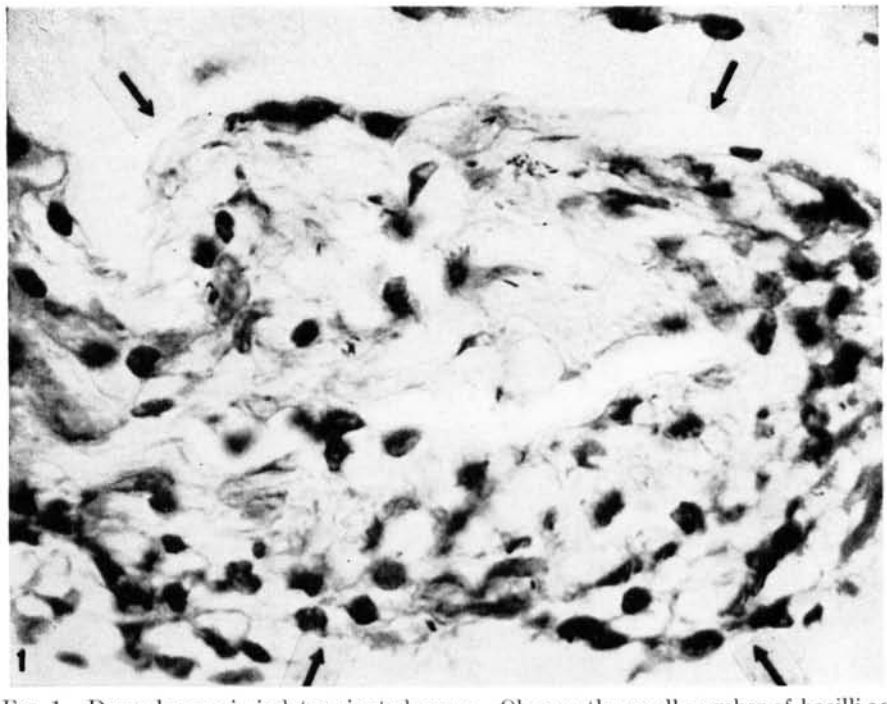


FIG. 1. Dermal nerve in indeterminate leprosy. Observe the small number of bacilli accompanied by some increase in intraneurial nuclei. There was very mild round cell infiltration about a few vessels and appendages in the dermis.

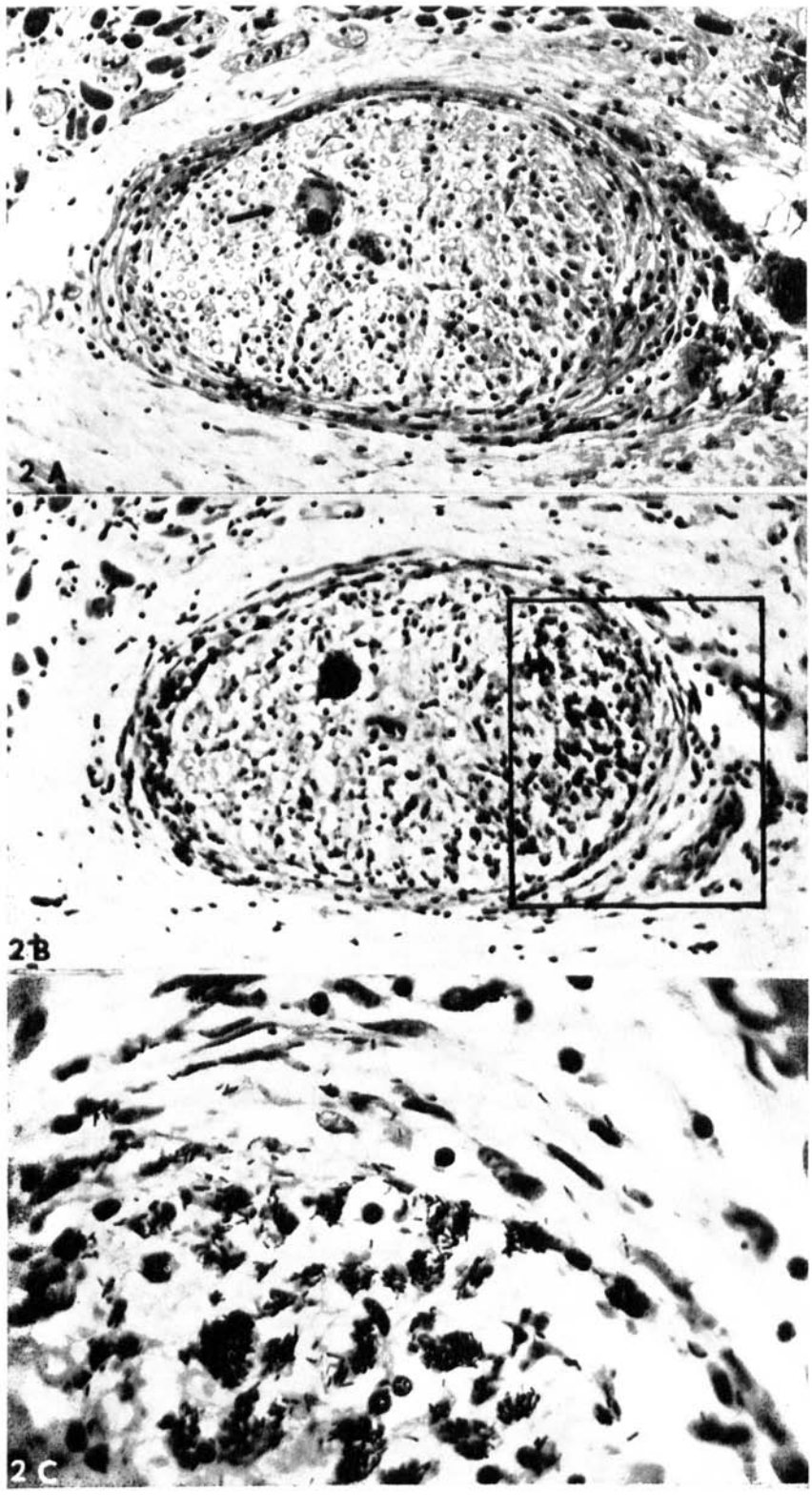
The correct diagnosis on this slide was made 8 years after the biopsy had been taken and after the patient was admitted to a leprosarium with advanced lepromatous leprosy. On review of the slide made 8 years before with special attention to the small nerves, the diagnosis of indeterminate leprosy was made. The original histopathologic diagnosis had been dermatitis, chronic, mild. AFIP Accession No. 320211. Neg. No. 59–257. Fite-Faraco stain. × 900.

inoculated were routinely studied histopathologically by hematoxylin and eosin and acid-fast stains. In order to obtain uniform histopathologic sampling of the ears, immediately on removal from the animal they were placed between two peices of cardboard held together by a paper clip. The ears thus flattened were placed in Zenker's formalin fixative.

After fixation, from each of the uniformly flattened ears three strips of approximately 3 mm. width were taken transversely, the line of sectioning being parallel to the base of the ear. The strips from each ear were placed in a single paraffin block with a cut edge down. Routinely, one hematoxylin and eosin and one Fite-Faraco stained slide were examined from each block. The histopathologic sampling, therefore, consisted of studying, for each ear, three sections taken at right angles to the skin surfaces and containing skin layers, striated muscle, and cartilage. Generally each section contained one or more cross, tangential, or longitudinal sections of small dermal nerves.

RESULTS

With heat-killed inoculum.—In control animals examined 12 months post-inoculation bacilli were rarely demonstrated and when


2A
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present were usually poorly stained and within the giant cells and histiocytes that contained ground glass particles from the Ten Broeck all-glass tissue grinder. In none of the sections from the control animals was there intraneurial or perineurial involvement by bacilli. After one year in none of the control animals did the heat-killed bacilli resemble in number, staining qualities, and morphology, the *M. leprae* that were seen in the animals inoculated with nonheated bacilli.

With nonheated inoculum.—In the histopathologic sections of ears from many animals in 20 of the 21 groups of hamsters inoculated with suspensions of nonheated bacilli, there were usually varying numbers of well stained bacilli located away from the site of the inoculation debris. Often these bacilli were long and solid and frequently formed intracellular globular masses and packet-like bundles within histiocytes.

Nerve involvement.—Invasion of nerves of the ear by *M. leprae* was observed in 93 (30.9%) of the 301 hamsters in the 21 experiments. Positive results were obtained in some of the animals in 20 experiments of this group. In animals inoculated with material from the patient from Surinam who was clinically diagnosed as having borderline leprosy no growth was observed, but very few bacilli had been observed in the inoculum prepared from the biopsy specimen obtained from this patient. In some nerves only a few bacilli were seen, but in others the intraneurial bacilli were very numerous and were distributed in phagocytes throughout the nerves (Figs. 2 and 3). The nerve sheath cells were frequently involved (Fig. 4).

Significance of nerve involvement.—Predilection of *M. leprae* for peripheral nerves is a characteristic feature of all forms of human leprosy. No other mycobacterium has been shown to have this special preference. The nerve involvement seen in these experimental animals resembled very much that seen in lepromatous leprosy, inasmuch as there was usually very little intraneurial cellular infiltrate, and some nerves were encircled by several layers of infected perineurial spindle cells, a feature commonly observed in lepromatous leprosy. The histopathologic reaction in the nerves resembled in many cases that seen in indeterminate or in the early stages of lepromatous leprosy, but in some the degree of involvement equalled that seen in moderately advanced lepromatous leprosy.

The predilection of *M. leprae* to involve nerves as demonstrated in

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FIG. 2. Photomicrographs of small nerve in ear of a hamster 23½ months after inoculation with a suspension of *M. leprae* from a lepromatous skin lesion. **A.** Hematoxylin and eosin stain × 210. Observe the perineurial sheathing that resembles that occurring in lepromatous leprosy. There is a slight increase in intraneurial nuclei. A Schaumann body is indicated by an arrow. AFIP Neg. No. 61–1704. **B.** A section similar to that shown in A, stained by the Fite-Faraco method. AFIP Neg. No. 61–1702. × 210. **C.** The numerous bacilli are well demonstrated in C, which is an enlargement of the area outlined by the rectangle in B. AFIP Neg. No. 61–1706. × 755.

FIG. 3. **A.** Photomicrograph of nerve (between arrows) in hamster ear near cartilage. This specimen was from an animal killed 17 months after inoculation with a suspension prepared from the ears of a hamster that had been inoculated 24 months previously with a suspension of human material. Observe the numerous intraneurial bacilli. AFIP Neg. No. 63–2025. Fite-Faraco stain. × 265. **B.** Higher magnification (× 850) of a part of nerve shown in **A.** AFIP Neg. No. 63–2026.

20 of these 21 experiments was in distinct contrast to the behavior of the "NQ" mycobacterium in the ears of hamsters ([2]). This cultivable mycobacterium, obtained from the skin of patients with lepromatous leprosy showed no tendency to invade nerves.

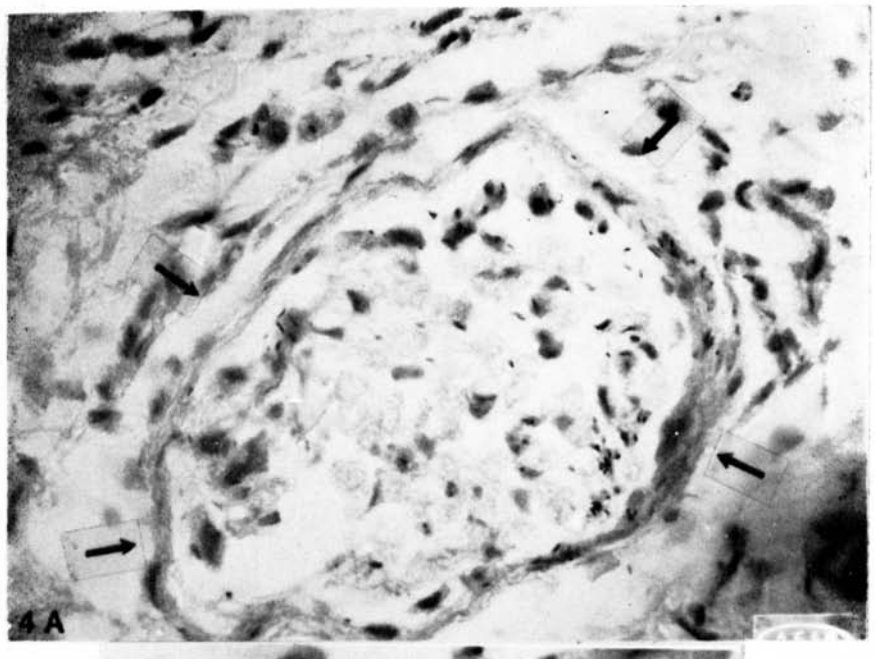
Inoculation of the testes.—In the foregoing groups of experiments the testes were regularly inoculated, but histopathologic sections taken from each testis yielded no evidence of lesions or multiplication of *M. leprae*. After one year ground glass residue from the Ten Broeck grinder could still be found in the testis, but the few bacilli observed were poorly stained and granular. In a few animals, however, several oil immersion fields of the tunica vaginalis contained histiocytes filled with well stained intact bacilli.

Transfer experiments of M. leprae *hamster to hamster.*—A natural second step in these transmission experiments was the transfer of *M. leprae* from the small lesions in the hamster ears to the ears and foot pads of other hamsters. Because lesions transferred were microscopic, in order to harvest the bacilli entire ears, including cartilage and epidermis, were homogenized. These transfers were usually made 12 to 24 months after the human to hamster inoculation, and the hamster to hamster transfers were made after similar time intervals.

Final evaluation has not been made of the attempts to produce in the hamsters, by continued hamster to hamster passage (at 12 to 24 month intervals), an infection with *M. leprae*. In some of the experiments, a second hamster to hamster transfer has been carried out successfully.

The usual method for accomplishing these transfer experiments was that of homogenizing in saline the ear of a hamster and, with that suspension, inoculating the ears of 10 or more hamsters. Because the microscopic lesions in the hamsters' ears were small, the number of bacilli in the suspension used for the hamster to hamster transfers has been small, frequently being only one or two in 10 to 20 oil immersion fields. Therefore, in the transfer experiments, the number of bacilli in the inoculum was considerably smaller than in the original suspensions made directly from infected human skin. In the transfer experiments the bacilli frequently appeared to be very well preserved and some were long and solidly stained, but the infection generally was not as extensive as had occurred in the ears of animals that were inoculated directly from human lesions.

Although in some of the hamster to hamster transfer experiments intraneurial invasion by *M. leprae* has been observed in a few animals, and growth of bacilli in some groups has been sustained for more than 5 years, it is doubted if, by the present methods, a well established infection due to *M. leprae* will be regularly reproducible in the ears by repeated hamster to hamster passages so as to furnish a satisfactory experimental model. Unless some way can be found for enhancing the growth of *M. leprae* in an animal a susceptible animal must be



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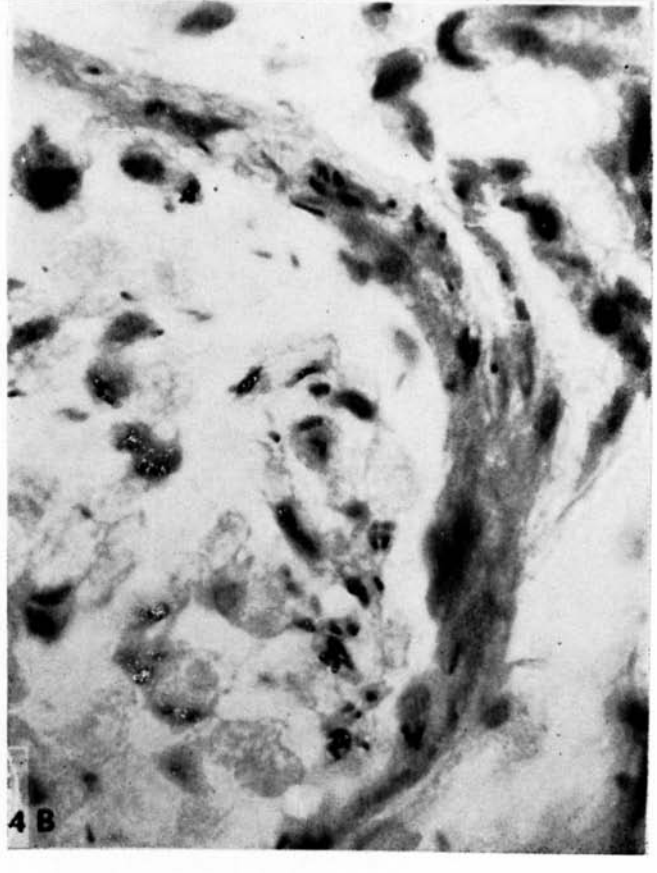

4 B

found that has a life span much longer than that of the hamster if macroscopic lesions are to be obtained.

SUMMARY

In 21 experimental groups of hamsters human leprosy material was inoculated into the ears and testes. Using nerve invasion by acid-fast bacilli as a criterion of growth, positive results were obtained in 20 groups and in 93 (30.3%) of 301 hamsters inoculated with non-heated suspension of *M. leprae*. Histopathologically the nerve lesions usually resembled those seen in indeterminate and early lepromatous leprosy, but in some sections the severity of the nerve lesion was comparable to that seen in moderately advanced lepromatous leprosy.

In these groups of experiments there was no evidence that growth of *M. leprae* occurred within the testis, but occasionally it appeared that mild growth was occurring in the tunica vaginalis.

No growth was obtained on Loewenstein-Jensen medium planted with the inoculum from patients or from the ears of hamsters that were used in the transfer experiments, hamster to hamster.

It was concluded that the infection of dermal nerves of hamsters by noncultivable acid-fast bacilli furnishes evidence that, although very mild, the lesions obtained in the ears of hamsters inoculated with material from human leprosy are due to infection with *M. leprae*.

RESUMEN

El material proveniente de lepra humana fué inoculada en las orejas y testículos a 21 grupos experimentales de hamsters. Usando la invasión nerviosa por bacilos ácido-alcohol-resistentes como criterio de crecimiento, fueron obtenidos resultados positivos en 20 grupos y en 93 (30.3%) de 301 hamsters inoculados con suspensión no calentada de *M. leprae*. Histopatológicamente las lesiones nerviosas usualmente recuerdan aquellas que se ven en la lepra indeterminada y lepromatosa temprana, pero en algunas secciones la severidad de la lesión del nervio es comparable a aquellas que se ven en las lepras lepromatosas moderamente avanzadas.

En este grupo de experimentos, no hubo evidencia de que el *M. leprae* creciera dentro de los testículos, pero ocasionalmente parecía que un crecimiento moderado estuviera ocurriendo en la túnica vaginalis.

No fué obtenido crecimiento en el medio de Loewenstein-Jensen implantado con el inoculum de pacientes o de las orejas de los hamsters que fueron usados en los experimentos de transferencia, hamster a hamster.

Se concluye que la infección de los nervios dérmicos de los hamsters por bacilos ácido-alcohol-resistentes no cultivables, provee la evidencia que, aunque muy moderada, las lesiones obtenidas en las orejas de los hamsters inoculados con material proveniente de lepra humana, son debidos a la infección con *M. leprae*.

FIG. 4. **A.** Small dermal nerve (between arrows) of hamster 17 months post-inoculation with *M. leprae* from human lesion. Observe the bacilli in the perineurium and within the nerve. Fite-Faraco stain. × 850. AFIP Neg. No. 63–2033. **B.** A part of same nerve × 1350. In this nerve both perineurial and intraneurial cells are involved. Note that the intraneurial involvement is greater adjacent to the segment of perineurium with many bacilli. This feature suggests that the bacilli gained entrance to the intraneurial tissue through the perineurium. AFIP Neg. No. 63–2032.

RÉSUMÉ

Du matériel de lèpre humaine a été inoculé dans les oreilles et dans les testicules d'une série de hamsters répartis en 21 groupes expérimentaux. L'invasion des nerfs par des bacilles acido-résistants étant prise comme critère de croissance, des résultats positifs ont été obtenus dans 20 groupes et chez 93 (30.3%) des 301 hamsters inoculés avec une suspension de *M. leprae* qui n'avait pas été passée à la chaleur. Histopathologiquement, les lésions nerveuses ressemblaient habituellement à celles notées dans la lèpre indéterminée ou dans la lèpre lépromateuse précoce. Néanmoins, dans certaines coupes la gravité de la lésion nerveuse était comparable à celle observée dans la lèpre lépromateuse modérément avancée.

Il n'y a pas eu, dans ces groupes d'expérience, d'évidence permettant de croire à une croissance de *M. leprae* dans les testicules. A l'occasion, toutefois, il est apparu qu'une croissance faible prenait place dans la tunica vaginalis.

Aucune croissance n'a été obtenue sur milieu de Loewenstein-Jensen ensemencé avec un inoculat provenant de malades ou d'oreilles de hamsters utilisés dans les passages expérimentaux entre hamsters.

Il a été conclu que l'infection des nerfs du derme chez les hamsters par des bacilles acido-résistants non cultivables fournit la preuve que les lésions obtenues au niveau des oreilles de ces animaux inoculés avec du matériel de lèpre humaine, quoi quelles soient très minimes, sont dues à une infection par *M. leprae*.

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