

THE INFECTIVITY OF *M. LEPRAE MURIUM* KEPT FOR  
VARIOUS PERIODS ON THE BENCH AND  
IN THE REFRIGERATOR

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Since the *in vitro* cultivation of *M. leprae murium* is at present impossible, the viability of the organism kept outside the animal body under various conditions has been investigated.

Marchoux (5) reported that when a pulp of infected tissue was kept in a 40 per cent glycerin medium in the refrigerator, this material kept its infectivity for as long as 51 months. When kept for 12 days at 37°C. on agar, or on the Shiga or Wherry media, it was no longer capable of transmitting the infection. He also claimed that the bacilli were killed by heating for 15 minutes at 60°C. Eisman, Gefic and Mayer (1) reported that the rat leprosy bacillus maintained its infectivity for periods up to 42 weeks when prepared from frozen lepromatous tissue stored in dry ice. Nakagawa and Nakamura (8) have claimed that lyophilized bacilli kept in various media and stored in the refrigerator remained infective as long as 2 years and 5 months, but they only demonstrated a local reaction at the site of inoculation, and no systemic disease developed in the infected animals. Goulding (2) has shown that viable bacilli can be satisfactorily preserved by freeze-drying for periods up to at least 6 months.

We have been doing experiments with human leprosy material, and were interested to know whether it would retain infectivity when sent from various places. As such information could not be obtained with human material, investigations were performed with murine leprosy. We, therefore, investigated the infectivity of the rat bacillus (1) in a leproma kept on the bench or in a refrigerator, without preservative, for periods up to 3 weeks, and (2) in suspensions in "Tween"-saline kept up to 6 days under the same conditions.

#### MATERIAL AND METHODS

The leprosy material was obtained from a rat leproma (Douglas strain) excised with sterile precautions and divided into two parts. One part was used, shortly after its removal, for preparing a suspension of the bacilli. The other part was subdivided into two portions, one of which was kept at room temperature on the bench and the other in the refrigerator at 0°C., both in sterile petri dishes.

The bacillus suspension was prepared by cutting the leproma into small pieces and grinding these down with a little silver sand and a few cubic centimeters of 0.05 per cent Tween 80 in normal saline. The material so obtained was first centrifuged lightly to bring down the coarse particles, and the supernatant fluid was recentrifuged at 5,500 r. p. m. for 20 minutes to bring down the bacilli, which were resuspended in Tween-saline. This final suspension was put in ampules which were sealed and kept on the bench or in the refrigerator to be used for corneal inoculation.

The animals used were albino mice of the C strain, weighing about 20 gm. at the beginning of the experiment. They were divided into groups of eight, and were inocu-

lated intracorneally with the various suspensions. Seventeen groups of mice were inoculated in all, as follows:

1. One control group (10 mice) was inoculated with the suspension prepared shortly after the removal of the leproma.
2. Eight groups were inoculated with suspensions also made at the outset but kept for 1, 2, 5 and 6 days, (*a*) on the bench or (*b*) in the refrigerator.
3. Six groups were inoculated with suspensions prepared, as described, from the parts of the leproma that had been kept intact either (*a*) on the bench or (*b*) in the refrigerator for 1, 2 and 3 weeks.
4. Two groups were inoculated with suspensions prepared from the parts of the leproma kept for 3 weeks under the conditions specified, but these suspensions were heated at 60°C. for two hours before inoculation.

#### RESULTS

In all of the mice of the different groups the intracorneal inoculation of the rat-bacillus suspensions produced a reaction in the form of an opacity involving a part of the cornea corresponding to the size of the inoculum. This opacity faded gradually but never disappeared completely, and ultimately it was replaced by a true lesion which developed and became superimposed upon it.

In the control group, lesions developed after an incubation period of about 3 weeks; they then grew larger until, in 6 out of the 10 mice, they involved the whole cornea and spread to the sclera in some 28 weeks after inoculation. In the other 4 of these mice the lesions at that time occupied one-half to two-thirds of the cornea. In addition, generalized infection, involving mainly the livers and spleens, was found in all the animals of this group when they were killed 35 weeks after infection.

The corneal lesions in the eight groups of mice inoculated with prepared suspension kept on the bench or in the refrigerator for 1, 2, 4 and 6 days, developed after the same latent period and progressed in a manner similar to those of the control, and generalized leprosy was found in all the mice of the different groups which were examined postmortem.

The numbers of bacilli in the two suspensions prepared from the parts of the leproma kept on the bench and in the refrigerator for a particular period (i. e., 1, or 2, or 3 weeks; see Item 3 above) were approximately the same, but were different from the numbers present in suspensions prepared after different periods. Consequently, a comparative study is here made between the groups inoculated on the same day.

In the mice inoculated with suspensions from one-week-old portions of the leproma, corneal lesions developed after an incubation period of 2 weeks and progressed rapidly, with no difference whatever between those produced by bench or refrigerator leproma suspensions.

In the other four groups inoculated with suspensions of the parts of the leproma kept for 2 weeks and 3 weeks, there was always an increase in the latent period, and a slight decrease in the rate of progress, of lesions which developed in mice inoculated with suspensions from the lepromas that had been kept on the bench as compared with the corres-

ponding animals inoculated with refrigerated leproma suspensions. For example, in the mice inoculated with the suspension of the leproma kept in the refrigerator for 2 weeks, corneal lesions developed in 4 out of the 8 mice after 4 weeks, in 2 more after 6 weeks, and in the last 2 after 10 weeks; whereas none of the mice in the corresponding group inoculated with the suspension of the leproma kept on the bench developed any lesion until after 12 weeks. All the mice in these groups in which a postmortem examination was performed had developed generalized leprosy.

The two groups of mice inoculated with heated suspensions also developed corneal lesions, but after very long latent periods of from 20 up to 59 weeks.

#### DISCUSSION

The results of these experiments show that *M. leprae murium* in a Tween-saline suspension stored for 1 to 6 days on the bench and in the refrigerator, and those in the leproma tissue kept for 1 to 3 weeks under similar contrasting conditions, remained viable and capable of producing both local and systemic infection in mice inoculated intracorneally. There was, however, a slight decrease in the infectious activity of the bacilli from the 2- and 3-weeks-old lepromas kept on the bench as compared with the corresponding organisms from lepromas kept in the refrigerator.

It has also been found that rat leprosy bacilli, in suspensions prepared from lepromas kept on the bench or in the refrigerator for 3 weeks and then heated for 2 hours at 60°C. before inoculation, remained viable and produced corneal lesions but after a long latent period. This confirms our previous findings (7), but is not in accord with those reported by Marchoux and Sorel (4), Muir and Henderson (6), Marchoux (5) and Hobby *et al.* (3).

If human leprosy bacilli behave in the same way as do the bacilli of rat leprosy, these findings suggest that human material kept at room temperature, and certainly in the refrigerator, will retain its infectivity for several weeks.

#### SUMMARY

*M. leprae murium* in a Tween-saline suspension stored for 1 to 6 days, and in lepromatous tissue kept for 1, 2 and 3 weeks, whether on the bench or in the refrigerator, remained alive and were capable of producing infection. This was also true of a suspension prepared from the 3-week-old leproma and heated for 2 hours at 60°C. before inoculation, although the latent period was greatly prolonged. These materials were tested by intracorneal inoculation in mice.

#### RESUMEN

El *M. leprae murium*, en una suspensión de Tween-solución salina conservada durante 1 a 6 días y en tejido lepromatoso guardado durante 1, 2 y 3 semanas, ya en el banco o en el refrigerador, permaneció vivo y capaz de producir infección. Esto

también rezó con una suspensión preparada de un leproma de 3 semanas de existencia y calentada por 2 horas a 60° C. antes de la inoculación, aunque se prolongó considerablemente el período de latencia. Estas substancias fueron comprobadas por la inoculación intracorneal en los ratones.

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