

## **Study of the use of nude mice in the cultivation of *Mycobacterium leprae* in a normal, non-specific pathogenic-free room at a temperature of 30–35°C, without air-conditioning**

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*Summary* From a study of cultivation of *Mycobacterium leprae* in nude mouse foot-pads in a normal, non-specific pathogen-free room at a temperature of 30–35°C, without air-conditioning, it was found that the nude mice in the trial could survive longer than 16 months, which is a sufficient period for laboratory and research activities with this model. Leprosy bacilli cultivated in nude mouse foot-pads could then multiply as well as those cultured in air-conditioned and specific pathogen-free conditions. The authors have some recommendations for the cultivation of *M. leprae* in nude mouse foot-pads and the care of the mice as follows:

- 1 The room for nude mice on the trial must be kept closed on all sides but an electric fan may occasionally be used to extract foul-smelling air.
- 2 Prevent ultraviolet rays from sunlight entering the room in order to protect mice from phototoxic dermatitis.
- 3 The room must be located on the upper floor so that contamination and changes in land temperature are avoided.
- 4 During breeding and feeding, nude mice must receive special close-care from birth in order to maximize survival.

The cultivation of leprosy bacilli in nude mouse foot-pads using the method described will—until *in vitro* growth is achieved—benefit research in chemotherapy, biochemistry, immunology, immunopathology, epidemiology and other related fields, at relatively low cost.

### **Introduction**

Since 1873 and Hansen's discovery of *Mycobacterium leprae*, there have been

many attempts to cultivate leprosy bacilli on laboratory artificial media and in animals such as normal mice, nude mice and nine-banded armadillos. In 1960 Shepard reported success in the cultivation of leprosy bacilli in normal mouse foot-pads;<sup>1</sup> in 1971 Kirchheimer & Storrs succeeded in cultivating leprosy bacilli in nine-banded armadillos,<sup>2</sup> and in 1976 success in cultivating leprosy bacilli in nude mouse foot-pads was reported.<sup>3-5</sup>

Nude mice naturally have neither hair nor thymus glands, and they lack cell-mediated immunity,<sup>6</sup> being sensitive to infections in normal conditions which may lead to death within 25 weeks.<sup>7</sup> Therefore, nude mice ordinarily have to be cared for in specific pathogen-free (SPF), air-conditioned rooms, involving much work and expenditure. In 1981, however, we started a trial to keep nude mice in a normal clean room, under non-SPF conditions at a temperature of 23–28°C, where nude mice could be kept from birth until the age of 16 months, or longer.<sup>8</sup> The present paper reports our continuing studies, aimed at the discovery of appropriate technology, at reasonable cost, to maintain nude mice in a non-SPF room at a temperature of 30–35°C without air-conditioning, and also to keep them alive long enough to be used for the cultivation of leprosy bacilli.

## Materials and methods

- 1 Leprosy bacilli were prepared from untreated lepromatous leprosy cases in Hank's balanced salt solution and bacillary counts performed.
- 2 Leprosy bacilli were inoculated into both hind foot-pads, 0.03 ml/foot-pad ( $6.5 \times 10^5$ /foot-pad) of 28 female nude mice, aged 5–6 weeks, weighing about 18–20 g each.
- 3 After inoculation of leprosy bacilli into foot-pads, 2 mice were sacrificed every month, and bacillary counts were made on the foot-pad tissue.

## Results

After inoculation of leprosy bacilli into both foot-pads ( $6.5 \times 10^5$ /foot-pad) of 28 female nude mice, they were kept in a normal room, not equipped with air-conditioning, at a temperature of 30–35°C. During the operation of the work there was no change of clothes and shoes by personnel, and related materials like mouse cages, water, and food were not sterilized in the usual way. From this study it was found that only one nude mouse got thinner, with bending of the backbone and death at 13 months, but the rest remained strong and lived longer than 16 months.

In month 13 after inoculation of  $6.5 \times 10^5$  leprosy bacilli per foot-pad, the remaining mice got lepromatoid lesions on the feet. Harvest counts showed that during the first 3 months of inoculation the numbers of leprosy bacilli decrease.

**Table 1.** Leprosy bacilli harvest from nude mouse foot-pads, after inoculation of  $6.5 \times 10^5$  leprosy bacilli per foot-pad, with solid ratio = 3:500

Month no.	AFB/foot-pad harvest	Solid ratio from foot-pad
1	$2.4 \times 10^5$	0
2	$3.0 \times 10^5$	0
3	$5.3 \times 10^5$	1:500
4	$3.8 \times 10^6$	2:500
5	$8.4 \times 10^6$	4:500
6	$1.8 \times 10^7$	10:500
7	$6.6 \times 10^7$	13:500
8	$1.2 \times 10^8$	18:500
9	$2.9 \times 10^8$	23:500
10	$4.2 \times 10^8$	28:500
11	$5.6 \times 10^8$	31:500
12	$1.3 \times 10^9$	33:500

They began to increase in the fourth month and this continued until the twelfth month (Table 1). The D-dopa oxidase test,<sup>9</sup> was used to confirm that the bacilli harvested from nude mouse foot-pads were *Mycobacterium leprae*.

## Discussion

This study on the cultivation of *Mycobacterium leprae* in nude mouse foot-pads has been conducted successfully in a normal room, not equipped with air-conditioning or permanent ventilation fan, at a temperature of 30–35°C, using non-SPF conditions for cages, bottles, water, mouse food and clothes. Our results show that in spite of these conditions bacilli could multiply effectively, as previously reported<sup>3–5</sup> with the loss of only 1 nude mouse at the age of 13 months. This indicated that at temperatures of 30–35°C when the weather was rather hot and the ventilation was not good, nude mice could mostly adapt themselves and live longer than 16 months, thus being of practical value in research work, at relatively low cost. During the course of this work, it has been noted that direct sunlight entering the room produces skin abnormalities and this may lead to weakness and death. Furthermore, we advise that nude mice should be kept in an elevated room away from the influence of the ground temperature and in an atmosphere which may be bacteriologically cleaner than that at ground level. It is important to pay very careful attention to newly-born mice and their feeding in the early stages of life so that they develop and grow up in good condition, capable of survival until 16 months or over.

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