

# Experimental Human Leprosy in the Footpad of Mice

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Multiplication of *Mycobacteria leprae* in the footpads of mice has been reported by Shepard.<sup>10</sup> The increase has been up to 1,000 fold. Other workers like Chatterji<sup>4</sup> and Bergel<sup>3</sup> have also reported successful transmission of *M. leprae* in rats and mice by using different strains of animals and different food and technique. Mukherji<sup>6, 7</sup> has not been able to confirm their works. This work was undertaken to find out if Shepard's method of transmission of *M. leprae* in mice could be produced.

## MATERIAL AND METHODS

Earlobe biopsies from untreated cases of lepromatous leprosy were obtained after treating them with tinct. iodine and alcohol. These were cut in small pieces and ground up with sea sand in pestle and mortar with a little Hank's balanced salt solution containing 0.1 per cent bovine albumin. The suspension was lightly centrifuged. The supernatant was pipetted off and counted for mycobacteria by the method of Shepard.<sup>9</sup> It was diluted with Hank's balanced salt solution containing 0.1 per cent bovine albumin to a con-

centration of approximately  $10^5$  mycobacteria per ml. This diluted suspension in doses of 0.03 ml was injected subcutaneously into a single footpad of a hind leg of each mouse. Three groups of 20 mice each were inoculated with suspensions prepared from earlobe biopsies from three untreated lepromatous patients. Ten mice in each group were likewise inoculated in the footpads with 0.03 ml of suspensions containing  $10^5$  organisms per ml of *M. leprae murium* and *M. phlei*. The animals were kept in a room where temperature was maintained at approximately 30 °C. One mouse from each group was sacrificed every month and the footpad examined for mycobacteria.

Mycobacteria in the mouse pads were counted as follows: The footpads were washed with soap and water, rinsed with sterile water and dried with sterile gauze. The footpads were removed aseptically and cut into fine pieces with a pair of scissors. Each footpad was then ground up with sea sand in pestle and mortar with a little Hank's balanced salt solution containing 0.1 of bovine albumin under aseptic condition. Any mass of

## Results:

TABLE I

### Number of Mycobacteria in mouse footpad

	No. injected	Number recovered			
		1st month	2nd month	3rd month	8th month
<i>M. leprae</i>	$10^5 \times 0.03$	$10^5 \times 0.03$	$10^5 \times 0.025$	$10^5 \times 0.025$	0
<i>M. phlei</i>	$10^5 \times 0.03$	$10^5 \times 0.03$	$10^5 \times 0.03$	$10^5 \times 0.025$	0
<i>M. lepraemurium</i>	$10^5 \times 0.03$	$10^5 \times 0.03$	$10^5 \times 0.03$	$10^5 \times 0.07$	$10^6 \times 2.3$

TABLE II

### Tissue changes in mice liver and spleen

Time after inoculation	<i>M. leprae murium</i>	<i>M. leprae</i>	<i>M. phlei</i>
1 month	No change	No change	No change
2nd month	Collection of macro phages surrounded by lymphocytes	No change	No change
3rd month	Collection of macro phages surrounded by lymphocytes	No change	No change
8th month	Collection of macro phages surrounded by lymphocytes	No change	No change

unground tissue was removed by leaving the suspension at room temperature for several minutes. The process was repeated several times with Hank's balanced salt solution containing 0.1 per cent bovine albumin. All the fluids were collected and the total number of mycobacteria estimated using Shepard's<sup>9</sup> technique. Average counts only are shown in the results.

#### DISCUSSION

Mycobacteria could not be recovered from the footpads of mice receiving *Mycobacteria leprae* after eight months. Some acid fast bacteria could however, be recovered from the foot pads of mice receiving *Mycobacteria phlei* and *M. leprae* during the first three months. Mycobacterium leprae murium however, grow well in the mouse footpads and their numbers increased to over 700 fold.

Shepard's<sup>9</sup> work claiming increase of *Mycobacterium leprae* in mouse footpads could not be confirmed. It is possible, however, that the patients from whom materials were obtained were suffering from rat leprosy infection as has been found in some cases by several workers like Balfour Jones<sup>1</sup> and Burnet.<sup>2</sup> Otherwise the mice used in Shepard's work might have latent Mycobacterium leprae murium infection or might have got it in the laboratory as has been reported by Mukerjee and Kundu.<sup>8</sup>

#### SUMMARY

*Mycobacterium leprae murium*, *Mycobacterium leprae*, and *Mycobacterium phlei* were injected into mouse footpads in doses of  $10^5 \times 0.03$  per footpad. None of the Mycobacteria could be recovered from the foot pads of mice nor histological lesions found in

the livers and spleen of mice receiving injections of *M. leprae* and *M. phlei* in the foot pads.

#### ACKNOWLEDGEMENT

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#### REFERENCES

- 1 BALFOUR JONES, S.E.B. (1937). The experimental transmission of rat leprosy to the golden hamster (*Cricetus auratus*). *J. Path. and Bact.*, **45**, p. 739-744.
- 2 BURNET, E. (1938). Inoculation positive de la lepre humaine au hamster. *Acad. Sc.*, **207**, p. 690-692.
- 3 BERGEL, M. (1957). Inoculation del Mycobacterium leprae a rattas alimentadas, a dietas pro-oxidantes, *Semana med.*, pp. 1148-1151, also *leprologia* (1957), **2**, p. 13-17.
- 4 CHATTERJI, K. R. (1958). Experimental transmission of human leprosy infection to a selected laboratory bred hybrid black mice. *Internat. J. Leprosy*, **26**, pp. 195-204.
- 5 KOOIJ, R. and GERRITSEN, TH. (1956). Positive lepromin reaction with suspensions of normal tissue particles. *Internat. J. Leprosy*, **24**, pp. 171-181.
- 6 MUKHERJI, A. (1961). Preliminary note on experimental human leprosy in hybrid black mice. *J. Sci and Indus. Res.*, **20c**, pp. 190-191.
- 7 MUKHERJI, A. and CHOWDHURY, B. L. (1962). Experimental human leprosy in mice and rats on pro-oxidant diet. *J. Sci and Indus. Res.*, **21c**, pp. 190-191.
- 8 MUKHERJEE, N. and KUNDU, S. (1959). Results of inoculation of white rats with human leprosy bacilli by intraneural route, preliminary report. *Internat. J. Leprosy*, **27**, pp. 355-59.
- 9 SHEPARD, C. E. (1960). The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice. *J. exper. Med.*, **112**, pp. 445-454.
- 10 SHEPARD, C. E. (1962). Multiplication of *M. leprae* in the foot pad of mouse. *Internat. J. Leprosy*, **30**, pp. 291-306.
- 11 SHEPARD, C. C. (1963). Immunological identification of footpad isolates as *Mycobacteria leprae* by lepromin reactivity in leprosy patients. *J. Exper. Med.*, **118**, pp. 195-204.