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,\textsuperscript{10} The increase has been up to 1,000 fold. Other workers like Chatterji\textsuperscript{4} and Bergel\textsuperscript{8} have also reported successful transmission of \textit{M. leprae} in rats and mice by using different strains of animals and different food and technique. Mukherji\textsuperscript{6,7} has not been able to confirm their works. This work was undertaken to find out if Shepard's method of transmission of \textit{M. leprae} in mice could be produced.

\section*{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.\textsuperscript{9} It was diluted with Hank's balanced salt solution containing 0.1 per cent bovine albumin to a concentration of approximately 10\textsuperscript{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\textsuperscript{5} organisms per ml of \textit{M. leprae murium} and \textit{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

\begin{table}[h]
\centering
\caption{Number of Mycobacteria in mouse footpad}
\begin{tabular}{llllll}
\hline
\textbf{M. leprae} & \textbf{M. phlei} & \textbf{M. leprae murium} & \textbf{No. injected} & \textbf{1st month} & \textbf{2nd month} \\
& & & \textbf{Number recovered} & \textbf{3rd month} & \textbf{8th month} \\
\hline
10\textsuperscript{5} x 0.03 & 10\textsuperscript{5} x 0.03 & 10\textsuperscript{5} x 0.03 & 10\textsuperscript{5} x 0.025 & 10\textsuperscript{5} x 0.025 & 10\textsuperscript{5} x 0.07 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Tissue changes in mouse liver and spleen}
\begin{tabular}{llll}
\hline
\textbf{Time after inoculation} & \textbf{M. leprae murium} & \textbf{M. leprae} & \textbf{M. phlei} \\
1 month & No change & No change & No change \\
2nd month & Collection of macrophages surrounded by lymphocytes & No change & No change \\
3rd month & Collection of macrophages surrounded by lymphocytes & No change & No change \\
8th month & Collection of macrophages surrounded by lymphocytes & No change & No change \\
\hline
\end{tabular}
\end{table}
ground 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’s9 technique. Average counts only are shown in the results.

**DISCUSSION**

Mycobacteria could not be recovered from the footpads of mice receiving *Mycobacterium leprae* after eight months. Some acid fast bacteria could however, be recovered from the foot pads of mice receiving *Mycobacterium 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’s9 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 Jones1 and Burnet.2 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.8

**SUMMARY**

*Mycobacterium leprae murium*, *Mycobacterium leprae*, and *Mycobacterium phlei* were injected into mouse footpads in doses of $10^4 \times 0.03$ 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.

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**REFERENCES**


