CULTIVATION AND ANIMAL-TRANSMISSION OF MYCOBACTERIUM LEPRAE (M. LEPRAE)

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1. Cultivation of M. leprae

In the past three decades, our various trials to cultivate M. leprae on bacteriological solid and liquid media favorable for the growth of M. tuberculosis have not shown any growth of the bacilli.

However, recently we were able to observe a limited multiplication of M. leprae in cultures of mouse foot-pad cells up to the third sub-cultures.

After 96 hr of incubation at 33°C for phagocytosis, infected cells were washed to remove unphagocytized bacilli and transferred to new culture vessels. There was a 4.9-fold increase in the number of bacilli during 70 days of successive cultures up to the third culture in flasks, giving an overall generation time of 30.4 days.

Acid-fast stained cover-slips taken from the culture in Leighton tubes revealed intracellular multiplication of M. leprae, frequently in bundles of hundreds of bacilli, and gave an average generation time of 16.4 days in the primary, 15.7 days in the secondary, and 8.1 days in the third culture.
All attempts to grow the acid-fast bacilli from the original bacillary suspension and the cell suspension at the end of the experiment on artificial culture media have failed. Subcutaneous inoculation of these materials to mice produced the typical lesions caused by rat-leprosy bacilli.

2. Animal-transmission of \textit{M. leprae}

Eighty-five CF#1 mice were inoculated with living leprosy bacilli from an untreated patient with lepromatous leprosy into the left hind foot-pads, and twenty with heat-killed ones, using $10^3$ to $10^4$ bacilli per foot-pat.

After three to eight months the mice were sacrificed and both left and right hind foot-pads, lungs, livers, kidneys, spleens, and left inguinal lymphnodes were examined for presence of acid-fast bacilli.

Multiplication of the leprosy bacilli in the left hind foot-pads of mice was confirmed in fifty-eight of eighty-five mice inoculated with living bacilli. Acid-fast rods were also found in the tissues other than the inoculated food-pads.

Discovery of acid-fast bacilli was 64.7 per cent in the mice inoculated with living ones and 50.0 per cent with killed ones.

And acid-fast rods were also found most frequently in the lymphnodes draining the inoculated foot-pads, but the number of bacilli were very small in most cases. The evidence presented suggests that the leprosy bacilli are carried from the site of inoculation to other sites of mice.

Cultures on bacteriological media favorable for the growth of most known mycobacterial species have not shown growth of the bacilli. The third passage of the bacilli is now under experiment.

\textbf{CHEMOTHERAPY OF HUMAN LEPROSY}
—\textbf{MAINLY, ON THE DDS-SENSITIVE STRAIN AND DDS-RESISTANT STRAIN OF MOUSE PASSAGES}—

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Shepard, et al. and Rees, et al. have described the use of the infection of mouse foot-pads with \textit{M. leprae} as a method for anti-microbial therapy of human leprosy, also, we have previously reported the activity of DDS against \textit{M. leprae} in this system using isolates from Japanese patients.

Therefore, a comparison of the activity of DDS and other drugs against DDS-sensitive, and DDS-resistant strain was carried out in mice inoculated into the foot-pad with mouse passages, and then the inhibitory effect of DDS against the multiplication of \textit{M. leprae} was performed in the mouse foot-pad system. The results obtained were as follows:

The multiplication of the so-called resistant strain was suppressed completely by DDS-administration with containing 0.5 mg per one gram of diet, these resistant