Observations on the Inoculation of *M. leprae* in the Foot Pad of the White Rat

G. R. F. Hilson, M.D.[1]

The characteristics of the infection that *Mycobacterium leprae* produces in the foot pads of mice have now become well-established ([1,2]). Shepard also injected hamster foot pads and testes with, at best, doubtful results. Since *M. lepraemurium* produces progressive infections in mice, hamsters, and rats, it seemed reasonable to discover whether the human organism would grow in the foot pads of rats. Some of the findings in this species are reported here; they show that it is in fact possible under these conditions to produce a limited infection resembling that in mouse foot pads.

Two isolates of human leprosy bacilli are referred to: one of these was a homogenate of leproma tissue from an untreated patient, and the other was mouse passage material containing one of Shepard's original isolates. White Wistar rats were used, and the technic of foot pad inoculation and assessment of bacterial growth were essentially similar to those described by Shepard ([1]). Portions of the inoculum suspensions were inoculated into the foot pads of Swiss white mice as well, and on appropriate bacteriologic media; the homogenates of rat tissues taken at intervals during the investigation were also tested for the presence of cultivable mycobacteria. Since none of the cultures yielded growth after one year's incubation, and the inocula reproduced the pattern of growth characteristic of *M. leprae* in the mouse foot pads, it seems highly likely that the findings to be described in the rats were indeed due to the human leprosy bacilli injected.

Figure 1 shows the results obtained following the injection of rats with bacilli taken directly from human leproma material. Part of the suspension used was heated at 60°C for 1 hour to kill the bacilli: the figure shows that in the case of the rats receiving this heated suspension, the numbers of bacilli per foot pad remain essentially unaltered for well over a year. This shows that the carcasses of the bacilli remain stainable in the tissues almost indefinitely, like those of many other mycobacterial species, and that clearance from the site of injection is negligible. The heated bacilli also provide a control for the possibility that the foot pad injections may stimulate a pre-existing latent infection due to a different mycobacterial species in the animals. The living inoculum, on the other hand, underwent a 200-fold increase (from an initial value of just under 10^5 bacilli per foot pad) during about 8 months, and then there was a gradual decline in numbers during the following year. There was also a gradual decrease in the proportion of solidly-staining bacilli in the homogenates over this period.

About one year after the primary inoculation, one of the foot pad homogenates was used to initiate passage to a new group of rats. The inoculum was diluted to contain just under 10^3 bacilli/foot pad, and about 50 per cent of the bacilli appeared solid on staining. The progress of this passage is also illustrated in Figure 1, the amount of bacillary increase being more than 1,000-fold over about 9 months. The aggregate increase over the two passages was thus of the order of 200,000-fold, or approximately 18 generations. The mean generation time derived from these two phases of growth is about 3 weeks, a figure similar to that which has been calculated for the growth of *M. leprae* in mice ([2]). In further serial passage of this strain under similar conditions, an approximate 100-fold increase was obtained (not shown in Figure 1).

This strain was also used to assess the influence of inoculum size on the progress of infection. At the time of the first passage already referred to, another group of rats was inoculated with passage material

[1]St. George's Hospital Medical School, London, S. W. 1, England.

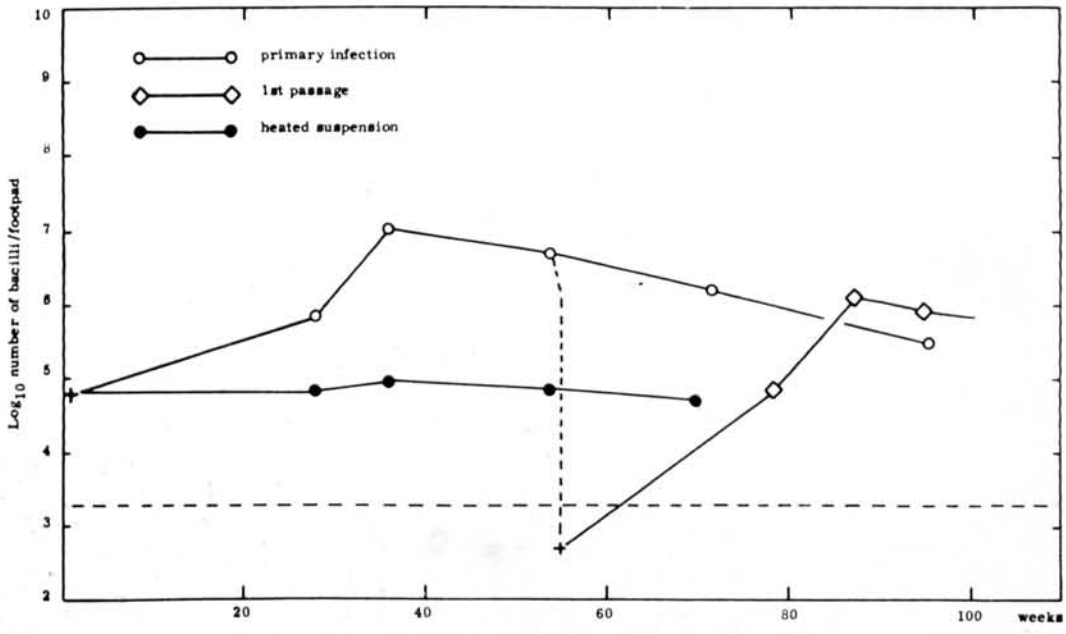

FIG. 1. The growth of acid-fast bacilli in the foot pads of white Wistar rats following the inoculation (as primary infection and first passage) of human leprosy bacilli obtained from an untreated patient. The horizontal dotted line in this and later diagrams shows the minimum number of acid-fast bacilli/foot pad detectable by the counting technic. The vertical dotted line indicates which homogenate from the primary infection group was used to initiate the passage.

at a concentration one hundred times as great as that used for the main passage group. Figure 2 shows how these two inocula of different size progressed. Both rose to reach the same "ceiling," though it took the smaller inoculum some 15 weeks longer to achieve the maximum, which was about 10^6 bacilli/foot pad.

Figure 3 shows the results of transferring a mouse passage strain of *M. leprae*. The data from one mouse passage are illustrated in the lower left-hand curve, and show a fairly characteristic pattern of growth. A mouse foot pad homogenate obtained about one year after initiation of infection was passaged into the foot pads and testes of both white rats and white mice. The two right-hand curves show the progress of the foot pad infections only, over a 30-week period. It can be seen that the amount of growth in the rat foot pads was approximately equal to that in the mice. In this experiment the progress of the testicular inocula was more favorable than that of the foot pad infections, a "ceiling" of over 10^5 bacilli/testis being reached in the case of both mice and rats. The top left-hand curve in Figure 3 (showing the growth of *M. lepraemurium* in mouse foot pads) is included to allow comparison between the human and rat leprosy bacilli on this time scale.

The histopathology of a few of the rats was investigated. These were animals taken at the peak of bacillary growth or a few months later. The findings were much the same as those reported for mice. The infection was localized to the injected organ. There was no change in the lymph nodes draining the area and no acid-fast bacilli were seen in them. Other organs showed no visible lesions and no bacilli. The foot pad itself showed no swelling or other naked-eye abnormality, and the microscopic changes were quite slight. They consisted of loose infiltrations with inflammatory cells mainly macrophages or histiocytes, lying at any depth from immediately under the epidermis down to the fascia of the plantar muscles. The acid-fast bacilli were found lying within the macro-

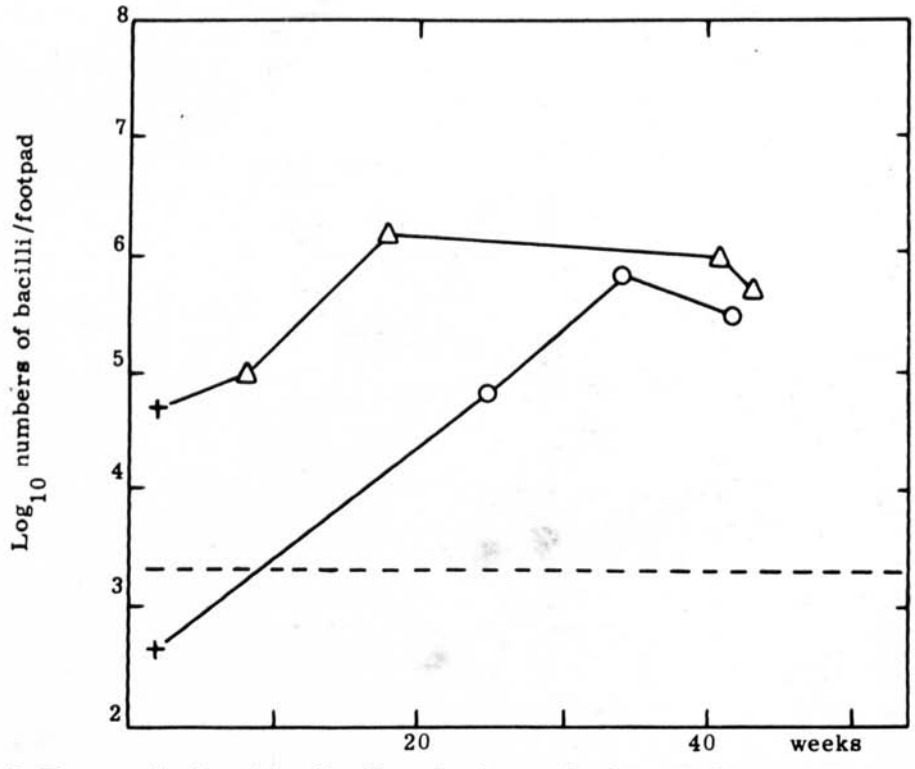

FIG. 2. The growth of acid-fast bacilli in the foot pads of rats following the inoculation of *M. leprae* from a rat foot pad homogenate, using inocula differing 100-fold in size.

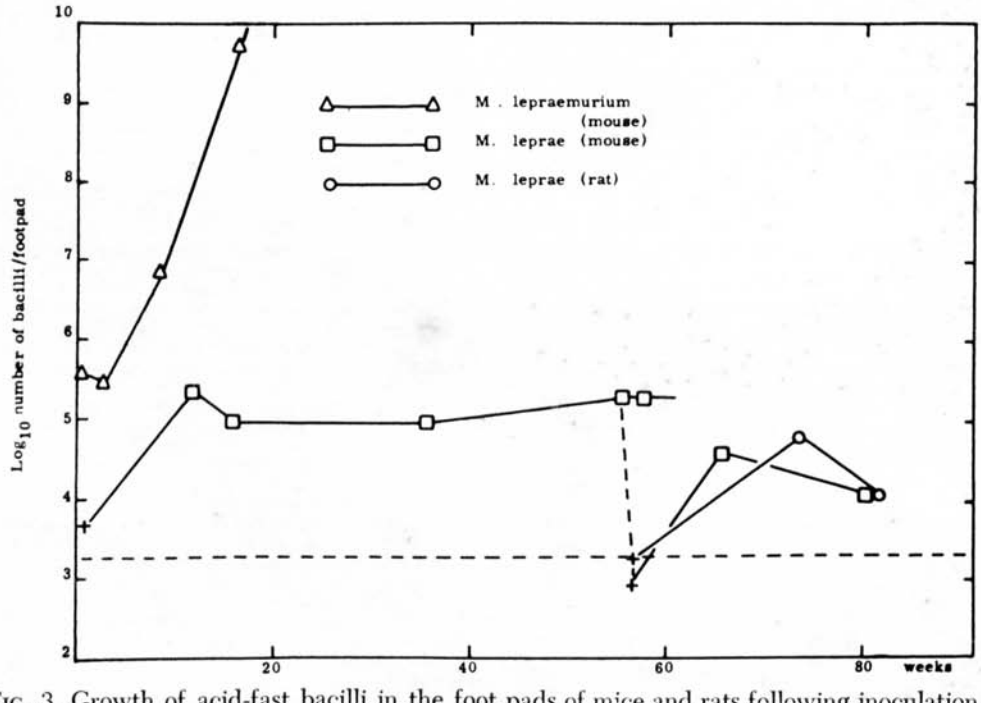

FIG. 3. Growth of acid-fast bacilli in the foot pads of mice and rats following inoculation with *M. leprae* (mouse passage strain) and *M. lepraemurium*.

phages. There was no apparent affinity for nerve fibers.

These results show that it is certainly possible to induce in rat foot pads a slow and limited growth of acid-fast bacilli after inoculation with human leprosy bacilli. The pattern of growth is similar to that in mouse foot pads, with the inocula of different sizes growing to a "ceiling" of between 10^5 and 10^6 per foot pad. One point of difference was seen, however: whereas in mice, when the phase of most rapid bacillary growth is over, there is a continued very gradual rise, in the rats this was not seen, and after the peak there was a downward trend in all the groups. More work will be needed, with additional strains, to assess the general validity of these observations and to discover if primary isolation from human sources can be made as regularly as in mice and whether or not passage can be continued indefinitely in this species.

SUMMARY

Using the Shepard technic, the foot pads of white rats were injected with human bacilli from two different sources. Microbial enumeration methods showed that in both cases limited multiplication of acid-fast bacilli occurred, generally similar to that seen in mouse foot pads, and the histopathology was also similar. Two further serial passages of one of the isolates were carried out.

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REFERENCES

1. SHEPARD, C. C. The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice. J. Exper. Med. **112** (1960) 445-454.
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DISCUSSION

Dr. Binford. I certainly appreciate Dr. Hilson's new information on the rat, because we have not heard previously on the use of this technic in that animal. His report was brought in really as an amplification of the discussion of Dr. Shepard's presentation. I am sorry we do not have time to open this now for further questioning. But before we break up, Dr. Cochrane, would you like to say a word about the International Leprosy Association? Dr. Cochrane has been carrying on as Acting President of the International Leprosy Association during the illness of Dr. José M. M. Fernández. (See footnote, page 399.)

Dr. Cochrane. Thank you, Dr. Binford. I do not know how many of you are familiar with the formation of the International Leprosy Association. We owe everything to the Leonard Wood Memorial, of course. In 1931 the Leonard Wood Memorial convened a round table conference in Manila. It was from it that the International Leprosy Association started, with Dr. Heiser as the President and myself as the first Secretary-Treasurer. During the second world war things didn't go as well as they should have gone. Dr. Wade and others struggled courageously, and Dr. Doull came through and helped out. Now I think we can say that if, not only this kind of conference, but the international congresses on leprosy are to maintain the high standing they have had in the past, we must have more members of the International Leprosy Association. Dr. Esmond R. Long and Miss delta derrom are doing splendid work with THE JOURNAL; it is now up to date. Therefore, the main objection to presenting papers for THE JOURNAL has disappeared. I trust that everyone here will support the International Leprosy Association, and if there are those here who have not yet joined, will you please join so that we really can bring the International Leprosy Association right up to standard. Unles we have a strong and vigorous International Leprosy Association, we will not have the high quality we desire

of leprosy research and leprosy clinical work, and will not have any real guidance for the future development of leprosy control. So I trust that what I have said now will prick some of your consciences, if you haven't joined, and like the recruiting sergeant I say please line up and join.

Dr. Mason. I do not feel that the next speaker needs any introduction whatever. Dr. Binford will discuss "The inoculation of human leprosy in the chimpanzee."