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Viability of *Mycobacterium leprae* Outside the Human Body

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It is important to recognise whether *Mycobacterium leprae* discharged from the body will remain alive after they settle down over articles of daily use, and if so the duration of their viability. The common belief is that the organisms die soon after they are discharged from the body, particularly in tropical countries. In order to verify this concept, an experimental procedure has been designed using the mouse foot-pad model. It has been found that the organisms remain alive for more than 9 days. This finding has an important bearing on the epidemiology of leprosy.

Introduction

Mycobacterium leprae are believed to be killed soon after they are expelled from the body since they are obligatory intracellular organisms. For this reason, the mode of transmission of leprosy was thought to be through direct skin contact, excluding the possibility of an indirect route through articles of daily use. Since it was not possible to culture the organisms, no experimental method could be designed to simulate the natural processes and check the viability of the organisms in the human discharges or excreta, either in a fresh state or after subjecting the material to drying and desiccation. However, with the advent of the mouse foot-pad model, it has been possible to grow the organisms in the experimental animal and verify their state of viability. This paper presents the results of an experimental study in which bacterial suspensions dried for different periods of time were inoculated into mouse foot-pads to assess their viability.

Material and Methods

Suspensions of *M. leprae* from two sources were prepared viz. from nose blows and from skin biopsy. (1) The patient was asked to blow his nose into a plastic bag. The bag was rinsed with normal saline and the contents transferred to a 50 ml flask. (2) A piece of skin obtained at biopsy was minced with scissors, homogenized and suspended in saline, carrying out all procedures aseptically at a low temperature over ice. The large particles were allowed to settle for 2 to 3 min. The supernatant fluid was collected.

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The bacterial suspension prepared by either of the above two methods was processed as follows: (a) About 1 ml of the suspension was processed for immediate inoculation. To the nose-blow material was added an equal volume of 2% NaOH. It was shaken vigorously, left for 10 min and then titrated with 2% sulphuric acid, using phenol red as indicator. The suspension was further sterilized by the addition of 200 I.U. crystalline penicillin per ml. (An earlier experiment wherein the nose blow material was inoculated without the addition of penicillin, resulted in the death of all the animals in 24 hours.) Enumeration of bacilli was done in this as well as in the skin tissue suspension. The material was diluted with Hanks' balanced salt solution (BSS) so that 0.03 ml contained 10^4 bacilli. A batch of 4 mice was inoculated with each specimen, the animals receiving 0.03 ml into each hind foot-pad. These animals served as positive controls. The remaining suspension was then autoclaved for 15 minutes at 15 lbs/in^2 pressure. The autoclaved material was inoculated into 4 mice, 0.03 ml into each hind foot-pad. These animals served as negative controls. (b) The rest of the original suspension was distributed to 5 sterile petri dishes. The petri dishes were left on the table in the laboratory and the material was allowed to dry. The dried material was scraped from the petri dishes after 15, 40 and 65 h, and 6 and 9 days. The scrapings were suspended in Hanks BSS to which crystalline penicillin was added. Bacterial counts were done and each specimen was inoculated into the hind foot-pads of mice so that 10^4 bacilli were introduced to each foot-pad.

The inoculated animals (controls and experimental groups) were housed in an air conditioned room at a temperature around 22° C. After 6 months, the mice were sacrificed, one at a time at monthly intervals. Harvests were performed from the inoculated foot-pads and bacterial counts were done. The method of inoculation, harvesting and bacterial counts were those described by Desikan and Venkataramanaiah (1976). All the observations were double blind, the smears prepared from the harvested material from several experiments being examined together after having been given coded numbers.

Results

The results of the experiment are given in Table 1.

TABLE 1

Viability in mouse foot-pads of M. leprae *derived from noseblows or skin and allowed to dry for varying periods**

Experiment number	Name/Source	Fresh specimen	Dried specimen					Autoclaved specimen
			15 h	40 h	65 h	6 days	9 days	
1	2	3	4	5	6	7	8	9
1	K.J./Nose blow M.I. 4%	2/4	0/4	1/4	1/4	2/4	1/4	0/4
2	K.P./Nose blow M.I. 7%	4/4	1/4	2/4	3/4	4/4	1/4	–
3	K.P./Skin M.I. 7%	3/4	2/4	2/4	1/4	0/4	4/4	0/4

* The figures indicate the number of mice showing multiplication of bacilli (numerator) out of the mice inoculated (denominator).

It could be seen that *M. leprae* remained alive and multiplied in the foot-pad even when the material containing the organisms was dried for 15, 40 and 65 h, 6 and 9 days. The negative findings in the foot-pads inoculated with autoclaved material showed that the positive finding was not due to any technical error. The infected material was allowed to dry inside a room in the shade but not in total darkness. The experiments were carried out under the fairly hot climatic conditions of a coastal town in South India. Table 2 is a weather chart showing the temperature and humidity on the days when the experiments were being carried out.

TABLE 2
Temperature and humidity chart at time of experiments

Date	Maximum (°C)	Minimum (°C)	Humidity (%)	Rain (mm)
May, 16	35.1	26.8	75	–
May, 17	36.0	27.2	79	–
May, 18	35.2	27.5	80	–
May, 19	36.9	26.5	77	–
May, 20	37.6	26.0	78	–
May, 21	37.2	26.8	74	–
May, 22	34.0	27.8	80	–
May, 23	35.0	27.6	77	–
May, 24	34.0	26.5	70	–
May, 25	37.4	26.5	73	–
May, 26	38.9	24.6	90	7.2
May, 27	37.6	24.8	78	–
May, 28	37.3	23.8	78	–
May, 29	37.4	25.8	70	–
May, 30	38.2	27.9	79	–
May, 31	38.6	25.4	83	–

Experiment 1 from May, 16 to 24
Experiment 2 from May, 20 to 28
Experiment 3 from May, 22 to 30

The mean maximum and minimum temperatures were 36.7° C and 26.3° C respectively and the humidity ranged from 70% to 90%. Since bacteria were found to be alive even on the 9th day of drying, the experiments have not established the end point of viability of the organisms outside the body. This apparently seems to be more than 9 days under the climatic and experimental conditions described above.

Discussion

In the experiment described above, nasal discharge and suspensions of *M. leprae* from skin lesions dried in the shade at an average maximum room temperature of 36.7° C and humidity of 77.6% were found to be viable up to 9 days. Since the bacilli were demonstrated to be alive even on the last day of the experiment, the end point was not established. The bacilli are therefore likely to resist conditions of drying for more than 9 days under the climatic conditions described. This was a very important finding and quite contrary to what was believed and expected. Davey and Rees (1974) first reported results of the experiments conducted in

London to demonstrate the viability of *M. leprae* after desiccation. They showed that nasal discharges allowed to dry for 24 h and 1.75 days contained viable bacilli. With one exception, no bacilli were found to be alive after drying the nasal discharges for 3 days or more. (In one instance, however, there was evidence of multiplication after drying for 7 days, the material containing less than 1% viable bacteria compared to the organisms in the fresh material.) It was therefore thought that in a tropical country, the organism would remain viable for a much shorter period outside the body. Chingleput, where the present experiments were carried out, has a much warmer climate, being located on a latitude of 13°N. The mean temperature at the time of our experiment was much higher than the temperature in London where the experiments of Davey and Rees were conducted. However, the mean humidity was 77.6% compared to 43.7% in London. The high humidity is perhaps responsible for the survival of *M. leprae* at a higher temperature.

The results of these studies are very significant and have an important bearing on the epidemiology of the disease. Since it has been shown that the organisms are viable in the discharges for several days, the possibility of infection due to indirect contact has to be seriously considered. Infection through inhalation is another possible route as shown experimentally by Rees and McDougall (1977).

It is regrettable that in this part of the country, as in many parts of the world, many people have the unhygienic habit of blowing their noses anywhere out of doors and wiping their fingers on any convenient nearby object. Bacilli could thus settle on such objects and also be sprayed over articles in daily use, from which they could be transmitted to other persons. Muir (1948) was of the opinion that indirect transmission occurs at times through the wearing of a patient's clothes, using his furniture and other appliances or living in a house vacated by a patient. The chances of indirect contact with a leprosy patient are quite high, particularly in a metropolitan city in India. It must be remembered, however, that while the possibility of transmission of the disease by an indirect method certainly does exist, there is no need to become unduly alarmed, since the frequency of clinical leprosy is much less than one would expect if this were the only factor involved. Obviously there are also other factors that influence the epidemiology of leprosy, especially in an endemic country like India.

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References

Desikan, K. V. and Venkataramanaiah, H. N. (1976). A modified method of harvesting *M. leprae* from foot-pads of mice. *Lepr. India* **48**, 157-162.

Davey, T. F. and Rees, R. J. W. (1974). The nasal discharge in leprosy: Clinical and bacteriological aspects. *Lepr. Rev.* **45**, 121-134.

Muir, E. (1948). *Manual of Leprosy*. E & S Livingstone Ltd., Edinburgh, p. 11.

Rees, R. J. W. and McDougall (1977). Air borne infection with *M. leprae* in mice. *J. Med. Microbiol.* **10**, 63-68.

Post-script

The question has been raised whether dilution of nose-blow material and decontamination with 2% sodium hydroxide might possibly destroy or dilute some constituents of nasal secretions detrimental to the survival of *M. leprae.* While such a hypothetical possibility might be considered, it would be relevant to mention in this connection that treatment with sodium hydroxide was considered necessary and was done for the fresh specimen in order to destroy the other organisms in the nasal secretions. However, this procedure was not adopted while preparing suspensions of dried material since the other organisms are likely to be killed during the process of drying. As such, the question of destruction of inhibiting factors in nasal secretions with sodium hydroxide does not arise as far as the dried material is concerned. The possibility of dilution may also be ruled out since the material was desiccated again.

The validity of the experiments is further confirmed by the results of inoculation with skin biopsy material which was not subjected to these procedures.