EFFECT OF ADVERSE ENVIRONMENTAL CONDITIONS ON *MYCOBACTERIUM LEPRAE*

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INTRODUCTION

It is well known that when a highly bacillated patient sneezes or coughs a large number of organisms are thrown out in droplets. Also, nasal discharges are often indiscriminately discarded. Bacilli may also be discharged from the skin of lepromatous leprosy patients through abraded or ulcerated skin lesions. In order that the organisms thus discharged should infect another individual it is necessary that they should remain viable on various objects, despite being subjected to adverse environmental conditions. The purpose of the present study is to find out if the bacilli do remain viable outside the human body and if so as to how long.

MATERIAL AND METHODS

Bacilli are collected from the skin of untreated patients of lepromatous leprosy and suspension prepared under sterile conditions by standard methods (1). A part of the suspension is used for immediate inoculation into mice. For this purpose the bacilli in the suspension are enumerated by standard methods and diluted so that 0.03 ml contain 10^4 bacilli. Six mice are inoculated into both hind foot pads by the diluted specimen. This serves as positive control. For a negative control, the diluted suspension is autoclaved and the autoclaved material is inoculated into both hind foot pads of 6 mice.

The remaining undiluted suspension is used for different experiments. It is subjected to different environmental conditions, retrieved later, suspended in saline. Bacilli enumerated in the suspension and diluted so that 0.03 ml contain 10^4 bacilli. Six mice are inoculated into both hind foot pads.

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Mice of the experiments, positive and negative controls are housed in air-conditioned rooms and fed with normal diet. After six months, the mice are killed one at a time at monthly intervals. Harvests from the foot pads are proceeded by the standard methods (loc. cit). Results of the harvest with the highest yield are expressed in the tables.

RESULTS

1) Drying in shade : Bacillary suspension is distributed to number of petridishes and allowed to dry at room temperature. At different intervals starting from day 3 to 12 months, one petridish is picked, the bacilli are retrieved, suspended in saline, suitably diluted and inoculated into mice. The results of harvest for one full year starting from June, are given in Table 1. It was found that bacilli remained viable for 5 months. In an earlier experiment started in the hot dry month of April, it was found that bacilli were alive only for 14 days. It is obvious from the experiment started in June after monsoon, that under humid and cooler conditions, the organisms could remain viable for a very long time.

2) Maintaining in wet soil : Local soil was sterilized and distributed to a number of sterile test tubes. Bacillary suspension was added to the soil and the soil kept moist by adding drops of sterile water. The tubes were left in room temperature. At weekly intervals, one test tube was taken and bacilli were retrieved by shaking the soil in sterile saline and collecting the supernatant. Mice were inoculated with the standard dose. The results of harvest are given in Table 2.

Harvest at the end of 6 weeks showed viable bacilli. The end point was not reached in the experiment. The experiment was therefore repeated, taking up the samples after 2, 3, 6, 9 and 12 months. No multiplication was found with any

Table 1 : Viability of *M. leprae* after drying in the shade (throughout the year)

Multiplication of <i>M. leprae</i> in footpads of mice														
Fresh Sample	Months of drying before inoculation													Autoclaved Sample (Control)
(0 days)	1.5	2	3	3.5	4	4.5	5	6	7	8	9	10	11	12
1x10 ⁶	1.7x10 ⁵	5x10 ⁴	1x10 ⁴	8x10 ⁴	2x10 ⁴	4x10 ⁴	9x10 ⁴	N	N	N	N	N	N	N

Inoculum - upto 10⁴ bacilli per footpad. N-Negative.**Table 2 : Viability of *M. leprae* in wet soil (Sept.-Oct., temperature, 25-30°C, Humidity, 66-44%)**

Multiplication of <i>M. leprae</i> in footpads of mice									
Fresh Sample	Days maintained in soil before inoculation								Autoclaved Sample (Control)
(0 day)	7	14	21	28	35		46		
1.2x10 ⁶	N	1.6x10 ⁵	1.3x10 ⁵	5.6x10 ⁴	N		1.8x10 ⁵		N

Inoculum - upto 10⁴ bacilli per footpad. N-Negative.**Table 3 : Viability of *M. leprae* in wet soil (Oct-Nov., temperature, 30-22°C, Humidity, 50-46%)**

Multiplication of <i>M. leprae</i> in footpads of mice								
Fresh Sample	Days maintained in saline before inoculation							Autoclaved Sample (Control)
(0 day)	3	7	14	21	28	35	43	
3.1x10 ⁵	1.1x10 ⁵	5.6x10 ⁴	7.5x10 ⁴	5.6x10 ⁴	2.7x10 ⁵	3.7x10 ⁴	5.6x10 ⁴	N

Inoculum - upto 10⁴ bacilli per footpad. N-Negative.

of the samples indicating that bacilli were not viable two months onward.

3) Maintaining in saline : Bacterial suspension in Hanks BSS was distributed to a number of test tubes. Viability was checked as in previous experiments. Results are given in Table 3.

It was noted that bacilli were viable in saline for 43 days, and the end point was not reached. So the experiment was repeated, following up for a longer time. Bacilli were found viable upto 2 months, but not later.

4) Exposure to Direct Sun light : Bacillary suspension was poured into several petridishes and allowed to evaporate. The material was exposed to direct sun-light every day from 9.30 a.m. to 12.30 p.m. by keeping the petri dishes open. They were kept back in the laboratory at other times. Bacilli retrieved from the dishes daily for 7 days were inoculated into mice. Results of harvest are shown in Table 4.

5) Maintainance at low temperature : Bacterial suspension in Hanks BSS was distributed to several test tubes and kept at 4°C, -20°C and -70°C. Viability at 4°C is shown in Table 5.

The bacilli remained viable for 60 days at 4°C, and also at -20°C. However, at -70°C, bacilli did not survive after 28 days.

6) Effect of "Savlon" and 70% alcohol : on exposure to 1% savlon, bacilli were killed after 24 hours, while 70% ethyl alcohol killed the bacilli in 30 minutes.

SUMMARY AND CONCLUSION

Transmission of leprosy to a susceptible host is generally by direct and intimate contact with an infectious case. The possibility of indirect transmission depends upon the viability of the organisms outside the human body, which the present study has attempted to assess, using the mouse foot pad model.

Drying under humid and cool atmospheric condition at room temperature, the bacilli were found to be alive for as long as 5 months. Results of the experiments have shown that *M. leprae* can withstand adverse environmental conditions for varying periods of time.

Findings like this were most unexpected since

Table 4 : Viability of *M. leprae* under direct sun light (May, temperature, 38-40°C, Humidity, 30-40%, exposure to sun daily for 3 h, from 9.30 am to 12.30 pm)

Multiplication of <i>M. leprae</i> in footpads of mice									
Fresh Sample		Days of drying in sun and shade before inoculation							Autoclaved Sample (Control)
(0 day)	1	2	3	4	5	7			
6.7x10 ⁵	1.2x10 ⁵	5x10 ⁴	N	N	3.5x10 ⁵	5x10 ⁴			N

Inoculum - upto 10⁴ bacilli per footpad. N-Negative.

Table 5 : Viability of *M. leprae* under refrigeration at 4°C

Multiplication of <i>M. leprae</i> in footpads of mice											
Fresh Sample	Days maintained at 4°C before inoculation										Autoclaved Sample (Control)
(0 days)	1	2	3	4	5	6	7	14	60	120	360
1x10 ⁶	1.8x10 ⁵	1.2x10 ⁵	1.1x10 ⁵	1.6x10 ⁵	2.1x10 ⁵	1x10 ⁵	2.6x10 ⁵	1.4x10 ⁶	5x10 ⁴	N	N

Inoculum - upto 10⁴ bacilli per footpad. N-Negative.

M. leprae are obligate intra-cellular organisms. Davey and Rees (2) first demonstrated that on drying in shade in London (humidity 43.7%) the bacilli survived for 7 days. Kaur et al (3) in Chandigarh also showed that the bacilli withstood drying for seven days. Our studies in Central India showed that survival is very greatly prolonged under humid conditions.

In view of the long survival of the bacilli under adverse environmental conditions, transmission of the disease indirectly is possible and the old theory of prolonged intimate direct contact with an infectious patient may not be required to spread the disease. However, it is also well known

that leprosy is a lowly infectious disease. While there is a good possibility of occurrence of viable bacilli in the environs in an endemic community, the presence of the causative organism is not the only factor behind the spread of the disease. The route of entry of the organism into the body of a new host is not clearly understood. Further, the innate immunity against leprosy in several ethnic groups like the Indians and Africans plays a major role in preventing the disease. While several factors are to be considered, the present study demonstrates an important epidemiological factor, namely the viability of *M. leprae* discharged from the patient.

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