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Temperature Optimum of *Mycobacterium leprae* in Mice

CHARLES C. SHEPARD
Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia

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ABSTRACT

SHEPARD, CHARLES C. (Communicable Disease Center, Atlanta, Ga.). Temperature optimum of *Mycobacterium leprae* in mice. J. Bacteriol. **90**:1271–1275. 1965.—*Mycobacterium leprae* multiplied most rapidly in foot pads of mice kept at an air temperature of 20 C. At air temperatures of 15 and 25 C, bacillary multiplication was slightly slower; at 10 and 30 C, distinctly slower; and at 4 and 35 C, no bacillary multiplication was detected. The temperature of the foot pad tissues of mice kept at an air temperature of 20 C averaged 27 to 30 C and that of mice kept at 10 and 30 C averaged about 25 and 36 C, respectively. These measurements indicate that the optimal temperature for the growth of *M. leprae* in mice is in the range several degrees above and below 30 C. The comparative effect of different air temperatures on the growth of *M. leprae* in foot pads was very similar to that found earlier for *M. marinum* in this site, thus indicating that the potential growth of *M. leprae* in vitro might have a similar optimum to *M. marinum* in vitro, i.e., 25 to 35 C. The optimal temperature for the growth of *M. leprae* appears to be the same in mice as in humans. It is pointed out that the temperature optimum of *M. leprae* may be a reflection of the fact that most of the bacilli being excreted into the environment, where they may reach new hosts, have multiplied in the nasal mucosa, a cool tissue.

In leprosy, the cooler tissues are more severely affected, as has been noted by many observers and described especially well by Brand (1959). The tissues most severely involved are skin, the superficial courses of the peripheral nerves, and the nasal passages. The viscera largely escape, except for microscopic lesions in bacillemic states or when there is secondary amyloidosis, which is, of course, not associated with the local presence of bacilli.

Mycobacterium leprae multiplies in the mouse foot pad (Shepard, 1960, 1963), a cool tissue, at ordinary air temperature. Modifications of the temperature of the foot pad tissue can be achieved by keeping the mice at different ambient temperatures, and we have described (Clark and Shepard, 1963) the effect of such modifications on the growth in foot pads of *M. marinum* (*balnei*), a rapidly growing species that causes human skin disease and has an optimal temperature range in bacteriological media of 25 to 35 C.

The present paper describes the effect of different ambient temperatures on the growth of *M. leprae* in mouse foot pads and gives improved estimates of the effect of different ambient temperatures on the temperatures of foot pad tissues of mice.

MATERIALS AND METHODS

The techniques for the experimental infection of mice have been described (Shepard, 1960). Acid-fast bacteria (AFB) were counted in suspensions of foot pad tissues by a microscopic method (Shepard, 1962). Environmental temperatures for the mice at 4, 20, and 25 C were obtained in rooms held at these temperatures. Water-jacketed incubators were used for 10, 15, 30, and 35 C, the former two being placed in the 4 C room. However, a controlled environmental room at 10 C was utilized for the mice in the experiments with primary clinical material as inoculum, and for the measurements of foot pad temperatures of mice kept at 10 and at 30 C. Temperatures were maintained usually within 1 C, except in the 25 C room where they were about 21 C in the first 2 months and about 23 C in the 3rd month. The mice were of the CFW strain maintained at the Communicable Disease Center. There were usually five to a cage. The diet was pelleted commercial chow.

The tissue temperatures were measured with a 22-gauge hypodermic thermistor probe used with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) by inserting the needle into the foot pad tissue. The temperature calibration of the probes was checked by placing the probes in clusters with standard mercury thermometers in water baths controlled at 0 and 37 C. In previ-

ous work we (Clark and Shepard, 1963) found the temperatures of the foot pad tissues to be within a few degrees of ambient temperatures between 10 and 35 C. In the present work, particular attention was paid to mice in their normal, unexcited state. At environmental temperatures of 25 C and lower, mice spend most of their time in huddles, and the rear foot pads and bedding are warmed by the huddle. At higher temperatures, they usually sit quietly and again the bedding is warmed by body heat. Once excited by the operator, however, the mice run about the cage, and contact with bedding at air temperature cools their foot pads. In the present series of measurements, the mice were caged in groups of five, in pairs, and alone; they were allowed to remain at the specified air temperature for at least 2 weeks. Rubber gloves were worn to pick up the mice, and hands were moved slowly. Only undisturbed mice were measured; those from huddles or those sitting alone were taken up directly before they had a chance to walk on surrounding bedding. The foot to be measured was not allowed to contact any solid objects, it was held by the toes, and the probe was inserted promptly. There was no noticeable difference in the temperatures for mice caged singly, in pairs, or in groups of five.

RESULTS

Experiments with mouse passage inoculum. In the first experiment (Fig. 1), the inoculum was the seventh passage of a strain (NSa) originating from a typical lepromatous patient in the Philippines. In the mice kept at 20 C after a lag phase of about 60 days, the AFB increased logarithmically to a level above 10^6; the curve was very similar to those seen in other experiments with *M. leprae* (Shepard, 1963; Shepard and McRae, 1965). The counts of AFB in mice kept at 10, 15, and 25 C were not much different, but, with a single exception, were lower than those kept at 20 C. In the mice kept at 30 C, the onset of bacillary increase was delayed until about 200 days, and AFB then rose to a level of about $10^{6.0}$. In the few mice that survived 35 C, no bacillary growth was detected in 200 days. Most of the mice kept at 4 C were lost by accident, but the single count at 120 days was very low.

A second experiment was carried out to compare a temperature of 4 with 20 C. The inoculum ($10^{3.7}$ AFB) was the sixth passage of a strain (B2415) originating from a Burmese whose disease was lepromatous with borderline features. AFB in the mice kept at 20 C rose, as expected, to $10^{5.7}$ at 118 days. AFB in the mice kept at 4 C were undetectable at 154, 236, 272, and 330 days, indicating counts of less than $10^{4.3}$. At 114 days, a count of $10^{4.7}$ was obtained; such a low count could well have arisen from the chance

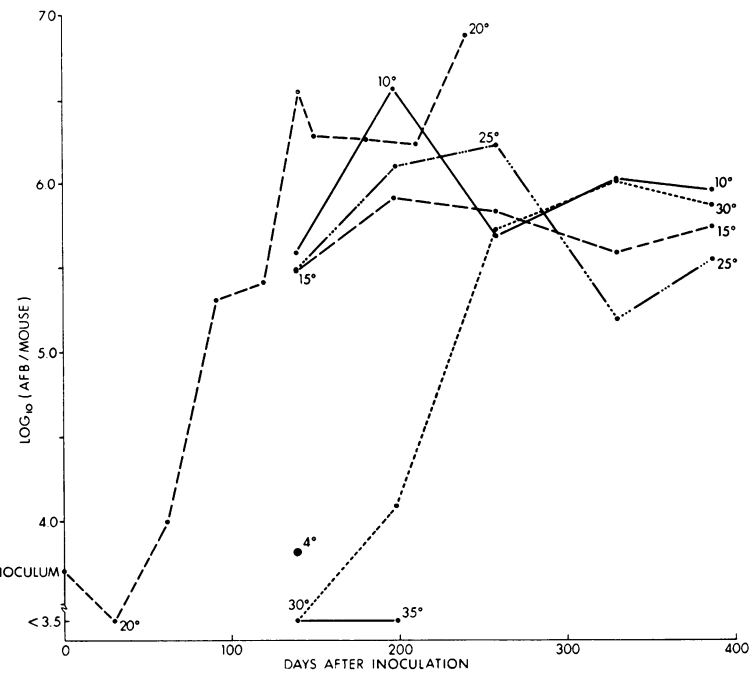

FIG. 1. *Effect of environmental temperature on growth of Mycobacterium leprae in mouse foot pads. Mice were inoculated with $10^{3.70}$ AFB from a passage strain and were placed at the various temperatures. The bacillary growth was observed by counts on pools of foot pad tissues of (usually) two mice.*

encounter of persisting inoculum. The counts were made on pools of tissue from two mice.

Attempts are being made to pass strains in mice kept at 10 and 30 C to see whether temperature variants of *M. leprae* can be selected, but the results are not yet clear.

Primary clinical material as inoculum. Since the passage strains had been maintained in mice at 20 C, it seemed possible that during passage bacilli might have been selected for growth in mice at this temperature. Accordingly, mice were inoculated with leprosy bacilli directly from human material (Table 1). The patients were typical lepromatous cases; 2592 and 2594 were from south Texas, and 2601 was from Puerto Rico. After inoculation, the progress of bacillary events was followed in histological sections of the feet of the 20 C mice killed at monthly intervals. When AFB appeared in the sections, counts of AFB were made on pools of four mice from each temperature.

TABLE 1. *Effect of environmental temperature on growth of Mycobacterium leprae in mouse foot pads**

Skin biopsy from patient	Inoculum		Temp mice were kept at	Harvest	
	No. of AFB†	Solidly staining bacilli		No. of AFB	Time
		%	C		days
2592	$10^{3.7}$	18	10	$10^{4.8}$	372
			20	$10^{5.9}$	371
			30	$10^{4.7}$	372
2594	$10^{3.7}$	18	10	$10^{4.6}$	216
				$10^{5.9}$	280
			20	$10^{5.7}$	191
				$10^{6.1}$	216
				$10^{6.1}$	223
			30	—‡	
2601	$10^{3.7}$	42	10	$<10^{4.5}$	149
				$10^{5.8}$	289
			20	$10^{5.5}$	147
				$10^{6.1}$	289
			30	$<10^{4.5}$	149
				$10^{5.8}$	289

* Mice were inoculated with bacilli from biopsy of patient indicated, and 20 mice were placed at each of the three temperatures.

† AFB = acid-fast bacteria.

‡ Mice died when incubator overheated.

Bacilli multiplied most rapidly in mice kept at 20 C. Bacilli also multiplied in mice kept at 10 and 30 C, but clearly more slowly than in mice kept at 20 C. Multiplication in mice kept at 10 C was more delayed than it had been in the experiment with passage material (Fig. 1), probably because the greater air circulation of the controlled environmental room cooled the cages more effectively. Thus, the results of the earlier experiment with mouse-passage material were confirmed. There was no suggestion that mouse passage had changed the temperature preference.

Temperatures in mouse foot pad tissues. In mice kept at 10 C (Table 2), a wide range of temperatures was recorded, but the mice in huddles had foot pad temperatures averaging 24.6 C. In mice kept at 20 C, the range of temperatures recorded was much smaller, and the temperature of those in huddles averaged 30.0 C. In mice kept at 30 C, the foot pad temperatures averaged 36.0 C for mice sitting quietly. These measurements indicate that in the mouse foot pad *M. leprae* multiplies most rapidly at about 27 to 30 C, and that it multiplies much more slowly at about 25 and 36 C.

DISCUSSION

The optimal temperature for *M. leprae* in humans has been estimated by measuring the temperature of the skin sites selected for their tendency to leprous involvement (*unpublished data*). The measurements were made on persons living in active endemic areas. The temperatures of skin sites most severely involved averaged 25 to 33 C, and the skin sites that escape leprosy averaged 35 to 36 C. Thus the optimal temperature for *M. leprae* in humans appears to be the same as it is in mice.

TABLE 2. *Temperatures of foot pads of undisturbed mice*

Air temp	Avg and range of temp of foot pads		
	Huddled	Sitting alone	Walking
C	C	C	C
10	24.6 (19.5–30.2), n* = 17	23.7 (19.5–29.5), n = 12	18.0 (15.8–22.0), n = 18
20	30.0 (27.0–32.5), n = 12	26.9 (25.5–27.9), n = 6	26.9 (25.8–28.2), n = 8
30		36.0 (34.4–36.9), n = 15	34.6 (32.7–36.0), n = 25

* Number of measurements.

The foot pad temperatures reported previously (Clark and Shepard, 1963) are corrected by the present measurements, which also bring into concordance the temperature optimum for *M. marinum* in mouse foot pads, in poikilothermic animals, and in bacteriological medium. Previously it had been thought that the optimum for *M. marinum* in mouse foot pads was lower than in the other two environments. The metabolism of the tissue and the activities of its component cells would be expected to increase in the temperature range studied, but these effects on tissue were not enough to change the temperature optimum of the pathogen noticeably.

The best ambient temperature for *M. leprae* in mouse foot pads was found to be the same as that for *M. marinum*. For both species, air temperatures 5 degrees above and below 20 C were moderately inferior, and air temperatures 10 degrees above and below 20 C were distinctly inferior. *M. marinum*, however, produced definite disease in mice at 4 C, whereas *M. leprae* did not multiply in mice at this temperature. The optimum for *M. marinum* in bacteriological medium (7H9 broth) was 25 to 35 C when the generation time was 4 to 6 hr. By analogy, then, one might expect that if *M. leprae* could be grown in bacteriological media, it would have a temperature range that is very similar to *M. marinum*, but perhaps would not extend quite so low. This expectation does not depend upon the correctness of the measurements of the mouse foot pad temperatures.

Palmer, Rees, and Weddell (1965) recently described the preferential location of *M. leprae* in striated muscle fibers of mice inoculated in the foot pad. They stated that the temperature will be high in the muscle and suggested that the growth of *M. leprae* in mouse foot pads depends on location in muscle cells rather than temperature. They also found that *M. leprae* will multiply when inoculated into thigh muscles. Since our routine involves viewing histological sections of foot pads of mice from most inoculated groups, we have seen many hundreds of sections containing *M. leprae* and can confirm that intramuscular growth is frequent. Growth has been distinctly more frequent in connective tissue, however, especially that located subcutaneously. Among the factors that appear to affect the location of the inoculum are the volume injected and the line of mice. Contrary to the expectation of Palmer et al. (1965), temperature measurements with the thermistor probe have not shown important differences between the subcutaneous tissue and the muscle of the foot. Measurements of the thigh muscle have shown temperatures averaging from 33 to 36 C, depending on the depth of the probe. Thus, depending on its location, the site of a thigh muscle injection in mice kept at 20 C might average cooler than the foot pads of mice kept at 30 C, in which multiplication did occur after a sufficient interval.

One might wonder at the example of a human pathogen with a temperature optimum of 27 to 30 C, since so many human pathogens have optimal ranges that include 37 C, the normal core temperature. Certainly in lepromatous leprosy, in which bacillemia is frequent, bacilli are repeatedly offered the opportunity of multiplying in tissue at core temperature. However, in lepromatous leprosy, which is the important infectious form of the disease, nasal involvement is very frequent, and in most cases at least 10^5 to 10^7 leprosy bacilli are excreted from the nose daily (Shepard, 1962). Although ulcers discharging bacilli may also be found at other sites, non-nasal ulcers are much less frequent. Thus, the pertinent temperature feature of the life history of *M. leprae* may be that the bacilli being excreted into the environment, where they may reach new hosts, have usually multiplied in the nasal mucosa. This could lead to selection for maximal growth at nasal temperatures. Temperatures of nasal mucosa at ambient temperatures of 20 to 30 C have been found to range several degrees above and below 30 C (*unpublished data*).

One might expect to find that other pathogens would have similar temperature optimum if their life histories depend upon reproduction in or on the nasal mucosa. Rhinoviruses, causing human coryza, probably reproduce chiefly in the nasal mucosa, and their temperature optimum in tissue culture is about 33 C (Tyrrell et al., 1960). They reproduce poorly in tissue cultures at 37 C. Their temperature optimum in tissue culture may be affected by the rate of metabolism of the cells, which increases in the interval from 30 to 37 C. There are bacteria also, e.g., *Staphylococcus aureus*, whose normal habitat is probably the nasal passages, but their temperature optimum on primary isolation has apparently not been studied.

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