INTERNATIONAL JOURNAL OF LEPROSY

Volume 42, Number 2
Printed in the U.S.A.

Studies of the Mouse Foot Pad Technic for Cultivation of *Mycobacterium leprae*

1. Fate of Inoculated Organisms[1, 2]

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Shepard has shown that inoculation of the foot pads of many strains of mice with small numbers of viable *Mycobacteria leprae* is regularly followed by limited multiplication of the organisms ([8]). The important variables that determine the characteristics of the resulting bacterial growth curve include the size of the inoculum, the proportion of viable organisms in the inoculum, the duration of the lag phase, and the doubling time during logarithmic multiplication. One may readily plot the logarithmic phase of the growth curve, because the number of organisms present during the latter portion of this phase may be counted with precision. The size of the inoculum may also ordinarily be determined with precision, and the proportion of viable *M. leprae* in the inoculum may be taken to be that proportion that is stained uniformly and brightly by a standard acid-fast stain. By extrapolating the logarithmic phase of the growth curve back to the number of viable organisms inoculated, one may estimate the duration of the lag phase.

This method of constructing the growth curve depends upon the assumption that the organisms present during logarithmic multiplication are generated by all of the viable organisms inoculated. That this assumption may be invalid was suggested by the results of a recent study in which only about 30% of a large inoculum was recovered in harvests made 24 hours after inoculation ([3]). In an attempt to elucidate the fate of the inoculated organisms, a series of experiments has since been carried out in which the recovery of inoculated *M. leprae*, *M. marinum* and radioactive particles of ^{99m}Tc-sulfur colloid was studied.

MATERIALS AND METHODS

Locally-bred BALB/c mice were used in most experiments. In a few experiments, B6C3F$_1$ mice that had been subjected to adult thymectomy and lethal whole-body X-irradiation followed by infusion of syngeneic bone marrow were used; these mice were provided by C. C. Congdon, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The *M. leprae* had been originally isolated from a patient with lepromatous leprosy by C. C. Shepard, Center for Disease Control, Atlanta, Georgia, and had subsequently been carried in mouse passage both in Atlanta and in this laboratory. The *M. leprae* to be inoculated were harvested from mouse foot pad tissues and were either used immediately or stored for several weeks at 4°C before use.

To prepare the more concentrated inocula employed in these experiments, *M. leprae* were centrifuged, and the sediment was resuspended in a small volume. The *M. marinum*, supplied by A. Back, City-County Health Department, San Francisco, California, were stored on Lowenstein-Jensen medium at 4°C. The suspensions of *M. marinum* to be inoculated were prepared from cultures in Dubos 7H9 broth with oleic-albumin supplement incubated at 32°C. Harvesting and counting of *M. leprae* and *M. marinum* were carried out according to the methods described by Shepard ([7, 9]).

[1]Received for publication 1 June 1973.

[2]This work was supported in part by the U.S. Leprosy Panel of the U.S.-Japan Cooperative Medical Science Program administered by the Geographic Medicine Branch, National Institute of Allergy and Infectious Diseases (Grants AI 07801 and AI 10110).

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Enumeration of viable *M. marinum* was performed by plating tenfold dilutions of tissue homogenates in triplicate on Dubos 7H9 agar.

The ^{99m} technetium-sulfur colloid was prepared by a modification of the method of Stern *et al* ([11]), in which $Na^{99m}TcO_4$ was heated with Na_2HPO_4 and gelatin in an acid solution, producing a colloidal suspension in which the radioisotope is thought to be present as insoluble $^{99m}Tc_2S_7$ carried on the surface of colloidal sulfur particles ([5]). Because of the short half-life of ^{99m}Tc (6 hours), the colloidal suspension was prepared shortly before each use. Chromatography of each suspension on silica gel with 85% methanol revealed less than 1% of the isotope to be present as $^{99m}TcO_4^-$. After the suspension had been filtered through a nitrocellulose membrane with pore size 0.8 μ, the filtrate was centrifuged at 1000 × g for 30 minutes. The supernatant was discarded and the sediment was resuspended in a small volume of Hanks' balanced salt solution for inoculation. Determination of the amount of radioactive material present was accomplished with a Nuclear-Chicago "Autogamma" well scintillation counter. All samples were counted in a final volume of two milliliters; liquid samples were diluted to two milliliters with Hanks' solution, and samples of tissue were suspended in a volume of tissue plus Hanks' solution equal to two milliliters. Samples were counted repeatedly for ten minutes; counts were corrected for background and radioactive decay.

RESULTS

Recovery of inoculated M. leprae. The results of five experiments in which large numbers of *M. leprae* were inoculated into the foot pads of mice are summarized in Table 1. Mice were inoculated in one or both hind foot pads with $10^{6.14}$ to $10^{6.86}$ *M. leprae* per foot pad contained in a volume sufficient to distend fully the foot pad tissues (approximately 0.5 ml). Harvests of the pooled tissues of four to eight foot pads were performed at intervals of one hour to nine days. Recovery of inoculated *M. leprae* ranged from 8.6% to 39%; mean recovery was 25.3%. The percent of *M. leprae* recovered did not appear to depend on the strain of mouse, the nature of the inoculum, or the time elapsed between inoculation and harvest. These results suggest that a large fraction of the inoculated *M. leprae* is not recovered by harvest.

Recovery of added organisms. The loss of inoculated *M. leprae* could be inherent in the mouse foot pad method, if the standard harvest procedure underestimated the actual number of organisms in the foot pad by 50% to 90%. To explore this possibility, a suspension of *M. leprae* containing $10^{6.65}$ organisms per milliliter was used in place of Hanks'

TABLE 1. *Recovery of inoculated* M. leprae.

Experiment no.	Mice	Inoculum	Inoculum: No./foot pad (× 10^6)	Harvest: Time (hr)	Harvest: No./foot pad (× 10^5)	Harvest: %
1	BALB/c	Fresh	2.64	24	3.20	12.1
				120	9.24	35.0
				216	6.67	25.3
2	BALB/c vaccinated with BCG	Fresh	2.64	24	2.26	8.56
				120	7.73	29.3
				216	7.09	26.9
3	BALB/c	Stored	7.21	1	17.8	24.7
4	BALB/c	Stored	1.77	1	5.16	29.2
				24	1.62	9.20
				72	3.67	20.7
				120	4.17	23.6
5	$B63F_1$ thymectomized irradiated	Stored	1.39	1	4.56	32.8
				24	3.35	24.1
				48	5.35	38.5
				168	5.47	39.4

TABLE 2. *Recovery of mycobacteria added to homogenate of foot pad tissue.*

Experiment no.	Added No./foot pad ($\times 10^6$)	Recovered No./foot pad ($\times 10^5$)	%
6	2.24	13.1	58.5
	M. leprae	8.2	36.7
		17.7	79.0
		16.8	75.0
7	2.14	15.0	70.1
	M. marinum	16.1	75.2
		22.1	103
		21.4	100

TABLE 3. *Harvest of* M. leprae *from foot pad and from adjacent tissues.*

Experiment no.	Standard	Harvest No./foot ($\times 10^5$) Dorsal & Distal	Proximal
8	11.0	0.42	1.61
	8.68	0.85	4.59

solution as the diluent for mincing the pooled tissues of four foot pads from mice that had not been inoculated. The tissue mince to which *M. leprae* had been added was carried through the standard harvest procedure. The results of this experiment, summarized in Table 2, show that recovery of added *M. leprae* ranged from 37% to 79%, with a mean of 62% for four such "harvests."

A similar experiment was performed in which a suspension of *M. marinum* containing $10^{6.63}$ organisms per milliliter was substituted for the suspension of *M. leprae*. The results of this experiment, also presented in Table 2, show that recovery of the added mycobacteria ranged from 70% to 103%, with a mean of 87% for four "harvests." These experiments show that, although the recovery of added organisms is usually less than complete, the loss of *M. leprae* inoculated into the intact foot pad does not represent an artifact of the harvest procedure.

Results of extended harvest. A second possible explanation for the loss of inoculated *M. leprae* is the distribution of the organisms into foot tissues not encompassed by the harvest. To examine this possibility, two extended harvests were performed of the pooled tissues of four feet each, using mice from a group inoculated some months previously that had been shown by an earlier harvest to have about 10^6 organisms per foot pad. After the foot pad tissue was excised as for a standard harvest, two additional pools of foot tissues were made. A dorsal and distal pool included all of the soft tissue that could be removed from the dorsum of the foot and from around the phalanges. A proximal pool included the tissues around the tarsal joint and the five millimeters of the leg just proximal to the joint. The results of this experiment, shown in Table 3, demonstrate that, of the organisms recovered in the extended harvest, 73% of the total was contained in the tissues included in the standard harvesting procedure, 5% was recovered in the tissues of the dorsum of the foot and around the phalanges, and 22% was recovered in the more proximal tissues.

Even if the fraction of organisms recovered from the additional tissue pools represents multiplication *in situ* of a similar fraction of the inoculum, the distribution of inoculated organisms to tissues not included in the standard harvest does not account for the loss of inoculated *M. leprae*. If only 74% of the inoculated organisms actually present

in the harvested tissues are recovered by the harvest procedure, and if 73% of the organisms inoculated are actually in the tissues encompassed by the harvest, fewer than 50% of the inoculated organisms would be lost.

Study of leakage of the inoculum. The loss of inoculated *M. leprae* as the result of leakage from the injection site represents a third possible explanation of the failure to recover 75% of the inoculated organisms. This was examined by inoculating and harvesting anesthetized mice. Mice were anesthetized by the intraperitoneal administration of 35 mg sodium phenobarbital per kg, after which *M. leprae* were inoculated into both hind foot pads. The animals were then restrained in a supine position, and anesthesia was maintained for the next hour with ethyl ether. At the end of this time, the animals were sacrificed and two harvests, each of four foot pads, were performed. The results of this experiment (Table 4) appear to exclude leakage of the inoculum as the cause of the loss of inoculated *M. leprae*. Because the animals were restrained, leakage could not have occurred because of the animal's normal walking activity. As an additional means of minimizing leakage, the foot pads were not scrubbed before harvest as is usually done. Despite these precautions against leakage, an average of 68% of the inoculated organisms was lost during the hour between inoculation and harvest.

Inoculation of dead mice. If the inoculated *M. leprae* not recovered in the standard harvest are not found in the adjacent soft tissues and have not been lost by leakage, then it appears likely that they have been removed from the region of the foot by the lymph or blood circulation. To study the role of the circulation in the loss of inoculated organisms, dead mice were inoculated and the recovery of the inoculum was measured. The results of two experiments are presented in Table 5. In the first experiment, mice were first sacrificed and then immediately inoculated with a suspension of *M. leprae*. One hour later, a harvest was performed from the pooled tissues of four foot pads. In the second experiment, a suspension of *M. marinum* was used, and four harvests, each from four foot pads, were performed. On the average, only 38% of the inoculated organisms were recovered in the five harvests. This unexpected result may be interpreted to indicate that inoculated *M. leprae* are not lost by means of the circulation. Marked swelling of the dorsum of the foot and surrounding tissues was observed at the time of each of the harvests, whereas when living animals are inoculated, the swelling of the foot pad tissues produced by the injection

TABLE 4. *Recovery of inoculated* M. marinum *from anesthetized mice.*

Experiment no.	Inoculum No./foot pad ($\times 10^6$)	Harvest No./foot pad ($\times 10^5$)	%
9	2.27	8.90	39.2
		5.50	24.2

TABLE 5. *Recovery after inoculation of dead mice.*

Experiment no.	Inoculum No./foot pad ($\times 10^6$)	Harvest No./foot pad ($\times 10^5$)	%
10	0.51 *M. leprae*	2.92	57.0
11	2.87 *M. marinum*	13.3	46.3
		8.10	28.4
		7.00	24.4
		10.1	35.2

mass disappears within a matter of minutes and swelling of the dorsum of the foot is never marked. Thus, the results of inoculation of dead mice may not be directly applicable to this study of the mechanism by which *M. leprae* inoculated into living mice are lost. On the other hand, the rapid disappearance of the distention of foot pad tissues after inoculation of living mice suggests that some of the liquid portion of the injection mass is removed by the circulation; perhaps some of the inoculated organisms are removed at the same time.

Systemic distribution of inoculated organisms. If the loss of inoculated *M. leprae* occurs by way of the circulation, one might expect to find organisms at distant sites. Two experiments have been performed to test this hypothesis. If the organisms were removed as large clumps, they might be filtered in the pulmonary capillaries. Table 6 shows that less than 0.25% of the inoculum was recovered from the pooled lung tissue of two mice given a large inoculum in each hind foot pad one hour earlier.

Another study of the systemic distribution of inoculated mycobacteria used *M. marinum,* to enable the detection of smaller numbers of organisms. In this experiment, the results of which are also shown in Table 6, a small inoculum was administered to each hind foot pad of several mice. Harvests of the pooled tissues of four foot pads and four inguinal lymph nodes were performed after one and two hours. In addition, the spleens of the two mice sacrificed after one hour were pooled for harvest. Aliquots of the suspension resulting from each harvest were plated, and the number of colony-forming units in each tissue calculated. The harvests of foot pad tissue reveal that more than 90% of the inoculum was lost within the first two hours following inoculation. No viable *M. marinum* were detected in the spleen and lymph node homogenates.

Experiments with ^{99m}Tc-sulfur colloid. Because of the difficulties of detecting small numbers of *M. leprae* and of preparing a large inoculum of *M. marinum* without clumping of organisms, we carried out experiments with an inoculum consisting of radioactive colloidal particles smaller than 0.8μ in diameter. The results of four experiments are summarized in Table 7. In the first two experiments, mice were sacrificed one and two hours after inoculation and harvests from the pooled tissue of four foot pads were performed at each time interval. An average of 14% of the inoculum was recovered in the foot pad harvests. No radioactivity could be detected in the two pools of four inguinal lymph nodes obtained from the animals receiving the smaller inoculum; only a trace could be detected in the nodes obtained from the animals administered the larger inoculum.

In the third experiment, mice were killed by exsanguination one hour after inoculation of both hind foot pads. After the foot pad tissue had been excised for harvest in the standard manner, the remainder of each foot was removed at the tarsal joint; foot pad tissues were pooled for counting as were the disarticulated feet from which the foot pad tissue had been excised. The blood, spleens, livers, and inguinal nodes were al-

TABLE 6. *Distribution of inoculated mycobacteria.*

Experiment no.	Inoculum No./foot pad[a]	Harvest Time (hr)	Organ	No./organ[a] ($\times 10^5$)	%
12	7.21×10^6 *M. leprae*	1	Foot pad	1.78×10^6	24.7
			Lung	$<1.82 \times 10^4$	<0.25
13	1.03×10^3 *M. marinum*	1	Foot pad	100	9.7
			Spleen	<1	<0.10
			Node[b]	<1	<0.10
		2	Foot pad	80	7.8
			Node	<1	<0.10

[a] Number of acid-fast bacilli when *M. leprae* inoculated; number of colony-forming units when *M. marinum* inoculated.

[b] Inguinal lymph node.

TABLE 7. *Distribution of inoculated ^{99m}Tc-sulfur colloid.*

Inoculum		Harvest			
Experiment no.	cpm[a]/foot pad	Time (hr)	Organ	cpm[a]/organ	%
14	44,100	1	Foot pad	6,550	14.9
			Node[b]	<1	<0.002
		2	Foot pad	9,340	21.2
			Node	<1	<0.002
15	11,483,000	1	Foot pad	909,040	7.9
			Node	28	0.00024
		2	Foot pad	1,193,300	10.4
			Node	16	0.00014
16	495,500	1	Foot pad	242,625	49.0
			Remainder of foot	63,525	12.8
			Liver	115	0.02
			Blood	16[c]	0.003
			Spleen	3[d]	0.0006
			Node	<1	<0.0002
17	84,330	1	Dead foot pad	9,530	11.0

[a] Counts per min. [b] Inguinal lymph node. [c] Cpm per ml. [d] Actual count of two spleens about twice background; count corrected for background 64 per 10 min, 95% confidence limits 48 and 80 counts per 10 min; thus, value of 3 cpm per spleen significantly greater than 0.

so pooled for counting. The data of Table 7 show that slightly more than 50% of the radioactive inoculum was not recovered by the standard harvest. That portion of the inoculum recovered by the standard harvest represented 79% of the total of radioactivity in the foot. Only traces of radioactivity were found in the liver and blood, and none could be detected in the spleen and nodes.

In a final experiment, recovery of inoculum after inoculation of dead foot pads was measured. Harvest of the foot pad tissue of two mice inoculated immediately after sacrifice resulted in recovery of only 11% of the inoculum. Thus, by three criteria—loss of inoculum after inoculation of living mice, detection by an extended harvest of only a fraction of that portion of the inoculum which was lost, and loss of a large fraction of the inoculum even when mice were sacrificed before inoculation—use of the ^{99m}Tc-sulfur colloid yielded results very similar to those obtained when mycobacteria were inoculated. Because minute fractions of the inoculum can be detected with considerable precision, the use of the radioactive colloid permits a more intensive study of the distribution of the inoculum beyond the foot pad. These studies, then, confirm those made with mycobacterial suspensions; the inoculated organisms not found in the foot are not to be found in the blood, liver, spleen or inguinal lymph nodes.

DISCUSSION

Recovery of inoculated *M. leprae* from the mouse foot pad has not been thoroughly studied. Rees and his co-workers ([12]) claimed complete recovery of an inoculum of 10^4 *M. leprae* per foot pad by individual harvests from four foot pads. Although Rees' technic may require the microscopic examination of a larger fraction of the harvested tissue than does Shepard's technic, the number of bacilli counted in the recovery of 10^4 bacilli could not have been very large, and such a determination could not have been made with great precision. Shepard's technic requires the examination of about 0.2 μl of tissue homogenate, approximately 1/10,000 of the total volume; when only one organism is actually seen in a harvest of four foot pads, the number of *M. leprae* per foot pad is in the range $10^{3.5}$ to 10^4. When a harvest yields 10^6 organisms per foot pad, 100 to 300 *M. leprae* are actually counted. If the organisms in the stained preparation from the homogenate are randomly distributed, the standard deviation (S.D.) of the

estimate of the number of organisms (n) is simply $\sqrt{n}$ (4). Therefore, if only one organism is counted, S.D. = 1, and the 95% confidence limits, $n \pm t_{0.95}\, n$, range from 0 to 13 organisms. However, if 100 organisms are counted, the 95% confidence limits range from 80 to 120, proportionately a much smaller range. Although the organisms in the preparation are probably not randomly distributed, the argument that larger numbers of organisms can be counted with greater precision appears intuitively to hold. For this reason, the measurements reported by Rees and his colleagues may not be inconsistent with the measurements reported here. It is also for this reason that the experiments described here employed larger inocula. Both Desikan (1) and Matsuo (6) have also reported their inability to recover large fractions of inocula of *M. leprae* from the mouse foot pad, confirming the observations reported here.

The results of the experiments reported here suggest that recovery of *M. leprae* inoculated into the mouse foot pad range from 10% to 40% with a mean of 25%. If the failure to recover 75% of the inoculated organisms was merely an artifact of the harvest procedure, we should expect to lose no more than 50% of the inoculum. The efficiency of the harvest procedure averaged 62% and 87% in two experiments. Distribution of the inoculum to foot tissues not included in the standard harvest procedure accounted for the loss of about 27% of the inoculum. The loss of inoculated *M. leprae* is obviously greater than can be accounted for by either of these two factors, and another explanation for the loss must be sought.

Where do the missing *M. leprae* go? Evidence for their loss via the circulation is the demonstration by Rees of late multiplication of *M. leprae* at sites distant from the foot pad (12). One may argue that dissemination of these organisms to the distant sites occurs after multiplication in the foot pad has already occurred. Alternatively, the late appearance of numbers of *M. leprae* at distant sites is consistent with multiplication from a very small number of viable organisms that arrived at the distant site soon after inoculation of the foot pad. Our inability to demonstrate organisms or radioactive colloidal particles at distant sites must be examined. The opportunity for such an examination is furnished by the data in Table 7.

According to Dunn (2), the combined axillary and inguinal nodes account for about 0.1% of the body weight of the CBA mouse, and the spleen accounts for 0.3%. If the radioactive material not found in the foot at the time of sacrifice of the mice in experiment 16 were uniformly distributed throughout the remainder of the animal, an inguinal node would be expected to contain 0.01% of the total, and the spleen 0.11%. The actual measurements reveal the presence of much less of the isotope in these organs. If the colloidal particles (and *M. leprae*) are not uniformly distributed, to which organs are they primarily distributed? No clues are apparent in these data. The presence of the particles in the blood confirms that organisms do indeed enter the circulation from the foot pad; one may only speculate regarding the sites to which they are preferentially distributed. The bone marrow appears to be a good possibility.

The loss of 75% of the inoculum has not been considered in the calculation of the data that result from experiments in which multiplication of *M. leprae* in the mouse foot pad is observed. Because most of these data relate to the logarithmic phase of bacterial multiplication, and usually depend on the counting of the relatively large numbers of *M. leprae* observed toward the end of the phase of logarithmic growth, no error has been introduced as the result of ignoring the loss of a large fraction of the inoculum. Loss of 75% of the inoculum is important only in those calculations that require the extrapolation of the logarithmic phase back toward the time of inoculation. Obviously, estimates of the duration of the lag phase and the proportion of the inoculum that is viable would be very much in error if the loss of organisms equivalent to two doublings were ignored. An example of such a situation is the measurement by Shepard and McRae (10) of the minimal number of viable *M. leprae* required to produce multiplication in the mouse foot pad. Correcting the estimate of 3 to 30 organisms for loss of inoculated *M. leprae* suggests that as few as one viable organism may be infectious for the mouse foot pad.

SUMMARY

The possibility of loss from the mouse foot pad of a large fraction of an inoculum of *M. leprae* was suggested by a preliminary ex-

periment, and a systematic investigation of this problem was undertaken. A series of experiments with various inocula, including freshly harvested *M. leprae, M. leprae* stored at 4°C, *M. marinum,* and suspensions of ^{99m}Tc-sulfur colloid all yielded much the same result: 60% to 90% of the inoculum could not be recovered by a harvest performed soon after foot pad inoculation. The recovery of organisms added to foot pad tissue harvested from uninoculated mice was nearly complete, excluding the possibility of an inherent deficiency of the harvesting procedure. Recovery of the inoculum was improved somewhat when a more extensive harvest was done, but much of the inoculum remained unaccounted for. Inoculum was lost even when dead mice were inoculated, and when anesthetized mice were inoculated to minimize the possibility of leakage. When radioactive colloidal particles of about the same size as *M. leprae* were inoculated, traces were found in the blood, liver, spleen and inguinal lymph nodes, but the quantities of the radioactive material in these organs were smaller than expected if that fraction of the inoculum lost from the foot pad were distributed uniformly among all of the tissues of the mouse.

Loss of a large fraction of inoculated *M. leprae* from the mouse foot pad occurs regularly, does not represent an artifact, and is not the result of leakage of the inoculum. Loss occurs probably by way of the circulation. The organisms lost from the foot pad do not appear to be uniformly distributed in the mouse, suggesting that they may be taken up preferentially from the blood by some organ such as the bone marrow.

RESUMEN

Un experimento preliminar sugirió la posibilidad de que se perdiera una gran parte del inóculo de *M. leprae* desde la almohadilla de la pata del ratón y, por lo tanto, se comenzó una investigación sistemática de este problema. Una serie de experimentos con diversos inóculos, incluyendo *M. leprae* recientemente cosechado, *M. leprae* mantenido a 4°C, *M. marinum* y suspensiones de coloide ^{99m}Tc-azufre, dieron todos más o menos el mismo resultado: al hacer una cosecha poco después de la inoculación en la almohadilla de la pata del ratón, dejaba de recuperarse entre 60 y 90% del inóculo. La recuperación de microorganismos que se añadieron a tejidos de la almohadilla de la pata de ratones no inoculados fué casi completa, excluyendo la posibilidad de una falla inherente al proceso de recolección. La recuperación del inóculo mejoró algo cuando se hizo una recolección más extensa, pero siempre hubo una gran parte del inóculo que desaparecía. El inóculo se perdía aún cuando se inoculaban ratones muertos y cuando se inoculaban ratones anestesiados para disminuir la posibilidad de escape. Cuando se inocularon partículas coloidales radioactivas de más o menos el mismo tamaño que el *Mycobacterium leprae,* se encontraron trazas en la sangre, en el hígado, en el bazo y en los ganglios inguinales, pero las cantidades de material radioactivo en estos órganos fueron menores que lo que se esperaría si la fracción perdida del inóculo puesto en la almohadilla de la pata estuviera distribuída en forma uniforme entre todos los tejidos del ratón.

La pérdida de una gran fracción del *M. leprae* inoculado en la almohadilla de la pata se observa en forma regular, no representa un artefacto y no es el resultado de un escape del inóculo. La pérdida se produce probablemente por vía circulatoria. Los microorganismos que se pierden de la almohadilla de la pata no parecen estar distribuídos en forma uniforme en el ratón, sugiriendo que pueden ser tomados preferencialmente de la sangre por algún órgano tal como la médula ósea.

RÉSUMÉ

Une expérience préliminaire a suggéré qu'une proportion importante des bacilles de la lèpre inoculée dans la coussinet plantaire de la souris, pourrait être perdue. On a entrepris de mener une investigation systématique de ce problème. Une série d'expériences au moyen de divers inoculats, comprenant entre autre des *M. leprae* récoltés récemment, des *M. leprae* conservés à 4°C, *M. marinum,* ainsi que des suspensions de ^{99m}Tc-souffre colloïdal, ont toutes livré le même résultat. Entre 60 et 90 pour cent de l'inoculat ne peut pas être récupéré lorsque l'on récolte les bacilles peu de temps après l'inoculation dans le coussinet plantaire. La récupération de microorganismes ajoutés a des tissus de coussinet plantaire prélevés chez des souris non inoculées, était presque complète. Ceci exclut l'éventualité d'une déficience inhérente du procédé de récolte. La récupération de l'inoculat était légèrement améliorée lorsque l'on procédait à une récolte plus étendue. Néanmoins, la plus grande partie de l'inoculat ne parvenait pas à être récupérée. On perdait même l'inoculat lorsque l'inoculation avait eu lieu chez des souris mortes, et lorsque des souris anesthésiées étaient inoculées afin de diminuer autant que possible la possibilité de perte au cours de l'inoculation. En procédant à l'inoculation de particules colloïdales radioactives, approximativement de la même dimension que *M. leprae,* on a pu trouver des traces de ces particules dans le sang, le foie, la rate et les ganglions lymphatiques inguinaux. Toutefois, les quantités de matériel

radioactif dans ces organes étaient plus faibles qu'on aurait pu s'y attendre si une fraction d'inoculat perdue lors de l'inoculation du coussinet plantaire était distribuée de façon uniforme dans tous les tissus de la souris.

La perte d'une fraction importante des *M. leprae* inoculés, a là suite de l'inoculation dans le coussinet plantaire de la souris, survient régulièrement. Cela ne répresente pas un artéfact, et n'est pas le résultat d'une perte lors de l'inoculation. Cette perte survient probablement par le truchement de la circulation. Les organismes perdus au niveau du coussinet plantaire, ne semblent pas être distribués uniformément chez la souris, ce qui suggere qu'ils pourraient être captés de façon préférentielle, dans le sang, par l'un ou l'autre organe telle que la moelle osseuse.

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