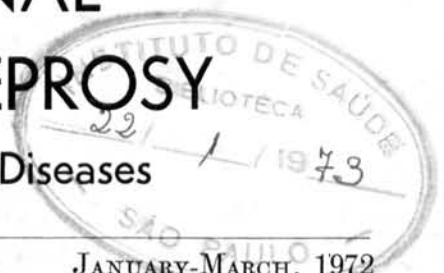


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Studies in the Viability of *Mycobacterium leprae* in Human Liver and Bone Marrow Using Thymectomized Mouse Foot Pad Technic¹

A. B. A. Karat, H. Harmer, A. S. Kumar and J. R. Albert²

Since the introduction of the mouse foot pad technic for the cultivation of *M. leprae* all the results reported to date have been limited to experiments with bacilli obtained from skin biopsy. We have been investigating the viability of bacilli found in bone marrow and liver, especially since the temperature in these organs is known to be at least 2°F greater than the skin temperature. Further, we wanted to determine whether the bacilli in the bone marrow and liver represented only dead bacilli phagocytosed by macrophages or whether the bacilli in these organs were viable bacilli capable of multiplication and dissemination in the body.

MATERIALS AND METHODS

Bone marrow aspirates and liver biopsies were obtained from lepromatous patients by standard technics already described (1, 3). The patients were classified by clinical, histologic and immunologic criteria. The

bone marrow aspirate was homogenized in Mickel's tissue disintegrator using 4 mm glass beads and 3 mm vibration for ten minutes. The bacilli in the supernatant were counted according to Shepard's technic (4), and 5,000 organisms were inoculated into the foot pads of mice in the usual way.

Liver biopsy specimens were homogenized using the same technic as that for homogenization of skin biopsy and 5,000 organisms were inoculated into the foot pads of mice.

The skin biopsy and bone marrow aspirate were obtained on the same day and the liver biopsy was obtained the following day.

The experimental animals were harvested every four weeks from five months after inoculation in view of the lag period of five months already reported (2). When the harvests were positive for acid-fast bacilli, part of the homogenate was submitted to culture in the Lowenstein-Jensen medium.

RESULTS

The maximum harvest counts from four experiments in thymectomized CBA mice are summarized in Table 1.

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² A. B. A. Karat, B.Sc., M.B., M.R.C.P. (Lond.), M.R.C.P. (Edin.), Consultant Physician & Director of Department of Experimental Medicine, Church of India Hospital, Bangalore-1A, S. India; H. Harmer, F.I.M.L.T., Medical Technologist; A. S. Kumar, M.Sc., Research Technologist, Exp. Medicine, C.S.I. Hospital, Bangalore-1A; J. R. Albert, B.Sc., Junior Research Technologist, Exp. Medicine, C.S.I. Hospital, Bangalore-1A.

TABLE 1A. Experimental inocula sources.

Expt. no.	Bacillary load					
	Skin		Bone marrow		Liver	
	Homogenate count per ml	MI (%)	Homogenate count per ml	MI (%)	Homogenate count per ml	MI (%)
1	9.2×10^7	2.0	7.0×10^6	5.0	5.3×10^6	0.0
2	1.9×10^7	1.0	2.6×10^5	0.0	2.0×10^7	4.0
3	3.3×10^8	2.0	8.8×10^5	16.0	3.0×10^7	6.0
4	7.5×10^7	8.0	4.4×10^5	0.0	7.2×10^6	2.0

TABLE 1B. Peak bacillary harvest from thymectomized CBA mouse foot pads following a standard inoculum of 5×10^3 AFB obtained from skin biopsy, bone marrow aspirate and liver biopsy.

Expt. no.	Foot pad harvest count								
	AFB from skin			AFB from bone marrow			AFB from liver		
	Peak harvest	MI of harvest bacilli (%)	Incubation period in days ^a	Peak harvest	MI of harvest bacilli (%)	Incubation period in days ^a	Peak harvest	MI of harvest bacilli (%)	Incubation period in days ^a
1	8.3×10^5	0.0	267	1.9×10^7	1.0	338	1.6×10^6	2.5	283
2	1.4×10^6	1.5	310	1.3×10^5	14.0	474	7.9×10^6	1.5	454
3	1.4×10^6	0.0	353	1.5×10^6	0.0	280	2.2×10^5	0.0	515
4	2.9×10^5	12.0	282	6.8×10^6	9.0	213	1.6×10^7	5.5	502

^a Incubation period is defined as the number of days between inoculation and peak harvest.

DISCUSSION

These experiments clearly substantiate the idea that despite the higher temperature in human bone marrow and the liver, lepra bacilli in these organs remain viable by their ability to multiply in the foot pads of mice. Since none of the organisms from the homogenates were cultivable in the conventional media, it would seem reasonable to assume that they were lepra bacilli. Thus the importance of liver and bone marrow (and other organs of the reticulo-endothelial system) as reservoirs of viable lepra bacilli needs to be borne in mind in determining therapeutic policies for various types of leprosy, especially since it has been already demonstrated that long after the skin smears have become negative,

solid organisms can be seen in casual bone marrow aspirates and liver biopsy specimens. Too hasty cessation of specific treatment can be expected to result in recrudescence or relapse of leprosy, especially in the bacillated types of the disease. Prolonged treatment of bacillated types of leprosy thus becomes a rational therapeutic policy instead of being empirical.

SUMMARY

Preliminary findings of a study of viability of *M. leprae* in human bone marrow and liver using the thymectomized mouse foot pad technic are described and compared with viability of *M. leprae* obtained simultaneously from skin biopsies of the same patients. The acid-fast bacilli obtained from

the bone marrow and liver biopsy homogenates of lepromatous leprosy patients are found to be viable judging from their ability to multiply in the foot pads of mice. The therapeutic implications of this finding are discussed.

RESUMEN

Se describen los hallazgos preliminares de un estudio de la viabilidad del *M. leprae* de la médula ósea e hígado humanos utilizando la técnica de la almohadilla de la pata en ratones timectomizados, comparándola con la viabilidad del *M. leprae* obtenido en forma simultánea de biopsias de piel de los mismos pacientes. Se encuentra que los bacilos ácido-resistentes obtenidos de homogenados de médula ósea y de hígado de pacientes con lepra lepromatosa son viables a juzgar por su capacidad para multiplicarse en la almohadilla de la pata del ratón. Se discuten las implicaciones terapéuticas de estos hallazgos.

RÉSUMÉ

On rapporte ici les observations préliminaires recueillies à la suite d'une étude de viabilité de *M. leprae* dans la moëlle osseuse humaine et dans la foie, menée par la technique du coussinet plantaire de la souris thymectomisée. Ces observations sont comparées avec la viabilité de *M. leprae* observée simultanément à partir

des biopsies de peau chez les mêmes malades. Des bacilles acido-résistants recueillis à partir de la moëlle osseuse et d'homogénéisats de biopsies hépatiques pratiquées chez des malades atteints de lèpre lepromateuse, ont été trouvés viables, si l'on en juge d'après leur capacité à se multiplier dans les coussinets plantaires de la souris. Les implications thérapeutiques de ces observations sont discutées.

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