# A Comparative Evaluation of Bacteriologic and Morphologic Indices of Mycobacterium leprae in Skin, Lymph Node, Bone Marrow, Nerve and Muscle<sup>1</sup>

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Although skin and nerves bear the main brunt of involvement in human leprosy, M. leprae can be detected in almost every system of the body with special relation to the reticuloendothelial system (<sup>5</sup>). In view of the higher temperature of the internal organs, as compared to skin, it may be of significance to assess the status of viability of the organisms as well as the degree of involvement (Bacteriologic Index) of tissues other than the skin. Though recent research has confirmed the viability of leprosy bacilli obtained from bone marrow and liver by mouse foot pad inoculation (4), as yet there is no report available eliciting the comparative evaluation of the Morphologic Index (MI) and Bacteriologic Index (BI) of different tissues. The present study was, therefore, carried out to assess the status of the BI and MI of M. leprae in various tissues; e.g., skin, lymph node, bone marrow, nerve and muscle obtained from untreated cases of leprosy.

#### **MATERIALS AND METHODS**

tissue smears were stained by a modified Ziehl-Neelson method for leprosy bacilli (<sup>2</sup>). The BI was calculated according to Ridley's (<sup>7</sup>) logarithmic scale by examining the smear under 1/12 inch oil immersion objective. The MI was calculated by the method of Waters and Rees (8) indicating the percentage of solidly staining bacilli out of total bacillary population (Fig. 1). On an average, about 300-500 individual bacilli were counted.

#### RESULTS

Of 15 patients, 13 were classified as lepromatous leprosy, including four with erythema nodosum leprosum (ENL) reaction. Two patients were suffering from dimorphous leprosy in reaction. The comparative BI and MI of skin, lymph node, bone marrow, nerve and muscle tissues from these patients are shown in Table 1. As compared to the BI of skin, the BI of lymph nodes was found to be similar in six, higher in six and lower in two patients. In the case of the bone marrows, it was similar in four and lower in the rest of the patients. Only in one patient having a very low BI of skin (1+) were no bacilli detected in the bone marrow. The BI of nerves

Fifteen untreated patients suffering from bacillary positive type of leprosy were selected for this study. All of the patients were classified by clinical examination and histologic evaluation of skin biopsies. In each case, a palpable axillary or inguinal lymph node was excised for bacteriologic (impression smears) and histologic studies. Bone marrow aspirates were obtained by the standard technic (<sup>3</sup>). Sural nerve biopsy and muscle biopsy from the quadriceps were obtained and tissue smears were prepared. The

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FIG. 1. Showing solid staining ( $\rightarrow$ ) and fragmented ( $\cdots$ ) bacilli in skin.

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Sr. no.	Age	Sex	Diagnosis	S BI	kin MI%	Lymj BI	ph node MI%	Bone BI	marrow MI%	BI N	Nerve MI%	M BI	uscle MI%
1	22	Μ	Lepromatous	3+	14	2+	2.8	1+	0				
2	20	F	Lepromatous	1+	2	1+	1.5	0	0			1+	0
3	50	Μ	Lepromatous	6+	14	6+	28	6+	1 <u></u>			1+	0
4	40	Μ	Lepromatous	5+	18	—	_	3+	1.5	3+	3.5	1+	0
5	45	Μ	ENL	6+	0	6+	5.2			4+	0		
6	30	Μ	Lepromatous	6+	11.4	6+	13.4	6+		4+	4.75	3+	
7	45	Μ	Lepromatous	3+	4.5	6+	4.5	3+		3+	2.5	3+	0
8	34	Μ	Dimorphous										
			reaction	2+	0	3+	6.5	2+	0	3+	0	0	0
9	18	Μ	Lepromatous	5+	3.8	6+	4	3+		3+	3.8	0	0
10	48	Μ	Lepromatous	5+	4.1	6+	2.5	3+	0	4+	3.5	0	0
11	60	Μ	ENL	4+	0	6+	5	—					
12	22	Μ	ENL	4+	1.25	4+	3	3+	0		_	0	0
13	34	Μ	ENL	3+	0	5+	2	2+	0	4+	0.9	3+	0
14	65	Μ	ENL	3+	0	3+	0	2+	0	2+	0	0	0
15	35	Μ	Dimorphous										
			reaction	4+	0	3+	0.3	3+	0.1	1+	0		

TABLE 1. Showing comparative BI and MI of skin, lymph node, bone marrow, nerve and muscle tissues.

and muscle tissues were generally lower than the skin, except in one case each of ENL and dimorphous leprosy in reaction, which presented higher BI's in the nerve. The MI's of lymph nodes were found to be similar to the MI's of the skin in five, higher in seven and lower in two patients. As compared to totally negative MI's of skin in patients suffering from ENL and dimorphous leprosy in reaction, the MI's of lymph nodes ranged from 0.3% to 6.5% respectively. In a majority of bone marrow aspirates the MI's could not be determined accurately because of the presence of bacilli in clumps, except in one patient in whom it was much lower than in the skin. The MI's of nerves were similar to the MI's of skin in five patients, higher in one and lower in the others. Nerve tissues from the patients in reactive phases also showed totally negative MI's as in the skin except in one case of ENL. None of the smears from muscle tissues showed viable solid staining bacilli (MI 0%) although M. leprae were demonstrated in six of them.

their ability to multiply in foot pads of mice. The Morphologic Index, i.e., the percentage of solidly staining bacilli, is considered to be a dependable index for viability of M. leprae in tissues (6). In the present study, the MI of lymph nodes of patients suffering from leprosy, has revealed certain interesting observations. It was either the same as the MI of the skin of the same patient or even higher. In the five patients suffering from reactional phases of leprosy, a significant number of viable bacilli were observed in the lymph nodes, whereas the MI of the skin was negative. This indicates that in certain situations examination of skin tissue alone may not reflect the actual status of viability of the organisms in the body. Since it has been well established that the MI of the skin becomes negative in three to six months following treatment with standard dosage of dapsone (1), it will be of further interest to know the MI of lymph nodes in such a situation, especially in view of the recent findings of a few solid staining organisms in casual bone marrow aspirates and liver biopsy specimens, long after the skin smears have become negative (4). The findings in other tissues, e.g., bone marrow, nerve and muscle, have not elicited better results than the skin. In bone marrow aspirates, evaluation of the MI was not possible in most of the cases because of the formation of clumps of bacilli. Nerve tissue has shown either com-

#### DISCUSSION

It is apparent from this study that *M. lep*rae thrive in tissues other than skin and nerves, especially in lymph nodes and bone marrow. Recently, Karat et al (<sup>4</sup>) reported that despite the higher temperature in human bone marrow and liver, leprosy bacilli in these organs remain viable as judged by parable or lower MI's than skin in most cases, whereas muscle has been found to be unsuitable for assessment of the MI because of consistent findings of nonviable bacilli (MI 0%) in all the specimens.

#### SUMMARY

This study elicits the comparative evaluation of the Bacteriologic Index (BI) and Morphologic Index (MI) of the skin, lymph node, bone marrow, nerve and muscle tissues of 15 untreated leprosy patients. The findings of comparable or even higher BI's and MI's in lymph nodes than skin are of considerable interest, especially the presence of a significant percentage of viable bacilli during reaction states when MI of skin and nerve tissue was found to be negative. Evaluation of the BI's and MI's of lymph nodes has been suggested to be of utmost importance for follow-up studies of patients being treated with antileprosy drugs.

#### RESUMEN

Este estudio se refiere a la evaluación comparativa del Indice Bacteriológico (IB) e Indice Morfológico (IM) de la piel, ganglios linfáticos, médula ósea, nervio y tejido muscular de 15 pacientes con lepra lepromatosa no tratados. El hallazgo de IB e IM comparables, o aún mayores, en los ganglios linfáticos que en la piel, es de considerable interés, especialmente la presencia de un porcentaje significativo de bacilos viables durante los estados reaccionales cuando el IM de la piel y el tejido nervioso se encontró negativo. Se ha sugerido que la evaluación de los IB e IM de los ganglios linfáticos puede ser de gran importancia para el seguimiento de pacientes que están bajo tratamiento con drogas antileprosas. phologique, aient présenté des valeurs comparables, ou même plus élevées, au niveau des ganglions lymphatiques, que dans la peau, est d'un intérêt considérable, spécialement si l'on considère qu'une proportion significative des bacilles présentait des caractéristiques de viabilité au cours des états réactionnels, alors que l'Index Morphologique des tissus cutanés et nerveux se révélait négatif. On suggère que l'évaluation de l'Index Bactériologique et de l'Index Morphologique des ganglions lymphatiques, est d'une importance considérable pour suivre le "devenir" des malades traités par des médicaments antilépreux.

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## RÉSUMÉ

Dans cette étude, on présente une évaluation comparative de l'Index Bactériologique (BI) et de l'Index Morphologique (MI) de la peau, des ganglions lymphatiques, de la moëlle osseuse, des tissus musculaires et des tissus nerveux, chez 15 malades de la lèpre non traités. Le fait que l'Index Bactériologique, de même que l'Index Morcability of experimental murine leprosy to the study of human leprosy. *In:* The Pathogenesis of Leprosy, G. E. W. Wolstenholme and M. O'Connor, eds., London: Churchill, 1963, pp 39-60 (Ciba Foundation Study Group No. 15).

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