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Demonstration of *Mycobacterium leprae* and its Viability in the Peripheral Blood of Leprosy Patients*

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Two methods for demonstrating *Mycobacterium leprae* in the blood of leprosy patients are described. On screening the blood of untreated lepromatous patients it was observed that all had bacteraemia. Bacteraemia was also observed in some patients with borderline and in some with treated lepromatous leprosy. Bacteraemia was not found in any one of the 15 patients with tuberculoid leprosy. High bacterial loads and the morphological integrity of the organisms in the skin were correlated with the degree of bacteraemia. The *Myco. leprae* in the blood were found to be viable. The evidence presented allows the conclusion that blood-sucking arthropods can take up viable *Myco. leprae* during a blood meal.

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Introduction

Mycobacterium leprae has been demonstrated not only in the Schwann cells of the nerves and histiocytes of the skin, but also in lymph nodes (Desikan and Job, 1966; Sharma and Srivatsava, 1958), spleen (Powell and Swan, 1955), liver and kidney (Mathur, Gupta and Singaravi, 1961), bone marrow (Gass and Rishi, 1934; Karat, 1966; Low and Dharmendra, 1937), muscle (Convit, Arvello and Mendoza, 1960), nasal mucosa and endothelial cells of capillaries (Fite, 1941). This implies that the bacilli must have disseminated through the blood stream into various organs and tissues. The final objective of our study is to throw light on the rôle of arthropods in the transmission of leprosy. In this connection an effort was made to determine the extent of bacteraemia in patients with untreated lepromatous leprosy and in those under drug treatment for varying periods.

Materials and Methods

The patients studied were selected at random from untreated and treated lepromatous, borderline, and tuberculoid cases. They were attending either

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leprosy clinics around Pondicherry or the out-patient department of the Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry. They were classified clinically according to the type of leprosy from which they suffered. Skin-slit smears were taken from various parts of the body. In collecting the blood samples the syringe was moistened with heparin solution (1000 units per ml) and 4 to 7 ml of blood then drawn from the cubital vein. None of the patients was in a reactional phase or in a febrile state at the time of the collection of blood.

Demonstration of Acid-Fast Bacilli

Two methods were used to demonstrate acid-fast bacilli in the macrophages of the blood.

1. THE BUFFY COAT METHOD

In this method heparinized blood is transferred into a test tube which is kept upright in a refrigerator for 5 h. The plasma is then pipetted off and the leucocytes from the buffy coat are transferred on to a clean glass slide with a capillary pipette. A leucocyte smear is prepared by the usual haematological technique, and air dried. The smear is fixed in formaldehyde vapour and stained by the Ziehl-Neelsen method.

2. THE LEUCOCYTE ADHERENCE METHOD

For this method approximately 4 ml of heparinized blood is kept in the refrigerator. The red blood cells settle down within an hour, leaving most of the leucocytes still in suspension. A drop of leucocyte-rich plasma is then transferred to a clean dry slide and the slide placed in a Petri dish with moist filter paper at the bottom to prevent drying out of the plasma drop. The leucocytes settle down and stick on the slide in about 30 min. The plasma is then gently washed off, using 0.5% phenol saline with the slide held at a slant. Finally the slide is air dried, fixed in formaldehyde vapour, and stained.

Samples of the plasma containing the leucocytes were inoculated in Löwenstein-Jensen medium and into the footpads of mice.

It was observed that in buffy coat smears the plasma with the remaining red cells formed, on drying, a wrinkled film which when stained interfered with the clear visualization of the acid-fast bacteria. Preparation of the smear, staining, and examination took a relatively long time (about 5 to 6 h). To overcome these shortcomings the leucocyte adherence method was employed. With this method the smears were uniform and clear, and the acid-fast bacilli when present could be demonstrated with ease. The total time taken for the examination of blood was also much less (2 to 3 h).

Results

Heparinized blood from 186 leprosy patients was examined. They comprised 151 cases of lepromatous, 15 cases of tuberculoid and 20 cases of borderline leprosy. The total incidence of bacillaemia was 41.4% (77 cases). All 38 untreated lepromatous patients had acid-fast bacilli (AFB) in the peripheral blood (Table 1).

TABLE 1

Incidence and degree of bacteraemia in patients with different types of leprosy

Type of case	Total no. of cases	Total no. of bacteraemic cases	No. of AFB in 500 fields of leucocyte smear 5–20	21–40	41-80	>81
Lepromatous						
(a) Untreated	38	38 (100%)	14	13	4	7
(b) Treated for 6 months	31	14 (45.16%)	6	5	3	
(c) Treated for one year	12	4 (33.3%)	3	1		
(d) Treated for more than one year	70	16 (22.86%)	9	4	2	1
Borderline	20	5 (25%)	5			
Tuberculoid	15	nil				
Total	186	77 (41.4%)	37	23	9	8

Of the 43 lepromatous patients treated for one year or less, 18 (41.86%) had bacteraemia. In 70 lepromatous patients who were treated for more than one year, only in 16 (22.86%) were bacteria seen in the blood. None of the 15 patients with tuberculoid leprosy had AFB in the peripheral blood, while only 5 (25%) of the 20 patients in the borderline group had visible evidence of bacteraemia.

The AFB were seen predominantly in macrophages (Fig. 1). In several instances, particularly among untreated lepromatous cases, the bacilli could be demonstrated also in the polymorphonuclear leucocytes (Figs 2 & 3). Extracellular AFB were present in a few instances. In most cases the bacilli were solidly stained and could be counted with ease, even when inside the macrophages. In

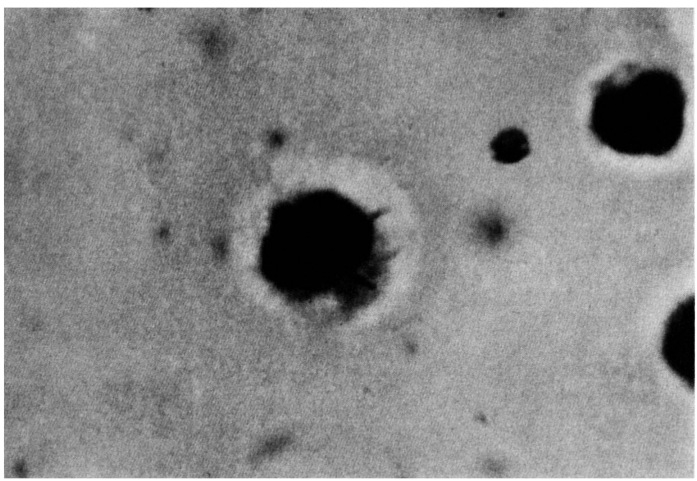

Fig. 1. Solidly stained *Myco. leprae* in a macrophage.

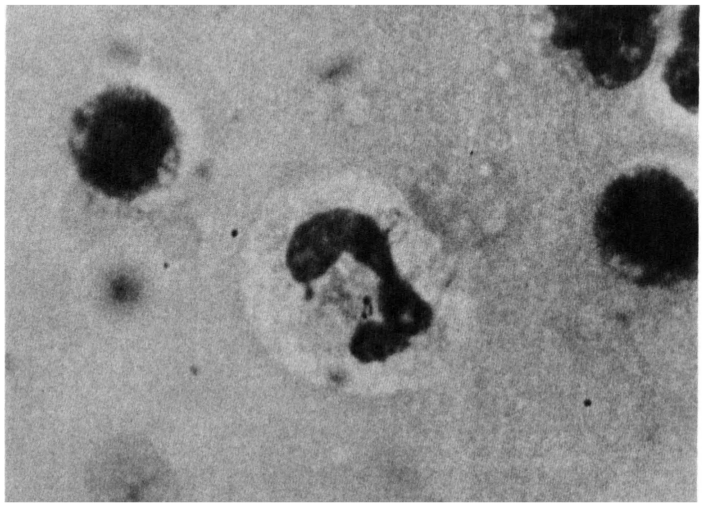

Fig. 2. Granular *Myco. leprae* in a polymorphonuclear leucocyte.

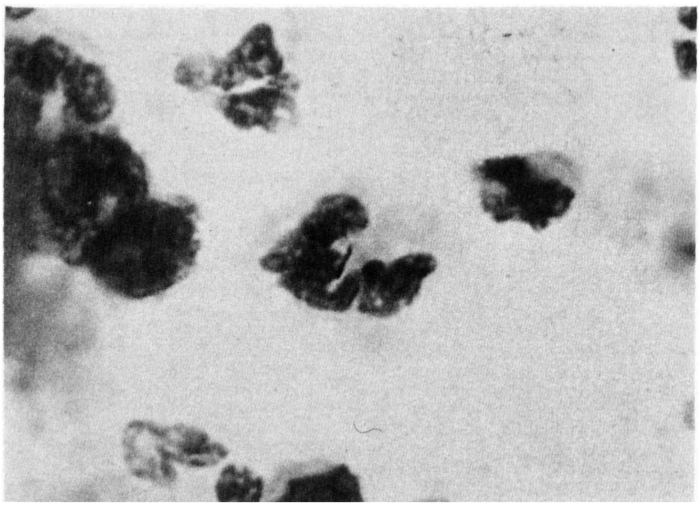

Fig. 3. Solidly stained *Myco. leprae* in a polymorphonuclear leucocyte.

some of the advanced lepromatous cases the macrophages were so packed with bacilli that it was difficult to count them (Fig. 4), particularly in those cases where the macrophages of the blood contained 30 or more bacilli per cell.

The number of bacilli varied from 5 to 120 per 500 fields of leucocyte smears, or expressed as bacteria per macrophage, it ranged from 2 bacilli per 100 cells to 2 bacilli per cell. From these data it was calculated that there were from 5×10^3 to 5×10^5 bacilli per ml of blood.

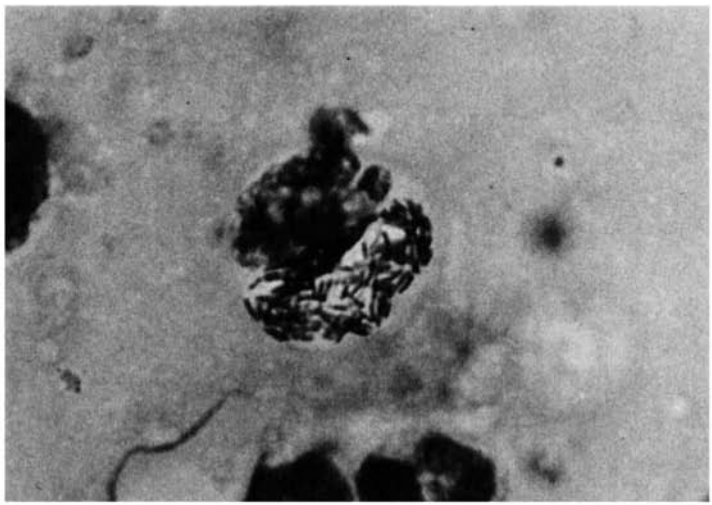

Fig. 4. Macrophage loaded with solidly stained *Myco. leprae.*

Skin slit smears from these patients were routinely examined and the bacterial index on the Ridley scale was found to be between 1 and 4. The morphological index varied from less than 2 to 40%. Aliquots of plasma containing leucocytes, cultured on Löwenstein-Jensen medium did not yield any growth of mycobacteria. Out of 77 samples of blood which were positive for AFB 15 were inoculated into the footpads of mice, and so far multiplication of AFB in the footpads has been obtained in 7 of them. The results for the remaining samples will become available later.

Discussion

Montel (1949) demonstrated leprosy bacilli in the blood of lepromatous patients, especially during febrile states. More recently Drutz and Levey (1970) have reported the continuous nature of bacillaemia in lepromatous leprosy. Our present investigation, besides confirming bacillaemia in lepromatous leprosy, also reports the occurrence of bacillaemia in borderline leprosy and its absence in tuberculoid leprosy. There is evidence of a continuous bacillaemia in all untreated lepromatous patients. The degree of bacillaemia is proportional to the bacterial and morphological indices of organisms in the skin. Moreover, bacilli from the blood of 7 patients were shown to be viable (infectious) in the mouse, but it is not known whether *Myco. leprae* can retain viability in blood containing high concentrations of DDS (dapsone).

According to Beiguelman (1967) macrophages of lepromatous patients do not digest *Myco. leprae,* whereas those of tuberculoid patients do. This may be one of the reasons for the absence of bacteraemia in tuberculoid patients. Although patients in the group with borderline leprosy had bacteraemia less frequently on the whole than those in the group with lepromatous disease, no histopathological classification of the borderline group was made.

There are two possible explanations for the persistence of the bacteraemia in

TABLE 2

Correlation between bacteraemia and bacterial and morphological indices of Myco. leprae *in leprosy*

Type of cases	No. of bacteraemic cases	Bacterial index				Morphological index (%)					No. of AFB in 500 fields			
		1	2	3	4	<2	2–10	11–20	21–30	>31	5–20	21–40	41–80	>81
Lepromatous														
(a) Untreated	38		2	19	17		4	20	9	5	14	13	4	7
(b) Treated six months	14		1	8	5			9	3	2	6	5	3	
(c) Treated for one year	4	1	1	1	1	1	2	1			3	1		
(d) Treated for more than one year	16		1	8	7	3	2	5	3	3	9	4	2	1
Borderline														
Untreated	3			2	1		1	1	1		3			
Treated	2		1	1		1		1			2			

lepromatous patients treated for more than one year. On the one hand the patients might not have taken their drugs regularly, or on the other hand the persistent bacteraemia might signify the emergence of drug-resistant bacilli.

The present investigation has helped in the selection of patients for feeding experiments with laboratory-reared insects. The constancy of bacteraemia in lepromatous patients together with the high prevalence of blood-sucking insects in this leprosy endemic area make it quite possible that arthropods could take up bacilli from patients during a blood meal.

Finally, bacteraemia should be taken into consideration by those in charge of blood banks where there may be the possibility of taking blood from persons with *undiagnosed* leprosy.

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References

Beiguelman, B. (1967). Leprosy and genetics. A review of past research, with remarks concerning future investigation. *Bull. Wld Hlth Org.* **37**, 461.

Convit, J., Arvello, J. J. and Mendoza, S. (1960). Lepromatous myositis. *Int. J. Lepr.* **28**, 417.

Desikan, K. V. and Job, C. K. (1966). Leprous lymphadenitis. Demonstration of tuberculoid lesions. *Int. J. Lepr.* **34**, 147.

Drutz, D. J. and Levey, L. (1970). The viability of blood borne *Mycobacterium leprae.* Abstract in *Int. J. Lepr.* **39**, 98.

Fite, G. C. (1941). Vascular lesions in leprosy. *Int. J. Lepr.* **9**. 193.

Gass, H. H. and Rishi, D. P. (1934). Examination of bone marrow for *Myco. leprae. Lepr. India* **6**, 8.

Karat, A. B. A. (1966). Acid-fast bacteria in bone marrow of leprosy patients. *Int. J. Lepr.* **34**, 415

Low, J. and Dharmendra (1937). Sternum puncture in leprosy: a study of 50 cases. *Lepr. India* **9**, 121.

Mathur, M. P., Gupta, B. K. and Singaravi, R. M. (1961). A preliminary study of hepatic involvement in leprosy. *Int. J. Derm.* **4**, 111.

Montel, (1949). Bacillaemie lépreuse. Affinités tinctoriles du "bacille de Hansen". *Bull. Acad. Méd.* **130**, 165. Abstract in *Int. J. Lepr.* **17**, 361.

Powell, C. S. and Swan, L. L. (1955). Leprosy: pathological changes observed in 50 consecutive necropsies. *Am. J. Path.* **31**, 1131.

Sharma, K. D. and Srivatsava, J. B. (1958). Lymph nodes in leprosy. *Int. J. Lepr.* **26**, 41.