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Arthropod Feeding Experiments in Lepromatous Leprosy*

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Laboratory-reared *Culex fatigans* and *Cimex hemipterus* were fed on untreated lepromatous leprosy patients. The presence of acid-fast bacteria in a high proportion of these insects after feeding showed that they can take up the bacilli from the patients' blood. The dependence of infestation of fed insects on the degree of bacteraemia in patients and the detection in *Cimex* of bacteria-laden leucocytes suggest that the insects took up the bacilli along with the blood rather than from the skin. Results of mouse footpad harvests showed multiplication of *Myco. leprae*, and therefore one must conclude that the leprosy bacilli in the insects were viable.

Introduction

The occurrence of mycobacteria in human parasitic arthropods in the field and the possibility of at least some of these being *Myco. leprae* has been shown in our laboratory (Narayanan *et al.*, 1972). The epidemiological significance of this observation is at the present time not clear. To be an effective vector an arthropod must be able to pick up the required number of bacilli from a patient and should contribute to the inoculation of the bacilli into another susceptible person. In addition, the bacilli must be able to survive inside the arthropod for a sufficiently long time, this depending on the feeding habits of the arthropod. This paper deals with feeding experiments conducted with mosquitoes and bed-bugs to investigate the ability of these arthropods to take up the bacilli from untreated lepromatous leprosy patients.

Materials and Methods

For the feeding experiments volunteer untreated lepromatous leprosy patients were selected from both roadside clinics and out-patients of the Department of Skin and Venereal Diseases of the Jawaharlal Institute at Pondicherry and admitted to the infectious diseases ward of the hospital. The patients' blood was investigated before commencing the experiment for the presence of acid-fast

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bacilli. The methods used for this study have been reported separately (Shankara Manja *et al.*, 1972).

Colonies of *C. fatigans* and *C. hemipterus* were established in the laboratory for feeding experiments. Field collected females of *C. fatigans* were allowed to oviposit in the laboratory to start the colonies; the larvae were reared in pans, using yeast powder as food. Laboratory-reared adult females were fed on mice for further propagation. The method adopted by Wattal and Kalra (1961), using wooden traps, was followed for the maintenance of *C. hemipterus* colonies.

Laboratory-reared female mosquitoes about 5 days old were used for the experiments. Female mosquitoes randomly drawn from laboratory colonies were examined microscopically and culturally to ascertain that they were free of acid-fast bacilli. For the feeding experiments with bed-bugs, nymph-stage *Cimex* or fresh adults from laboratory colonies starved for 14 days or more were used. Nymphs and adults drawn at random from laboratory breeding cages were examined microscopically and culturally for contaminating acid-fast bacilli.

The feeding experiments with mosquitoes were done at night in insect-proof rooms of the infectious diseases unit. The volunteers were given 65 mg of Gardinal (phenobarbitone) to ensure undisturbed sleep. A mosquito net was lowered over the bed and secured at the lower sides after the subjects were asleep; known numbers of mosquitoes were then released into the net. Two workers kept watch over the patient throughout the night to render him any assistance if he wanted to come out of the net and to ensure that the mosquitoes did not escape during the process. All mosquitoes fed, unfed, or dead were recovered and accounted for in the morning, and those which had fed were separated for further examination.

Feeding experiments with bed-bugs were conducted during the day. For this purpose a screw cap of a McCartney bottle with perforations on top was used. Four pieces of black paper with slits cut along the sides were snugly fitted inside the cap with spaces between the layers. Known numbers of bugs were released into the cap, using a fine artist's brush. The bugs readily entered the cap through the slits in the black paper and rested inside. The mouth of the cap was covered with fine voile cloth, fastened with rubber bands, and the cap then placed on the body of the volunteer, with the voile side in contact with the skin and secured with bandage cloth. After about 2 h the bugs were taken out for examination.

Fed mosquitoes and bed-bugs were killed by chilling. The wings and legs of the mosquitoes were removed before making a homogenous suspension. Then the mosquitoes and bugs were ground separately in a cold mortar, using 1 ml of 0.1% bovine albumin (fraction V) in Hanks balanced salt solution. This suspension was examined microscopically for acid-fast bacilli (AFB). As the numbers of bacilli in the suspension were too low to be counted, 0.03 ml of the suspension was directly inoculated into the footpads of Swiss albino mice (Rockefeller strain).

Results

The results of the feeding experiments with *Culex* showed that, out of 38 successful feeding experiments, mosquitoes in 27 experiments had taken up AFB, as seen by microscopy (Table 1, Fig. 1). Of the 35 experiments conducted with *Cimex* 18 were microscopically positive for AFB (Table 2, Fig. 2, 3).

The blood samples from volunteers 1 to 5 were not investigated for bacteraemia, but it is reasonable to assume that they were positive because all

TABLE 1

Results of feeding experiments with mosquitoes

Patient No.	Degree of bacteraemia (AFB per 500 fields)	No. of feeding experiments	Nature of feeding	AFB in mosquito homogenate Positive	Negative
1	Not recorded	1	Nil		1
2	Not recorded	1	Nil		1
3	Not recorded	4	1 Nil 3 Fair	3	1
4	Not recorded	1	Poor		1
5	Not recorded	2	Poor	1	1
6	73	3	Poor	3	
7	0	1	Poor		1
8	23	3	Fair	2	1
9	18	1	Fair		1
10	28	3	Good	3	
11	21	3	Fair		3
12	24	3	Fair	2	1
13	16	1	Nil		1
14	48	3	Fair	2	1
16	26	3	Fair	2	1
17	100	3	Fair	3	
19	83	3	Fair	2 + (1)	
20	120	3	Fair	3	

Figures in brackets indicate impression smears.

TABLE 2

Results of feeding experiments with bed-bugs

Patients No.	Degree of bacteraemia (AFB per 500 fields)	No. of feeding experiments	Nature of feeding	AFB in bed-bug homogenate Positive	Negative
10	28	2	Good	0	2
11	21	1	Good	0	1
12	24	2	Good	1	1
12	Not significant[a]	2	Good	0	2
13	16	1	Good	0	1
14	48	4	Good	3	1
15	18	2	Good	0	2
16	26	3	Good	2	1
17	100	3	Good	3	0
18	35	4	Good	1	3
19	83	3	Good	1 + (2)	0
20	120	3	Good	2 + (1)	0
21	63	2	Good	1	1
22	11	3	2 Good 1 Poor	1	2

[a] After about 4 months of treatment with DDS.

Figures in brackets indicate impression smears.

untreated lepromatous leprosy patients have been shown to be bacteraemic (Shankara Manja *et al.*, *loc. cit*). The results of the feeding experiments showed that the microscopical detection of acid-fast bacteria in the insects depends on the degree of bacteraemia of the patient.

In one of the 38 successful feeding experiments with *Culex* mosquitoes, a homogenate of mosquitoes was prepared 48 h after feeding and 0.03 ml of the suspension was inoculated into mouse footpads. The footpad harvest 6 months after inoculation yielded less than 3×10^4 AFB per mouse; after about 9 months the total yield went up to $5 \pm 0.5 \times 10^5$ AFB per mouse. Finally, in about 14 months, it reached a maximum of $1.05 \pm 0.1 \times 10^6$ AFB per mouse. This shows that there was a steady increase in the number of bacilli in the footpads of mice. On re-inoculation almost the same results were obtained in the new group of mice showing a 100 to 300-fold increase of bacilli in between 6 and 9 months. In another experiment with a different volunteer, *Culex* mosquitoes yielded $1.2 \pm 0.2 \times 10^5$ AFB per mouse in about 14 months after inoculation. In this experiment the suspension was prepared immediately after feeding. These bacteria have now been re-passaged into a new batch of mice and the results are awaited. None of the AFB obtained in these experiments grew on Löwenstein-Jensen (L-J) medium. A typical growth pattern in the footpads of mice, together with the inability to grow on L-J medium, are highly suggestive of *Myco. leprae*.

Examples of acid-fast bacilli in homogenates of mosquitoes and bed-bugs are shown in Figs 1 and 2 and the presence of bacilli in a leucocyte in a smear prepared from a bed-bug is shown in Fig. 3.

Discussion

It is a well-known fact that the incidence of viable *Myco. leprae* in patches on the skin of untreated lepromatous leprosy patients is much higher than in the skin

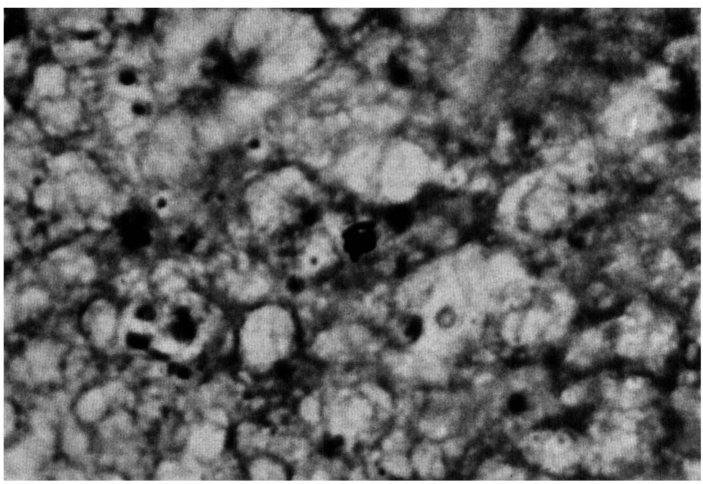

Fig. 1. A clump of solidly stained *Myco. leprae* in ground suspension of mosquitoes fed on a leprosy patient.

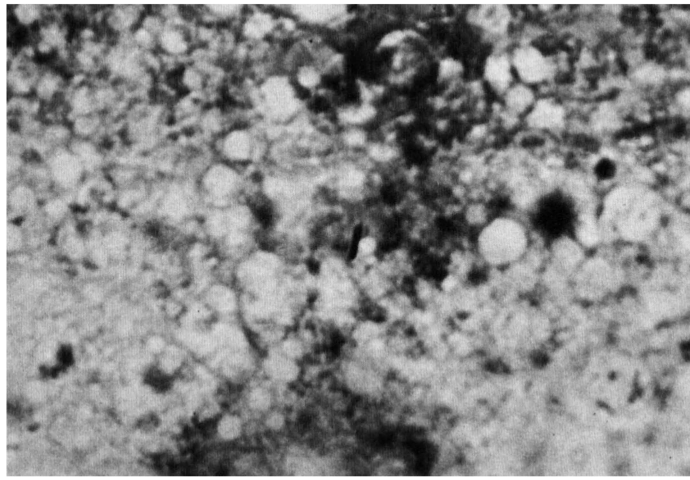

Fig. 2. A single solidly stained *Myco. leprae* in a ground suspension of bed-bugs fed on a leprosy patient.

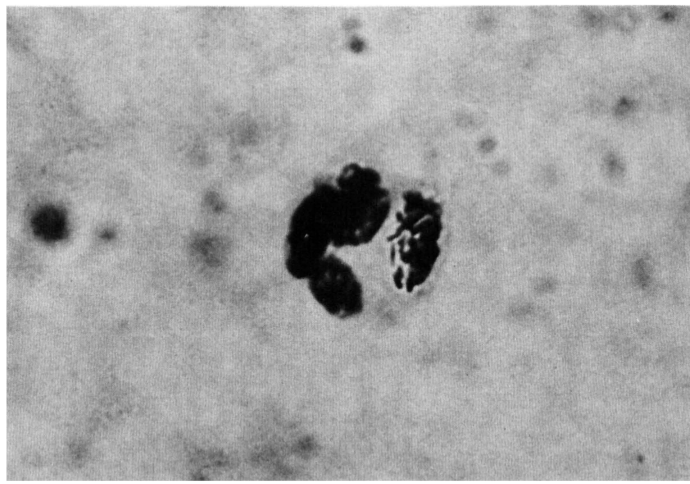

Fig. 3. A partially digested leucocyte loaded with solidly stained *Myco. leprae* in a smear made from a bed-bug after feeding on a leprosy patient.

elsewhere. It has been shown in our laboratory that the degree of bacteraemia increases in relation to the bacterial and morphological indices of the bacteria in the skin-slit smear. It also has been shown in our laboratory that blood-borne *Myco. leprae* are viable. An arthropod feeding on a leprosy patient can ingest *Myco. leprae* either while its mouth parts are penetrating the skin containing the bacteria or along with the blood it draws. While the method of mosquito-feeding experiments used in these studies permits the mosquitoes to suck blood from any

convenient point in the body, the bed-bugs were mostly confined to patches on the body for their feeding. There was, however, no indication that the bugs had ingested more bacilli than the mosquitoes, and this may be because the bacilli are taken up along with the blood by both these insects and not from the skin at the time of penetration. The finding of intact leucocytes containing bacilli in bugs examined after feeding supports this view. Suspensions prepared from fed *Culex* mosquitoes immediately after feeding and again after 48 h were inoculated into mouse footpads and yielded $1.2 \pm 0.2 \times 10^5$ and $1.05 \pm 0.1 \times 10^6$ bacteria respectively in 14 months, showing that the insects had indeed taken up viable organisms.

Twenty pools of laboratory-reared *Culex* mosquitoes and 10 pools of laboratory-reared bed-bugs were quite free of AFB. This makes it very likely that the AFB found in the insects which had fed on patients were taken up during the feeding process. McFadzean and Macdonald (1961) allowed mosquitoes and bed-bugs to feed on lepromatous leprosy patients with highly positive skin smears and then re-fed the insects on patients with tuberculoid leprosy. On the basis that no microlepromin reaction was produced in the tuberculoid patients, they doubted the ability of these insects to pick up the bacilli from the skin. The results of the present study however show that *C. fatigans* and *C. hemipterus* can take up the bacilli along with the blood meal.

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