Attempts to Find Disease Germs in the European Bed Bug (*Cimex Lectularius*) after Feeding Experiments in Various Diseases: Leprosy, Lymphadenoma, Carcinoma, Etc.

David Thomson

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ATTEMPTS TO FIND DISEASE GERMS IN THE EUROPEAN BED BUG (*CIMEX LECTULARIUS*) AFTER FEEDING EXPERIMENTS IN VARIOUS DISEASES: LEPROSY, LYMPHADENOMA, CARCINOMA, ETC.

BY

DAVID THOMSON, M.B., CH.B. (EDIN.), D.P.H. (CANTAB.)
GROCERS' SCHOLAR

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I. INTRODUCTION

In recent years, the important part played by blood-sucking insects in the transmission of disease has been brought strongly into evidence. Mosquitos carry the pathogenic agents of malaria, yellow fever and filariasis. Tsetse-flies transmit various forms of sleeping sickness. Bugs and fleas are able to transmit plague germs, Manning (1912), whilst the former may be responsible for the transmission of kala-azar, Patton (1907, 1912), and possibly of leprosy, Long (1911). By feeding experiments Patton (1911) and Wenyon (1912) were able to get some development of *Leishmania* and allied flagellates in bed bugs, fleas and mosquitos. Dewèvre

(1892) found tubercle bacilli in bed bugs living in the bed of a tuberculous host. These diseases, the latter excepted, are confined chiefly to the tropics, but it is not known whether the blood-sucking insects which occur in temperate climates may not transmit some of the diseases which occur in the temperate zone. Bugs, fleas and lice are all very common in the civilised countries of temperate climes, in spite of the great advances made in sanitation and personal cleanliness. It appears, therefore, that those engaged in research on non-tropical diseases, many of which are still shrouded in mystery, have neglected this department of research, which is being so thoroughly investigated by those engaged in the study of diseases peculiar to the tropics. No one appears to have attempted to investigate such diseases as lymphadenoma and the various leukaemias, from this point of view. These are diseases in which the blood is affected, and, moreover, they resemble certain of the tropical diseases mentioned, in the enlargement of the spleen and lymphatic glands. Recently I was much impressed by the remarkable resemblance between a case of advanced lymphadenoma and kala-azar. This case was a young child five years of age. She had enlarged glands and a very large spleen, extending below the umbilicus. There was extreme emaciation, anaemia and lassitude, and a few days before death she developed an ulcer on her cheek, which rapidly perforated, forming a kind of cancrum oris, all of which features are also characteristic of kala-azar. This induced me to commence feeding experiments with bed bugs on this case of lymphadenoma and on other obscure blood diseases, which constantly come into hospital in this country. Crithidia have been found in fleas, Porter (1911), and it has been recently shown by Fantham (1912) that 1 per cent. or more of body lice in England contain *Herpetomonad* flagellates, the cysts of which are found in their faeces. Fantham did not succeed in infecting either himself or animals with these parasites, but their presence in these insects is suggestive. No one appears to have observed protozoal flagellates in the bed bug, but they harbour certain organisms which I shall describe below. It appears to me that a considerable research should be conducted with blood-sucking insects regarding the obscure diseases occurring in temperate climates. Although it has not been ascertained whether or not these obscure diseases are

due to organisms, yet so long as they are obscure, we must consider the possibility of organismal causes. Yellow fever was found to be transmitted by *Stegomyia fasciata*, although no organism could be discovered in the blood of the patients, so that the inability to demonstrate organisms in an obscure disease should not debar an investigation with blood-sucking insects. It is always possible that a phase in the development of a pathogenic organism might appear in some of these insects fed on patients suffering from disease, a phase more easily seen through the microscope than in the blood of the patient. In malaria some of the parasitic phases in the mosquito are much more gross, so to speak, than the phases in the blood of the human host. Finally, the blood sucked by the insect into its gut becomes digested and changed, and may be considered as a kind of culture medium in which an obscure organism may develop. J. G. Thomson and Sinton (1911) have shown that the growth and morphological changes in human trypanosomes in culture media, are exactly similar to those occurring in the gut of the tsetse-fly which has sucked blood infected with these organisms. I have ventured to make these remarks as a plea for further research with insects regarding the obscure diseases which occur in temperate climates, and which have so far baffled all other methods of scientific investigation.

II. ORGANISMS NORMALLY FOUND IN THE BED BUG

The number of control bed bugs examined in this research was 184. Of these, 107 were examined for acid fast bacilli, by staining with the Ziehl-Neelsen stain. The remaining 77 were stained by the Giemsa method. Eight of these latter were fed on a normal person. The remaining 69 were examined as soon as they had been obtained from their natural haunts. I speak of these as controls. The majority of them were adult bugs and had undoubtedly fed on human beings, but whether any of these human beings had been suffering from disease it is impossible to say. The only method of obtaining perfect controls would be to hatch out the bugs from the eggs and rear them to the adult stage, feeding them at times on a normal person. I have not done this. However, I will now describe the various organisms which I have found in these so-called controls.

(a) *Bacterial organisms*: Bacilli were found in about 25 per cent. of the bugs. Usually these bacilli were scanty, but occasionally I have found them in masses, in the intestinal contents, and in the lining cells of the gut. They were, as a rule, long, slender bacilli. Stout bacilli of the coli type were practically never found, and I have seldom been able to detect cocci. I was unable to detect any acid-fast bacilli in the 107 controls stained by the Ziehl-Neelsen method.

(b) *Yeasts and Moulds*. These were more commonly found in the gut than bacteria. Sometimes a mycelium was found sprouting from the spores. On one occasion I transferred the intestinal contents of a bed bug as aseptically as possible into a blood agar tube. The only growth obtained from this was an abundant white mould with numerous spores and mycelium. Bacteria were seldom found in the faeces of these bugs, but on some occasions I have found fungal spores, and if the faeces are kept for some time in a moist atmosphere, a copious mouldy growth, as a rule, sprouts from them.

(c) *Protozoal organisms*. I have been unable to find any definite protozoa in the total 455 bugs examined, and never any flagellates such as have been found in fleas and lice.

(d) *Other bodies, possibly organismal*:

(1) Indefinite bacilliform bodies occurred in about 10 per cent. of the bugs. These bodies occurred in masses in the protoplasm of the gut cells and were also found in the intestinal contents. They appeared to me to be a species of organism attacking the cells of the intestine.

(2) 'Chlamydozoon-like bodies': These occurred in about 25 per cent. of the controls. They were found chiefly in the cells of the oesophagus, less commonly in the cells of the mid and hind gut, and only occasionally did they occur free in the intestinal contents.

I have called them 'Chlamydozoon-like bodies' because they resemble the bodies to which Prowazek has applied the name of Chlamydozoa, and which are believed to be organismal. Similar bodies have been described by Leishman (1912). I am in considerable doubt as to whether these similar bodies found in oesophageal cells of the bug are organismal or whether they are of

the nature of cell secretions. These bodies vary in appearance as follows:—

(1) A minute red staining point (after using Giemsa), almost invisible, within a clear circular area or vacuole.

(2) A minute red staining point with a clear halo around it. This clear halo, in turn, is bounded by a red staining ring.

(3) A red granule without any halo around it.

(4) A small spherical red body, paler in the centre than at the periphery, and without any surrounding halo.

These bodies, as a rule, stain red in colour, but where the tissue is thick, so that the stain is too dark, they show up as dark-blue bodies, varying in size from a minute granule up to 2μ and even 3μ in diameter. They frequently occur in pairs partially fused together, as if they were dividing into two.

III. THE FEEDING OF BED BUGS ON PATIENTS

This is very easily done. I have found the following procedure very suitable. Procure some glass tabloid phials with their metal caps.* The metal caps which I used were about $\frac{3}{4}$ inch in diameter and about $\frac{1}{2}$ inch deep. Transfer about six bugs into one of these metal capsules. The bugs are easily transferred from one vessel to another, by using a wire with a loop at one end. With this turn the bug on its back and then place the loop among its legs. It will cling to the loop and can then be shaken off into the metal cap. Enclose the bugs in this metal capsule by stretching a single layer of surgical gauze, such as is used for dressings, over the mouth of the capsule. This is secured in position by a piece of plaster wrapped round the side of the capsule. Shake the bugs from the bottom of the capsule on to the gauze and place it face downwards on the patient's forearm. Secure it in that position against the patient's skin with a piece of plaster. If this is left undisturbed for 5 to 10 minutes, it is almost certain that the bugs will be found well engorged with blood. They bite very readily through the gauze, provided it is stretched tightly across the mouth of the capsule. In this fashion I have frequently fed 12 bugs in 5 minutes.

* I used the phials which are frequently sent out by the Wulffing Co. with samples of formamint tabloids.

The patient does not feel much, and, in the majority of cases, the bugs scarcely leave any visible mark. On only one of my patients they produced slight blisters. On several occasions I was able to feed these insects without waking the patient, especially in the case of young children. After feeding the bugs, they should be transferred to the glass phial to which the capsule belongs. A piece of cardboard should be placed in the phial to act as a foothold for them, and they deposit their excreta and eggs on this cardboard. I fed them only once, and killed and examined them at intervals varying from 4 to 19 days after feeding. A large number should be fed on several cases of a given disease, before coming to any conclusion. It has been found, for example, that only about 5 per cent. of Anophelines fed on a malarial patient become infective, and there are many cases of malaria which would never infect anophelines, as they do not always contain gametes. Similarly, only about one in twenty of tsetse-flies fed on cases of sleeping sickness become infective. Again, conclusions cannot be drawn from the microscopical examination of the bugs alone, for although no abnormal organism can be found in them after feeding on a given diseased patient, yet they may be carrying some invisible virus, as in the case of yellow fever. To complete the experiment, therefore, insects which have fed on a given disease should be allowed to bite other men or animals at varying periods after their feed.

The feeding experiments which I am about to describe have been very incomplete, as I have not been able to employ sufficient numbers and also because I have not allowed the bugs to bite other men or animals. Hence, although my results have been so far negative, there is no room for dogmatic statements that they do not carry any of the diseases on which I have experimented.

IV. RESULTS OF THE FEEDING EXPERIMENTS ON DISEASED PATIENTS

(a) *Nodular Leprosy*. Twenty-one bed bugs, *Cimex lectularius*, caught in Liverpool, were fed directly on the nodules of a patient in the Royal Southern Hospital. They were killed and examined for acid-fast bacilli at periods varying from one to ten days after feeding. Two bugs were examined two months after feeding.

Result.—No acid-fast bacilli could be found in the salivary glands, intestines or faeces. Twenty-one controls were examined in a similar fashion and no acid-fast bacilli were found.

I made further investigations in Panama and also in Trinidad. Eighty-six bed bugs (*Cimex rotundatus*) were caught in Ancon Hospital, Panama, and examined as controls, but no acid-fast bacilli could be detected in them (Ziehl-Neelsen stain used). Eighty-four bed bugs (*Cimex rotundatus*) caught in Ancon Hospital were fed on seven lepers at Paloseco leper asylum, Panama. In six of the lepers chosen the disease was advancing rapidly, and one exhibited a recent crop of small nodules. The bugs were fed directly on the nodules, but not on open sores. The seventh case exhibited a scaly condition of the skin. The bugs were allowed to feed on the patients for a period of half an hour and were engorged with blood after the application. These bugs were killed and examined for acid-fast bacilli at periods varying from one to fifteen days after the feeding. No acid-fast bacilli could be detected in their alimentary tract or faeces, nor in their salivary glands.

Seventeen bed bugs caught in Paloseco leper asylum were similarly examined and no acid-fast bacilli were detected. These latter bed bugs were caught on the mattresses of the beds of lepers. They contained blood and had presumably fed frequently on leper patients.

Eighteen bed bugs (species not determined) were caught on the mattresses of the beds of lepers in the Trinidad leper asylum. The examination of these insects for acid-fast bacilli was also negative.

In a total of 107 control bugs no acid-fast bacilli were found. This agrees with the investigations of Long (1911) and Sandes (1911).

In a total of 105 bed bugs fed on lepers and 35 caught on the mattresses of leper patients, I was unable to detect acid-fast bacilli. In this respect I have been unable to confirm the results of Long and Sandes.* It seems to me, therefore, that bed bugs are not so

* Sandes (1911) and Long (1911) found acid-fast bacilli in twenty-six per cent. of the bed bugs which they fed on leper patients.

Skelton and Parham (1913) were unable to find acid-fast bacilli in 175 bed bugs caught in the beds of lepers in Zanzibar.

Vide also the experiments of Paldrock; Smith, Lynch and Rivas (1913).

liable to ingest lepra bacilli from patients as has been maintained. However, it is conceivable that they might ingest these bacilli by feeding on very acute cases of leprosy, as in such cases the bacilli have been found occasionally in the circulating blood.

(b) *Lymphadenoma (Hodgkin's Disease)*. Five bed bugs were fed on a very advanced case of this disease three days before the death of the patient. All of these developed in 8 days a greyish, mouldy growth over their bodies, and microscopic examination showed that they were infected with a mycelium and spores. Twenty-two bugs were fed on an early case of this disease. These were examined at varying periods up to 13 days after feeding. They did not show anything different from what was found in the controls, except that 63 per cent. of them contained 'Chlamydozoon-like bodies.' The peculiar condition of the first five bugs may have been a coincidence, as occasionally mould-like spores and even mycelial threads have been found in the controls, but always very scantily.

(c) *Spleno-Medullary Leukaemia*. Forty bed bugs were fed on one typical case of this disease and examined at varying intervals of from 4 to 19 days afterwards. All of them showed one persistent abnormality from the control bugs. This was the constant presence of Charcot-Leyden crystals in their intestinal contents and even in their faeces. These crystals were practically always abundant. They were colourless, pointed at both ends and varied in size from about 7μ to 40μ in length. They occurred singly and in small groups, and were, apparently, very brittle and easily fractured. They stained a faint greenish colour with the Giemsa stain.

Fifty per cent. of these bugs contained numerous 'Chlamydozoon-like bodies,' principally in the cells of the oesophagus.

(d) *Chronic Lymphatic Leukaemia*. A very typical case was used, which presented a leucocyte count of 800,000 per c.mm., of which 97 per cent. were small or medium sized lymphocytes. None of the 22 bed bugs fed on this case showed any apparent abnormality, except that the majority showed very large numbers of pale blue bodies in the intestinal contents. These were undoubtedly the remains of the ingested lymphocytes. Sixty per cent. of them showed numerous 'Chlamydozoon-like bodies' in the

cells of the oesophagus. Not one of them showed Charcot-Leyden crystals. These bugs were killed at intervals of from 4 to 13 days after feeding.

(e) *Sarcoma*. Twenty-eight bugs were fed on a patient suffering from sarcoma of the ileum. These were examined at intervals varying from 5 to 16 days after feeding, but they revealed nothing abnormal. Forty-three per cent. showed 'Chlamydozoon-like bodies' in the oesophageal cells.

(f) *Carcinoma*. Six bugs were fed on an advanced case of cancer of the bowel, but nothing abnormal was found in them.

(g) *Malaria*. Eight bugs were fed on a case of malignant tertian malaria, with crescents. They revealed no development of the malarial parasite within them.

I am much indebted to Dr. Macalister of the Royal Southern Hospital, Liverpool, for his kindness in allowing me to perform these insect feeding experiments on several of his patients.

V. SUMMARY AND CONCLUSIONS

(1) Four hundred and fifty-five bed bugs were employed in this research, of these, one hundred and eighty-four were used as controls.

(2) Insufficient research has been carried on with regard to the possibility of the transmission of disease in temperate climates, by blood-sucking insects occurring in these climates.

(3) Protozoal parasites do not appear to exist normally in the European bed bug.

(4) No acid-fast bacilli were found in 105 bed bugs fed on lepers, nor in 35 bed bugs caught on the bed mattresses of leper patients.

(5) In the few experiments conducted, nothing abnormal was found in bugs fed on cases of lymphadenoma, chronic lymphatic leukaemia, sarcoma, carcinoma and malaria.

(6) Forty bed bugs fed on a case of spleno-medullary leukaemia all developed numerous Charcot-Leyden crystals in their intestines.

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