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No. 27.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

PRELIMINARY REPORT ON THE DEVELOPMENT OF THE LEISHMAN-DONOVAN BODY IN THE BED BUG.

BY

CAPTAIN W. S. PATTON, M.B., I.M.S.

(On special duty.)

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA.

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Price eight annas or 9d.

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PRELIMINARY REPORT OF THE DEVELOPMENT OF THE LEISHMAN-DONOVAN BODY IN THE BED BUG.

AN important contribution to our knowledge of the Leishman-Donovan body was made by Major Rogers,[1] I.M.S., when he announced that he had succeeded in observing the development of the parasites into flagellates, in splenic blood taken from a case of Kala Azar, after he had added a small quantity of sterile citrate of sodium solution to the blood and incubated it at a low temperature.

In a later communication,[2] he describes the development at 22° C. At the end of the first day the parasites had increased considerably in size, the protoplasm staining a bluish colour and appearing finely granular; the macro-nuclei were much enlarged, while the micro-nuclei remained unaltered. By the end of the second day the parasites had further increased in size, particularly the macro-nuclei; and he observed double forms of the large oval parasites in varying degrees of contact up to nearly complete fusion of the two cells, which he concluded represented a process of conjugation.

On the third day he observed the conjugating pair of organisms as elongating, and noted the first appearance of flagellated forms. In these pyriform bodies the macro-nuclei were seen in the thick ends of the parasites, and the micro-nuclei had passed to the thinner ends from which the flagella arise. On the fourth day numerous double pyriform organisms were seen, only a small percentage having developed flagella.

Soon afterwards, Captain Christophers,[3] I.M.S., working in Madras, confirmed Major Rogers' observations. He saw most of the forms described above and has added considerably to our knowledge of this stage of the parasite. On the fourth day of his cultural experiments, he observed the large forms resembling those described by Rogers. They occurred in groups and had apparently arisen by a process of fission showing no evidence of conjugation. On the fifth day there appeared in the enlarged parasites a new structure which was seen as a pink stained vacuole-like area, of the size of the macro-nucleus and lying in close apposition to the micro-nucleus. Further, a peculiar red staining substance was noticeable as a tail-like process projecting from the end of the parasite in which the small chromatin mass is situated; this substance, increasing in volume, surrounded this end of the parasite and fusing with that of adjoining parasites bound the group together. A ragged filament, of nearly the same colour as the pink stained area, was seen protruding from the flagellar end of the parasite; this probably represents the first appearance of

the flagellum. On the sixth day each group of elongated parasites contained one or more flagellate forms in which the flagella could, as a rule, be traced to the micro-nucleus which thus appears to be a blepharoplast. On the seventh and eighth days fission produced both large and elongate forms, and on the ninth day and later fission of the elongate forms proceeded rapidly, resulting in smaller as well as irregular bodies which Christophers terms secondary fission forms. Throughout the earlier stages many of the small oval parasites appeared to be undergoing development whilst included in the altered substance of a cell.

Some of the observations of Christophers deserve special attention. He is the first to describe the vacuole-like area which develops in close relation to the micro-nucleus and is associated with the later development of the flagellum. Another important observation made by him is that the parasites undergo development whilst included in cells.

Lieutenant-Colonel Leishman,[4] R.A.M.C., and Captain Statham, R.A.M.C., who next studied the extracorporeal stage of the parasite, have confirmed in the main the observations of Rogers and Christophers. Describing the earlier changes, Leishman notes that these may take place up to, and even beyond, the formation of the large circular or oval parasites, while embedded in the splenic macrophages. He also describes a process of unequal longitudinal fission in some of the mature flagellated forms, in which the nuclei of the parent do not play any part. They were split off from one side of a mature flagellate and appeared as thin spirillum-like organisms and, in some cases, more than one such form was seen split off from the same parent. These attenuated forms included two or more chromatin dots and the protoplasm contained characteristic vacuoles. At first they were destitute of flagella, which, however, developed later, when these spirilla-like flagellated forms were much more active than the larger parasites.

Rogers[5] has recently improved his method of cultivating the parasites by adding a small quantity of citric acid to the citrated splenic blood, and in his latest communication[6] describes the developmental changes in splenic blood under these conditions. The development appears to be much more uniform than that obtained by his earlier methods, so that the majority of the parasites can be found in nearly the same stage on a given day; and most of the parasites on the third day had developed flagella. In the acid medium the abundant development of flagellates results in the formation of rosettes, the individual organisms then elongate and the rosette begins to break up, owing to the increasing motility of the flagella, and free swimming forms are produced.

Rogers discusses the nature of these parasites, and after examining many of the fully developed free forms, finds that there is no trace of an

undulating membrane and that the micro-nucleus does not pass from the anterior end towards the macro-nucleus. From this he concludes that the parasite belongs to the order *Herpetomonas* and is not a true trypanosome, naming it the *Herpetomonas* of Kala Azar. This conclusion seems to be premature, especially when it is considered how little is yet known of the life-histories of the different species of this genus (*Herpetomonas*) of the Flagellata.

Before describing the experiments carried out with the various blood-sucking insects, it is important to consider how the human body might be primarily infected by the parasite, and, in order to answer this question, it is necessary to examine the theories which have been advanced to explain the channels by which the parasite leaves the human body.

Sir Patrick Manson[7] and Dr. Low, studying a case of Kala Azar in the Seamen's Hospital, London, were unable, after a prolonged search in films of the finger blood, to find any of the parasites, and concluded that the disease could not be transmitted by means of the peripheral blood. As they found the parasite in the liver as well as in the spleen, they thought it possible that the biliary ducts were the channels by which the parasite escaped into the fæces. They were, however, unable to find any parasites in this situation. On the death of this patient, they[8] examined sections of his organs and were able to confirm Christophers' observation as to the presence of the parasites in the ulcers of the large intestine; they suggest that the parasites may escape by the intestinal tract.

Captain Statham,[9] R.A.M.C., has carried out a large series of examinations and cultural experiments with the fæces from a case of Kala Azar, and has failed to find the parasite in this situation; and I have on many occasions searched for parasites in the mucus in the fæces of cases of Kala Azar, also in the sanious discharge from cases with *cancrum oris*, but in neither case have I ever found parasites. Captain Statham[9] has also pointed out that he was unable to recover the parasites from (1) sterile tap water, (2) sterile pond water and (3) ordinary pond water which were inoculated from splenic cultures.

Several observers have noticed that the Leishman-Donovan bodies readily die out in a cultivation of splenic blood if it becomes bacterially contaminated, and fæces of Kala Azar patients suffering from diarrhœa swarm with bacteria of many kinds.

The occurrence of the Leishman-Donovan bodies in ulcers of the skin has led Sir Patrick Manson[10] to suggest that the parasite leaves the human body in the discharges from ulcerated surfaces, intestinal or cutaneous, and is ingested by some foul-feeding fly in which it undergoes multiplication, and is then implanted into the human host by this insect by a bite or by contact with broken skin surface.

Failure to recover the parasite from the discharges, and the fact that it requires for its growth, in cultures at least, approximately sterile conditions, makes it probable that it does not leave the human body by the means suggested.

The other hypothesis, suggested by Christophers and Rogers as the most probable means by which the parasite leaves the human body, is that it is ingested by a blood-sucking insect, Rogers[6] mentioning the bed bug as a possible intermediate host. This hypothesis requires the presence of the Leishman-Donovan bodies in the peripheral blood in a suitable condition for further development and in sufficient numbers. Donovan and Christophers appear to be the only observers who have found the parasite in the peripheral circulation. Rogers,[6] referring to the relationship of the leucocytes to the parasites in cultures, still doubts whether the Leishman-Donovan bodies can be found in the peripheral blood; he, however, notes that Donovan and Christophers have found this stage of the parasite within leucocytes during high fever, the latter having on two occasions found a number of them nearly all within the polymorphonuclear cells in a differential count of 500 leucocytes.

Early in December 1905 Major Donovan showed me these parasites in the leucocytes in a film made from the finger blood of a case of Kala Azar, and I have since been able to confirm the observation of Christophers that numbers of parasites are occasionally present in the peripheral circulation. In 38 out of 45 cases of Kala Azar examined in the General Hospital, Madras, parasites were present in the peripheral blood. It is true it was necessary in some of the cases to examine more than one film, whilst in one instance as many as eleven films were searched before a parasite was found; but with the technique employed it was as a rule easy to find them. Six of the 38 cases were suffering from severe diarrhœa, general œdema, and *cancrum oris;* the remainder were still in an early stage of the disease, though many of them have since died.

These results show that in order to diagnose the disease it is not always necessary to puncture the spleen. In such cases splenic puncture is always a dangerous operation and accidents have happened in the hands of most careful physicians. Sir Patrick Manson [11] has urged more frequent blood examinations before resorting to splenic puncture, and has suggested puncture of the liver as a substitute. This, though a somewhat safer operation, is not altogether devoid of danger, and it often happens that the puncture has to be repeated before the parasites are found. For these reasons a method of diagnosis which is safe and sure has been greatly needed, and I have shown that careful examination of the finger blood is very often all that is necessary.

In making the film it is important to attend to one or two details. No pressure must be applied to the finger and the drop of blood used must be a large

one. The edges of the film should, if possible, be straight as this facilitates considerably the rapid examination of a large number of leucocytes. Such a filsm stained deeply by Romanowsky's method can be satisfactorily searched in a little over an hour. After some practice I was able to find the parasite with a Leitz 1-6th objective even before the stained film was dry.

It may be asked, 'is it necessary to examine a film at some particular hour during the day or night?' In order to settle this point I have on two separate occasions examined films taken every hour from a patient suffering from Kala Azar in whose peripheral blood I had found the parasites. The record of these observations will be found on the chart appended which also shows the temperature taken hourly. It will be seen on examining this chart that the number of the parasites in the peripheral blood bears no relation to the temperature, and that there is no particular hour when parasites are especially abundant. The observations are of interest in showing that the number of parasites in the peripheral blood is not dependent on any definite periodicity in their discharge into the circulation, but varies from hour to hour throughout the day with each specimen taken.

One of the first cases of Kala Azar I studied was a boy of 14 (Case 1), in Major Donovan's ward, suffering from uncontrollable diarrhœa; his spleen was one inch and his liver one inch and-a-half below the costal margin, and on palpating his abdomen great pain was complained of all over the large intestine. Two blood films were examined, one contained 34 parasites in the leucocytes and the other 60; in the latter they were nearly all in the polymorphonuclear leucocytes which were distinctly increased in numbers.

I have since examined eight similar cases in each of which diarrhœa was the most marked symptom. One of these cases was particularly interesting, for the patient, a boy of 17 (Case 10), had been ill for only four months. The disease began with fever, which was followed by diarrhœa that persisted till death; the spleen could not be felt below the costal margin although the liver extended four inches below the normal limit, and on palpation of the abdomen great pain was complained of in the region of the large intestine, even down to the first part of the rectum. The motions, varying in number between ten and sixteen, were of a greenish yellow colour and contained flakes of necrosed tissue, some mucus and undigested food, and often small quantities of blood. The temperature never rose above 102° F. during the twenty-four hours. In a film of finger blood made seven days before death I found 75 parasites, most of which were in polymorphonuclear leucocytes, though a few were in the mononuclear cells. Fig. 1 on the plate shows a large mononuclear leucocyte containing seven parasites, and Fig. 2, a polymorphonuclear leucocyte containing four parasites; both these cells were found in thesame film. The para sites

have the same appearance as those seen in films of splenic blood. The two following blood counts were made from this case on March 19th :—

Count I.—

Total leucocytes counted	500
Polymorphonuclear leucocytes	75·6 per cent.
Large mononuclear leucocytes including transitional and intermediate cells	11·4 „
Small mononuclear leucocytes	6·8 „
Myelocytes	2·6 „
Eosinophyl cells	3·6 „

I found one parasite in an eosinophyl cell, two in a large mononuclear cell and sixty in the polymorphonuclear leucocytes, many of which contained as many as three parasites.

Count II.—

Total leucocytes counted	500
Polymorphonuclear leucocytes	78·4 per cent.
Large mononuclear leucocytes including transitional and intermediate cells	9·6 „
Small mononuclear leucocytes	9·8 „
Myelocytes	1·4 „
Eosinophyl cells	·8 „

In this film there were 98 parasites in polymorphonuclear leucocytes and 12 in the large mononuclear leucocytes.

These two counts show distinctly an increase in the polymorphonuclear cells which is probably due to the ulceration of the large intestine, and it is of interest that under these conditions the majority of the parasites are in the polymorphonuclear cells. Two days before the death of the patient I found 128 parasites in a blood count of 500 leucocytes, this being the largest number of parasites I have yet found in a film of peripheral blood.

At the autopsy, the hepatic flexure of the large intestine was found firmly bound down to the lower surface of the liver; the large intestine was ulcerated throughout, and in the liver there were two abscesses the size of oranges and several small ones containing glutinous pus.

All these observations show that, if careful search is made, the Leishman-Donovan bodies can be found in the leucocytes in the peripheral circulation, and further, that the parasites occur in large numbers in those cases in which the large intestine is markedly ulcerated.

Rogers [6] has recently shown that the development in his acid medium of the parasites embedded in the polymorphonuclear leucocytes is in every respect typical, but somewhat slower than that seen in the free forms; therefore, since the parasites occur in the peripheral circulation and that they are

capable of further development while in the leucocytes, it is not difficult to understand that infection may be acquired by some blood-sucking insect.

We may now consider if there is any other evidence in support of the view that some blood-sucking insect acts as the carrier of the parasite.

Rogers,[12] tracing the spread of the disease from village to village in Assam, found that when a person suffering from Kala Azar went to reside in an uninfected village the next case occurred in the house in which this person had lodged. He[12] also records instances of Europeans contracting Kala Azar by coming in contact with natives who subsequently died of the disease; in two instances there was no other case for miles around.

In Madras many of the Kala Azar patients give very clear histories of having lived with or near persons who have either died of, or are still suffering from the disease, and I have often noted that the first case in a house terminated with very severe diarrhœa.

Dr. Bentley [13] records in "Notes on Kala Azar in Assam" that he has frequently noticed when the epidemic was at its height that cases showing dysenteric symptoms, especially when bed-ridden, were followed by fresh cases in the same household or among the attendants; and that when the intestinal form of the disease is in abeyance the epidemic assumes a milder type.

That cases of Kala Azar with extensive ulceration of the bowel are a source of danger to others living in close contact with them is in accordance with my observations on the blood of such cases. A blood-sucking insect feeding on these cases could easily take in leucocytes containing the parasites, and if the insect was a suitable host and there was nothing to prevent the development of the parasites, infection later of a healthy individual could follow.

Two instances of a multiplicity of cases occurring in one house have recently come to my notice, and I am indebted to Dr. Vigayaraghavelu for the notes of the first series of cases, which are given below:—

Case No. 1, R. . .	Son of No. 2 . .	Age 20 years . .	Disease began February 1902, patient still living.
Case No. 2, A. . .	Father of No. 1 . .	Age 42 years . .	Disease began May 1902, died January 1904.
Case No. 3, S. . .	Son of No. 5 . . .	Age 9 years . .	Disease began June 1903, died March 1905.
Case No. 4, K. . .	Daughter of No. 5 . .	Age 15 years . .	Disease began December 1903, patient still living.
Case No. 5, R. . .	Father of Nos. 3 and 4, brother-in-law of No. 2.	Age 39 years . .	Disease began April 1904, patient still living.
Case No. 6, P. . .	Daughter of No. 2 .	Age 13 years . .	Disease began September 1904, patient still living.
Case No. 7, N. . .	Nephew of No. 5 . .	Age 11 years . .	Disease began October 1905, patient still living.

I visited the house occupied by this family, and saw those who are still suffering from the disease. They all had enlarged spleens, were getting fever regularly, and had œdema of the lower extremities. Case No. 4, a girl of 15, was very much reduced owing to diarrhœa which was evidently getting worse.

The house in which the family lived had a central courtyard in to which all the rooms opened. Some of the family were accustomed to sleep in the verandah along the courtyard, and the others in the rooms. On searching for blood-sucking insects I found only bugs, which were in large numbers in crevices in the doors and roof.

I was unable to find a case of Kala Azar in the adjoining houses, and was informed by the occupants that they had not heard of one. I have every reason to believe the statements of these people who were intelligent and knew the disease quite well.

The second instance of a number of cases occurring in one house is also of interest. Most of the cases had been in hospital and were diagnosed either by splenic puncture or by examination of the peripheral blood. The family lived in a small detached house which consisted of two rooms with a verandah on each side. The first case in this house (Mr. T.) had been for some time in hospital where the disease had been diagnosed as Kala Azar after splenic puncture. His friends removed him during the last stages of the disease, when he was bed-ridden and suffering from acute diarrhœa, and he died at home on August 2, 1905, about a week after he left the hospital. His mother-in-law (Mrs. C.) died on August 24, 1905, in hospital, where her case was not diagnosed as Kala Azar, but as anæmia and diarrhœa. The next case (William C.) was diagnosed after examination of splenic blood in hospital, as one of Kala Azar. He died at home on October 29, 1905. Nelson C., who was ill during his brother's lifetime and had also been in hospital several times, when the parasite was found in the splenic blood, died on December 8, 1905. Mrs. W., whose illness began in October, died in hospital on December 14, 1905, four days after admission. Many parasites were found in films of splenic blood. In March 1906, Mrs. T. came to hospital suffering from fever and an enlarged spleen, in which parasites were present in large numbers. Mabel W. came to hospital with a history of irregular fever and diarrhœa which had persisted for nine days. Her spleen was only slightly enlarged, but I found a parasite in a film from her finger blood. The house occupied by this family was in a very insanitary condition and bugs were plentiful in the furniture and also on the walls.

These two instances show very clearly that the infection tends to spread through a household.

In Madras among the blood-sucking insects which might be concerned in the transmission of the disease are the louse, mosquito, house flea, bed bug, and

the human tick (*Ornithodoros savignyi*), recently found in Madras. No experiments were carried out with the flea, as I was unable to find it in any number in the houses.

Pediculus capitis.—Finding that *pediculus capitis* was common on the heads of patients, I began my experiments with this insect.

Experiment I, Case 1.—On December 2, 1905, I found 34 parasites in a film made from the finger blood of this boy. On the same day about 50 l ce were placed on his head. The next day eight lice were dissected, their mid-gut contents were carefully examined and in one slide a single free parasite was found which showed no change either of development or degeneration.

On December 4th the boy died, and the remaining lice were removed and placed on the head of a second patient (Case 2), in whose peripheral blood parasites were found. On the same day ten lice were dissected, and in two slides four parasites were found which were unaltered, and, if anything, showed commencing degeneration, evidenced by their somewhat ill-defined outlines. The remaining lice were dissected on December 5th, 6th, and 7th, but I failed to find any parasites in the films. Altogether 47 lice were dissected.

Experiment II, Case 2.—On December 8th, ten parasites were found in a blood film taken from the finger of this patient, and shortly after this 32 fresh lice were placed on his head. On December 10th, 11th, and 12th, these lice were dissected and examined, but I was unable to find a single parasite. After dissecting 102 lice, no further experiments were undertaken with them, as I could find no evidence of development of the parasites in their mid-guts.

Pediculus corporis.—Experiment I, Case 3.—About 15 of these lice were placed on the patient on December 12th, on which day 10 parasites were found in a blood film. On the 14th, 15th, and 16th, the lice were dissected and examined, but I was unable to find any parasites. No further experiments were carried out with these lice.

Culex fatigans.—This is one of the commonest mosquitoes in Madras City, being very abundant in George Town where it breeds in wells and gutters. A large number of the larvæ of this species were kept in the laboratory, and the adult mosquitoes were used for the experiments. On selecting a suitable case, a mosquito net was fixed on the bed, the mosquitoes being placed inside the net at night and collected in the morning.

Experiment I, Case 4.—A large number of these mosquitoes were placed in the net on the evening of December 15th, and next morning ten fully gorged female mosquitoes were recovered. A blood film examined on December 15th contained seven parasites.

On December 18th, the seven remaining mosquitoes were dissected and

in the mid-gut of the sixth in the series a flagellate was seen in the fresh condition, but on smearing out the film it was lost. At first I was inclined to think that this flagellate represented a fully developed Leishman-Donovan body, as I was not then aware of the common presence of flagellates in mosquitoes bred out from larvæ in the laboratory. I was, however, soon after informed by Christophers that he had found similar flagellates in the mid-guts of the males of this species, and that on examining the gastro-intestinal tract of the larvæ he found the early stages of development of the parasites. I have since been able to examine these flagellates, which are long slender spindles tapering posteriorly possessing a long wavy flagellum. They are probably a species of *Herpetomonas*.

Similar, if not identical, parasites were found by Major Ross[14] in 1898 at Ootacamund, and he has recently called attention to these observations on the presence of the parasites in the larva, pupa, and imago of *Culex fatigans*. Novy, MacNeal and Torrey[15] in a short paper on mosquito trypanosomes, say they have found flagellates in 15 per cent. of caught mosquitoes subsequently fed on clean animals, rats, guinea-pigs and pigeons. These parasites appear to be of several distinct species, the most common being *Herpetomonas* (probably *Herpetomonas subulata*) and *Crithidia fasiculata*. These observers have been able to cultivate the parasites on solidified blood agar, and found that the cultural forms conformed in every respect with the parasites found in the mosquitoes. I have since found similar parasites (*Herpetomonas*) in the mid-guts of *Culex pipiens*.

One hundred and fifty-seven female mosquitoes (*Culex fatigans*) which were fed on the patient were dissected between December 18, 1905, and January 17, 1906, but I was unable to find any Leishman-Donovan bodies in their mid-guts. Blood films were taken daily from the patient, and with three exceptions, parasites were always found. Two of the mosquitoes contained normal flagellates (*Herpetomonas*).

In most of the dissections of these mosquitoes, after examining the fresh preparations, the films were smeared out and stained by Romanowsky's method. Besides the flagellates mentioned above, many of the films contained bacteria and a few spirilla. As nothing to suggest the development of the Leishman-Donovan bodies was seen, I decided to abandon any further experiments with this mosquito.

Ornithodoros savignyi.—Captain Christophers[16] has recently drawn attention to the presence in the Madras Presidency of this tick which was first brought to his notice by Dr. Oliver who found it in the gravelly floors of the third-class passenger sheds at Tirapaty. Specimens have been obtained also from Kalahasti, Tirapati West, Yerfed, Gekakur, Vendod, Tanjore, Conjeeveram, and

lately, in Madras itself. In Madras they are found in the sand under the trees in the People's Park and on the outskirts of the town, but I have not been able to find any evidence of the presence of the tick in the houses in George Town. I have myself searched on the floors of some of the houses and also along the streets, but without success. Boys suffering from Kala Azar have, however, told me that they were bitten by ticks when playing under the trees on the outskirts of the town.

Experiment I, Case 5.—On January 16, 1906, ten parasites being found in a blood film, four adult female ticks were placed on the patient's abdomen where they soon began to suck blood and when fully gorged dropped off. On the 27th, seven parasites were found in a blood film, and the ticks were again fed.

On the 29th, one of the ticks was dissected and its gut contents being smeared out on several slides were stained by Romanowsky's method and carefully examined, but with negative results. On the 31st, the second tick was dissected and this time, in addition to examining the gut, the ovaries were also smeared out and stained, but in none of the films could I find any parasites. The two remaining ticks were allowed to ovulate in order later to examine the larvæ and nymphs.

Seven more ticks were fed on another patient (Case 6) and examined between February 5th and 17th, but nothing was found in the dissections. In each case films of blood, examined shortly before feeding the ticks, contained parasites. Larvæ and nymphs were examined later with negative results.

No more experiments were carried out with this tick as there were no evidence of the development of the Leishman-Donovan body in their guts.

Anopheles stephensi.—This is the only species of anopheles that has been, so far as I am aware, found in George Town. I have had occasion to examine many larvæ from the wells in this part of Madras, and they have always been those of *Anopheles stephensi.*

Captain James,[17] I.M.S., reports having found the larvæ of this species in Madras City in unused wells, and I have often satisfied myself that the larvæ can live in wells in constant use. I noted that larvæ could be found in one particular well although it was used constantly by the occupants of four adjacent houses. This observation is of considerable interest as showing that the larvæ of this mosquito can live in spite of adverse conditions.

A large number of the larvæ of this mosquito were collected early in March 1906, and when the mosquitoes had hatched out they were kept in bottles, the mouths of which were covered with netting. These bottles were taken to the ward at night and placed under the flexed knee of the patient, a method of feeding these mosquitoes which I have found satisfactory.

Experiment I, Case 7.—On March 11th a film of blood from this patient contained 14 parasites, and six adult mosquitoes were fed on the same night. They were again fed on March 12th, when I found four parasites in a blood film. On the 15th the remaining five mosquitoes were dissected and examined, and in four of the films there were many flagellates, which on staining appeared to be a species of *Crithidia*. They were short organisms measuring about 4·8μ with their anterior ends truncated or concave.

Thirty-four female mosquitoes were fed on two patients (Cases 8 and 9) between March 12th and 28th, and on dissection they were found to contain no Leishman-Donovan bodies, though 23 of the mosquitoes contained normal flagellates (*Crithidia*). On examining the gastro-intestinal tracts of the larvæ of this mosquito these normal flagellates were found in varying stages of development.

Leishman-Donovan bodies were found in large numbers, in films of the finger blood of one of these patients, on one occasion as many as 75 being found. No more experiments were carried out with *Anopheles stephensi*.

Stegomyia sugens.—This mosquito is common all over Madras, being specially prevalent in the wells in George Town.

Experiment I, Case 10.—Ten female mosquitoes were fed on this patient during the day on March 15th, a blood film taken shortly before feeding contained 78 parasites. On March 17th and 19th the mosquitoes were again fed, blood films taken on these dates contained respectively 32 and 21 parasites. A few bugs were also fed on the patient on the 19th, but they were eaten by ants which had got into the pill boxes. The mosquitoes were dissected on March 22nd, but I was unable to find any parasites. After having been fed, several times, 21 female mosquitoes were dissected between March 20th and 26th, but no parasites were found in the smears. On each of these days blood films taken before the mosquitoes were fed contained many parasites.

On account of all these failures no other species of mosquito was experimented with.

Bugs.—At first, some difficulty was encountered in keeping and feeding these insects. They were confined in pill boxes, the lids of which were perforated to allow for ventilation, a few pieces of crumpled filter paper being placed at the bottom of the boxes. When required for feeding, the boxes were opened and inverted on the arm or leg of a patient. Owing to the facts that it was often difficult, especially at night, to recover all the bugs, and that ants could readily enlarge the holes in the lid, I adopted another plan for keeping them. Small glass specimen tubes, such as are used for storing mosquitoes, were found to answer the purpose. A few pieces of filter paper were put into the tubes and the bugs on being dropped in hid themselves in a short time in the

folds of the paper. These tubes were then placed in a small dish standing in a basin of water. No corks are necessary if the paper is pushed well down to the bottom as the bugs cannot climb up the glass. On taking the tubes to the bedside, the paper containing the bugs was gently drawn up to the mouth of each tube with a pair of forceps and the tubes inverted over the patient's skin, shortly after which the bugs came out and began to suck blood, and when fully gorged returned readily to the folds. Bugs fed in this way live and breed as in nature, and can be fed either by day or night.

On March 28th, a large number of bugs were obtained from many sources and were placed in a jar containing some crumpled filter paper; the next day suitable specimens were removed with a fine pair of forceps and placed in a tube ready for use.

Experiment I, Case 11.—On March 29th, eleven bugs were fed on this patient, in a film of whose finger blood nine parasites had been found. On March 30th, 31st, and April 2nd the bugs were again fed although no parasites had been found in blood films on those dates. On April 2nd one of the bugs was dissected and the mid-gut contents which contained fresh and altered blood were examined in two separate films under cover slips. In one of the preparations I saw a pyriform-shaped flagellate actively moving about among the corpuscles. Its structure was difficult to make out in the fresh condition, but a hyaline nucleus situated about the centre of the body was visible. I was unable, however, to make out the blepharoplast or any granules in the protoplasm. Its movements were less active than those of the flagellates found in *Anopheles stephensi*, its flagellum exhibiting slow lashing movements.

The flagellates of *Anopheles stephensi* are extremely active, darting across the field of the microscope, then suddenly altering their course and moving in the opposite direction; their flagella exhibit vibratile rather than lashing movements. The movements of this flagellate of the bug were more like those of the flagellates of *Culex fatigans* and *Culex pipiens*. I was at first in doubt where this flagellate came from, for, on examining the saline solution used in my dissections, I could find no organisms of this nature. On attempting to make a stained preparation the parasite was unfortunately lost, so I was unable to study it further. Thinking that it might possibly prove to be the fully developed Leishman-Donovan body, I determined to go on with my experiments with bugs.

It is not necessary to give details of the dissections of 41 bugs examined between April 2nd and 13th, as I was unable to procure a suitable case in hospital and all the dissections proved negative.

On April 14th I was shown by Mr. Haller a case of Kala Azar in a European, in the last stages of the disease, suffering from very severe diarrhœa. No blood film was examined in this case. Six bugs were removed from the

patient's bed, and I asked the relatives to send me any others they could find, but, owing to the death of the patient, they were never sent.

On April 15th I dissected one of the bugs, and on staining the film found a large mononuclear leucocyte containing three parasites (Fig. 4). From the appearance of the mid-gut contents, it was most probable this bug had last fed on the patient on April 13th; the parasites had therefore been in the bug about 40 hours. They stained well with Romanowsky's stain, their outlines being distinct, and in every way they were similar to those found in smears made from splenic blood. Another bug dissected on the same day showed a free parasite (Fig. 5), which also stained well, no leucocyte being found anywhere near it. The remaining four bugs contained no parasites.

From April 16th to May 14th I was unable to obtain a good case in hospital. A few bugs, however, were fed on a patient on April 20th, on which day a single parasite was found in a blood film. One of the bugs dissected four hours after the last time it was fed, contained a parasite in a polymorphonuclear leucocyte (Fig. 3) which was lying unchanged in the protoplasm of the cell.

On May 16th a suitable case, a boy of 16 with *cancrum oris*, general œdema and severe diarrhœa, was admitted into hospital.

Experiment I, Case 12.—On May 16th a film of blood was examined and found to contain 32 parasites, and on this day 22 bugs were fed on the patient. They were fed again on the 17th, a blood film taken shortly before the feeding containing 52 parasites. On May 18th, 15 parasites were found in a blood film, and the bugs were again fed. On the 19th I dissected one of the bugs, and found two free parasites in the film (Fig. 8). From the appearance of the cells in the mid-gut contents, this bug probably fed last on the 18th. Both parasites showed the earliest stages of development, their protoplasm stained faintly blue and the macro-nuclei, though still towards the periphery, appeared granular. In one, the macro-nucleus and protoplasm contained a single vacuole, while in the protoplasm of the other there were three.

The bugs were fed daily from the 19th to the 31st, and on each day a blood film was examined and found to contain parasites in varying numbers. On June 1st I dissected an adult female bug which had last fed between 6 and 7 P.M. on the 31st, and found 15 free parasites in the film. Two of the parasites are seen in Fig. 6, the larger showing distinct signs of development. Its protoplasm has increased in volume, the macro-nucleus much enlarged is granular, and though still touching the periphery is extending towards the centre of the parasite. The micro-nucleus, somewhat thicker, is lying on the periphery of the cell and adjacent to it a vacuole is seen. The smaller parasite is stained feebly throughout and seems to be degenerating. What appears to be the remains of a leucocyte is seen lying close to the parasites,

its nucleus is represented as a faintly stained mass, and the protoplasm is granular and indistinct.

Fig. 7 represents another parasite from the above film at about the same stage of development. Its macro-nucleus, more central, is much enlarged, and the micro-nucleus, somewhat thicker, lies near it. The protoplasm, stained deep blue, contains two large and two small vacuoles, but there are no granules in it. No sign is seen of the commencing formation of the vacuole-like area, or any evidence of the division of the parasite.

On June 4th another female bug was dissected, and in the slide a parasite showing further development was found (Fig. 9). This parasite, measuring 3·8μ in its greatest breadth, is much rounder than the one described above (Fig. 6); its protoplasm, stained deep blue, contained three vacuoles. The macro-nucleus, much enlarged, is pear-shaped and lying well within the parasite, while the micro-nucleus, which is slightly enlarged, lies about the centre of the cell. A circular shaped body which represents the vacuole-like area, stained pink by Romanowsky's method, is seen lying between the micro-nucleus and the periphery of the parasite. An irregular mass, the remains of a leucocyte, is lying adjacent to the parasite. The bug from which this parasite was recovered was fed last on May 31st between 6 and 7 P.M., and was dissected at 7 A.M. on June 4th, the parasite had therefore been in the mid-gut of the bug about 82 hours.

On June 4th I found it necessary to feed the remaining bugs on another case of Kala Azar, but the bug from which the above parasite was obtained had apparently not fed, as there were no fresh red cells in its mid-gut. Four bugs were dissected on the same day with negative results. On June 5th another female bug was dissected, and eleven parasites were found in the slide, six of these are shown in Figs. 10, 11 and 12. In Fig. 10, two elongated pyriform parasites measuring 5μ and 3·5μ are seen lying in close apposition. In the larger the macro-nucleus, much enlarged and containing a vacuole, occupies about the whole of the posterior pole, while in the other it is smaller and lies to one side touching the periphery; both the micro-nuclei, somewhat thickened, are curved, one being concave towards the macro-nucleus, the other convex. The protoplasm of both the parasites is stained a deep reddish blue and contains neither vacuoles nor chromatin granules. A brush-like structure consisting of many pink staining filaments is seen protruding from the anterior end of the larger parasite and represents the early stage of the flagellum. In the smaller parasite it is only just protruding and appears as a blunt rod, stained pink. Both the flagella pass out from the vacuole-like areas which are shaded in the figure.

Fig. 11 represents a parasite from the same film showing signs of longitudinal division. The macro-nuclei, circular in shape and finely granular, are

seen lying on each side of the central line. The micro-nuclei are lying on the outer sides of each parasite, adjacent to the macro-nuclei. The two vacuole-like areas are clearly seen, and protruding from them is a small rod, which stains pink, and represents the commencing formation of the flagella. A large vacuole lies between the two parasites, showing that separation is beginning at this point. The protoplasm of both the cells, staining dark pink, contains no vacuoles or granules. Each parasite measures 4·8μ in length and 1·5μ in breadth.

Fig. 12 shows two attenuated parasites attached by their posterior poles only. The two macro-nuclei, considerably elongated, are situated about the centre of each cell, while the micro-nuclei are in close proximity to them. The protoplasm of both are stained reddish and contain no granules or vacuoles. Each cell measures 5μ in length and ·8μ in breadth. In the same film there were two other parasites showing early signs of development and three elongated forms without flagella.

The remaining bugs were dissected on the 5th and 6th with negative results.

During the months of June, July and August a large number of bugs were fed on persons suffering from Kala Azar, in whose peripheral blood only a few parasites could be found, but the dissections of all these bugs proved negative.

In September three cases of Kala Azar suitable for feeding experiments were admitted into hospital. The first of these, a young woman aged 22, was suffering from *cancrum oris*, general œdema and diarrhœa; I made a film from her finger blood and found 21 parasites, after which a number of bugs were fed. I was unable, however, to continue these feeding experiments as the patient's friends removed her from hospital. No parasites were found in the bugs.

The second case was a boy of 17 with general œdema and diarrhœa, in a film of whose peripheral blood I found 11 parasites. A number of bugs were fed on three successive days, blood films taken before feeding showing many parasites; as the patient died suddenly I was unable to feed the bugs again and the examination of their mid-gut contents showed no parasites.

The last case was that of a boy aged 12 who had been in hospital on a previous occasion when I had found the parasite in his peripheral blood; he was now very ill suffering from uncontrollable diarrhœa. On the 10th, three days after his return to hospital, when examining him I found in his bed a large number of bugs in all stages of development, showing they had been in the bed for some time; a blood film examined on this day contained 27 parasites. The patient who occupied the bed before this boy was admitted, had been suffering from pleurisy. I removed some of these bugs for dissection and the rest were destroyed; on the 14th an adult female bug was dissected and in the film I found three parasites, two of which resembled the attenuated forms shown in Fig. 12.

They were attached from their posterior poles to about the centre of their bodies; the circular macro-nuclei were granular in appearance and situated about the centre of each parasite. The micro-nuclei were lying close beside the macro-nuclei, touching the periphery of each parasite. There were no vacuole-like areas or flagella. The third parasite was oval in shape containing a large circular macro-nucleus with the micro-nucleus in close apposition to it. There was no distinct vacuole-like area to be seen, but a diffused pink stained mass was situated at some distance from the macro-nucleus; it was difficult to decide from these appearances what change was taking place in the parasite.

The remaining bugs taken from the bed were dissected but no parasites were found. On the 11th six bugs were fed on the patient for the first time; shortly before this 30 parasites having been found in a film of finger blood. The bugs were again fed on the 12th and 13th at approximately the same hour as on the 11th and on these days thirty-two and sixteen parasites respectively were found in blood films. The patient died on the 13th. The bugs were fed daily from the 13th to the 17th on a young monkey and were all dissected on the 17th and in two of the films three parasites practically unchanged were found. Assuming that the parasites had been ingested by the bugs on the 13th, they had been in their mid-guts about 96 hours. I was unable to find any parasite showing development.

In the bug I could find no large granular blue forms which are so characteristic of cultures at 22°C. All the most advanced forms were considerably smaller than those seen in cultures and in each parasite the micro-nucleus lay close up to the macro-nucleus.

Further work on the development of the parasites can alone confirm these observations, and it would be unwise to be too dogmatic regarding the precise mode of their development.

The discovery of the development of the Leishman-Donovan body in the bed bug has led to my endeavouring to ascertain the distribution of the species of bug used in my experiments. Through the kindness of medical officers, assistant surgeons and hospital assistants I have been able to examine a large number of specimens of bed bugs from various parts of India, Assam and Burma, and I have found, so far, without exception that they are identical with the Madras bed bug. In order to compare this bug with the typical *Cimex lectularius*, L. I obtained some living specimens of this species from the slums of London and found that the Indian bed bug is *Cimex macrocephalus*, Fieb. Recently I sent some specimens of Indian bed bugs to Mr. Distant who confirmed my observation that they are *Cimex macrocephalus*.

It is generally believed, as is stated by Mr. Distant, that *Cimex lectularius*, L. is distributed throughout India, while the presence of *Cimex macrocephalus*, Fieb.

has only been recorded from Burma (Bhamo). Further investigations regarding the distribution of the two species are in progress.

CONCLUSIONS.

1. The Leishman-Donovan body occurs frequently in the peripheral circulation in cases of Kala Azar in Madras, the parasites being seen in the leucocytes but never in the red cells or free in the plasma.

2. In certain cases with extensive ulceration of the large intestine there occurs an increase of the polymorphonuclear leucocytes and many of these cells then contain parasites.

3. Though the Leishman-Donovan body has been recovered from the mid-guts of lice, *Pediculus capitis*, it was never found in *Pediculus corporis*, *Culex fatigans*, *Anopheles stephensi*, *Stegomyia sugens*, or the tick (*Ornithodoros savignyi*).

4. The parasites can be recovered from the mid-guts of bugs (*Cimex macrocephalus*), fed on cases of Kala Azar, and kept at room temperature (80–82° F.), and these parasites have in a few cases shown considerable development.

I take this opportunity tc thank Majors Robertson, Donovan, Foulkes, and Captain Long for their kindness in permitting me to examine the cases of Kala Azar under their treatment.

NOTE.

Since writing the above I have found in the bed bug (*Cimex macrocephalus, Fieb.*), all the intermediate stages of development and numerous fully developed flagellates similar to those seen in cultures of splenic blood. These will be described in detail in another paper.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Photogravure.

Survey of India Offices, Calcutta, July 1906.

No. 27.

(NEW SERIES.)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

PRELIMINARY REPORT ON THE DEVELOPMENT OF THE LEISHMAN-DONOVAN BODY IN THE BED BUG.

BY

CAPTAIN W. S. PATTON, M.B., I.M.S.
(On special duty.)

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.

CALCUTTA:
OFFICE OF THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.
1907.

Price eight annas or 9d.