

## Original Articles.

ON A HERPETOMONAS FOUND IN THE GUT OF THE SANDBLY, *PHLEBOTOMUS ARGENTIPES*, FED ON KALAZAR PATIENTS.

A PRELIMINARY NOTE.

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*Introduction.*—Before giving an account of the experiments which form the essential subject matter of this paper, it seems desirable to give a brief résumé of the events leading up to them.

At an informal discussion between Majors H. W. Acton and R. Knowles and Dr. Napier in 1922 it was resolved that the difficult and important problem of kala-azar transmission should be the first to be investigated by the various workers at the School who were in a position to undertake this work. It was shewn by Acton and Knowles (1914) in their studies of the Halteridium parasite of the pigeon that the degree of infection varied very much with the time and season of the year, and in connection with kala-azar the first point upon which information was needed was to ascertain if possible at what time of the year new infections occur in man. It seemed likely that the following information would be of assistance:—(1) a frequency distribution table shewing the date of commencement of the fever, as recorded from the patients' own statements; and (2) an examination of the wet bulb thermometer readings during the year. In this way it could be seen whether there was any correlation between any peak in periods of greatest incidence of the disease and the wet bulb temperature record. Any such correlation might indicate the most favourable time of the year during which to carry out investigations; otherwise a great deal of useless work might be carried out at seasons when the temperature and humidity conditions were unfavourable for transmission.

At the same time Lt.-Colonel T. C. McCombie Young, I.M.S., then Director of Public Health, Assam, was approached, and the suggestion made that he should carry out a similar investigation in the far wider field of that

province. The results of his enquiry were published in 1923, and shewed that in the most heavily infected areas the greatest number of new cases appeared to occur during the cold weather, and that probably the season most favourable for infection was the few months prior to the onset of the cold weather.

Lastly, Napier's aldehyde test does not become positive until about the fourth month of the disease:—a fact which is of still further assistance in determining roughly for any given case the approximate date of infection. During the period November to April many early cases come for treatment, whose aldehyde reaction is still negative. During the rest of the year negative reactions in kala azar cases are rare.

From a study of these issues, the conclusion was reached that the three or four months before November appear to be those when new infections are most likely to occur in Calcutta city, and would be the most favourable period for the proposed enquiry.

In 1923 at a second meeting between the same workers, Napier pointed out that there appeared to be a difference in the incidence of kala-azar among permanent residents of Calcutta city between the northern and southern parts of the city. An analysis of all such cases among permanent residents who had come under treatment in Napier's clinic was carried out by him, and the results published in 1923—(Knowles, Napier and Das Gupta 1923, p. 334). It was there shewn that there exists in Calcutta city an endemic focus of kala-azar in and around ward 14, where the infection is spreading among the local residents, "area B": whereas in northern Calcutta, "area A", although cases of kala-azar are very frequently imported in the form of patients who come into the city in considerable numbers from outside for treatment at the large hospitals, yet the disease here fails to spread, or to establish itself.

This finding made obvious the need for a very careful, full and detailed survey of area B, and of area A simultaneously as a non-endemic control. This survey has now been carried out in detail by the second author, (L.E.N.), and its results will shortly be published in a separate memoir.

In the meantime every effort was made by Napier to obtain the services of a trained entomologist for this particular research, but he was absolutely unsuccessful in all his attempts to obtain such help, and the most favourable period of the year in 1923 passed by without any further enquiry being possible.

In 1924 Major H. W. Acton, I.M.S.—then acting Director of the School—approached the Indian Research Fund Association, and through the kindness of Lt.-Colonel J. D. Graham, C.I.F., I.M.S., Secretary to its Scientific Advisory Board and the Governing Body, a grant was



made, and the third author (R.O.A.S.) was deputed for special duty at Calcutta on this enquiry. In what follows, it may be remarked that the experimental work to be recorded was entirely carried out by the third author (R.O.A.S.), the first two authors only directing and supervising. We are also indebted to Temporary Assistant Surgeon B. M. Das Gupta, Assistant Professor of Protozoology at the School, for valuable help in the examination of blood films, taking of cultures, etc., in connection with the work. The enquiry was thus finally started on the 28th June, 1924.

It had been pointed out to the senior writer by Major J. A. Sinton, v.c., I.M.S., in a private communication in 1922, that the known distribution of the sandfly, *Phlebotomus argentipes*, in India appeared to coincide with that of kala-azar. Without Major Sinton's most willing help and assistance, this enquiry would not have been possible. He has taken endless trouble in identifying sandfly species for us, preparing type mounted specimens, supplying information with regard to identification and distribution of species, and has made several valuable suggestions; and our most grateful thanks are due to him in this matter.

On the 21st June, 1924, Mr. T. Bainbrigge Fletcher, M.A., F.E.S., F.Z.S., Imperial Entomologist, Agricultural Research Institute, Pusa, very kindly accompanied the senior writer and Dr. Chandler of the School on a tour of investigation throughout areas A and B respectively. It was felt that the observations made by a distinguished entomologist who was unbiassed in favour of any one particular view of the transmission problem would be of very great help and value. At this tour it was noted that sandflies occurred in both areas, but they appeared to be more numerous in the endemic area; whilst breeding grounds for Culicoides were almost completely absent from the non-endemic area, but very prevalent in the endemic area. It appeared that the two most likely classes of blood-sucking insects upon which to concentrate attention were sandflies and Culicoides. In connection with the latter group, we have to acknowledge most gratefully the very great kindness of Mr. Bainbrigge Fletcher in identifying species, preparing type mounted specimens, and in helping the enquiry in every possible way.

It is not the purpose of this paper to record the earlier experiments and work carried out by the ancillary enquiry. Three species of sandflies have so far been encountered in Calcutta; *P. minutus*, together with its variety *niger*, *P. argentipes*, and *P. squamipleuris*, the last in very scanty numbers. *P. minutus* has been present in both areas A and B up to the end of October, but *P. argentipes* did not appear upon the scene until late in July, and was especially prevalent in the endemic area

in September and October. Of the Culicoides, seven species have been encountered to date, two of which are apparently new. A new technique for the breeding and feeding of sandflies, suitable for use in the hot, damp climate of Calcutta during the rains, has been worked out by the third author and will form the subject of a separate communication by him later. The work has been very heavy, and observations are steadily accumulating.

In view of the work by Acton (1919) on the correlation of the sites of Bagdad boils with those of sandfly bites, and the work by the Sergeant brothers, Parrott, Donatien and Béguet (1921) on the transmission of oriental sore by sandflies, also in view of Major Sinton's observation with regard to the apparent identity of the geographical distribution of *P. argentipes* and of kala-azar in India, special attention was paid to this insect. The earlier work with *P. minutus* had entirely negative results; in fact we have not yet succeeded in getting this species to feed on man at all. Its favourite host appears to be the gecko.

The following positive results with *P. argentipes*, however, appear to be worthy of record, even at this early date. In passing it may be noted that, in examining the patient's blood to determine the intensity of the infection in the peripheral circulation, the technique for thick blood films advocated by Knowles and Das Gupta (1924) was adopted. The seven patients upon whom the flies were fed were unselected cases in hospital which had not yet received any treatment.

*Experiment 1.*—20th September 1924. Six female laboratory bred out *P. argentipes* were fed upon the arm of a patient with kala-azar, whose thick blood films subsequently shewed scanty parasites present. They were kept at room temperature, and were dissected on the 24th, approximately 72 hours after the feed. In the fore-gut of one, typical and motile herpetomonad protozoa were found, some 5 to 6 in number. A culture was attempted on N.N.N. medium, but unfortunately became septic. The remaining five fed flies were negative.

*Experiment 2.*—26th September 1924. Twelve laboratory bred female *P. argentipes* were fed upon the arm of the same patient as in experiment 1. Four only fed during the night. One was dissected on the 29th, with negative results. The other three were found dead inside the mulmul netting on the morning of the 30th and were at once dissected. In all three, herpetomonad flagellates, in every way resembling *L. donovani* flagellates, were found in the fore-gut, in number varying from 6 to 10.

Attempts were made to obtain stained preparations, but were unsuccessful. A white mouse was injected intraperitoneally with the contents of two of these guts. It died upon the 15th day after injection; examination of the viscera failed to shew any Leishmania; the spleen, however, was enlarged, and there were areas of necrosis in the liver and kidneys. Result; inconclusive.

*Experiment 3.*—23rd October 1924. Four laboratory bred female *P. argentipes* were fed upon the arm of the same patient as in experiments 1 and 2, on the night of the 23rd|24th. On dissection on the 27th, some 72 hours later, active herpetomonads were found in the fore-gut of two, some 6 to 18 in number in each. Again, attempts to secure stained specimens and cultures were with negative results. The remaining two flies, dissected on the 28th, were negative.



*Experiment 4.*—24th October 1924. A batch of eight laboratory bred *P. argentipes* was put to feed at night on the arm of a patient with kala-azar, in a thick blood film from whom only one parasite had been encountered. Of these, 4 were males, and did not feed; the other four were females, of whom two became engorged with blood, and two shewed only traces of blood in the mid-gut. The two engorged flies were dissected on the 29th. Both shewed from 40 to 50 motile herpetomonads,—many rather sluggish in movement—in the fore-gut and anterior part of the mid-gut. The other two flies, dissected on the 30th, shewed no parasites.

Stained preparations from the first two flies were obtained by osmic acid fixation and Giemsa's staining, and shewed typical herpetomonads, exactly resembling those of *L. donovani* as seen in preparations from a culture on N.N.N. medium.

*Experiment 5.*—25th October 1924. A batch of laboratory bred female *P. argentipes* was fed upon the arm of the same patient as in experiment 4. Four fed. One was dissected upon the fourth day with negative findings. The remaining three were dissected upon the fifth day. One shewed very scanty herpetomonads in the fore-gut. The second shewed an intense infection, with masses of herpetomonads in both the fore-gut and mid-gut, and even a few in the hind-gut. The contents of the dissection were aspirated into a hypodermic syringe and administered subcutaneously to a monkey, *M. rhesus*, three-quarters of whose thyroid gland had been removed at a previous operation in order to lower its resistance to disease, and to experimental infection. The third fly gave negative results. The monkey is still under observation.

*Experiment 6.*—26th October 1924. A batch of laboratory bred *P. argentipes* was fed upon the arm of a patient with kala-azar. Thick films of this patient's blood did not shew any parasites, but parasites were found on spleen puncture. Two females fed. One was dissected on the 31st and shewed scanty herpetomonads present; the other, dissected the same day, gave negative findings.

*Experiment 7.*—28th October, 1924. A batch of laboratory bred *P. argentipes* was fed upon the arm of a newly admitted case of kala-azar, in which the diagnosis had not then been confirmed. Next morning two engorged females and one which had taken a small blood meal were collected. All three were dissected on the fourth day. Two shewed scanty herpetomonads present; the third was negative. The case was subsequently proved to be kala-azar upon spleen puncture.

*Experiment 8.*—10th November 1924. A batch of laboratory bred *P. argentipes* was fed on the arm of a kala-azar patient. The next morning 10 fed flies were found. On the 14th, 3 were dissected; of which 1 shewed herpetomonads present. Four were dissected on the 15th, of which one shewed a heavy infection with herpetomonads present, one a scanty infection; the other two being negative.

*Experiment 9.*—11th November 1924. A batch of laboratory bred *P. argentipes* was fed on the arm of a kala-azar patient. The next morning one fed female was found. This was dissected on the 16th, but with negative findings.

*Experiment 10.*—A batch of laboratory bred *P. argentipes* was fed on the arm of a kala-azar patient. The next morning 14 fed females were found. Of these, for some reason or another, 11 died the next day, leaving only 3 available for dissection. These were dissected on the 17th, and 2 of them shewed scanty herpetomonads present.

*Experiment 11.*—14th November 1924. A batch of laboratory bred *P. argentipes* was fed upon the arm of the same patient as in experiment 10. The next morning 18 fed females were found. Of these 11 were dissected on the 18th, of whom 5 shewed herpetomonads, the infection being a very heavy one in one instance; 7 were dissected on the 19th, of which 2 shewed herpetomonads, one of them having a heavy infection.

The question immediately arises as to what was the source of the herpetomonads seen in the gut of these 25 fed female *P. argentipes*. It may be mentioned that all eleven experiments were consecutive, and that they had been preceded by a series of negative experiments when it is possible that the technique was less satisfactory. It will be seen that the percentage of positive findings is extraordinarily high; no less than 25 out of 56 fed flies being infected, and in six instances heavily infected. Were these herpetomonad forms developmental forms of *L. donovani* or natural herpetomonads of the sandfly species concerned?

According to Shortt (1924), the natural protozoa of sandflies hitherto encountered are as follows:—

(a) A Monad, which is probably not a protozoon at all, but a mistaken interpretation of the spermatozoa of the sandfly.

(b) A Crithidia, which is probably a developmental phase of *Trypanosoma hemidactyli* of the gecko; found in *P. minutus*.

(c) *Herpetomonas phlebotomi* Mackie (1914); found in 7 out of 65 female *P. minutus* (identified by Patton as such), and in considerable numbers; although this author failed to find any corresponding herpetomonad in the gecko lizard which is the natural host of *P. minutus*. As far as we are aware, up to the present date and from a careful search of the literature, this finding of Colonel Mackie's remains an isolated one. It is also to be noted that it occurred in the heavily kala-azar infested district of Nowgong, Assam.

*Controls.*—The parasite encountered in these 25 positive female *P. argentipes* was an undoubted herpetomonad, both in its movements, its characters under dark ground illumination, and in stained preparations, and morphologically similar to that of *L. donovani*. It had been realised from the very outset of the enquiry that a very extensive examination of the insects worked with regard to their natural protozoa would have to be carried out. Up to the date of writing (22nd November 1924), the following sandflies have been dissected and examined as controls. (By "dissected" is meant that the entire alimentary tract was dissected out and all its parts examined.)

"Sandflies", nothing recorded as to species and sex	103
<i>P. minutus</i> , males	207
<i>P. minutus</i> , females	94
<i>P. argentipes</i> , males	317
<i>P. argentipes</i> , females	90
Total	811

Of these, 38 of the *P. argentipes* were males and 15 females bred out in the laboratory, all the remainder being flies captured in the wild state. Of the 94 female *P. minutus* captured and examined, 4 contained ingested reptilian blood; and of the 75 female *P. argentipes* captured and examined, 8 shewed



ingested blood, in each case of mammalian origin. The habits of the two species, indeed, appear to be entirely different, *P. minutus* preferring the lizard as its natural host, and *P. argentipes* preferring mammalian blood.

The protozoal and other parasites encountered in the dissection and examination of the above 811 flies were as follows:—

(a) A form resembling a Rickettsia in the rectum of a male *P. minutus*.

(b) On three occasions a typical Bodo in the rectum of male *P. minutus*; on two of which occasions the parasites were grown in Boeck's medium and gave cultures of a typical Bodo, with a large nucleus, a kinetoplast, a short anterior flagellum and longer trailing posterior flagellum, as seen under the dark ground and in stained preparations.

(c) On one occasion what appeared to be a spirochæte in the rectum of a male *P. minutus*, but which failed to shew up in the stained preparations from this material.

In none of the 811 sandflies examined as controls has anything resembling a natural herpetomonad been found; and this includes 407 *P. argentipes*, identified as such.

On the other hand, the eleven experiments were consecutive and included only one failure. In six of the flies the infections were surprisingly heavy. In experiment 7 a tentative diagnosis of kala-azar was made as the result of the sandfly feed, even before it had been confirmed by finding the parasite on examination of the patient's spleen puncture film.

*Fed Controls.*—In addition to examination of wild flies and of unfed laboratory bred ones, the following control experiments were carried out with female, laboratory bred out *P. argentipes*, fed upon persons not suffering from kala-azar:—

*Control Experiment (a).*—4th November 1924. Flies fed on a patient with malaria. 3 fed. One was dissected on the 7th, and 2 on the 8th. No protozoa found.

*Control Experiment (b).*—5th November 1924. Flies fed upon a patient suffering from dysentery. 3 fed, and were dissected on the 9th. No protozoa found.

*Control Experiment (c).*—7th November 1924. Flies fed upon a patient with asthma. 11 fed, and were dissected on the 12th. No protozoa found.

*Control Experiment (d).*—15th November 1924. Flies fed upon a case of rat bite fever in hospital. 3 fed, and were dissected on the 19th. No protozoa found.

*Control Experiment (e).*—16th November 1924. Flies fed upon the same patient as in Experiment (d). One fed, and was dissected on the 20th. No protozoa found.

*Control Experiment (f).*—17th November 1924. Flies fed upon a patient with malaria. 8 fed, and were dissected on the 20th. No protozoa found.

*Control Experiment (g).*—18th November 1924. Flies fed on a patient suffering from endocarditis. 17 fed, and were dissected on the 22nd. No protozoa found.

Thus no protozoa and no herpetomonads were found in any of the 46 female *P. argentipes* fed upon patients suffering from diseases other than kala-azar. These 46 may be added to the previous 811 control flies,

bringing up the total number of the controls to 857.

The conclusion appears to be irresistible that this herpetomonad is the herpetomonad form of *L. donovani*. If this is so, the gut of the female *P. argentipes* forms a cultural environment for *L. donovani* far and away more favourable than any artificial culture medium that we know, and very much better than the mid-gut of the bed bug.

This report, however, is of a purely preliminary character only, and it is not intended to lay down any hypothesis as to the mode of transmission of kala-azar. The observed facts are recorded in the hope that they may be of use to other experimental workers in the field. It only remains to add that, during the period covered by these investigations, the wet bulb thermometer readings were approximately as follows:—

*Experiments 1 and 2.* 20th to 30th September, 80° F. with a standard deviation of less than 1° F.

*Experiments 3 to 7.* 23rd October to 1st November, 70° F. to 75° F.

*Experiments 8 to 11.* 10th to 19th November, 65° F. to 75° F.

Our most grateful thanks are due to Major H. W. Acton, I.M.S., for constant help and assistance. Also to Lt.-Colonel S. R. Christophers, C.I.E., I.M.S., Director of the Kala-azar Commission, in association with which the ancillary enquiry is being carried out, for his very kind permission to publish this preliminary note in the *Indian Medical Gazette* in order to ensure its early publication.

#### Conclusions.

1. In ten out of eleven consecutive experiments, female laboratory bred *P. argentipes*, fed upon the parasite-containing blood of kala-azar patients, shewed typical herpetomonad forms in the fore-gut or mid-gut at the third to the fifth day after the feed. No less than 25 out of 56 such fed flies shewed herpetomonads, and in six instances the infection was a heavy one, although the patient's blood films had shewn only scanty *L. donovani* present.

2. On dissection and examination of 811 control sandflies, including both *P. minutus* and *P. argentipes*, the only natural infections encountered have been once a Rickettsia, three times a Bodo, and once a doubtful spirochæte. Nothing resembling a herpetomonad has been encountered.

3. On dissection and examination of 46 other control female *P. argentipes* fed upon persons not suffering from kala-azar, no protozoa were found.

4. It is concluded that *L. donovani* passes into its flagellate form in the gut of *P. argentipes* under suitable conditions of atmospheric temperature and humidity. Any further conclusions with regard to the kala-azar transmission problem, however, are at present carefully avoided. What is wanted is further work, which is now in progress, rather than speculation.



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## ANKYLOSIS OF JOINTS.\*

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I AM showing to-night a series of cases which are examples of a class of surgical work for which there is almost unlimited scope in India. One can hardly drive down a busy street of any Indian city without seeing some examples of crippled limbs, the result of disease or injury, which are capable of restoration to useful function by orthopædic methods. It is work which consumes a great deal of time, involves repeated operations, and necessitates infinite care and attention to small details of treatment if success is to be attained. The results, however, are so striking and gratifying, both to patient and surgeon, that they are well worth the trouble involved. I have often been told that Indian patients will not submit to prolonged treatment and repeated operations, and to a certain extent this is true, so that it is advisable to take the shortest cuts which will lead to a reasonably good functional result, rather than to adopt more tedious methods which may possibly in the end give a more perfect result. My personal experience is that these patients will submit to prolonged treatment, provided that it is not too painful, and that they have visible evidence of steady progress. It is this latter point which is so important and which makes it imperative to study each case as an individual problem and to select such methods as will provide easily demonstrable improve-

ment from the very start of treatment. In this branch especially the psychology of the patient must be considered, and the great value of a few convalescent cases walking about the ward in caliper splints to impress the new comers must be over-emphasised.

In Europe, prior to the war, joints ankylosed in bad position were not very common, as care was taken that, when a joint had perforce to be allowed to ankylose, it did so in good position. When ankylosis in faulty position occurred it was usually the result of neglected tuberculosis, and the methods of dealing with the condition had become stereotyped and gave satisfactory results. In India, however, we have to deal with neglected cases of other inflammatory and traumatic conditions besides tuberculosis, and it was not easy to obtain satisfactory guidance as to how to proceed in these cases. The war, however, gave a tremendous impetus to orthopædic surgery, and the study of enormous numbers of cases of infected compound fractures and infective arthritis led to great improvements in methods of dealing with stiff joints. Among those who have contributed to the recent advances in orthopædic methods the name of Sir Robert Jones stands out pre-eminent; it is impossible to over-estimate the debt which this branch of surgery owes to him; no matter what kind of orthopædic case one undertakes to treat, one will find that some original idea or method of his holds the field and is almost universally accepted.

*Classification.*—The old classification of ankylosis of joints into "true" and "false," according as to whether they were dependent on intra-articular fibrosis or extra-articular contracture, has given place to a much more elaborate classification, based on the true pathology of the condition and differentiating the various structures which may be involved, from the skin which may be contracted from ulceration or the healing of septic wounds, down to the bones which may be ankylosed, deformed by a mal-united fracture or obstructed in their movements by masses of callus. This classification is both accurate anatomically and useful for teaching purposes, but in practice it is difficult to apply, as the majority of the cases present lesions which fall under two or more headings. A slight modification of the old classification, which I find useful clinically, leaves old fractures and dislocations on one side for special treatment, and divides up stiff joints as follows:—

1. Cases in which the pathological process (disease or injury) started outside the joint. This comprises all cases of contractures of skin and muscle and periarticular fibrosis, the results of septic compound fractures. The primary attack will have to be made on the structures outside the joint, the adhesions in the latter being light do not usually present

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