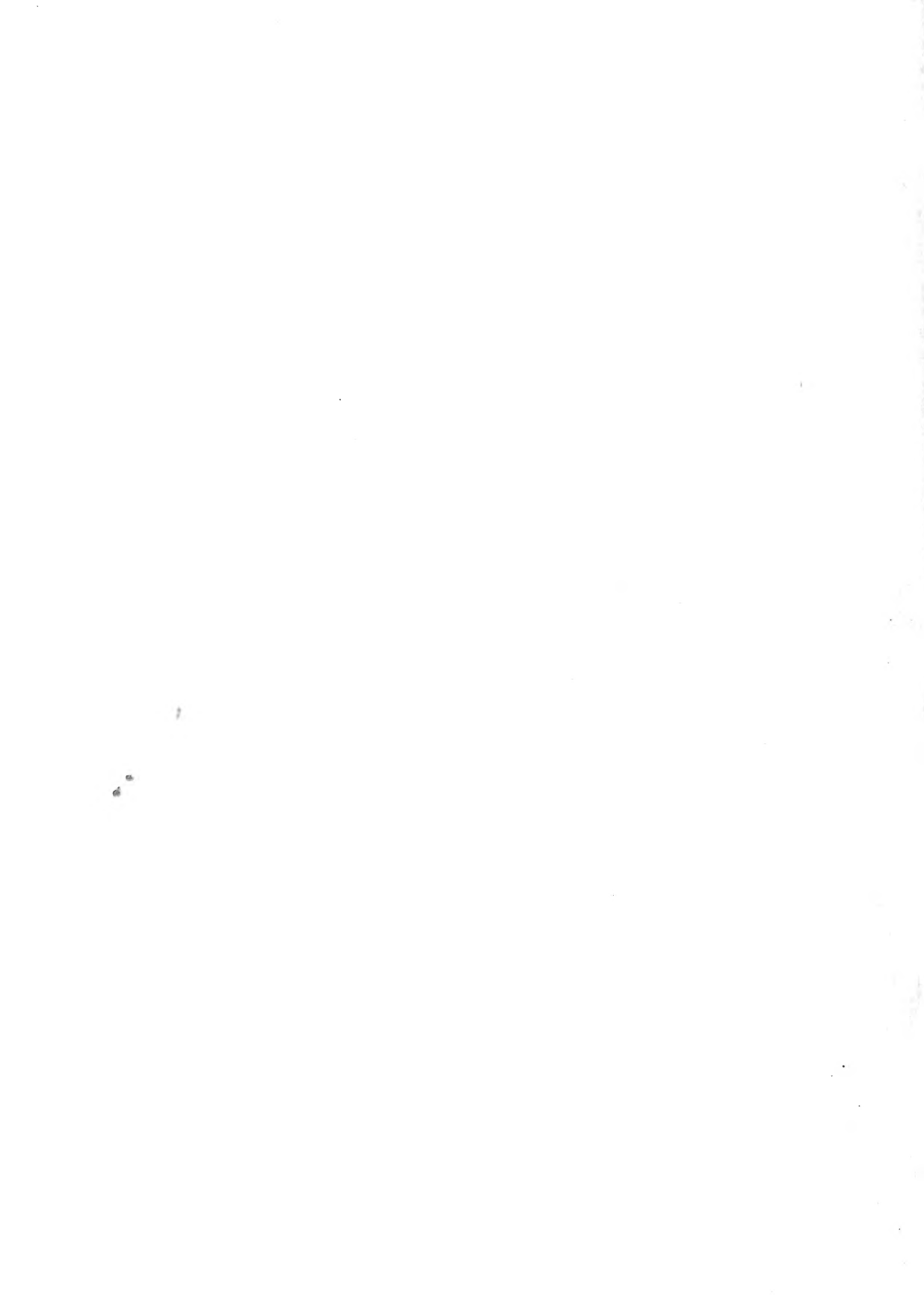


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ANNALS OF TROPICAL MEDICINE
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(THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND
PARASITOLOGY

ISSUED BY THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

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VOLUME VI

(March 29, to December 30, 1912)

With twenty-six plates, forty-four figures in text, and eighteen charts

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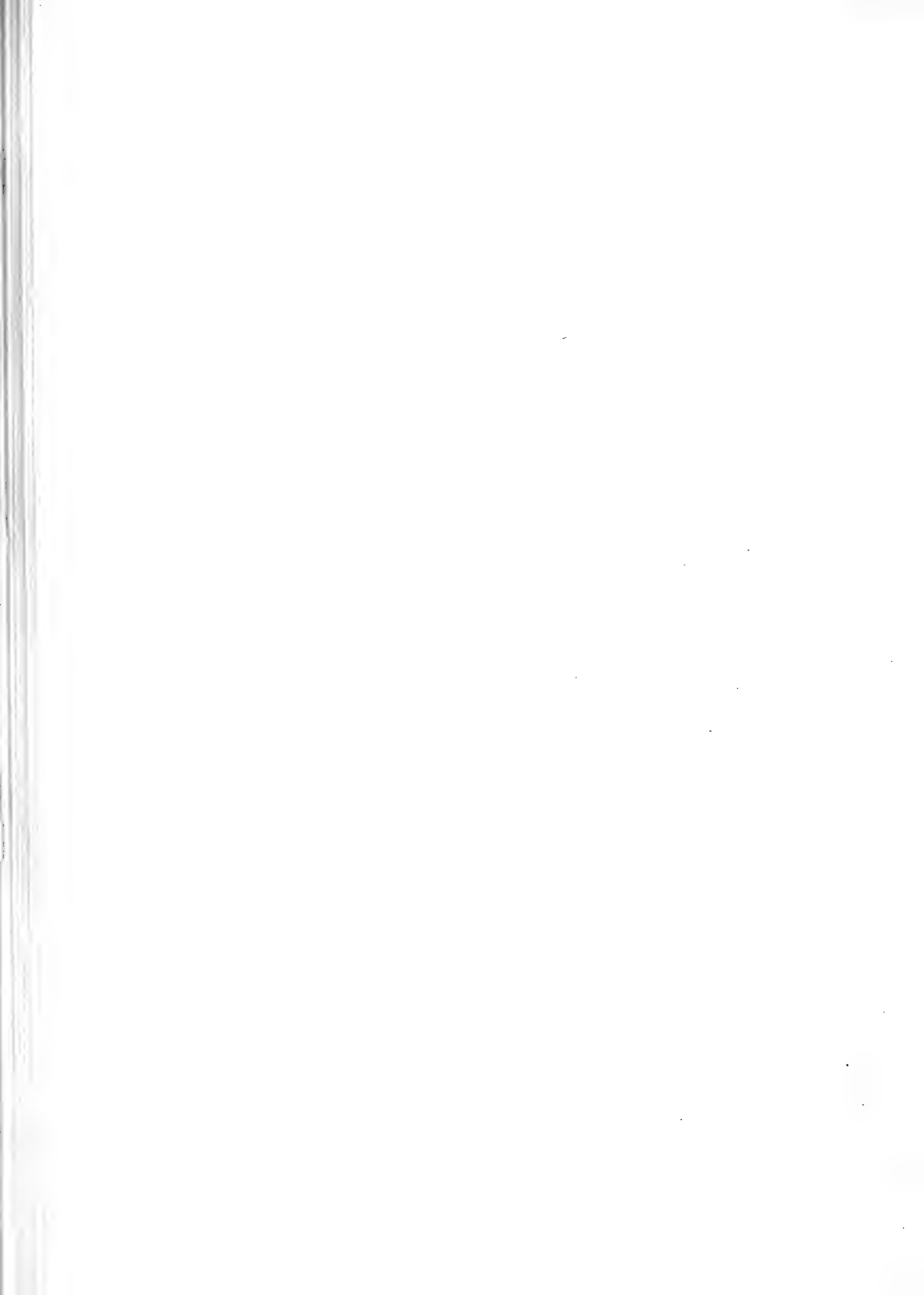
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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is Part A of the first number of the sixth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.



ON THE TRANSMISSION OF HUMAN TRYPANOSOMES BY *GLOSSINA MORSITANS*, WESTW.; AND ON THE OCCURRENCE OF HUMAN TRYPANOSOMES IN GAME

BY

ALLAN KINGHORN, M.B. (TORONTO),

AND

WARRINGTON YORKE, M.D. (LIVERPOOL)

(*First interim report of the Luangwa Sleeping Sickness Commission, British South Africa Company*)

(*Received for publication 3 March, 1912*)

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I. INTRODUCTION

The experiments detailed below, on the transmission of human trypanosomes by means of *Glossina morsitans*, Westw., have been carried out at Nawalia, N. Rhodesia, situated approximately 12° 25' S., and 32° 2' E., on the right bank of the Nyamadzi River, a tributary of the Luangwa. In a direct line, the laboratory is about fourteen miles west of the Luangwa River, and lies at a height of about 150 feet above the level of that stream, which is, in this locality, roughly 1,900 feet above the sea.

Owing to various unforeseen circumstances, including a delay in

the receipt of equipment, it was impossible to commence work until the middle of June, 1911, so that during the greater portion of the time covered by the experiments the meteorological conditions have been those of the dry season, notably a high mean temperature, combined with a very low percentage of relative humidity. These conditions obtained until the end of November, when the rains broke.

A further delay in the completion of our results has been caused by the fact that during the hotter months the breeding flies died comparatively rapidly, and produced, also, a large percentage of abortions.

II. STRAIN OF HUMAN TRYPANOSOMES EMPLOYED

Quite recently, Stephens and Fantham (1910) have described some peculiar morphological features which they observed in a strain of trypanosomes derived from the Luangwa Valley, the chief of which consisted in a markedly posterior displacement of the macronucleus.

The animal reactions of the same strain, worked out by Bevan (1911), Yorke (1910), and others, have shown it to be particularly virulent for all the animals employed, and accordingly, on morphological and pathogenic grounds, the species *Trypanosoma*

* In Stephens' and Fantham's original paper they write: 'It may be stated at once that the peculiarity of this Rhodesian trypanosome is that among the short or stumpy forms some have the nucleus at the posterior (non-flagellar) end the position of this posterior nucleus varies. Starting from the stumpy forms in which the nucleus is in the middle, we have all transitions up to that in which the nucleus is actually terminal and posterior to the blepharoplast.' While in the Transactions of the Society of Tropical Medicine and Hygiene for November, 1911, they say: 'He quotes Bevan as stating "that he has noted that in short forms the macronucleus may be situated slightly posterior to the centre of the parasite" With regard to this neither we nor anybody else would attach importance to this fact alone the nucleus may actually lie on the posterior side of the blepharoplast. This we regard as the essential characteristic.'

If this second statement is intended to limit the meaning to be attached to the term 'posterior nucleus,' it appears to us that some confusion may result. The meaning which has been attached by one of us (W.Y.) to the term in other papers is that conveyed in Stephens and Fantham's original paper, and that is our interpretation in the present communication. Forms in which the macronucleus is actually posterior to the blepharoplast are of extremely rare occurrence, in our experience. On the other hand, those forms in which the macronucleus is displaced within the posterior quarter or fifth of the body of the trypanosome so that it comes to lie in contiguity to the blepharoplast, are of very common occurrence, often forming an appreciable percentage of the parasites found in an animal's blood on certain days. This displacement constitutes, in our opinion, the chief morphological peculiarity of this strain. Such forms have not been described, as yet, in any of the mammalian trypanosomes other than the human strains obtained from the Luangwa Valley and Nyasaland. We except, of course, *Trypanosoma transcaaliense*, which could give rise to no confusion.

rhodesiense was created by Stephens and Fantham (1910). A similar parasite has been described from a case of human trypanosomiasis originating in Nyasaland by Stannus and Yorke (1911).

The exact relationship which this trypanosome bears to *Trypanosoma gambiense* is still an open question, and without discussing the validity of the species, we desire at present simply to record the fact that we have inoculated rats from twelve cases of human trypanosomiasis, eleven of which were isolated from villages in the Luangwa Valley, for the most part situated on the main roads, and in every instance have observed the peculiar displacement of the nucleus already mentioned. The animal reactions of such of these strains, on which work has been done, agree with those of the Armstrong strain, as described by Yorke (1910).

It will be understood, therefore, that in our transmission experiments the strains of human trypanosomes utilised answer in all respects to the description of *T. rhodesiense*.

III. TRANSMISSION OF THE TRYPANOSOME

A. BY LABORATORY-BRED *Glossina morsitans*.

Certain general conditions which attach to all the experiments may be mentioned.

The identity of the flies has been controlled both by direct examination of the external characters, and by the preparation of the male genitalia, as recommended by Newstead (1911), so that we can state with some degree of confidence that we have been dealing only with *Glossina morsitans*, Westw.

All the experimental animals have been kept in fly-proof cages, the fronts of which were protected by a double layer of wire gauze, the inner composed of coarse, and the outer of mosquito meshing. The two layers were separated by a space of one inch in order to obviate the possibility of an animal being bitten while pressing its body against the front of the cage.

The feeding of the flies, and the changing into fresh bottles daily was done personally by one of us. The flies were kept in such a manner that they had no opportunity of obtaining food from other than the animals used in the actual experiments. Each fly was

preserved in a separate bottle, and had a special number, so that an exact history of its life, the number of meals it had, the animals on which it fed, and other particulars, were available.

Experiment 1. Commenced August 20th, 1911.

It is somewhat difficult to tabulate this experiment, owing to the fact that it was not started with a definite number of flies. Between August 20th and September 29th, twenty-six flies had hatched out, and each, as it did so, was given its first meal on an animal showing numerous parasites in the peripheral blood, so that on any given date the periods which had elapsed since the infecting feeds of the flies varied considerably. In Table 1 the main facts in connection with the flies are given.

Twenty-four hours after each fly had fed on an infected animal, it was afforded an opportunity of feeding on a clean monkey (No. 41); after forty-eight hours on a second (No. 42); and from the third day onwards on a third (No. 52). The schedule of feedings is given in Table 2.

From this table it will be seen that neither of the first two monkeys on which the flies were allowed to feed became infected, whereas No. 52 did so on the 27th of September.

A reference to Table 1 will show that up to, and including, the 26th of September, twenty-three flies had fed on an infected animal more than three days previously, and had, accordingly, been fed on Monkey No. 52. The three flies A 24, 25, and 26, had never fed on this animal, and therefore had not to be considered in the attempt to isolate the infected fly. Moreover, six of the flies, A 1, 3, 9, 11, 13, and 20, had died prior to the 26th of September, and of these, three proved to be negative throughout on examination. The other three, namely A 3, 11, and 20, were found to show a heavy intestinal infection of trypanosomes. Fly A 3 died on September 6th, A 11 on September 12th, and A 20 on September 13th, while the monkey did not become infected until September 27th, much too long an incubation period for one of these flies to have been the infecting one. We have additional proof for this conclusion in that the abdominal contents (gut + salivary glands) of flies A 3 and 20, on inoculation into monkeys, did not determine an infection.

On the 26th of September there were, then, twenty flies with which to deal, amongst which was at least one infected one. As

TABLE 1.—Giving date of infecting meal, date of death, and duration of life after infecting meal.

No. of fly	Date of infecting feed	Date on which fly died	Duration of life from date of infection
A 1	20/8/11	22/9/11	33 days
A 2	21/8/11	20/10/11	60 "
A 3	25/8/11	6/9/11	12 "
A 4	26/8/11	23/10/11	58 "
A 5	27/8/11	28/10/11	62 "
A 6	27/8/11	17/10/11	51 "
A 7	28/8/11	11/10/11	44 "
A 8	29/8/11	11/11/11	74 "
A 9	30/8/11	12/9/11	13 "
A 10	31/8/11	20/10/11	50 "
A 11	31/8/11	12/9/11	12 "
A 12	3/9/11	27/9/11	24 "
A 13	3/9/11	5/9/11	2 "
A 14	5/9/11	28/9/11	23 "
A 15	6/9/11	25/10/11	49 "
A 16	7/9/11	4/11/11	58 "
A 17	8/9/11	3/10/11	25 "
A 18	8/9/11	14/10/11	36 "
A 19	9/9/11	19/10/11	40 "
A 20	9/9/11	13/9/11	4 "
A 21	13/9/11	23/10/11	40 "
A 22	16/9/11	14/11/11	59 "
A 23	17/9/11	27/10/11	40 "
A 24	25/9/11	3/11/11	39 "
A 25	26/9/11	29/9/11	3 "
A 26	26/9/11	29/10/11	33 "

stated above, three flies, A 24, 25, and 26, had never fed on Monkey No. 52, so that the enquiry was limited to seventeen, and this was further reduced, by the death of flies A 12 and 14 on

TABLE 2.—Showing transmission of human trypanosomes by laboratory-bred *Glossina morsitans*.

Date	Animal	No. flies fed	Result	Remarks
Aug. 21-Sept. 18 ...	Monkey 41	5*	Negative	Flies fed 24 hours after infecting feed
.. 22 .. 28 42	14*	..	Flies fed 48 hours after infecting feed
.. 23 .. 26 52	23	Infection	Flies fed 72 hours and onwards after infecting feed
	.. 68	5	Negative	(a) Infecting feed over 30 days before
Sept. 27 and 28 69	6	..	(b) Infecting feed between 20 to 30 days before
	.. 70	5	Infection	(c) Infecting feed less than 20 days before
Oct. 4 72	16	..	All the flies fed
.. 4	White rat 77	16	..	All the flies fed
.. 5	Monkey 68	14	Negative	Infecting fly did not feed
.. 6-9 58	16	Infection	All the flies fed
.. 9 61	1	..	Infecting fly, only, fed
.. 11-12 68	14	..	Infecting fly fed on 13th and 14th as well
.. 13-16 69	13	Negative	Infecting fly did not feed
.. 16-19 83	12	Infection	Infecting fly commenced feeding on 16th, others on 17th
.. 21-28 69	10	Negative	Infecting fly did not feed
.. 29-Nov. 11 56	4	..	Infecting fly did not feed

* The remaining flies refused to feed.

September 27th and 28th—both flies negative on examination—to fifteen. These were accordingly split into three groups, based on the length of time which had elapsed since the date of the

infecting feed, and each group was allowed to feed for two days on a clean monkey, Nos. 68, 69, and 70.

Group (a). Infecting meal over thirty days previously.

Group (b). Infecting meal between twenty and thirty days previously.

Group (c). Infecting meal less than twenty days previously.

Of the three monkeys, No. 70 was the only one to become infected, and the transmitting fly was thus located in Group (c), consisting of A 19, 21, 22, and 23.

While waiting to ascertain which of the three monkeys would become infected, all the flies were fed from September 29th to October 3rd on Monkey No. 72, and on October 4th on White Rat No. 77. Both of these animals became infected in due course.

On October 5th all the flies, with the exception of A 19, were re-fed on Monkey No. 68, and from the 6th to the 9th all were fed on Monkey No. 58, except on October 9th, when Fly A 19 alone was fed on Monkey No. 61. Of these animals, No. 68 did not become infected, while Nos. 58 and 61 did. The Fly A 19 (σ) was thus proved to be the infecting one.

No particular interest attaches to the further experiments. From the Table it will be seen that all those animals on which Fly A 19 fed became infected (Nos. 68 and 83), whereas those on which it did not feed remained quite healthy (Nos. 69 and 56).

When it had been definitely ascertained which was the infecting fly, it was possible to determine fairly accurately the duration of the cycle of the trypanosomes in the insect. Fly A 19 had its infecting meal on September 9th, and Monkey No. 52, the first to become infected, showed trypanosomes in the peripheral blood on September 27th. On the 26th, therefore, the last day on which the flies were fed on this animal, a period of eighteen days had elapsed since A 19 fed on the infected animal. The average incubation period of the local strain of human trypanosomes in monkeys is four or five days, and subtracting this from the eighteen days, it is evident that the fly must have become infective in thirteen days.

This fly, A 19, lived for forty days from the date of the infecting meal, and between the time of becoming capable of transmitting the parasite and the date of death, had fed on eight animals, all of which became infected.

The other flies were fed continuously from the date of the possible infecting meal to that of death, which occurred at varying periods from two to seventy-four days, but none of them became infective.

Experiment 2. Commenced November 14th, 1911, with sixteen laboratory-bred *Glossina morsitans*.

In this experiment the flies were infected directly on a case of Human Trypanosomiasis, each fly being allowed to feed on one occasion only. Ten fed on the 14th of November, when the patient showed three trypanosomes per field in the blood, and the remaining six on the 15th, when there was, on an average, one trypanosome to seven fields (Zeiss Oc. 4, Obj. DD.).

The subsequent meals are shown in Table 3.

TABLE 3.—Showing transmission of human trypanosome by laboratory-bred *Glossina morsitans*.

Days after infecting feed	Animal	No. flies fed	Result	Remarks
1st to 5th	White rat 116	15	Negative	
6th to 11th	" 118	15	"	
11th to 15th	" 124	15	Infection	
16th to 20th	" 116	13	"	
21st to 22nd	" 118	12	"	
23rd to 27th	Monkey 137	6	Negative	Flies divided into two groups to separate the infective one
" "	" 138	5*	Infection	
28th to 42nd	" 148	Varied	Negative	

* One fly of this group (6) refused to feed, and died on the 26th day of the experiment.

Rat No. 124 became infected on December 4th, five days after the flies had fed last, and as the incubation period of the trypanosome in these animals, on an average, is five days, it seems probable that the fifteenth day was the one on which the infecting fly became capable of transmitting the parasite.

On the 7th of December (twenty-third day after infecting meal), the twelve flies then alive were divided into two groups, in order to effect an isolation of the infective one, and were fed on Monkeys

Nos. 137 and 138, as indicated in the Table. On December 12th, the fly numbered B 23 (♂) died, and on examination proved to be heavily infected throughout the alimentary canal, and in the salivary glands. No infection of the proboscis, however, was observed.

The other flies were fed on a clean monkey until the forty-second day, but without any result.

B. BY 'WILD' *Glossina morsitans*

Experiment 3. Commenced on November 14th, 1911, with ninety-eight 'wild' flies.

Prior to infecting these flies with the human trypanosome, they were fed for three days, November 14th to 16th, on a healthy monkey (No. 95), and for the next four days on a native fowl. The monkey never became infected. From the 21st to the 24th of November the insects then alive, fifty-seven in number, were fed on an infected monkey showing twenty to thirty trypanosomes per field in the peripheral blood, and were afterwards fed on healthy animals, as in Table 5.

TABLE 5.—Result of feeding 'wild' *Glossina morsitans* on clean monkeys, after a preliminary meal on an animal infected with the human trypanosome.

Days after first infecting feed	Animal	No. flies fed	Result	Remarks
4th to 6th	Monkey 125	48	—	Monkey died on 7th day
7th to 9th	" 127	41	Negative	
10th to 13th	" 130	34	Infection	
14th to 16th	" 119	31	"	
17th to 18th	" 140	7	—	Monkey died on 19th day
17th to 23rd	" 141	1	Infection	Flies divided into two groups to isolate infective ones
20th to 25th	" 144	4	"	

This experiment was finished after the flies had fed on the twenty-fifth day, the flies being then killed and embedded.

The duration of the cycle of the parasites in the flies, in this instance, would appear to be slightly over eleven days. The first

infecting meal was taken on November 21st, and Monkey No. 130 showed parasites in the peripheral blood on December 7th, a difference of sixteen days. As stated already, the incubation period of this trypanosome in monkeys is about five days, and by subtracting this from the sixteen days, we obtain eleven for the duration of the cycle.

IV. TRANSMISSION OF THE HUMAN TRYPANOSOME BY *GLOSSINA MORSITANS*, IN NATURE

The following experiments were two of a series undertaken to ascertain what varieties of trypanosomes were being transmitted by *Glossina morsitans*, in Nature. In these, varying numbers of freshly-caught flies were fed on monkeys immediately on reaching the laboratory, and while, in several cases, it was found that monkeys were successfully infected by the bites of such flies, these two experiments were the only ones in which the parasite concerned corresponded to the human organism.

Experiment 4. Commenced October 30th, 1911, with sixty freshly-caught flies, to which were added twenty-two additional ones on the next day. The flies were fed as indicated in Table 6.

TABLE 6.—Showing the transmission of the human trypanosome by naturally-infected *Glossina morsitans*.

Date	Animal	No. flies fed	Result	Remarks	
Oct. 30-Nov. 4	Monkey	96	60 - 22	Infection	
Nov. 6	"	125	29	"	
Nov. 7-10	"	128	19	"	
Nov. 11-12	"	113	7	Negative	Flies divided into two groups to isolate the infected fly.
"	"	114	6	Infection	

On November 13th, the thirteen flies still alive, were killed and embedded. In the sections, numerous parasites were found in the gut and salivary gland of only one of them.

Experiment 5. Commenced January 7th, 1912.

In this experiment, all the 'wild' *Glossina morsitans* brought into the laboratory from day to day were fed on the one monkey, No. 210. This animal became infected on January 16th, and, allowing four days only as the incubation period of the parasite (the average, as stated earlier, is five days), must have been infected on the 11th or 12th. Including the 12th of January, 269 flies had fed on the monkey.

The conclusion that the trypanosome transmitted by the flies in these experiments is the human one, is based on (1) the morphology, and (2) the animal reactions.

(1) MORPHOLOGY.

This is identical with that of the strains we have isolated from infected natives, and with that of the strain described by Stephens and Fantham (1910). It shows the same marked dimorphism; short, non-flagellated forms, with the decided posterior displacement of the macronucleus, and the peculiar arrangement of the granules in the anterior portion of the body, viz., a row along either side of the trypanosome with a clear strip of protoplasm intervening; and long, free-flagellated forms, many of them of the prominent 'snout' type. The measurements, also, correspond with those of known human strains.

TABLE 7:—Comparison of the measurements* of the 'fly' trypanosome with those of strains obtained from cases of Sleeping Sickness.

Strain	No. measured	Length in microns		
		Average	Maximum	Minimum
Human	50	21.32	32.25	13.27
'Fly'	50	22.58	36.25	14.5

* The method we have adopted in measuring the trypanosomes is essentially that described by Bruce. The blood smears were dried in the air, fixed in absolute alcohol, and stained with Giemsa. Five hundred parasites, in each case, taken as they came, were drawn with the camera lucida at a magnification of 2000 diameters, and the length measured along the middle line. Only 25 were drawn from a preparation made on any one day, and dividing forms were not included.

The curves obtained by plotting out the distribution of the various lengths, expressed in percentages of the number measured, will be found in the chart on page 20.

(2) ANIMAL REACTIONS.

Table 8 gives a synopsis of the course of the disease in the animals.

TABLE 8.—Pathogenicity of the 'fly' trypanosome.

	Animal	Incubation period in days	Duration in days
Monkey	96	6	47
..	105	4	54
..	108	4	9
..	114	6	42
..	210	4	—
Rat	103	3	24

The number of animals is very small, but is sufficient to demonstrate the virulence of the strain.

DISCUSSION OF THE RESULTS

In these transmission experiments, there are at least three sources of error which must be considered, (1) accidental infection of the experimental animals by other than the experimental flies, (2) hereditary transmission of trypanosomes from infected female flies to their progeny, and (3) natural infection in the experimental animals.

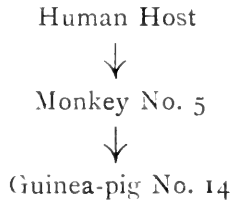
(1) With regard to the first of these, the conditions under which the experimental flies and animals were kept have been mentioned already, and it seems more than improbable that accidental infection would account for the unfailing regularity with which the animals became infected after the infective flies had fed on them. Moreover, in all our experiments to date, well over 200, such an occurrence as the unexpected infection of an animal has not been observed.

(2) The number of bred flies which we have been able to obtain has been too small to permit us to examine many of them prior to use in the experiments, but such as were, have been found uniformly free of infection. Stuhlmann (1907), Kleine (1909), and Bruce

(1911, a) with his colleagues, have examined large numbers of bred flies belonging to the species *Glossina brevipalpis*, *Glossina morsitans*, and *Glossina palpalis*, and are unanimous in the opinion that a hereditary transmission of trypanosomes does not occur amongst the tsetse flies.

(3) With reference to the third point, we have used in the course of our work well over 100 monkeys, and have never yet seen a naturally-occurring trypanosome infection in any of them. *Plasmodium kochi* has been observed occasionally, but beyond this, nothing. Experiment 2 was specially devised to obviate the possibility of error through the use of local monkeys (*Cercopithecus pygerythrus*). The flies, as they were obtained, were fed on healthy, imported rabbits, which showed no signs of infection throughout; they were infected directly from the human host; and they were then fed on white rats.

The genealogy of the strain used in Experiment 1 was as follows:—



The monkey, before inoculation, had been carefully watched for fifteen days, and during this time had showed no signs of an infection, while the monkeys Nos. 41, 42, and 52, on which the flies were afterwards fed, had been examined daily for a week before they were used in the experiment, and had thus been proved to be quite healthy.

The trypanosomes transmitted by these bred flies were identical with the human one, both morphologically and in their animal reactions.

There are certain points in connection with the experiments which appear to be worthy of emphasis. The number of bred flies which has been used in each is strikingly small, very much more so than in any other similar work of which the records are available. In the two experiments a total of forty-two was employed, and of

these two only became infective, a percentage of 4.76, or, considering only the number (twenty and fifteen) which lived over the duration of the cycle of the parasites in the insects, a percentage of 5.71.

With regard to Experiment 3, in which wild flies were used, no definite statement can be made as to the percentage infected, other than at least two were transmitting the trypanosome out of a total of fifty-seven. Freshly-caught flies have been shown to be transmitting other species of trypanosomes, and the finding of an intestinal or salivary gland infection in a fly which had not actually been proved to be transmitting the human parasite, would therefore be of no particular significance.

The time occupied by the trypanosomes in completing their cycle in the flies is also strikingly short, approximately two weeks (thirteen, fifteen, and eleven days). That of *Trypanosoma gambiense* in *Glossina palpalis* is ordinarily over twenty-one days, and in Taute's (1911) recent work with *Glossina morsitans*, the same time was taken by the trypanosome with which he was working. Kleine's (1909) experiments with the same species of *Glossina* were without result, though a very large number was used, and the experiments continued over a long period of time.

How to account for these different results is a problem which immediately presents itself. Are they due to different meteorological conditions? Or are they due to the use of different strains, or species, of human trypanosomes? Kleine's experiments were made on the shores of the Victoria Nyanza, at an altitude of 3,700 feet; Taute's on Lake Tanganyika, the height of which is 2,680 feet; and ours at Nawalia, at an altitude of, roughly, 2,000 feet; while the results may be epitomised as failure, flies infective after twenty-one days, flies infective after eleven to fifteen days. In this connection, we hope to have an opportunity of repeating the transmission work on the Congo-Zambesi watershed, which varies between 4,000-5,000 feet above sea-level.

So far as our results go, we have seen no indication of late infection in any of our flies, although some of them have lived as long as seventy-four days after the potentially infecting meal.

All our results go to show that mechanical transmission of the trypanosomes does not occur, that is, if a period of twenty-four hours has elapsed since the infecting meal. We have not made any

experiments to learn whether infection could be accomplished by interrupted feeding. This has been proved with various insects, but practically would account for very few, if any, cases of the disease.

The infective flies have been found to retain the power of transmitting the parasites during life, and do not require to feed more than a single time on any animal in order to infect it, neither do they require, prior to becoming infected, to feed more than once on an animal suffering from trypanosomiasis.

Our investigations on the manner in which an infective fly transmits the disease are incomplete, but seem to indicate that the explanation advanced by Bruce is the correct one. The infective flies have been found, both by direct examination of the various organs, and by cutting sections of the whole abdomen, to harbour trypanosomes in the gut and salivary glands. Three flies in Experiment 1, which died four, twelve, and twelve days after the infecting meal, were found to contain parasites in the gut, but the abdominal contents (including the salivary glands) of two of these, dead after four and twelve days, when injected into monkeys, did not result in infection. On the other hand, a portion of the anterior gut only of an infective fly, containing many trypanosomes, determined an infection in a white rat six days after inoculation. This would tend to corroborate Bruce's statement that the flies are non-infective until the cycle of the parasites has been completed. With the exception of the three flies mentioned, in addition to the permanently infected ones, all the others, dissected as they died, have been uniformly free of infection in the gut, salivary glands, and proboscis. An infection of the proboscis has never been observed in any of the bred flies which were transmitting the trypanosome.

Experiments 4 and 5 are of particular interest in that they dispose of the criticism that the successful transmission of the trypanosome in the laboratory does not necessarily mean that it occurs in nature. In all, 1,340 freshly-caught *Glossina morsitans* have been fed on nine monkeys, and while infection resulted in several, only two groups proved to be transmitting the human organism.

V. OCCURRENCE OF THE TRYPANOSOME IN GAME

The possibility that game might act as a reservoir of infection in Sleeping Sickness areas has been recognised almost since the inception of work on the disease, but up to the present it would appear that the trypanosomes have never been demonstrated in such animals under natural conditions. In Uganda, Bruce, Hamerton and Bateman (1911) have proved that certain species of buck, notably waterbuck, bushbuck, and reedbuck, can readily be infected with *Trypanosoma gambiense* by allowing infected *Glossina palpalis* to feed on them, and that healthy flies, in turn, may be infected from diseased game. They were unable, however, to examine a sufficiently large number of head to ascertain whether a natural infection was present.

The importance of the question is obvious, and the results of our investigations on the point afford a striking commentary on the potential danger involved in the infection of the game.

The Luangwa Valley is particularly rich in a widely-varied fauna, and owing to the fact that in the dry season the great bulk of the game tends to collect in the vicinity of the few permanent streams, it has been comparatively simple to shoot buck for the purposes of experimentation. The animals examined, and the findings, are given in Table 9.

It will be seen from this Table that ninety-eight animals, comprising nineteen genera, have been examined directly, and that inoculations have been made from fifty. The sub-inoculated animals have lived sufficiently long to enable us to determine their susceptibility to the various game trypanosomes.

In this vicinity there appear to be at least three well-differentiated trypanosomes affecting game: one which is closely allied to *Trypanosoma pectorum*; a second, to which monkeys and rodents are refractory, recalls, morphologically, *Trypanosoma vivax*; and a third which is identical, morphologically and in its animal reactions, with the human parasite isolated from local cases of the disease.

From the fact that *Trypanosoma vivax* has been found, it will be apparent that, by the use of monkeys and rats for the initial inoculations, a source of error in the exact estimation of the

percentage of game harbouring trypanosomes has been introduced. This has been unavoidable, owing to our inability to procure clean sheep or goats for the purpose. The percentage of game, 30·6,

TABLE 9.—Results of examination of game for trypanosomes.

Animal	Number examined	Number found infected by direct examination of blood	No. of inoculations	Number of positive inoculations in which parasites were found in peripheral blood	Number of positive inoculations in which no parasites were found in peripheral blood	Total number found infected by direct examination and by inoculation
1. Elephant (<i>Elephas africanus</i>)	1	0	1	0	0	0
2. Rhinoceros (<i>Rhinoceros bicornis</i>)	1	0	1	0	0	0
3. Hippopotamus (<i>Hippopotamus amphibius</i>)	1	0	0	0	0	0
4. Zebra (<i>Equus burchelli</i>)	3	0	2	0	0	0
5. Roan (<i>Hippotragus equinus</i>)	5	1	1	0	0	1
6. Wildebeest (<i>Connochaetes taurinus</i>)	2	0	1	0	0	0
7. Kudu (<i>Strepsiceros kudu</i>)	7	3	3	1	1	4
8. Hartebeest (<i>Bubalis liechtensteini</i>)	2	0	1	0	1	1
9. Waterbuck (<i>Cobus ellipsiprymnus</i>)	26	15	14	5	1	16
10. Puku (<i>Cobus vardoni</i>)	17	1	6	0	0	1
11. Mpala (<i>Aepyceros melampus</i>)	18	1	11	1	1	2
12. Bushbuck (<i>Tragelaphus scriptus</i>)	7	4	4	1	1	5
13. Bushpig (<i>Potamochoerus chaco-potamus</i>)	2	0	1	0	0	0
14. Warthog (<i>Phacochoerus aethiopicus</i>)	6	0	3	0	1	1
15. Lion (<i>Felis leo</i>)	2	0	0	0	0	0
16. Hunting dog (<i>Lycacon pictus</i>)	1	0	1	0	0	0
17. Giant rat (?)	1	0	0	0	0	0
18. Genet (<i>Genetta rubiginosa</i>)	2	0	0	0	0	0
19. Squirrel (?)	1	0	0	0	0	0
Totals	98	25	50	8	6	31

found to be infected with trypanosomes, with the means at our disposal, represents, therefore, the minimum for the country around Nawalia.

We reserve a discussion of the two varieties first mentioned for a future date, and shall deal here only with that one which appears to be the human parasite. Of the fifty buck from which inoculations were made, eight have proved to harbour this organism, a percentage of 16.

In Table 10 the animal reactions of the strains obtained from the various buck are given.

TABLE 10.—Pathogenicity of the *human* trypanosome from various species of game.

Animal	Subinoculations	Incubation period	Duration of infection
Waterbuck 1	Monkey	71	6 days
	"	82	11 "
	"	99	4 "
	"	117	6 "
	Rabbit	79	4 "
	Guinea-pig	80	11 "
Waterbuck 2	Rat	81	3 "
			21 "
Waterbuck 3	Monkey	170	5 "
	Rat	160	5 "
Waterbuck 4	Monkey	201	6 "
	Rat	178	6 "
Waterbuck 5	Monkey	181	7 "
	Rat	213	5 "
Hartebeest	Monkey	112	8 "
	"	120	7 "
	"	150	4 "
	"	168	5 "
	Rat	128	4 "
Mpala 1	Monkey	169	5 "
	Rat	157	6 "
Mpala 2	Monkey	199	4 "
	Rat	176	5 "
Warthog	Rat	195	5 "
Native Dog	Monkey	131	5 "
	Rat	129	4 "

For the purpose of comparison, we give the incubation period and duration of infection in similar animals inoculated from known human strains.

TABLE 11.—Comparison of the pathogenicity of the *human* trypanosome from game with that of two strains obtained from cases of Sleeping Sickness.

Animal	Trypanosome from game		Local human trypanosome		'Armstrong' strain	
	Incubation days	Duration days	Incubation days	Duration days	Incubation days	Duration days
Monkey 14	4-11	7-4	2-7	4-42	3-5	8-14
Rabbit 1	4	3	4	16-61	3-14	19-45
Guinea-pig 1	11	23	12-19	65-81	3-15	39-82
Rat (8)	3-6	11-46	2-8	15-43	1-7	6-45

The figures in parenthesis refer to number of each animal inoculated from game.

The extreme virulence of the strains derived from buck is most marked, and the correspondence between these reactions and those of known human strains is equally pronounced.

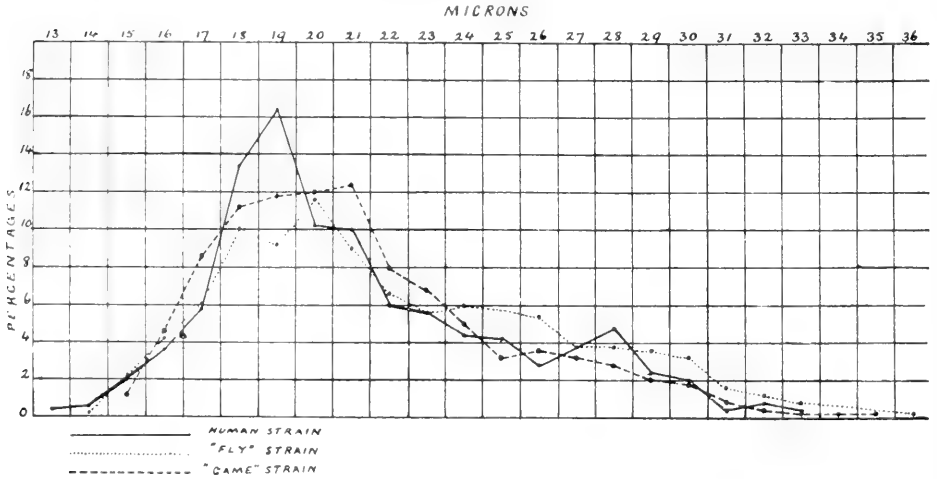
The measurements of the game strain of parasites are given in Table 12.

TABLE 12.—Comparison of measurements of *human* trypanosome from game with those of a known human strain.

Strain	No. measured	Length in microns		
		Average	Maximum	Minimum
Human	500	21.32	32.25	13.27
'Game'	500	21.35	35.5	15

The curves obtained by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total number measured, is given in the Chart for the human, 'game,' and 'fly' strains.

CHART, giving the percentages of the various lengths of the parasites encountered in an examination of 500 of each of the three strains, human, 'game,' and 'fly.'



The very striking resemblances in these curves is apparent at a glance, and a further similarity in the three strains of trypanosomes is evidenced by a comparison of the percentages of the 'stumpy and short,' 'intermediate,' and 'long' forms mentioned by Bruce (1911,^b) in connection with *Trypanosoma brucei*.

TABLE 13.—Comparison of the percentages of 'short,' 'intermediate,' and 'long' forms of the human, 'game,' and 'fly' strains.

Strain	Short forms 13-21 μ	Intermediate forms 22-24 μ	Long forms 25-36 μ
Human	62.4	16	21.6
'Game'	61.8	19.8	18.4
'Fly'	52.2	18.2	29.4

The curves of the strains which we have given above correspond more or less to that of *Trypanosoma brucei*, and it may be asked whether the parasites we have isolated from game, and from the animals infected by naturally-infected 'wild' flies, are not identical with this trypanosome. A comparison of the curves, and of the

percentages of the 'short,' 'intermediate,' and 'long' forms with those of *Trypanosoma brucei*, shows that there is a marked tendency for the trypanosomes to collect towards the 'short' and 'long' poles in the case of *Trypanosoma brucei*, whereas in our strains the bulk of the parasites are disposed at the 'short' pole.

TABLE 14.—Percentages of 'short,' 'intermediate,' and 'long' forms of two strains of *Trypanosoma brucei**.

Strain	Short forms 13-21 μ	Intermediate forms 22-24 μ	Long forms 25-36 μ
<i>T. brucei</i> , Zululand ...	53.0	5.5	41.5
<i>T. brucei</i> , Uganda ...	39.3	21.8	38.9

* Compiled from tables in Reports of S.S. Comm. Roy. Soc., No. 11, 1911.

This distinction is, perhaps, rather a fine one, more particularly when one considers the comparatively small numbers of parasites from which the curves have been constructed.

We might state here that we are of the opinion that to be of any real value such curves must be plotted out from the measurements of a large number of trypanosomes, more especially when dealing with a dimorphic parasite of the human type, and we regret that time has not permitted us to measure a larger number than we have done. In any experimental animal infected with the human trypanosome, the proportion of short and long forms found in the blood on any one day is not equal; on one day practically nothing but short forms may be seen, while on another, only long forms may be present. It is obvious, therefore, that unless a limited number only of parasites be measured on any one day, and unless other preparations on later days be utilised, a source of error may be introduced, and false conclusions drawn.

Reference has already been made to the morphology of the human trypanosome in connection with that of the 'fly' strain. With regard to that of the 'game' strain, there is nothing to add except that it is identical with the other two, both in fresh and stained preparations. When the parasites are plentiful in

sub-inoculated animals, the short forms, with no free flagellum, exhibit the same posterior displacement of the nucleus, and the same arrangement of the granules, while among the long, free-flagellated forms, individuals of the 'snout' type are of frequent occurrence.

The presence, among the short forms, of parasites displaying the marked nuclear displacement, is confined, so far as our present knowledge extends, to the human strains originating in this part of Africa, and we must therefore conclude that these strains derived from the fly and game, exhibiting as they do the same morphology, measurements, and animal reactions, are identical with the local human trypanosome.

In addition to the game, the parasite was isolated from a native dog, found to be naturally-infected, in a village some fifty miles from the laboratory, where, so far as we are aware, the human disease has not been diagnosed.

VI. SUMMARY

1. The human trypanosome, in the Luangwa Valley, is transmitted by *Glossina morsitans*, Westw.
2. Approximately 5% (4.76) of the flies may become permanently infected, and capable of transmitting the virus.
3. The period which elapses between the infecting feed of the flies and the date on which they become infective, is approximately fourteen days.
4. An infected fly retains the power of transmitting the disease during its life, and is infective at each meal.
5. Mechanical transmission does not occur if a period of twenty-four hours has elapsed since the infecting meal.
6. Some evidence exists to show that in the interval between the infecting feed and the date on which transmission becomes possible, the parasites found in the flies are non-infective.
7. *Glossina morsitans*, in nature, has been found to transmit the human trypanosome.

8. Certain species of buck, viz., waterbuck, hartebeest, mpala, and warthog, have been found to be infected with the human trypanosome.

9. A native dog has been found to be infected with the human trypanosome.

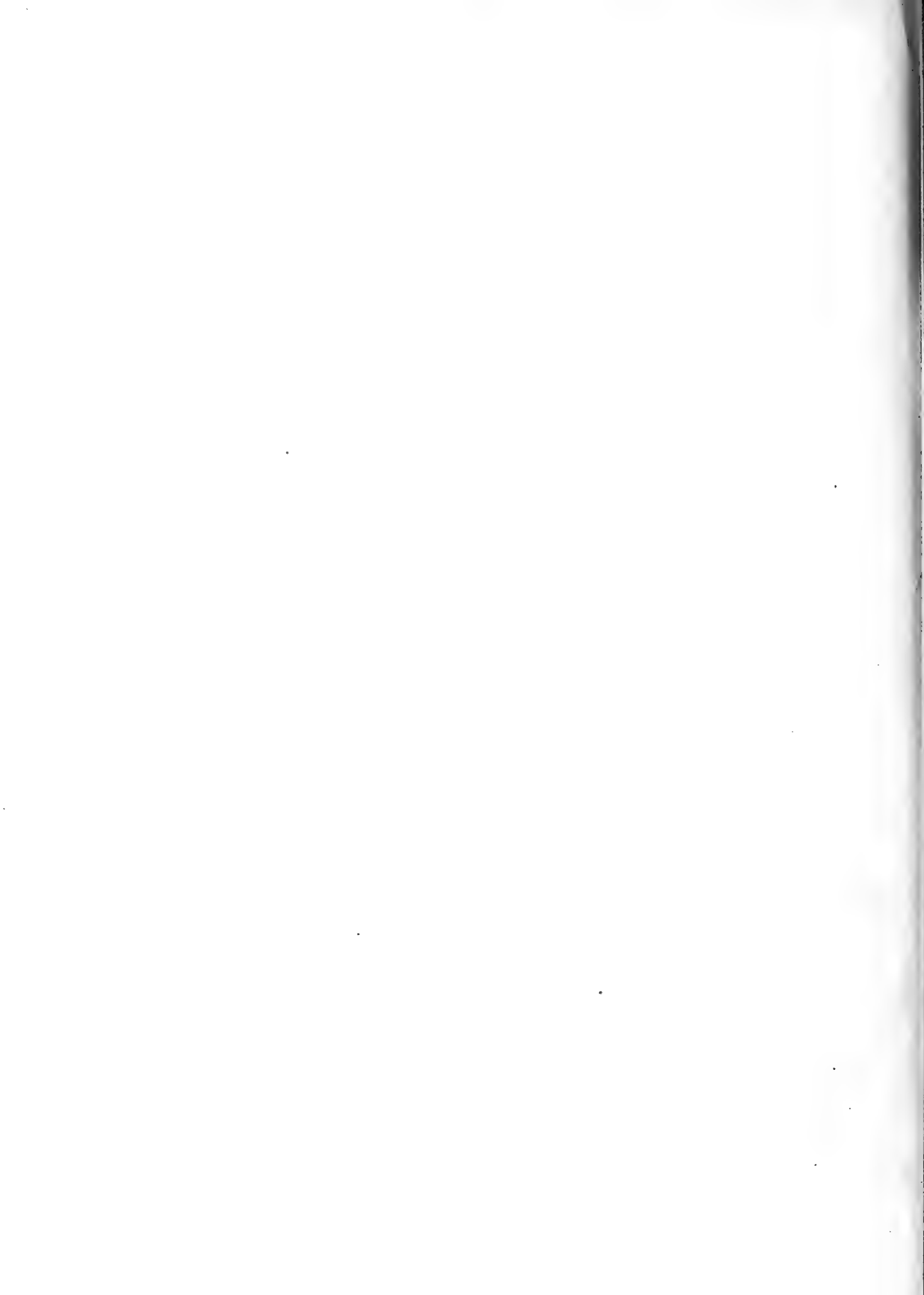
In conclusion, we desire to express our thanks to Dr. A. F. Wallace, M.O., N. Rhodesia, and to Mr. Ll. Lloyd, Entomologist to the Administration, for assistance during the course of the experiments.

NAWALIA, N. RHODESIA,

18 *January*, 1912.

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HERPETOMONAS PEDICULI, NOV. SPEC.,
PARASITIC IN THE ALIMENTARY
TRACT OF *PEDICULUS VESTI-
MENTI*, THE HUMAN BODY LOUSE*

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(Received for publication 24 November, 1911)

PLATE I

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INTRODUCTION

The organism which forms the subject of this paper is a small flagellate Protozoön occurring in the alimentary tract of the body louse, *Pediculus vestimenti* (*P. corporis*). The flagellate belongs to the genus *Herpetomonas*, and is, I believe, now recorded for the first time. I propose for it the name *Herpetomonas pediculi*, using the name *Herpetomonas* in the sense of Saville-Kent (1881), the founder of the genus.

The parasite was first seen by me nearly three years ago, when working in Cambridge. At that time, material being scanty and

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apparently difficult to obtain, there seemed to be no special reason for recording the presence of yet another species of *Herpetomonas*, though the possibility of lice acting as carriers of disease was realised. Other researches were then in progress, but I have given *H. pediculi* intermittent attention ever since I discovered it. Lately it has been suggested that flagellates belonging to the genera *Herpetomonas* and *Crithidia*, occurring in the digestive tracts of blood-sucking insects, are really stages in the life-history of vertebrate trypanosomes. In consequence of the importance of the subject in relation to the transmission of trypanosomes, and of the increasing attention devoted to leishmaniasis (or human herpeticomoniasis), I have resumed my study of *Herpetomonas pediculi*, and have found its complete life-cycle.

I hope to show that, although *Herpetomonas pediculi* might easily be confused with dangerous parasites transferable to man by the bite of the insect hosts, yet it is really a harmless flagellate of the gut of the louse. Bred lice, fed on my own blood, have been used in the research.

MATERIAL AND METHODS

The hosts of *Herpetomonas pediculi* were common body lice, *Pediculus vestimenti*. The lice were originally obtained from the bodies of infested children, and from verminous clothing recently removed from the body. Some of the lice so obtained were dissected, and found to contain *Herpetomonads*. The adult lice were isolated into glass tubes, containing small fragments of white woollen material. They clung to the material, and excrement from them adhered to it. Examination of the said excrement in a very few cases showed non-flagellate stages of the parasite. Lice, thus known to be infected, were then paired, as also were lice that were apparently uninfected. The parent lice were kept in closed tubes in contact with my body, and were fed twice daily, either on my arm or on the back of my hand. They fed greedily, often evacuating faeces as they fed, and at times even unaltered fresh blood. The eggs from these lice were kept also in tubes, and, when the larvae hatched, they were treated in the same way as the adults.

Detailed examination was made of the eggs, larvae, and adult

lice, and it was found that bred lice from parents known to be infected were parasitised by *H. pediculi*. Though clean lice bred clean, the young ones became infected when they came in contact with the faeces of infected lice.

Again, faeces known to be infected were smeared on spots at which uninfected young lice were allowed to feed. Three days later, control insects showed no flagellates or other form of the parasite, while those fed at infected spots contained active flagellates when dissected.

The sole food supply of the bred lice was my own blood. Cultures of my blood on Mathis' modification of Novy and MacNeal's medium have never yielded any flagellates of any sort.

The percentage of infected lice is not a high one—I have dissected as many as fifty lice at a time without finding *Herpetomonads*—nor can the infection be described as heavy; three hundred *Pediculi* were examined, of which twenty-five were found to be infected. Much time has been devoted to the examination of fresh material, which is absolutely essential if the life-cycle is to be followed with exactitude. The gut of the louse was divided into small serial portions, each of which was finely teased and examined in physiological salt solution. Observation of the living organisms was supplemented by examination of thin smears of the gut contents, teased intestinal wall, and other organs, and long search was made for possible intracellular stages.

Wet fixation with osmic vapour, followed by absolute alcohol, was used, and coloration with Giemsa's or other modification of the Romanowsky stain. Very dilute solution of methylene blue was of use as an *intra vitam* stain.

It may be noted that lice dissected when several hours had elapsed since the last feed showed the parasites better than recently-fed ones. The presence of much blood in the gut interferes with the study of the living organism, as well as adds to the difficulty of staining.

OCCURRENCE OF THE PARASITES IN THE HOST

Herpetomonas pediculi exhibits three typical stages in its life-history—the pre-flagellate, flagellate, and post-flagellate stages. So far the examination of the mouth parts of the lice has shown no

parasites. The pre-flagellate stage of the parasite occurs chiefly in the oesophagus and proventriculus of the adult louse, and throughout the alimentary canal of the immature insect or larva. The flagellate stage is found at its best in the mid-gut of the mature louse, while the extreme intestinal and rectal portions of the gut contain the post-flagellates. The latter are also found in the excrement. The late larva may contain a few flagellate parasites.

Detailed examination was made of the reproductive organs and ova of the lice, but no parasites were found therein. The possibility of the hereditary infection of the lice with *H. pediculi* was, in my opinion, excluded so far as the specimens that I examined were concerned. The parasites have not been found in organs other than those of the alimentary tract. The diverticula of the alimentary canal apparently are uninfected.

MOVEMENTS

Movements of *H. pediculi* are most easily observed in the fully developed flagellate stage. They are also very vigorous during division, but as the object of the said movements then is to complete the fission of the organism, they are of a somewhat exceptional character.

Usually movement is brought about by waves of contraction followed by relaxations passing down the body, while progression is aided by the lashing of the flagellum, which is forwardly directed. The type of movement is somewhat euglenoid, and the occasional concentration of the cytoplasm into the anterior end produces a 'peg-top' effect that is very characteristic. The progression is somewhat spasmodic, consisting of alternate slow movements and rapid darts forward, accompanied by a slight rolling from side to side. Reversal of the direction of motion is easily accomplished. The organism either swings as a whole in a semi-circle, the posterior end acting as a centre of rotation, or the flagellum bends back parallel to the body, which then swings suddenly in a semicircle, and so comes to lie in a straight line with the flagellum, after which the parasite moves away in the opposite direction from what it was traversing previously.

The flagellum often lashes vigorously, and when obstacles are

encountered appears to test them by touching them rapidly in different spots.

Rotatory movements occur when the *Herpetomonas* becomes fixed to *débris* in the lumen of the gut. The organism lashes its flagellum violently, and the flagellum describes a series of circles and spirals, producing marked currents in its neighbourhood.

A very common movement is that of partial rotation of the body of *H. pediculi*. The posterior end of the body remote from the nucleus is chiefly concerned, and this portion often twists over, so that the dorsal surface becomes ventral, and *vice versa* (Plate I, fig. 10). Sometimes a small cluster of parasites may be observed, twisting simultaneously and producing a general shimmering effect.

Entanglement of two organisms by their flagella is sometimes seen. The movements of the parasites then are most violent. On one occasion I saw the flagellum of a small parasite torn from its body by the vigorous movements of a much larger *Herpetomonas* with which it had become entangled. Such intensely vigorous movements as the last-mentioned are rare.

LIFE HISTORY IN BRIEF OF *HERPETOMONAS PEDICULI*

It is of interest to trace the course of the development of the parasite throughout its life in one host. The following development has been observed in the living organism, and afterwards corroborated by examination of stained preparations.

In the excrement of lice infected with *H. pediculi* are small, oval bodies, well adapted for resisting desiccation or other adverse condition. Similar bodies occur in the hind gut of the lice, where they are formed before passing out with the excrement. Other lice, feeding at spots contaminated by their predecessors, ingest some of these small oval post-flagellates, which consist of a 'varnish-like' thin cyst wall, enclosing some granular protoplasm, a nucleus and a blepharoplast (Plate I, figs. 27-29). Such cysts may also be ingested by larval *Pediculi*. Passing with fresh blood into the fore gut of a new host, the post-flagellates commence a new development, and as this leads to the formation of the flagellate, it has been termed the pre-flagellate stage (fig. 1).

At first round or oval, the parasite rapidly commences to

elongate, the end first lengthening becoming, as a rule, the flagellar end of the organism. At this period, a somewhat more refractile area can be seen in life, and the finely granular chromatophile contents of this area concentrate, forming a thread which ultimately reaches the surface. The thin ectoplasm of the parasite is pushed forward still more by the thread, which ultimately becomes free of the body and protrudes as a short flagellum (fig. 2). The flagellar origin is the chromatophile area, or so-called 'flagellar sac,' which is usually in the neighbourhood of the blepharoplast, which, in the fully developed organism, is always anterior to the nucleus. Growth of the non-flagellar end is continuous, and by the time that the flagellum has reached its full length, the non-flagellar (or posterior) end has elongated and become fully developed.

During the actual flagellate stage (figs. 8-18) little in the way of actual development occurs, but the vital activities of the organism are displayed by vigorous multiplication. Increase in numbers in *H. pediculi* takes place by longitudinal division. The parasites about to divide seem to grow broader just prior to the act, and to become more granular. The first indication of division is shown by the concentration of the substance of the blepharoplast into two masses, which are connected together by a very narrow neck. The dumbbell-like body so formed (fig. 19) gradually separates into two distinct blepharoplasts, placed slightly obliquely, one on either side of the body (fig. 20). The intra-cellular part of the flagellum (or rhizoplast) commences to divide just after the blepharoplast, and the split appears to extend forwards. Concentration of the nuclear material occurs simultaneously, and the nucleus becomes constricted, usually in the median line and parallel to the long axis of the body, but occasionally markedly to one side. The constriction deepens, and ultimately two nuclei are produced. These migrate to the sides of the organism, and fission of the general cytoplasm commences (figs. 20, 21). The flagella lash about very much at this time, and their vigorous action aids in the separation of the daughter organisms. The latter gradually diverge until they come to lie in a straight line, and finally become separated from one another at the apex. The fission is practically always followed by active swimming movements of the daughter organisms. Consequent on this great activity of the daughter forms immediately after division,

rosettes of Herpetomonads, due to repeated longitudinal division and non-separation of the resultant parasites, are exceptional. Division is best seen in the mid-gut of the louse.

After a series of longitudinal divisions, resulting in the production of a number of flagellate individuals, a reaction sets in, and the parasite prepares for life outside the body of its host. As the Herpetomonad passes backwards into the very dark semi-digested blood in the hind gut of the louse, the chromatin of its flagellum dwindles, and appears to be absorbed (figs. 22-25). The cytoplasm concentrates around the nucleus, to which the blepharoplast also is drawn nearer. The parasite becomes more or less rounded or oval, and proceeds to secrete a thin, gelatinous wall, which rapidly hardens to a 'skin-tight' coat around the organism, which, in this sense, may be said to encyst (figs. 26-28). Thus prepared and protected, the oval bodies, now known as post-flagellates, pass from the gut of the host, mingled with the dejecta, to recommence the life-cycle if ingested by a new host.

MORPHOLOGY

A. THE PRE-FLAGELLATE STAGE

The pre-flagellate stage (figs. 1-4) of *H. pediculi*, at its earliest, takes the form of small oval or rounded bodies, measuring 6μ to 7μ by 4μ to 5μ . They have a marked resemblance to the Leishman-Donovan bodies. The pre-flagellate shows a thin ectoplasm and endoplasm containing refractile granules. The nucleus (fig. 1) is oval, or occasionally rounded, while the deeply staining blepharoplast (kinetic nucleus) may be bar-like, oval, or occasionally rounded, and is well marked (figs. 1, 2). The position of the blepharoplast varies somewhat, as would be expected in a developing organism. It may lie to the side of the nucleus or above it, and occasionally the blepharoplast is apposed to the nucleus. The chromatophile area (fig. 1) from which the flagellum differentiates is also present. Division occurs in the pre-flagellate stage (fig. 5), more especially when the organism is beginning to elongate, and possesses a short flagellum. Isolated chromatoid granules are sometimes present in the pre-flagellates (fig. 2). The appearance of inter-

mediate forms (figs. 2-4, 6, 7) between the pre-flagellate and the flagellate has been indicated in the section dealing with the life-history.

B. THE FLAGELLATE STAGE

The flagellate form (figs. 8-18) of *H. pediculi* is an active organism relatively small compared with other Herpetomonads, its body length varying from 11μ to 26μ in the specimens examined. The inclusion of the flagellum doubles the length of the organism, for the flagellum itself may be 30μ in length (fig. 16). The cytoplasm of the organism is finely alveolar (figs. 8-18), and very refractile in life. Chromatoid granules (figs. 15, 18) are present in some cases. The protoplasm rarely presents marked vacuoles in stained preparations, but in life a clearer area is sometimes seen near the origin of the flagellum.

The nucleus (figs. 8-18) is round or oval, and crowded with very fine granules. A karyosome is seen in some cases (figs. 11, 15), but is not visible in all, doubtless being masked by the numerous fine granulations present. A nuclear membrane apparently occurs, but is not so chromatic as when the nucleus is of a vesicular type. The blepharoplast (kinetic nucleus) may be oval or rod-like, lying transversely across the body, or somewhat obliquely. Occasionally it is curved, and it often presents a bowed appearance prior to division. It stains deeply, taking a purplish tint with Giemsa. Before the onset of the multiplicative phase the blepharoplast presents no differentiation. The free flagellum tapers finely at its free end. It originates as a rhizoplast near the blepharoplast, and occasionally a minute basal granule can be distinguished with difficulty.

Aggregation rosettes. Just as division rosettes are infrequent, so aggregation rosettes, or clusters, are uncommon. It is noteworthy that the members of an aggregation rosette (fig. 13) may be of different sizes and ages. In such rosettes, the parasites either mass themselves around some food particle with which they are in contact by their flagella, or else several organisms intertwine their flagella and so form a sort of bouquet or ball of living organisms, all vibrating slowly from a common centre provided by their interlaced

flagella. These rosettes in time break up into the component units. One after another, the slow-moving organisms manage to detach themselves and swim away until the last two separate. The object underlying these simple rosette formations is not fully understood. Possibly it enables the flagellates to withstand better any currents in the gut, and so gives them a somewhat longer lease of life as flagellates, before encystment overtakes them.

C. THE POST-FLAGELLATE STAGE

The post-flagellate stages of *H. pediculi* (figs. 25-29) when fully formed are small bodies (less than the pre-flagellates) containing protoplasm and a nucleus, to which the blepharoplast may be apposed, or in which nucleus and blepharoplast can be distinguished as separate entities. The blepharoplast is often somewhat smaller than in the other phases of the parasite. The cyst wall is extremely thin, staining pinkish after Giemsa (figs. 27, 28). The blepharoplast is not always easy to demonstrate in stained preparations, but that it must be present is obvious when one has taken the trouble to watch the process of post-flagellate formation in the living animal, and has studied a series of stained preparations of intermediate forms (figs. 22-26). Cysts with thick, radially-striated walls (fig. 29) have very rarely been encountered, and I am inclined to think that the presence of the swollen gelatinous wall containing striations, and enclosing a parasite with chromatoid granules, is a sign of degeneration.

HERPETOMONAS PEDICULI* IS A NATURAL PARASITE OF *PEDICULUS VESTIMENTI

Recently much controversy has arisen from statements made to the effect that flagellates found in sanguivorous insects must be regarded as developmental stages of trypanosomes. Accordingly—ignoring the evidence of life-cycle and morphology—*H. pediculi* would be regarded by some as a phase of a trypanosome. Sweeping statements such as that quoted are rarely logical, and when they are based upon a series of speculations and single instances, instead of on an accumulation of facts, they are usually unsound.

While it is quite true that certain trypanosomes, e.g., *T. lewisi*, assume a *Herpetomonas*-like form in cultures, yet they are not then under exactly natural conditions. Further, they may be considered as reverting to the type from which it seems probable that they have originated, namely, primitive *Herpetomonads* which have undergone morphological changes and in process of time have evolved the trypanosome type when inoculated into the vertebrate host.

With regard to *H. pediculi*, I do not think that there is any doubt that it is a flagellate, natural to and parasitic in the insect host, and that it has no connection with a human trypanosome, pathogenic or non-pathogenic. In support of this conclusion, I cite the following facts and experiments:—

(1) At various times during the past three years I have fed lice on my blood from the time of hatching until they died. A tsetse fly transmitting *Trypanosoma gambiense* is at first limited in its period of infective inoculation. Lice might also be similarly limited, but, owing to the method of feeding adopted, no question of the lice not having fed at their infective period can be entertained. In spite of repeated feedings of lice, my blood shows no signs of trypanosomes, whether tested by ordinary microscopical examination of films, by thick films, or by cultural methods, and the period covered by the experiments is ample to have allowed of full development of trypanosomes, were *H. pediculi* a phase of one.

(2) Artificially infected lice have been fed simultaneously. The result of mass feeding surely should have been sufficient to produce some indication of trypanosomes, were any present. No such indications have been found, even after inoculation of my blood into susceptible animals like white rats. (Animals examined for six weeks after inoculation.)

(3) The experiment of inoculating rats with the contents of the gut of lice containing *H. pediculi* has given no positive results whatever. The rodents remained perfectly healthy, nor did cultures of their blood, or thick film examinations, yield any trace of trypanosomes.

(4) Cultures of the gut contents of infected lice showed no further stage in the life-history of the parasite.

(5) The methods of infecting larvae and adults of *P. vestimenti* with *H. pediculi* have been briefly indicated in a preceding section.

The same contaminative method of infection has been observed under natural conditions, and resembles that found in the case of some other insects, such as *Pulex irritans* (adult and larva), infected with *Crithidia pulicis* (Porter, 1911) and *Nepa cinerea*, harbouring *Herpetomonas jaculum* (Léger, 1902; Porter, 1909).

Further, the well defined development of *H. pediculi*, with its pre-flagellate, flagellate and post-flagellate forms, presents a cycle complete in itself, and there is no evidence to show that there is any connection with the life-cycle of any other organism.

Contamination of experimental *P. vestimenti* by feeding on other vertebrates has been rigorously excluded, so that no fallacious results can accrue from outside sources.

From the foregoing considerations, the conclusion obviously must be that if *H. pediculi* be a stage in the life-history of a vertebrate trypanosome, the said trypanosome should most probably be present in my blood, and should have revealed itself by now. Repeated cultures, thick-film blood examinations, and ordinary smears, examined continuously during this research, have all proved negative. Hence, all the evidence available points to the fact that *H. pediculi* is a parasite of the insect *Pediculus vestimenti*, and has no connection with any trypanosome of persons on whom lice may feed. Were such a trypanosome to exist, it is surprising that it has not been recorded ere this, considering the number of blood examinations undertaken in various scientific institutions.

Further, I do not think that *H. pediculi* has any connection with *Leishmania*, as no symptoms of leishmaniasis have developed in me, and England is a country free from the disease. However, the possible occurrence of such a natural *Herpetomonas* in lice must be remembered in experimenting with *Pediculi* as possible transmitters of *Leishmania*.

'Wild' lice—the term commonly used to denote lice that were not bred for purposes of investigation, but collected at random—obtained from several widely different districts in England, have also yielded the flagellates when dissected. Doubtless, were more lice available from other areas, some also would be infected. The inference is then, I think, fairly justified that *H. pediculi* occurs in a few body lice throughout England.

Some Continental authorities would, perhaps, place *H. pediculi*

in the genus *Leptomonas*. However, I have followed most English workers in considering that members of the genus *Herpetomonas* are really uniflagellate, as originally defined.

**NOTE ON THE BIOLOGY AND LIFE-HISTORY OF
*PEDICULUS VESTIMENTI***

The study of parasitic Protozoa demands a good knowledge of the life-history and habits of the host. In dealing with lice, great difficulty was at first experienced, as the literature on the subject is very scattered and unsatisfactory. Since the commencement of this research, a valuable paper by Warburton (1909) has appeared, which gives details as to the length of life of the lice, time of incubation, and rearing of the larvae. I can fully confirm all that Warburton has recorded.

The eggs of *P. vestimenti* vary in their incubation period. I found that while a few eggs hatched in four to five days, others matured as much as six weeks after laying. Warburton found the same kind of variation. The larvae were pale coloured, and fed as soon as they left the egg, if placed on the back of the hand. Moulting occurred every four days, the new skins being slightly darker than the previous ones. The larvae fed very greedily, and were much more active than the adults. When feeding, a larva has sucked blood for as long as twenty-five minutes, peristaltic waves being clearly visible in the gut the while. Usually ten to fifteen minutes' feed was sufficient.

The imaginal stage is attained about eleven to twelve days after hatching, sexual maturity about four days later. Copulation is intermittent, but frequent. In several cases it occurred shortly after feeding, particularly when the insects fed greedily, so that unchanged fresh blood occasionally passed from their bodies after the semi-solid digested blood *débris* had ceased to be voided. Egg-laying at the rate of four or five per day occurs during the rest of the life of the female, who is longer-lived than the male. Warburton found that the adult life of a male was about three weeks, that of a female four weeks. In my own experiments similar results were obtained, but I also found that the length of life was sometimes about a week less, in each sex.

The mode of feeding of adult *P. vestimenti* is of interest. After settling down on the hand, often clinging to the scrap of cloth on which they usually rest, a fairly sharp stab is made, and immediately the blood begins to flow into the alimentary canal, which becomes bright red. As feeding proceeds, the louse gradually raises its abdomen, until it is almost vertical in extreme cases. As fresh blood passes into the gut, defaecation occurs, much excrement being produced. If an attempt be made to remove a louse before it has finished its feed, the pull of the ring of hooks near the lower lip can be felt. Lice fed in a somewhat restricted area showed no hesitation in sucking blood at spots fouled by themselves or their neighbours. Adult lice would feed for twenty to thirty minutes. If feeding were neglected, the lice died in about three days. I found it necessary to feed them at least twice daily, though I have succeeded in keeping two females alive for three weeks when fed only once a day. Larvae perish if not fed within thirty-six hours of hatching, and even then there is great loss during the larval stage.

Lice are also very sensitive to changes of temperature. Body heat seems necessary for them, though eggs can withstand great extremes of temperature.

Death of *P. vestimenti* appears to occur very suddenly. I found that a fair number of those adults that died did so within a short time after a meal, their alimentary canals containing much unchanged blood.

Regarding the specific name of the body-louse there is much uncertainty. Neumann, in a recent paper (July, 1911), suggests that *P. vestimenti* is a sub-species of *P. capitis*, and would then be called *P. capitis vestimenti*. However, a discussion of such a difficult matter of nomenclature is quite outside the scope of this paper.

SUMMARY AND CONCLUSIONS

1. *Herpetomonas pediculi* is a parasite of the body-louse, *Pediculus vestimenti*. The parasite appears to be confined to the alimentary tract and faeces of its host (adult and larva), one phase of it having been recovered from the dejecta. The parasite is spread from louse to louse by the contaminative method, cysts of the parasite being swallowed by the insect. The whole life-cycle has been followed in the living material.

2. Movements of the flagellate are very rapid and somewhat spasmodic, and are easily accomplished by the aid of the flagellum. Rotatory motion and movements of flexion occur.

3. The parasite exhibits three well-marked developmental phases, united by a continuous series of intermediate forms:— (i) the pre-flagellate, which produces a flagellum and elongates (figs. 1-7), and becomes (ii) the flagellate (figs. 8-18), which, after a growing and multiplicative phase by longitudinal fission (figs. 19-21), forms (iii) the resting, 'encysted' post-flagellate form, adapted for extra-corporeal life (figs. 22-29).

4. Pre-flagellate stages, best found in the oesophagus and proventriculus of the louse, or in the larva, strongly resemble Leishman-Donovan bodies. They are 6μ to 7μ long and 4μ to 5μ broad. The nucleus and blepharoplast are well defined. A chromatophile area, from which the flagellum develops, is present.

5. The flagellate forms occur chiefly in the mid-gut. The body-length is from 11μ to 26μ in those I have examined. The cytoplasm is finely alveolar. The nucleus is round or oval, and the blepharoplast stains deeply. Aggregation rosettes of flagellates of various ages and sizes are occasionally found (fig. 13).

6. Post-flagellate forms are oval, usually provided with a 'skin-tight' cyst. The blepharoplast seems smaller than that of the flagellate or pre-flagellate. This stage is best observed in the rectum of the louse, and can be recovered from the faeces.

Radially striated, thick-walled cysts occur very rarely (fig. 29).

7. My experiments show that *H. pediculi* is not a stage of a vertebrate trypanosome, for I have fed the infected lice from the time of hatching to the time of death on my own body, and have made detailed examinations of my own blood by smears, thick films, and by cultures, as well as by sub-inoculations into white rats, none of which has ever given indication of trypanosomes during three years of experiments. Animals inoculated with *H. pediculi* from the gut of lice have also shown no parasites.

8. *H. pediculi* is a parasite of the louse, *Pediculus vestimenti*, and shows no connection with any vertebrate trypanosome. Also, it is not connected with *Leishmania*.

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EXPLANATION OF PLATE I

All figures outlined with Abbé-Zeiss camera lucida, after wet fixation with osmic vapour and absolute alcohol and staining with modified Romanowsky solution; 2 mm. apochromatic objective (Zeiss) and compensating oculars 8 and 12 used. Magnification 1500 diameters.

Figs. 1 to 7 represent pre-flagellate and intermediate forms of *H. pediculi* from the digestive tract of the larva or the fore-gut (oesophagus and proventriculus or anterior lobe of the stomach) of the adult *Pediculus vestimenti*.

In fig. 1 note the chromatophile or pink-staining finely granular area from which the flagellum arises.

In figs. 2, 3, 4, 6, 7 note the gradual lengthening of the flagellum, and elongation of the body.

Fig. 5 represents a late pre-flagellate organism dividing.

Figs. 8 to 18 show flagellate parasites from the stomach and anterior part of the intestine (or mid-gut) of the adult louse.

Fig. 8. Young flagellate.

Fig. 10. Flagellate showing body twisted or folded over about the middle of its length.

Fig. 13 represents an aggregation rosette, or cluster of flagellates, of different sizes and ages.

Figs. 14, 15, 16 show parasites whose bodies are thrown into characteristic undulations. Note the chromatoid granules shown in fig. 15.

Figs. 17, 18 represent stout flagellates from the anterior part of the intestine. The latter figure shows the stoutest parasite seen during the research. The parasite contained chromatoid granules.

Figs. 19 to 21 represent dividing forms.

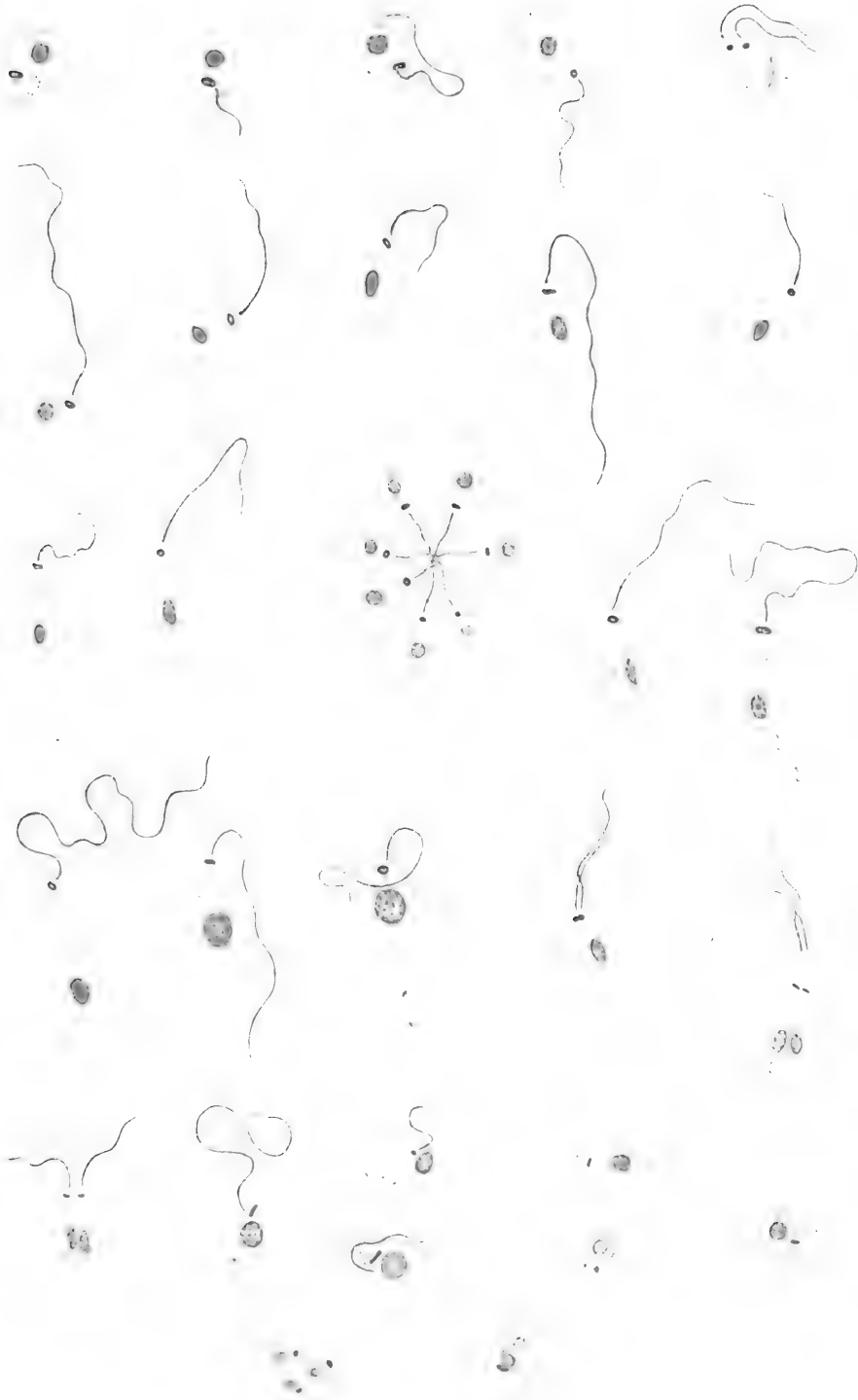
Figs. 22 to 25 show stages of the parasite leading to post-flagellates and cysts (figs. 26-29), as seen in the hinder part of the intestine, including the rectum and the faeces.

In figs. 22-25 note the gradual shortening and absorption of the flagellum, and the contraction and rounding of the body. In fig. 25 only the rhizoplastic part of the flagellum remains. Chromatoid granules occur in these parasites.

Figs. 27 to 29 represent truly encysted forms, as found at the extreme posterior end of the gut, or voided in the faeces with semi-solid, black blood remains.

In figs. 27, 28 the cyst-wall is thin and varnish-like, and closely apposed to the parasite. It stains pink after Giemsa.

Fig. 29 represents a gelatinous, thick-walled cyst with striations. Such cysts were very rare.



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NOTE

Dr. Stannus wishes to make the following acknowledgments regarding the paper on "A Case of Human Trypanosomiasis in Nyasaland, with a note on the Pathogenic Agent," by Drs. H. S. Stannus and Warrington Yorke. (Annals of Tropical Medicine and Parasitology, Vol. V, No. 3 (1911), page 443.)

Through an oversight, failure was made to acknowledge our indebtedness to Dr. William Murray of Mvera, and to Dr. Meredith Sanderson in charge of the Sleeping Sickness Investigation, for clinical notes and record of temperature of the case therein recorded.

ON THE PRESENCE OF *LEISHMANIA* IN THE DIGESTIVE TRACT OF *ANOPHELES MACULIPENNIS*

BY

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(Received for publication 5 February, 1912)

PLATES II, III, IV

In a previous paper, published in the 'Lancet' (1911, *a*) and 'Pathologica' (1911, *b*), I have described the different methods employed in my researches on *Anopheles maculipennis*. I have in this paper given the reasons on which my statement was based that the Leishman bodies could live for a certain length of time, and in some cases multiply, in the digestive tract of the above mosquito. I also stated that I believed the results to be of great interest, as I had never found the Leishman bodies in smears (fixed in methyl-alcohol and stained with Giemsa) from the intestines of *Cimex lectularius* and *Pulex serraticeps* and *P. irritans*, which had, in my presence, fed on artificial cultures containing numerous parasites.

As regards *Pediculus capitis*, the conditions of experiment were rather unfortunate owing to the difficulty experienced in inducing the insects to feed on the cultures, and owing to the short time the insects live outside their natural habitat. I did not find any Leishman bodies in the smears made from a few of these insects (*P. capitis*), although they appeared to me to have taken up some of the cultures.

My experiments comprised several hundred, with numerous controls (i.e., with insects which had not been fed on cultures), in order to see what other parasites normally inhabit the intestine of *A. maculipennis*, knowing that flagellates had previously been found in the digestive tract of mosquitos.

As regards *A. maculipennis*, I have examined more than 300

smears, and I have convinced myself that the parasites (*Leishmania*) found by me could not have been mistaken for other similar parasites, as they can be distinguished by their nucleus, different staining, and generally much larger size. Besides, these flagellates, as far as I was able to ascertain, are rather scarce.

The reasons which lead me to believe that the Leishman bodies can live in the digestive tract of *A. maculipennis* are as follows:—

1. The conspicuous staining of the different parts of most of the parasites.

2. The frequent presence of large numbers of parasites.

3. The fact that, if the parasites had not been alive, they would in a short time have been destroyed by the digestive juices as well as by the numerous cocci, bacteria and other parasites found in the intestine.

Objection has been raised to my work on this subject, that the parasites found by me were well-stained degeneration forms. To say the least, it would be strange that degeneration forms should stain as well as the living organisms. A comparison between the parasites, illustrated in Plates II, III, IV, and drawn from stained preparations, and those of living cultures containing numerous parasites, will show no impaired development of the former. I do not deny that there may perhaps be degeneration forms amongst the bodies found by me—but does this never occur in cultures? Further, objection has also been made to my statement that the parasites were living in the digestive tract, that the mosquitos had, together with the parasites, also taken up some of the culture medium. It may be possible that the culture medium does assist the development of the parasites in the digestive tract of *A. maculipennis*, although I have not found this to be the case in *P. serraticeps* and *P. irritans*, which likewise had taken up the culture medium together with the parasites.

Nor is it possible that the *Leishmania* could live for a greater length of time in a culture medium outside the test-tube, or exposed to the action of bacteria. It has, therefore, to be admitted that there exist in the digestive tract of *Anopheles maculipennis* and other Culicidae including *Stegomyia fasciata*, special conditions favourable to the life of *Leishmania*, and that these conditions do not exist in the other insects experimented upon.

In all the experiments on the different insects, I have used well-developed cultures containing numerous parasites. It seemed to me that the number of the parasites found in the digestive tract of the mosquitos was greatest, and the forms most varied in cases where the insects were still partially filled with blood from a previous meal on man or on animals. In some preparations nuclear changes (spindles) were observed. Is it not reasonable to suppose that that which occurs when mosquitos have fed on cultures, will also happen when they feed on men or animals? If we suppose that the *Anopheles* is the transmitting agent of the disease, would it not also ingest the blood in which the parasites are contained? It is well known that these mosquitos are very bloodthirsty. As it has been suggested that the dog might be the intermediate host (though I doubt the correctness of this hypothesis since the percentage of infected dogs is too high in localities where Kala-Azar is extremely rare, at least in Italy), the mosquito would ingest the parasites together with blood, and the same would be the case in the transmission from man to man during the period in which the parasite is present in the circulation. The *Leishmania* thrive on nutritive media containing blood, and one may infer that the blood is a necessity for their life and development.

I believe I am justified in saying, now, after more numerous experiments, that the parasites not only live but also develop, which is evident from the figures in the Plates.

Plate III shows clusters of parasites and rosette forms; it has hitherto been doubted that development took place because these forms did not occur. According to my experience the groups and rosettes demonstrate very little, owing to the great tendency of the *Leishmania* to form clusters and adopt most varied shapes. But the important points of my researches are to have found:—

1. Among the parasites derived from the intestine of mosquitos very numerous forms of division, into two or three, as generally found in well-developed cultures (see Plates II and III).

2. Modifications in the shape and structure of the parasites derived from the intestine of mosquitos as compared with those obtained from the culture on which the insects had been fed.

3. Karyokinesis of the nucleus occurring in some of my preparations.

I have already stated in another paper (1911, *b*) that I found in preparations from the intestines of mosquitos, fed on cultures of from ten to fifteen days, small round forms with nucleus and blepharoplast identical with those contained in the internal organs of individuals infected with Kala-Azar. These forms can also be found in cultures, but only after some considerable length of time.

In more recent experiments, I have employed, instead of flagellate forms, the round, non-flagellate forms found in culture tubes after some time had elapsed. Attention must be paid to the fact that these forms were not only capable of life, but also of multiplication, for flagellate forms developed in culture tubes from the non-flagellate forms used for experiment.

In this way I obviated the objection made that the flagellate forms found by me were not *Leishmania*. I obtained also from the intestine of the mosquitos numerous parasites which were well preserved, partly flagellated and, in some instances, so numerous as to resemble actual cultures. Therefore, the non-flagellate forms can live in the intestine of *A. maculipennis* as well as the flagellate forms.

Besides the modifications in the shape of the parasite and nucleus, there appeared to me to be also modifications in the structure of the protoplasm which stained more intensely, and at times appeared granular, as I found it only in the parasites from the intestine of the mosquitos.

As regards the presence of granules in the parasite, this is a point which merits still further researches, considering the immense numbers of cocci present in the digestive tract of mosquitos as seen in Plate II. It might be that it is simply a case of superposition of the cocci over the parasites, although the number of the granules within the periphery of the parasite is often much greater than that of the cocci in the field outside the parasite. The examination of fresh preparations does not help very much in answering the question.

In the faeces of mosquitos excreted at various times after the insect had been fed on the cultures, fair numbers of parasites of various forms were found, partly isolated and partly in groups. They were, to a great extent, well preserved, as seen in Plate IV.

This shows to perfection that the *Leishmania* can pass the digestive tract of *A. maculipennis* without being destroyed, and sometimes not even altered. Therefore, more or less the same occurs here, as in the case of *A. maculipennis* and the malarial plasmodium. In fact, true excretions of malarial parasites have been described from infected mosquitos.

I had wished to obtain cultures from the *Leishmania* found in the digestive tract of *A. maculipennis*, more especially as objections have been raised that I had not made these cultures, an experiment which is certainly most difficult.

It is not always possible to obtain cultures from the splenic juice in which there are numerous *Leishman* bodies, and other blood parasites or bacteria. In the digestive tract of *A. maculipennis*, however, there are very numerous cocci, which in Novy-MacNeal-Nicolle's medium develop so rapidly during the first twelve hours that they impede the life and development of the *Leishmania*. It must also be remembered that the *Leishmania* obtained from the splenic juice and inoculated on the above medium require from at least three to four days for development, and that the parasite does not grow on a non-sterile medium, and dies as soon as other bacterial growth sets in.

There is, consequently, all the more reason to believe that, if the *Leishmania* can live in the digestive tract of *A. maculipennis*, there must exist, in the intestine, exceedingly favourable conditions which do more than compensate the possible destructive action of other germs present, or of the digestive juices themselves. The virulence and behaviour of the numerous cocci, in the intestine of the insect and in cultures, is certainly not the same; and I, therefore, did not waste any time in attempting to make cultures, but I fixed the mosquitos and embedded them in paraffin in order to cut sections, and examine especially the salivary glands. I have already made numerous sections, stained with haematoxylin and eosin, and the results seem to be encouraging, but, owing to the importance of the argument, I do not wish to draw hasty conclusions before more numerous experiments have been made.

The experiments had to be discontinued at the beginning of November, when, owing to the fall of temperature, no insects could

be obtained. They will be resumed on a large scale next spring, especially the animal experiments. It is necessary to transmit the infection from the mosquito to animals susceptible to infection with the parasite of Kala-Azar, such as monkeys, dogs, etc.

The experiments considered in this paper are, as I stated previously, only the beginning of a series of researches up till now made in the laboratory, and I have never attached more importance to them than merited by the results obtained.

I do not wish to discuss the question of the identity of the Kala-Azar of India and that of the Mediterranean, as this does not come within the range of my argument. I am of opinion that, in Italy, the possibility of a transmission of the Leishman parasites by means of the Anopheles does not meet with any difficulty. In many parts of Italy, where Kala-Azar is present as well as malaria, cases of both diseases have occurred to my knowledge in one and the same family. There are other countries round the Mediterranean where malaria and Kala-Azar co-exist. Nicolle has seen many cases of Kala-Azar in Tunis and suggests experiments on the mosquito. A study of the distribution of the Anopheles mosquito in countries infected with Kala-Azar is not only useful but also necessary.

It has been stated that the yearly recurrence of Kala-Azar in spring, which has been observed in some parts of Italy, is contradictory to the transmission of the Leishmania by means of the Anopheles mosquito.

I am, however, of opinion that the recurrence of the disease at a certain time of the year cannot be a valid objection considering our complete ignorance about the incubation period (I may also mention that it is difficult to recognise the onset of the disease). I would like to mention that the period of incubation varies greatly in different animals, dogs, monkeys, etc. We cannot, therefore, draw a parallel between the number of cases of Kala-Azar and the number of bites of the Anopheles mosquito. It has been stated that cases of Kala-Azar occurred with greatest frequency in spring, and that the Anopheles bites most frequently in July, August, and September, and this may very well be so. Might it, however, not also be possible that the incubation period in man was as long as the time elapsing between these months and spring? If we admit the

transmission of Kala-Azar by the *Anopheles* mosquito, it seems to me most probable that such would explain the occurrence of cases in countries far away from primary foci.*

I have made experiments on other mosquitos, and found that the *Leishmania* can also live in the digestive tract of some *Culicidae*, including *S. fasciata*, although not as frequently and abundantly as in *A. maculipennis*.

CONCLUSIONS

After numerous experiments on *Pulex irritans* and *P. serraticeps*, as well as *Pediculus capitis* and *Cimex lectularius*, which had been fed on cultures of *Leishmania*, no parasites were found which could be identified with *Leishman* bodies, and I am of opinion, therefore, that further observations should be made with mosquitos, especially *Anopheles maculipennis* where this mosquito exists.

The *Leishman* bodies are present in the digestive tract of *A. maculipennis* in great numbers and in various stages of development, as seen in Plates II-IV.

The staining of the different parts of the parasites, viz., nucleus, protoplasm and blepharoplast, is so evident that it must be admitted that the parasite is alive and capable of reproduction.

In some of the parasites true nuclear karyokinesis has been observed.

Wenyon (1911) of the London School of Tropical Medicine, in an exhaustive and interesting monograph on the Oriental Sore of Bagdad, has found that *Leishmania tropica* can live and develop in the intestine of *Stegomyia fasciata*. From the non-flagellate form of Oriental Sore he obtained the flagellate form in the intestine of *S. fasciata*, that is to say, the same occurred in cultures of *Leishmania tropica* as in the case of *L. infantum*.

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* In Italy we find examples of cases of Kala-Azar which are far apart and there has been absolutely no contact with infected patients. Infection by bugs and fleas could be definitely excluded because there was no contact with either the actual patient or with the clothes of an infected patient, and in some cases there were no animals in the houses, so we can therefore exclude the possibility of these acting as carriers of the disease in these cases.

EXPLANATION OF PLATE II

The figures have been drawn with Zeiss. Obj. 6, Comp. oc. 8, and show two microscopic fields with numerous *Leishmania* derived from the digestive tract of *Anopheles maculipennis*, fixed in absolute methyl-alcohol, and stained with Giemsa.

Fig. 1 shows, besides the Leishman bodies, numerous bodies and other parasites which stain red with Giemsa. The other parasites were also met with in fleas and bugs. The *Leishmania* were very numerous and present all the different stages of development, from the small, non-flagellate forms found in the haematopoietic organs of patients with Kala-Azar to the flagellate forms of cultures. It will be noted that numerous dividing forms are present.

Fig. 2 shows numerous *Leishman* bodies, nearly all non-flagellate, from the more or less rounded to the oval forms. The smears containing these parasites were obtained from mosquitos which had lived for a much longer period than those from whom the parasites in fig.1 were obtained. The size of the parasites varies exceedingly; forms are present which show more than one nucleus and some of which are filled with chromatin (?) granules. Some forms, which are rare, appear about to degenerate.





EXPLANATION OF PLATE III

Figures 1-19 have been drawn with Zeiss. Obj. 8, Comp. oc. 8; and Figures 20-35 with Zeiss. Obj. 6, Comp. oc. 8.

- Fig. 1. Rosette form with interesting modifications of the nuclei which are better seen with a higher magnification. Close by two small non-flagellate parasites with nuclei and blepharoplast as in the parasites found in the spleen.
- Figs. 2-5. Parasites about to divide; figs. 3-5 with two nuclei and two blepharoplasts.
- Fig. 6. Common flagellate form.
- Fig. 7. Globular form with central nucleus. The nucleus contains little masses of chromatin.
- Fig. 8. Large form showing cystic appearance, with numerous nuclei and blepharoplasts.
- Fig. 9. Form with two nuclei and numerous blepharoplasts.
- Fig. 10. Groups of parasites, some of which are non-flagellate.
- Fig. 11. Globular parasite with three nuclei and one blepharoplast.
- Fig. 12. Groups of parasites, some flagellate, others non-flagellate.
- Figs. 13-14. Dividing forms.
- Fig. 15. Dividing form (?).
- Fig. 16. Dividing form with polar grouping of chromatin; at a higher magnification chromatin filaments can be seen.
- Fig. 17. Group of well stained parasites.
- Figs. 18-19. Some modifications of the nuclei as in fig. 16.
- Figs. 20-24 and 26. Dividing forms with enormous numbers of chromatin granules in the protoplasm.
- Fig. 27. Group of parasites of various forms.
- Fig. 29-30. Small oval forms with flagellum just indicated and with protoplasm stained intensely blue. They are identical with the first stages of development found in cultures, neither nucleus nor protoplasm differentiated.
- Fig. 31-32. Globular forms with many nuclei.
- Fig. 33. Apparently a cystic form.
- Fig. 34. As figs. 31, 32.
- Fig. 35. Globular form.





EXPLANATION OF PLATE IV

Figs. 1-24 show parasites derived from the digestive tract of some culicids and from *Stegomyia fasciata*.

Figs. 2, 7, 8, 9, 12, 14, 16, 17, 21, 23 represent dividing forms.

Fig. 18. Parasites with their protoplasm partly rose and partly an intense blue, similar to some forms of the first stages of development.

Figs. 19, 20, 22. Groups of parasites of various forms, some with one blepharoplast and two nuclei.

Fig. 24. Probably a joined form of three parasites with short flagella.

The second part of the figure shows figures of *Leishmania* which have been derived from the faeces of *Anopheles*, which had fed on cultures. In many of the parasites the protoplasm, nucleus and blepharoplast are intensely stained.

Figs. 1-4 and 14. Parasites similar to those found in the internal organs of Kala-Azar patients.

Fig. 7. Group of four parasites, in one of which only is the flagellum preserved.

Fig. 16. Probably a dividing form.

Many of the other parasites have lost their flagellum and show two nuclei.





TREATMENT OF BERI-BERI

BY

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AND

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(Received for publication 14 February, 1912)

The result of treatment in the following cases appears sufficiently striking to be recorded. Three Lascars were admitted to the Royal Southern Hospital on November 8th, 1911, with symptoms of beri-beri.

HISTORY PRIOR TO ADMISSION

None of the three had had any similar condition before. They had been firemen on a voyage of about five weeks' duration. The food provided them was mainly rice and fish. The rice was of the white, polished variety. The fish had gone bad, and they were unable to eat it. The symptoms had come on about a week previous to admission.

CONDITION ON ADMISSION, TREATMENT AND RESULTS

Case I, Lascar B, age 60. Somewhat emaciated and feeble. Pulse 80, very weak. Scarcely able to walk. Pain and tenderness in the muscles of the arms and legs. Marked oedema of both legs below the knees, and of both hands. Two days after admission, oedema developed in the forearms. Knee jerks, supurator jerks, etc., absent.

Treatment. A full mixed diet, with the addition of yeast (one ounce daily) and Katjangidjo beans (200 grams daily), was commenced on the 14th November. One week later, oedema quite gone, no pain or tenderness in the muscles of the arms or legs, knee jerks present but sluggish, walking well. Discharged sixteen days

after commencement of treatment, walking perfectly, with knee jerks normal, and no pain or oedema.

Case II, E. A., age 20. Patient rather wasted, walked fairly well, but dragged his feet. Knee jerks and other reflexes absent. No oedema. Pain, on pressure, in muscles of calves and arms. Pulse regular, 86.

Treatment. Full diet, and yeast one ounce daily. Six days later, walking better, knee jerks still absent, but no pain in muscles on pressure. Discharged sixteen days after treatment, walking perfectly, knee jerks present, but still sluggish.

Case III, F. A., age 20. Considerably wasted. Pulse 100, temp. 100.7° F. Slight oedema of both legs. Pain in muscles of calf. Walked with a spastic gait. Knee jerks exaggerated. Ankle clonus present.

Treatment. Full diet, with sanatogen and yeast one ounce daily.

Six days afterwards, oedema quite gone. No pain, and walking well. Knee jerks about normal; ankle clonus absent.

Discharged sixteen days after treatment. No pain, no oedema. Increase of body weight. Knee jerks still a little brisk.

Remarks. The improvement in the condition of these patients was much more rapid than is customary, and would seem to show the marked curative power of the addition to the diets of Katjangidjo beans as recommended by Hulshoff Pol, and of yeast as recommended by Schaumann.

The yeast was of the variety used by brewers, and was administered in rice papers, the patients being able to swallow in this fashion about 1 drachm at a time.

THE VITALITY OF, AND CHANGES UNDERGONE BY, TRYPANOSOMES IN THE CADAVER OF THE ANIMAL HOST

BY

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*From the Runcorn Research Laboratories**(Received for publication 3 April, 1912)*

PLATE V

Yakimoff and Kohl (1907) found that *Trypanosoma evansi* preserved its vitality in the dead body of an animal for a maximum period of twenty-seven hours. Musgrave and Clegg, working with the same trypanosome, stated that motile parasites were not usually found two hours after death, but that in exceptional and rare cases, living parasites were found after sixteen hours. The same observers noted that blood was rarely infective after twenty-four hours, but that in one case the blood of an animal which had been dead forty-eight hours was still infective.

In regard to human beings who had died of trypanosomiasis, Gray (1907) observed that actively motile trypanosomes could generally be found somewhere in the dead body within a few hours after death, but that they were seldom found in the blood.

The experiments detailed below were carried out in order to determine how long trypanosomes remained alive and infective in animals (rats and mice) which had been killed during an infection with trypanosomes, and also to study the changes which the parasites underwent in such dead animals.

Method of carrying out the experiments. For the first five experiments the following procedure was adopted. An infected rat was killed when the parasites first became numerous in its blood. It was kept at laboratory temperature, the experiments being carried out during the months of July, August and September. At the time of death films were made, both fresh and stained preparations. After a stated interval the animal was opened, and in addition to making fresh and stained preparations from the heart blood, an

inoculation was made into a rat or mouse. At definite periods, fresh and stained films were made and inoculations done. Thus, several examinations and inoculations were made from each animal. The appearances in fresh film and stained preparation were noted, and the conditions found at each period were compared with the results of the inoculations made. In the last four experiments a somewhat different plan was followed. A number of rats or mice, generally six, of equal size, was inoculated on the same day with equal doses of trypanosome-containing blood. These animals were killed simultaneously as soon as the last of them had passed the incubation period and presented a fair infection. At the time of death, therefore, some of these animals were fairly, others heavily, infected. The dead bodies were then left at laboratory temperature for certain periods, after which they were opened, and blood, taken from the heart, was utilized to make fresh films, stained preparations, and inoculations into animals. In these experiments each animal was used only once for inoculation purposes. It was possible thus to observe how long living trypanosomes remained microscopically evident in the blood of the dead animals, to note changes in character of the parasites in fresh and stained conditions, and also to fix a definite time after which the blood containing the parasites was no longer infective on inoculation. Intraperitoneal injection was used throughout the experiments, a definite amount of blood mixed with 1/4 c.c. of a citrated physiological salt solution being injected on each occasion.

Strains of trypanosome used. The majority of the experiments were made with *T. gambiense* and *T. rhodesiense*, a smaller number being made with *T. brucei*, *T. equinum* and *T. pecorum*.

The vitality of the trypanosome in the cadaver. In the fresh films, a hundred and fifty fields (Zeiss objective DD, ocular No 4) were examined before the blood was considered negative. From Table I it will be seen that in the blood of dead rats living trypanosomes were discovered up to twenty-one hours in the case of *T. equinum*, in the case of *T. brucei* up to twenty hours, up to twenty-four hours in the case of *T. pecorum*, up to twenty-nine hours in the case of *T. rhodesiense*; in the blood of dead mice living trypanosomes were found up to forty-eight hours in the case of *T. gambiense*.

A. CHANGES IN THE TRYPANOSOMES AS SEEN IN FRESH FILMS

(a) *Decrease in numbers.* The first fact observed was the decrease in the numbers of the active living parasites after the death of the animal host. Taking for example Experiment I, *T. equinum*. At death, the note is, fifty living to a field, after four hours ten to a field, after ten hours five to a field, after twenty-one hours one to a field, after twenty-eight hours none found. This fairly steady decrease was not always noted. In one experiment, No 11, a rat infected with *T. rhodesiense*, a most markedly rapid decrease was observed. At death there were about two hundred living parasites to a field, after four hours only two to a field, after twelve hours one to five fields, after twenty hours one to twenty fields, after thirty-six hours none found. In this case it will be seen that the decrease is extremely rapid during the first few hours after death. There is a point of considerable interest about this particular decrease which will be returned to later, in referring to the stained films.

(b) *Changes in appearance.* The changes consisted chiefly of swelling up of the protoplasm, the posterior end being mostly affected, resulting in the production of club-like, skate-shaped, and globular forms. A granular appearance was also marked in these altered parasites, beginning immediately after death.

(c) *Changes in motility.* As the trypanosomes became bulbous and distorted, their movements became slower and finally ceased. Frequently, as they slowed down large clumps of agglutinated trypanosomes were found, many of the individuals still moving fairly actively. Many parasites, however, often of granular appearance, became immobilized without loss of shape, and could be seen floating amongst the corpuscles. In certain cases, although the number of active trypanosomes had decreased very greatly, those which persisted and retained their normal shape, moved with marked activity, even apparently in excess of the normal. This was particularly noted in *T. gambiense* in mice, after forty-eight hours.

Infectivity of the blood of dead animals. Table I shows the maximum period of infectivity of the blood from the cadaver of the trypanosomiasis animals. In the blood of dead rats *T. rhodesiense* was infective after forty-two hours, *T. gambiense* after twenty-nine hours. In the blood of dead mice both *T. rhodesiense* and

T. gambiense were infective after forty-eight hours. In Table II, where the experiments are analysed in greater detail, are given the period at which inoculation was done after the death of the animal host, whether live trypanosomes were seen or not, the incubation period, and time of death in those inoculated animals which became infected.

It will be observed that several inoculated animals died so soon from various causes, that the parasites had not time to develop. In this connection it may be noted that in no case where living trypanosomes were seen at the time of inoculation, did infection fail to occur, in an animal which lived for a reasonable time.

Number of animals infected after varying periods. In Experiment VI, *T. rhodesiense*, each of three rats inoculated with blood from three dead rats which had lain twenty-nine hours at laboratory temperature, became infected, and one out of three when the dead rats had lain forty-two hours. In Experiment VII, *T. gambiense*, two of three rats inoculated with blood of rats twenty-nine hours dead became infected, none of three rats became infected where blood was used from bodies which had lain forty-two hours. In Experiment VIII, *T. rhodesiense*, where the blood was taken from mice forty-eight hours dead, one mouse out of two which lived twenty days became infected. In Experiment IX, *T. gambiense*, the blood of mice dead forty-eight hours was used for inoculation. The only mouse which lived twenty days became infected. Had the others lived it is probable that several more would have been infected, as three out of the five original mice had living parasites in their blood after they had been dead forty-eight hours.

B. CHANGES IN THE TRYPANOSOMES AS SEEN IN STAINED PREPARATIONS

Soon after death a granular change was seen in many of the parasites. The granules appeared quickly, many forms being filled with granules even within one hour after death. The conditions present after the death of the animal host may be divided into two classes, both of which were observed in varying degrees.

(1) *Changes accompanied by loss of form.* Some trypanosomes became granular, the nucleus being broken up into small staining

globules, and the whole protoplasm being filled with dark stained granules, contrasting with the paler protoplasm of the parasite. Other forms were seen in which apparently these granules were greatly reduced in number, only one or two dots being seen in the region where the nucleus was.

Non-nucleated forms. Forms were seen in which, although the protoplasm, blepharoplast and flagellum, and outline of the parasite appeared perfect, there was no trace of nucleus or granules to be seen. These non-nucleated parasites were first observed in a rat infected with *T. pecorum*, which was dying swarming with parasites. Later a search for them proved their presence in the living rat infected with the strains of *T. gambiense*, *T. rhodesiense*, *T. equinum*, *T. brucei*, *T. dimorphon*, at similar times. They were then looked for and found in these experiments, but although found with ease soon after death, it was not easy to discover them later on. They did not appear to be parasites from which, as a result of fracture or pressure, the nucleus had been displaced, because in such cases one could generally discover traces of the fractured and displaced nucleus, and the parasites did not present a clear outline. Nor were they the result of imperfect staining, because trypanosomes immediately adjacent to them presented perfectly stained nuclei. It seems probable that they were the result of a process of granular disintegration of the nucleus, after which solution or extrusion of the granules had followed. This mode of degeneration, however, if it is degeneration, was by no means a common one in these experiments.

(2) *Changes accompanied by loss of form.* The majority of the trypanosomes soon after death became swollen and distorted. The component parts, still clearly visible and well stained, lost their relative positions, so that in many the nucleus and blepharoplast came to lie very close together, rounded bodies being formed, with the flagellum encircling them.

Definite degeneration forms. The term 'degeneration' is used here to signify only those changes which were present in trypanosomes in the blood of a dead animal, at a time when the blood containing such altered trypanosomes failed, on inoculation, to infect rats and mice. The test, therefore, made use of in the experiments, to decide the question whether changes observed in

the trypanosomes indicated degeneration or not, was the effect of inoculation into these animals.

The various parts of the parasite disappeared irregularly, giving rise to an infinite variety of forms, among which the round form predominated, a protoplasmic circular mass containing the nucleus and blepharoplast or possibly granules. In many no flagellum could be made out, in which case the smaller body often seen with the nucleus was probably granular in origin. A further step consisted in the isolation of those nuclei which persisted and the detachment of the flagellum with the blepharoplast. Masses of nuclei were found which represent the remains of agglutinated groups of trypanosomes. The variety of forms presented by the various degenerating portions of trypanosomes is enormous. A few of them are shown in Plate V. All the forms represented in this Plate, Part B, are taken from blood after it had ceased to be infective on inoculation, and are, therefore, 'degeneration' forms in the sense of the definition given above. That is to say, blood which contained, in many instances in enormous numbers, these rounded forms, somewhat resembling the appearance of described 'resistant' forms, failed to infect rats and mice.

In practically all the experiments, careful search of films taken after the blood had ceased to be infective, revealed the presence of a few fairly well-stained, well-shaped parasites.

The decrease of the parasites in Experiment II, T. rhodesiense. Above, in dealing with the conditions seen in fresh films in this case, it was noted that a very rapid decrease of the trypanosomes occurred within a few hours of death. Examination of the stained films showed that this decrease was remarkable in one respect. The stained film, taken at the time of death, when there were about two hundred parasites to the field in the fresh film, showed a very large preponderance of the usual long, free flagellated trypanosomes, and a small proportion of short, stumpy forms, some of these having the posterior nucleus which is characteristic of this strain. The proportion of long, free flagellated forms to short, stumpy forms, was at this time eighty to one. Within four hours, however, this proportion had been reduced to five to one. After twelve hours the proportion of long to short had sunk to one long to twenty short, and after twenty-two hours, of fifty trypanosomes seen, only two

were long forms. Further, it was observed that the short, stumpy forms were more perfect in their appearance and staining reaction than the long forms. They appeared, therefore, in this experiment, to have a markedly greater power of resistance to the process of disintegration than did the long forms. As regards the other experiments this peculiarity was not noticeable.

The incubation period in animals inoculated with blood from the cadaver. Yakimoff and Kohl (1907) found that the incubation period was increased when cadaver blood was used, but that the sooner after death the inoculation was made the less marked was the lengthening of the incubation time. Somewhat similar results were obtained in these experiments. A reference to Table II will show that the maximum incubation period for *T. gambiense* was twenty days in a mouse (blood forty-eight hours old), and for *T. rhodesiense* nineteen days in a rat (blood twenty-nine hours old). It will be observed, however, that one mouse, Experiment VIII B, inoculated with *T. rhodesiense* blood, forty-eight hours old, presented an incubation period of eleven days only.

In several individuals, after a prolonged period, the trypanosomes became numerous in the blood very rapidly, more so than in the usual infections with ordinary infected blood.

CONCLUSIONS

(1) *T. gambiense* and *T. rhodesiense* can remain infective in the blood of the dead animal host for forty-eight hours.

(2) This infectivity need not be attributed to any specially formed 'resistant bodies,' as living trypanosomes were found by the microscope (e.g., *T. gambiense*) in three out of five animals which had been dead forty-eight hours.

(3) In case of accident, in which all the animals holding a strain of trypanosomes died at once, it would be worth while inoculating up to forty-eight hours, probably even longer, after death. For such inoculations a large series of animals should be used, as several of them would almost certainly die before the incubation period was over.

REFERENCES

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- MUSGRAVE and CLEGG. 'Trypanosoma and Trypanosomiasis,' p. 46, *vide* Bull. Sleeping Sickness Bureau, 1909, No. 8, p. 294.
- GRAY and TULLOCH (1907). Reports of the Sleeping Sickness Commission of the Royal Society, VIII, p. 17.

TABLE I.—To show, for the experiments done, the maximum duration of infectivity in blood of dead trypanosomiasis animals, kept at laboratory temperature.

No. of Experiment	Strain	Animal	Maximum time (in hours) post mortem after which—		INOCULATIONS		
			(1) Living trypanosomes found in fresh film	(2) Blood infective	Animal	Incubation in days	Duration of disease in days
I	<i>T. equinum</i> ...	Rat ...	21	21	Rat	14	21
II	<i>T. rhodesiense</i> ...	Rat ...	22	22	Rat	8	11
III	<i>T. brucei</i> ...	Rat ...	20	29	Rats (1) (2)	11	38
IV	<i>T. gambiense</i> ...	Rat ...	11	11	Mice (1) (2)	7 5	29 10
V	<i>T. pecorum</i> ...	Rat ...	24	24	Rats (1) (2)	8 11	10 13
VI	<i>T. rhodesiense</i> ...	Rat ...	29	42	Rat	11	32
VII	<i>T. gambiense</i> ...	Rat ...	None seen	29	Rats (1) (2)	7 7	20 9
VIII	<i>T. rhodesiense</i> ...	Mouse ...	None seen	48	Mouse	11	20
IX	<i>T. gambiense</i> ...	Mouse ...	48	48	Mouse	20	31

TABLE II.—An analysis of the foregoing experiments showing the period after the death of the animal at which inoculations were done, and the results of the inoculations.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	*	Animal inoculated	Incubation in days	Day of death	Remarks
I ...	<i>T. equinum</i> — Rat ...	10	+	Rat ...	10	20th	Trypanosomes never found in blood. " " " " " "
				Rat ...	14	21st	
				Rats (1) ...	—	42nd	
				(2) ...	—	11th	
				Rats (1) ...	—	33rd	
II ...	<i>T. rhodesiense</i> — Rat ...	36	—	(2) ...	—	21st	
				Rats (1) ...	5	11th	
				Rat ...	8	11th	
				Rats (1) ...	—	1st	
				(2) ...	—	1st	

* + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.

— Signifies living trypanosomes not found.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	*	Animal inoculated	Incubation in days	Day of death	Remarks
III ...	<i>T. brucei</i> —						
	Rat ...	29	—	Rats (1) ...	11	38th	
				(2) ...	7	29th	
IV ...	<i>T. gambiense</i> —						
	Rat ...	11	+	Mice (1) ...	5	10th	
				(2) ...	5	10th	
		24	—	Mice (1) ...	—	7th	Trypanosomes not found.
				(2) ...	—	6th	" "
		36	—	Mice (1) ...	—	4th	" "
				(2) ...	—	4th	" "
V ...	<i>T. pecorum</i> —						
	Rat ...	24	+	Rats (1) ...	8	12th	
				(2) ...	11	13th	

* + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.

— Signifies living trypanosomes not found.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	*	Animal inoculated	Incubation in days	Day of death	Remarks
VI ...	<i>T. rhodesiense</i> —						
	Rats :—						
	A ...	29	+	Rat ...	19	24th	
	B ...	29	+	Rat ...	12	25th	
	C ...	29	—	Rat ...	10	23rd	
	D ...	42	—	Rat ...	—	—	Alive 35th day. Tryps. never seen.
VII ...	<i>T. gambiense</i> —						
	Rats :—						
	A ...	29	—	Rat ...	7	20th	
	B ...	29	—	Rat ...	7	9th	
	C ...	29	—	Rat ...	—	9th	Tryps. never seen.
	D ...	42	—	Rat ...	—	9th	
E ...	42	—	Rat ...	—	—	Alive 38th day. Tryps. never seen.	
F ...	42	—	Rat ...	—	18th	Tryps. never seen.	

* + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.
 — Signifies living trypanosomes not found.

No. of Experiment	Strain and Animal	No. of hours after death at which inoculations made	•	Animal inoculated	Incubation in days	Day of death	Remarks
VIII ...	<i>T. rhodesiense</i> —						
	Mice:—						
	A ...	48	—	Mouse	—	—	Alive 40th day. Tryps. never seen.
	B ...	48	—	Mouse	11	20th	”
	C ...	48	—	Mouse	—	12th	Tryps. never seen.
	D ...	48	—	Mouse	—	7th	”
IX ...	<i>T. gambiense</i> —						
	Mice:—						
	A ...	48	—	Mouse	—	—	Alive 40th day. Tryps. never seen.
	B ...	48	+	Mouse	—	2nd	Septicaemic infection.
	C ...	48	+	Mouse	—	2nd	”
	D ...	48	—	Mouse	—	9th	Tryps. never seen.
E ...	48	—	Mouse	—	3rd	”	
		48	+	Mouse	20	31st	”

• + Signifies that at the time when inoculation was done living trypanosomes were found in the blood in fresh film.
 — Signifies living trypanosomes not found.

EXPLANATION OF PLATE V

Magnification 2,000 to 2,100 diameters.

PART A

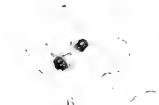
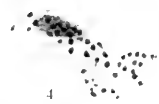
- Nos. 1, 2, 7, 8. *T. rhodesiense* in rat soon after death.
- No. 3. *T. gambiense* in mouse 48 hours after death.
- No. 4. *T. rhodesiense* in rat 29 hours after death.
- No. 5. *T. rhodesiense* in rat 48 hours after death.
- No. 6. *T. rhodesiense* in rat 5 hours after death.

PART B

- Nos. 1, 7, 17. *T. rhodesiense* in mouse 48 hours after death.
- Nos. 2, 5, 6, 16. *T. rhodesiense* in rat 48 hours after death.
- No. 3, 8, 9, 11, 12. *T. rhodesiense* in rat 42 hours after death.
- Nos. 4, 10, 13, 14, 15. *T. gambiense* in rat 42 hours after death.

PART A.

PART B.



THE DEVELOPMENT OF A LEUCOCY- TOZOON OF GUINEA-PIGS*

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PLATE VI

The presence of 'bodies' within the large mononuclear leucocytes of guinea-pigs was first noticed by Kurloff (1898). He described them as inclusions; for in a drop of guinea-pig's blood he noted that many of the large lymphocytes contained, within their cytoplasm, clear, spherical vacuoles which were distinct from the nucleus, and which had not been described before; and he suggested the possibility of these bodies being accessory nuclei. Since their discovery by Kurloff they have been subjected to much research; and papers describing various observations concerning them have been published by Burnett (1904), Staubli (1905), Goldhorn (1905), Ledingham (1906), Howard (1907), Pappenheim (1908), Patella (1908), Hunter (1909), and Schilling (1911).

Kurloff noticed that when the blood containing these bodies was fixed and stained, they contained a nucleus-like structure staining with nuclear dyes, but he believed them to be vacuoles formed by a secretion product of the cells which held them. Ehrlich (1906) also thought that Kurloff's bodies represented some 'Secretstoff.' Dr. Ledingham, to whom I am indebted for much information, seems to have been the first to suggest the possibility of their parasitic nature, and he mooted an analogy to the *Cytorryctes variolae* or *vaccinae*. Goldhorn (1905) boldly called them leucocytozoa. The most recent work published on the subject is that of Schilling (1911). He has examined these bodies by 'vital' staining with Azur, and he has described some of the earlier stages of their development while in the mononuclear leucocytes

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(lymphocytes). He believes that the rod stage precedes the granule stages, and this has caused him to adhere to the opinion that Kurloff's bodies must be classed with the Chlamydozoa, symbiotic structures, or vaccine inclusions.

Early in 1911, while examining a guinea-pig's blood by a new jelly method of examination of blood cells, H. C. Ross saw Kurloff's bodies, and pointed out to me that the method demonstrated the probability of their parasitic nature. The new method, which was devised partly at the suggestion of Sir Ronald Ross, K.C.B., has already been fully described (H. C. Ross, 1909); the bodies then seen were in the earlier stages of their development. But the inclusions stood out so clearly by this method that I determined to continue the observations, for this technique seemed to show details of structure which had not been described before; and since by the new process the bodies can be subjected to the action of various stains and chemical agents there was a possibility of the phases of their development being observed. I may state that I have now been able to convince myself that these bodies are living parasites of the mononuclear white corpuscles (lymphocytes), and henceforth in this paper I propose to call them such.

I use a modification of the original jelly method—it is as follows:—A 2% solution of agar in water is boiled, sterilised and filtered. To 5 c.c. of the filtrate is added 0.5 c.c. of a 10% solution of sodium chloride in water, and 0.5 c.c. of a 1% solution of Azur II in water. The total bulk of the mixture is made up to 10 c.c. in a test tube. When molten, a small quantity of the jelly is allowed to spread itself in a thin film on a microscope slide and to cool and set. Then a drop of guinea-pig's blood (or citrated blood) containing Kurloff's bodies (about 90 per cent. of the guinea-pigs examined by me, and which were obtained from dealers in England, are infected) is placed upon a cover-glass, and this is inverted on to the set jelly. The blood spreads out between the cover-glass and the surface of the jelly, and, after an interval of five minutes, during which the blood corpuscles come to rest, the specimen may be examined under the higher powers of the microscope. After a further interval of a few minutes—the exact period varying slightly with the temperature of the room—the granules of the leucocytes begin to stain, after which their nuclei gradually stain a deep blue;

the contours of the erythrocytes, as well as those of the leucocytes, show up clearly, and the method is a pretty example of *in vitro* staining. In some of the larger mononuclear cells the colourless parasites will be noticed at one side of the protoplasm. These parasites are inside the cell, because the shape of the nucleus of the lymphocytes is moulded according to the size of the parasite, which grows larger as it develops—in its youngest stages it is small, while in its last intracellular stages it bulges the lymphocyte cell wall and squeezes the nucleus into a small space; this point is of interest because, as Hunter has shown, Patella claimed that Kurloff's bodies lie upon and not in the lymphocytes. In cells containing the larger parasites smaller vacuoles can also be seen; these latter always remain clear and transparent even when examined on stain-containing jellies, and they vary in numbers, and slightly in size, in different examples. It has been suggested that these smaller, subsidiary vacuoles are polar bodies, but more probably they contain excretory products of the lymphocytozoa into the cytoplasm of their hosts, for they become larger and more numerous as the parasite grows.

When examined on the jelly, and immediately before the staining of the nucleus of the leucocytes, the contents of the parasites begin to stain*—the internal chromatin structure of the spherical sac embedded in the lymphocytes' cytoplasm becomes purple and remains stained for several hours, so that its examination is readily made. If the bloods of a number of infected guinea-pigs are watched in this manner from day to day what appear to be the successive stages of the growth of the parasite in the lymphocyte can be seen and drawn; but the leucocytes of a single animal at any particular moment contain, usually but not always, parasites in the same stage of development. The cycle, however, can be followed by observing the blood of one guinea-pig hourly.

*It must be emphasised that if the jelly contains excess of salts or impure stains, the wall of the parasite will stain in an irregular manner, and then patches of stain will hide its contents. Furthermore, if the blood on the jelly dries, or if the blood is fixed in any way, the same thing occurs. Similarly, patchy staining is obtained by the various fixed film methods in vogue, as, for example, Romanowsky's or Jenner's stains. Even Azur stain, when applied to the dried or fixed films of blood, will not demonstrate the details of the development of the parasite. No alkali should be added to the jelly.

The interpretation which I place upon the appearances I have seen are as follows:—The parasite presents itself, in the smallest phase of its intracorpuseular cycle, as a tiny translucent body embedded within the cytoplasm of the larger mononuclear blood corpuscles and near the periphery of those cells. Usually one of such bodies is present in any one cell, but occasionally two or even three parasites may occur in the same cell. The parasite, in this early stage, contains a double purple dot (Pl. VI, figs. 1, 2); in this phase it resembles the Leishman-Donovan bodies found in human leucocytes in cases of Kala Azar. When first seen the dot is motionless, but after a time on the jelly, as the lymphocyte host becomes disorganised, it may show some Brownian movement. In the next stage the parasite is larger, and the chromatin dot has divided into two or more dots until the sphere-like sac may be packed with them (fig. 3). Then each dot becomes dumb-bell shaped (fig. 4), and again, by a simple process of elongation, rod shaped (figs. 5, 6, 7). The parasite may contain one of these rods (fig. 9), or it may be full of them—the actual numbers varying in different examples. Sometimes a parasite may contain one or more rods, some dumb-bells, and some dots. But the size of the parasites increases steadily with these successive stages of the development of their contained chromatin (compare figs. 1 and 15). During the rod formation, the smaller subsidiary vacuoles already mentioned appear in the cytoplasm of the host cell (figs. 3, 5, 12); they never contain any chromatin and remain unstained. With its growth the parasite begins to compress the nucleus of the lymphocyte (figs. 13, 14), and the wall of the latter can be seen as a shell enclosing the parasite (figs. 14, 15, 16). The rods grow longer and thicker (figs. 8, 9, 10) until they stretch across the parasite, and their ends may be doubled against its wall, and they may then present in optical section an erroneous impression of flattening or a terminal bulging (figs. 8, 13, 14). In the next stage a stout flagellum grows out from both ends of the rod (figs. 8, 11, 12, 13), which becomes rolled up in a coil within the sphere (figs. 13, 14, 15). The rod with its two flagella splits longitudinally in its whole length (figs. 8, 12), and this process of splitting takes place again and again. The fission throughout is always lengthwise, never transverse. A specimen in this stage will show the parasite, now equal in size to

the original dimensions of its host-cell, bulging the wall of the latter, compressing the nucleus into a small space, and containing within its interior a mass of woven, twisted, and intertwined purple threads, a conglomerate maze of worm-like spirilla stained red by the Azur dye (figs. 15, 16).

Arrived at its maturity, the parasite breaks away from the shell of its host-cell and then bursts, setting free the threads into the plasma (fig. 17). But the flagellate forms, owing to the fact that they are stained, are dead and motionless, and they may remain attached to the shrunken sphere sac, their ends waving in the currents set up.

It was found very difficult to demonstrate the motile, flagellate forms of the parasite when free in the blood. They cannot be seen then by the jelly method, because, probably, they stain momentarily as the trypanosomes do, and immediately die and become achromatic, and unless stained they are not visible. By the examination of ordinary wet films of the blood I was unable to demonstrate the presence of these free flagella, although a disturbance of the corpuscles was frequently seen. But the blood of some infected guinea-pigs, drawn under all aseptic precautions and examined by the dark ground illumination, showed free-swimming spirochaete-like bodies. It was not until the blood of highly infected guinea-pigs containing full matured lymphocytozoa was treated with an equal part of a 1% solution of 'globin'* and incubated at 37° C. for eight hours that the free flagellate forms in the blood plasma could be fixed and stained by ordinary methods (fig. 18). Even by this process it is not always possible to demonstrate them, and the maceration involved gives them the appearance of spirilla with blunt ends. However, some of the spirilla obtained after the treatment with the 'globin' show the wavy outline of spirochaetes. Sir Ronald Ross was the first to suggest that these flagellate forms constitute the gametes of the parasite; this seems quite probable, though no separate female form has yet been noticed. It will be remembered that Lewis suggested that trypanosomes are sperms, and, perhaps, these spirochaete-like bodies are similar stages of a larger parasite.

*The filtrate of a solution of haemoglobin which has been precipitated by heat. H. C. Ross claims that this substance induces the division of certain cells.

What may possibly be the last phase of this parasite has occasionally been seen in preparations which had been submitted to the action of 'globin' for a further period of four hours. It is an object which resembles somewhat the trypanosome 'latent bodies' described by Moore and Breinl (fig. 19). Hunter has also mentioned the presence of amoeboid forms of this parasite being free in the plasma, but he does not picture them. These may be the form now drawn (fig. 19).

Dr. J. W. Cropper and I have repeated and can confirm the experiments of Ledingham (1906) and Hunter (1909), namely, that newly-born guinea-pigs do not show these lymphocytozoa in their blood. Although a pregnant animal may be markedly infected, the young, when born, possess no parasites. As has been already observed by these writers and by Schilling (1911), the number of parasites found in both the peripheral blood and in that of the internal organs of any one infected guinea-pig varies greatly from day to day. The parasites seem to appear in large numbers, to diminish, to disappear, and then, after a varying period of time, to reappear. Except for a slight anaemia, shown by the presence of an increased number of erythroblasts in the peripheral blood, the guinea-pigs do not suffer apparently. The livers of many of these infected animals show, however, single or multiple white patches of necrosis varying in size between that of a pin's head to that of a large pea, and extending into the substance of the organ. But we have no proof, as yet, of their direct relation to the parasite.

Fixed specimens of the various stages of the development of this parasite may be made by substituting an equal amount of a 1% solution of caustic soda in the jelly for the sodium chloride solution. By this means the red blood corpuscles are laked, but the nuclei of the leucocytes and the chromatin of the lymphocytozoa stain well. The cover-glass can then be lifted from the jelly and mounted while still wet in Canada balsam. Many of the leucocytes with the contained parasites will adhere to the cover-glass and will retain their stain.

Since writing this paper, Hindle has published a preliminary note (Hindle, 1911), 'On the Life-cycle of *Spirochaeta gallinarum*.' He asserts that these spirochaetes possess an intracellular stage within the cells of the Malpighian tubes of the tick, *Argas persicus*.

In view of the life-history of this lymphocytozoon of guinea-pigs his work is of great interest.

I have to express my indebtedness to Dr. J. W. Cropper and to Dr. H. Bayon for their help in these researches; the latter was the first to recognise the free-swimming, spirochaete-like bodies. I also wish to thank Professor Minchin and Dr. Martin for much help and advice and the interest they have taken in this work.

SUMMARY

Kurloff's bodies are parasites, lymphocytozoa inhabiting only the mononuclear cells of the guinea-pig's blood.

These lymphocytozoa have an intracorpuseular stage, and ultimately give rise to free-swimming, spirochaete-like bodies, which may be gametes.

The development of the spirochaete-like body is demonstrated.

The name *Lymphocytozoon cobayae* is suggested for this parasite.

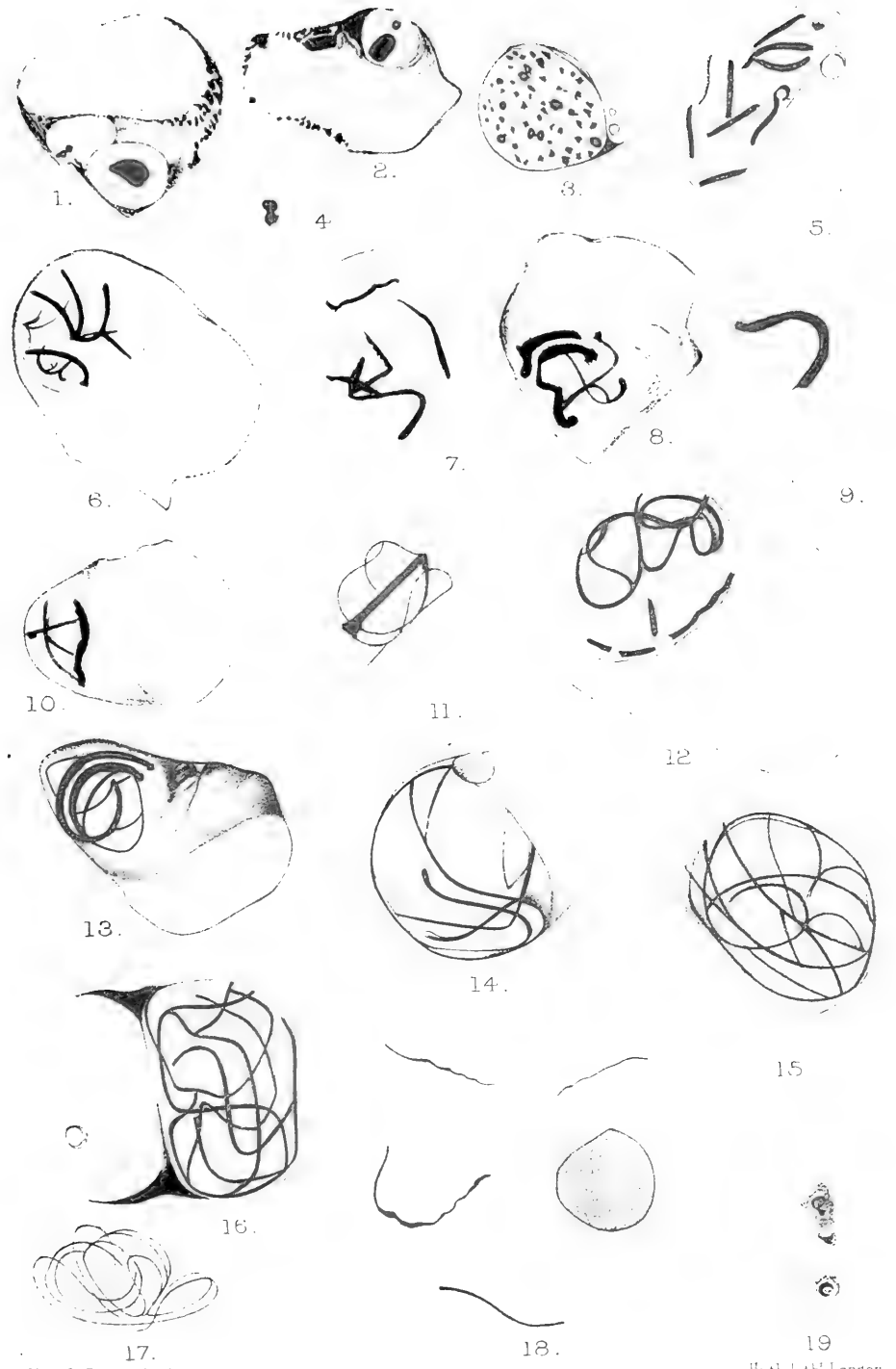
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PLATE VI

Lymphocytozoon cobayae.

For Explanation of Plate see text.



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DEVELOPMENT OF A LEUCOCYTOZOON OF GUINEA-PIGS.

A NOTE ON ANTI-SYPHILIS MEASURES IN UGANDA

BY

CAPTAIN G. J. KEANE, R.A.M.C., M.D., D.P.H., D.T.M.

(Received for publication 18 February, 1912)

PLATES VII, VIII, IX

Syphilis had become recognised to be so prevalent and its effects on the vital statistics of the native population so serious, that in 1907 Government took special steps to check the spread of the disease. The gravity of the situation will be realised when it is understood that the sterility of the Baganda women is remarkable, that abortion is very common, that of those children born alive, only the minority survive the first year of life.

The infantile mortality rate is apparently rarely below 50 %, and sometimes reaches as high as 80 %.

The general death-rate in many parts of the country exceeds the birth-rate.

In these circumstances the extinction of the Baganda race is obviously seriously threatened. In view of these facts the late Colonel F. J. Lambkin, R.A.M.C., was sent to Uganda to report on the subject. His investigations confirmed the reports of the extent of the ravages of the disease, and he made certain recommendations with regard to measures to be adopted. These included the sending of a certain number of special medical officers to treat syphilis on a large scale by means of intra-muscular injections. Their objects were, to report further on the prevalence of the disease, to see if intra-muscular injections could be successfully carried out on a large scale and report on their efficacy, and to observe whether a sufficient attendance of infected could be secured by appealing to the intelligence of the people through their chiefs.

The Baganda are unquestionably a most superior race, indeed they have been called, 'the Japanese of Africa'; their chiefs are very astute, and are keenly alive to the uncertainty of the future of their population.

RESULTS

The first conclusion established, was, that complete treatment, as we know it in Europe, i.e., 21 months, was not feasible with the native population, and that there was good ground for questioning how far it could be said to be immediately necessary amongst the Baganda. The chief explanation of native disinclination to long-continued treatment was that, in the majority of cases, all active signs had disappeared at the time of completion of the second course of injections. The native could not see the force of persisting in treatment when he no longer had symptoms.

Other reasons of impracticability of long attendance were: the native custom of 'visiting'; all Baganda like to take long journeys up country at times, hence a break in their attendance. Further, every native has to perform labour for his chief, and in the ordinary way for earning livelihood and payment of tax.

The remarkable feature of all cases was the immediate response to treatment that took place. Commonly, all signs had disappeared at the completion of six injections. The suppression of symptoms was seemingly permanent in many cases. I had the opportunity of observing certain cases of well-marked secondary syphilis, who had only received one or two courses of injections, who at the end of that time were free from signs, and who to my certain knowledge remained so for fifteen months afterwards, although receiving no further treatment.

These observations, if confirmed by further experience, would be of great importance for the prospects of the success of this work in Uganda. Such persons cannot be said to be entirely cured, that is to say, they are immune from fresh infection, and being free from signs are presumably incapable of directly transmitting the disease. The occurrence of congenital syphilis is not precluded, except in so much as the spread of acquired syphilis is militated against. But an important step in the work of prevention of the spread of the disease is taken. The completion of treatment is rather the affair of the individual than a matter of public concern.

The second point established was that, short of some legislative assistance, a sufficient attendance of sick could not be secured. Thus, after eighteen months' work at Kampala, the attendances

dropped off considerably, and all the efforts of the chiefs failed to bring up the numbers of the sick to such a level as to justify the officers engaged there continuing the work.

Just before this time, I had commenced work at an out-station, Masaka. Here there was an Administrative Officer, administering a very large population, in which there was reported to be a high prevalence of venereal disease, assisted by a very capable and intelligent chief, named the Pokino. I continued the work here for a further eighteen months. When the Pokino was informed of the proposed anti-venereal disease measures to be adopted amongst his people he expressed his pleasure, and extended hearty co-operation. He built the hospital buildings, shown in the accompanying photographs, at his own expense, also some 200 huts for the accommodation of sick. He arranged for food supply for the sick.

The attendance at Masaka was always numerous, but not as high as it ought to have been from the point of view of influencing the incidence of the disease per 1,000 population, nor as numerous as I had arrangements made for. I could easily have treated seven to ten times the actual numbers.

Treatment never seemed to be in any way resented by the native population. It was admitted that the white man had a wonderful remedy, and the cases that did attend invariably expressed astonishment and gratitude at their rapid recovery.

The explanation of non-attendance seemed to be indifference; indifference on the part of the sick to the consequences of the neglect of the disease, and indifference to the danger that they themselves constituted to the healthy.

Thus I found that it was common to find a case of advanced secondary syphilis, with a pustular rash, living in a crowded house, his presence in this condition seeming to be in no way resented by his companions.

TREATMENT

From the purely medical, therapeutic point of view, the work has been a complete success.

Nearly all cases treated were in the secondary stage.

Tertiary syphilis is relatively uncommon, and is confined chiefly

to cutaneous ulceration. Lesions of the nervous system and parasymphilitic conditions are rare.

Treatment in all adult cases consisted in intra-muscular (gluteal) injection of Lambkin's Mercurial Cream.

Six injections were administered at intervals of one week, then followed a rest-period of one month, then a further course of four weekly injections, followed by four weeks' rest and so onwards for as long as patient could continue attendance.

The intra-muscular method was entirely satisfactory clinically, as the accompanying photographs show. In practice, it is an extremely rapid and convenient method of treating large numbers. I found I was able, with trained native assistance, to inject and make entries in case-sheets at the rate of sixty patients per hour. During three years' almost daily use of the method I had only one case of gluteal abscess.

Jeyes' Fluid was used for the preliminary preparation of the skin.

All the routine work, the dispensing, preparation of stock solutions, writing up of case-books, dressings and local applications, were carried out by native assistants.

The Baganda are remarkably apt pupils, they write and speak English well; they make good type-writers.

The organisation of specially trained native hospital assistants will, I hope, be an important feature of the campaign against venereal disease in Uganda.

The dose given in all cases was $1/5$ th grain of the Lambkin Mercurial Cream. Higher doses constantly resulted in salivation without any other notable results.

I wish to express my indebtedness to Dr. A. D. P. Hodges, C.M.G., Principal Medical Officer, Uganda Protectorate, for kind permission to publish, and to Captain E. B. Place, District Commissioner, Masaka, for taking the accompanying photographs.

EXPLANATION OF PLATES

PLATE VII

- Fig. 1. General view of arrangement of a Venereal Treatment camp; below are seen lines of temporary huts for accommodation of sick.
- Fig. 2. Nearer view of sick lines.
- Fig. 3. An average morning attendance at Masaka.
- Fig. 4. Chancre penis; commencing rash seen.



FIG. 1.

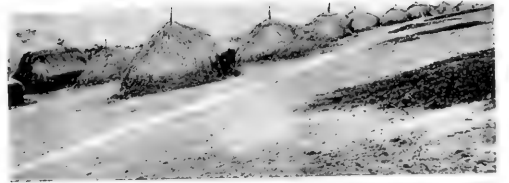


FIG. 2.



FIG. 3.



FIG. 4.



PLATE VIII

Fig. 5. Case 230; on admission.

Fig. 6. Case 230; after two injections only.

Fig. 7 and 8. Secondary and congenital syphilis; on admission and after treatment. The woman and child in Fig. 8 are the same as those on the left in Fig. 7. Fig. 8 is made from a photograph taken after the woman's head had been shaven.

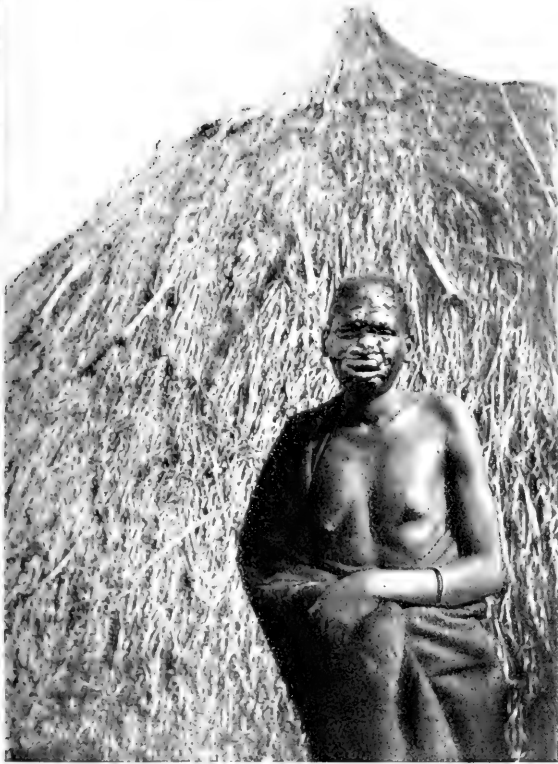


FIG. 5.



FIG. 6.



FIG. 7.



FIG. 8.



PLATE IX

- Fig. 9. Secondary syphilis.
- Fig. 10. Secondary syphilis.
- Fig. 11. Congenital syphilis.
- Fig. 12. Dispenser, dresser, clerk and interpreter at Masaka. A typical healthy Muganda 'Boy.'



FIG. 9.



FIG. 10.



FIG. 11.



FIG. 12.



NOTES ON SOME EARLY REFERENCES TO TROPICAL DISEASES

BY

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(Received for publication 19 March, 1912)

I. AN ELIZABETHAN HANDBOOK OF TROPICAL MEDICINE

The kinship of letters and national enterprise has never been more happily illustrated than in the closing years of Queen Elizabeth's reign. The world, grown more spacious, was daily yielding fresh material for the noble writers whose works form our inexhaustible national treasury. Within ten years around the turn of the sixteenth century there was a series of nautical adventures in close association with literary productions, showing that if the seaman exercised a moulding force on literature, literature in its turn was not without its influence on seacraft.

With all this interest in oversea attempts, attention was bound to turn to the *professional* needs of sailors, and a large literature intended for the use of seamen rapidly arose. These works have a literary as well as a scientific value and among them books on medicine adapted to the especial needs of sailors would naturally take a place (Note 1). New and strange lands yielded diseases equally new and strange, and we may therefore look to this period for the small beginnings of the special study of tropical medicine in this country. The little work which we here discuss has some pioneer claim in this regard, while it contributes an addition to the English medical works of literary merit.

In 1598 there appeared in London a small pamphlet of 25 pages by one G. W., printed by F(elix) K(ingston) for H(umphrey) L(ownes). The author has entitled his work 'The Cures of the Diseased in Remote Regions, Preventing *Mortalitie*, incident in Forraine Attempts of the *English Nation*,' the 'Attempts' being

clearly voyages to Tropical America. This is perhaps the earliest work on medicine intended for sailors published in Great Britain.

THE
CURES OF
the Diseased, in remote
Regions.

PREVENTING MORTALITY,
incident in Forraine Attempts,
of the *English* Nation.

*La honra mas vale,
merecerla que tenerla.*



At London
Printed by F.K. for H. L.
1598.

Photo by Donald Macbeth, London

G. W. was a layman in physic, and relates, in a note to the reader, that 'the cause that induceth mee publiquelie to expresse

the Cure of Diseases of such consequence ([which] every judicall conceite may perceiv to have been the only prejudice to our Nation in the expeditions of our time to the Southerne parts: from whence they have returned with renoued victorie, yet exceedinglie opprest withe extreame and penurious sicknesse, that hathe much more prevented the proceeding and performing of their pretensions than the power of Enemies) is not that I purpose, practitioner-like in Phisicke or Chirurgerie, to assume unto me anie knowledge in those Sciences and Faculties: But to possesse all men of remedies for such infirmities, as in my owne experience, have infinitelie impaired *English* Forces in intemperate Clymates, which I publish, for the good of those whom cause may compell to have use thereof, and would be so censured of all.'

The author had voyaged principally to the Spanish Main, and was captured by the enemies of his country. In his dedication '*To the Queenes most Excellent Majestie*' he writes of himself that 'in my unjust imprisonment in Spayne, it pleased God to afflict me with the *Tabardilla* Pestilence: whereof being in cure, by an especiall Phisition of the King, I observed his Methode for the same, and such other Diseases as have perished Your Maiesties people in the Southerne parts, Which Remedies have since, by my direction, taken the like good effects.'

The most pleasing feature of the little book is the verse with which our author opens and concludes his theme. The lines are here given in full.

THE WRITER'S INTENT

Man that is borne, not for himself is borne,
 But for his Prince, his Countrie, and his friends.
 To helpe the sicke, distressed and forlorne,
 Are works of Mercie, Man to man extends.
 Who hath the power, and meanes, and will not cherish,
 Shall with the Hider of his Tallent perish.

Transgression first did sinne in man beget,
 Sinne, sickness, death, and mischiefes many more:
 For as men's mindes on wickednes were set,
 So plagues increast, which were not knowne before.
 But God, whose mercie, iustice doth exceed,
 Sends helps for hurts, and salves for sore at need.

CONCLUSION

Let no man boast of beautie, strength, or youth :
 For, like to flowres we bud, we spread, we fade :
 Nothing is certaine, but the certaine truth,
 To-day a man, to-morrow but a shade.
 His last apparell, cut out with a spade,
 Of nature's coarsest stuffe (I meane) her molde,
 Must shroud the corps, that living shone in golde.



TO THE QUEENES
 MOST EXCELLENT
 MAIESTIE.

*S*Acres Soueraigne,
 in my iniust imprisonment in Spayne,
 it pleased God to afflict me with the Ta-
 bardilla Pestilence : whereof being
 in cure, by an especiall Phisition of
 that King, I obserued his methode
 for the same, and such other Disea-
 ses, as haue perished your Maie-
 sties people in the Southerne parts.
 Which Remedies haue since, by my
 direction, taken the like good effects.

A 3 And

Even in his Index the writer bursts forth into verse, and the book is prefixed by the following table:—

THE BOOKE'S CONTENT

The burning fever, calde the *Calenture*,
 The aking *Tabardilla* pestilent,
 Th' *Espinlas* prickings which men do endure,
Cameras de Sangre, Fluxes violent,
 Th' *Erizipila*, swelling the Pacient,
 The *Tiñoso*, which we the Scurvey call,
 Are truly here describ'd, and cured all.

English seamen of the period had learned much of their craft from the Spaniards, and G. W., like many contemporary writers on nautical subjects, uses Spanish words and quotes Spanish proverbs freely. A few extracts from the pamphlet are here appended, with identification and descriptions of the diseases with which it deals.

By the *Calenture* is probably meant the conditions now classed as heat-stroke or sunstroke. The word, which in Spanish means simply a heat or fever, was introduced into England from Spain about 1590. The *Calenture* was considered to be especially a disease of sailors in the Tropics, and by the popular fancy it was associated with a delirium in which the patient imagined the sea to be a green field and desired to jump into it. Cases are even recorded where the disease is said to have seized upon whole crews (Note 2).

Shakespeare, in his famous description of the facies Hippocratica in the death of Falstaff, was perhaps thinking of this condition. 'After I saw him fumble with the sheets and play with flowers and smile upon his finger ends, I knew there was but one way, for his nose was sharp as a pen, and 'a babbled of green fields.' (Henry V, Act II, Scene III.) This passage was first printed in 1598, and must have been written about the same date as our pamphlet. (The text is here given with the well-known emendation of Theobald.)

'The *Calenture*,' says one author, 'is the most usuall Disease, happening to our Nation in intemperate climats by inflammation of Blood and often proceeding of immoderate drinking of wine.' The treatment suggested would appear by no means irrational, even nowadays, for some types of the disease. 'So soone as the Pacient

is perceived to be possest of the Calenture, except the Chirurgion defer it for danger of the signe open the Median veine of the right arme, and take such quantitie of blood, as agree the to the abilitie of the bodie, which not asswaging the heate by the next day; to open the same veine in the left arme, and take so much more blood at his like discretion. And the bodie being Costive (for so commonly the Pacients are) to give him some meete Purgation; and not to permit him to drinke other than water coolde, wherein Barley and Anniseedes have been sodden with bruised Licorice And not to suffer the Pacient to drinke, seven dayes after he is perfectlie recovered, any other drinke, than such water.'

Tabardilla is Spanish for a 'burning fever,' but, unlike the Calenture, the word did not become naturalized in England. Under the term *Tabardilla*, our author is apparently describing cases of yellow fever, together with others of a haemorrhagic form of bilious remittent malarial fever, and, perhaps, some cases of Dengue. 'The Disease,' he says, 'is so exceedingly pestilent and infectious, as by the same whole kingdomes in both the Indias have been depopulated.'

He describes an initial headache, jaundice, and vigil which would well apply to yellow fever, where, however, the purpuric rash is a rare phenomenon. 'The *Tabardilla*,' he tells us, 'first assaults the Pacient very vehemently with pain in the head and backe, and the bodie seeming yellow, is some signe thereof, and within twenty-four howres it is so tortuous that he that is possest thereof cannot sleep or rest, turning himself on either side, back and bellie; and burning in the backe most extremely. And when it growes to perfection, there will appeare red and blew spots upon the pacient's brest and wrists.'

The treatment for the *Tabardilla* is purgation and phlebotomy, the patient is to drink water only, 'and to bee carefull of his diet: for if this *Tabardilla*, which we call here in England God's Tokens, come again unto the Pacient he can hardly escape it. And it is no lesse infectious than the usual English Plague.'

The symptoms and character of the plague and its 'tokens,' as the associated rash was called, would have been well remembered by the author, for in 1592 there occurred one of the worst outbreaks

of this epidemic recorded in London. The disease lingered there until the end of the century, and made necessary wide circulation by the Government of a leaflet, entitled 'An advise set doune upon her maiesties expresse commandement by the best learned in Physicke for the preservation of her Good Subjects from the plague.' Although G. W. treats the infectious nature of plague as assured, the discussion of this barren topic occupied a large field in medical literature for another two hundred and fifty years (Note 3).

The *Espinlas* we are unable to identify. We therefore give the author's description of it in his own words.

'The *Espinlas* is a very strange sicknes, and usual in those parts, to such as take cold in their breasts, after great heat or travell. And most times it comes to those that lye with their breasts upon the ground especially in the night.

' TO KNOW THE *Espinlas*.

'The partie having the *Espinlas* will be giddie in the head and have pain and pricking at his breast, as with many thorns; wherefore I thinke it is so called of Spina and Espina, the Latine and Spanish words for a Thorne. And there will be upon the Huesso radio [Anglicé—radial bone], or Focell, being the upper part of his arme, a hand breadth above the wrist, a little kernell by which it is certainly knowne. And he that hath this Disease will not have appetite to meate or drinke; nor cannot digest meate, although he bee procured to take it.

' TO CURE THE *Espinlas*.

'The *Espinlas*, appearing by the former signes: Take Olive oyle presently, and therewith chafe the Kernel upon the Pacients arme, using so to do twice every day until it be dissolved: and laying Oyle likewise upon his breast, stroke it upward somewhat hard with the hand: then spread fine flax upon it and the kernell, making it fast with a rowler: and within two or three days the diseased thereof will be recovered. Whereas els it is very dangerous to deprive them of life.'

By '*Cameras de Sangre*, which is the Bloodie Flux,' forms of

Tropical Dysentery are clearly indicated. 'The Patients,' we are told, 'often die suddenly without feeling much griefe They must be thoroughly purged of the sliminess engendered in the passages Give to purge him in the morning halfe a pint of White Wine coold, wherein $\frac{1}{2}$ ounce of Rubarb hath been sodden,' and for diet 'if it be on Land, the livers of Goates (especiallie), Sheepe, or Bullocks roasted: And if at Sea, Rice, only sodden in water until the infirmitie is perfectly asswaged.'

The word *Erysipelas* is as old as medicine itself, and although the *disease* was described by the father of medicine, the *term* acquired an exact significance only in modern times. Our author, however, under the heading 'Erizipila,' gives a good description of the disease in its modern connotation. 'He that hath the *Erizipila*, will bee swolne in the face, or some part of him, and it will be of yellow colour mixt with red. And when it is thrust with the finger, there will remaine a signe or dent of the same; and then by degrees it will fill againe to the former proportion. And it speedily infecteth the inwarde parts, because such swellings come sooner to perfection in hot places than in temperate Countries. And therefore the diseased thereof, must bee immediatlie provided of remedie.'

The treatment of Erysipelas is interesting. 'Some savage people have first found perfectly to cure this *Erizipila*, by brusing so much *tobaco*, as will yeeld spoonfuls of juyce and to drinke it presently after they are infected therewith. And to launce the places swolne; thereunto putting *Casada* wet (Note 4), and made in paste. Continuing in colde places and shadie, neere Rivers: and not to travell or labour, until they are recovered. But the Spaniards in India, set so many *Ventoses* (Note 5) upon the swolne places as they can containe, scarrifying them, and drawing out the corrupted Humour so congealed.'

'*The Tiñoso, or Scurvy*,' is described as 'an infecting Disease, sufficientlie known unto Seafaring men: who by putrified meates, & corrupted drinkes, eating Bisket flowrie, or foule crusted, wearing wet apparell and slothfull demeanour obtaine the same.' The disease, our author says, is 'so ordinary at Sea, as it hath seldomē seen, any Ship or Pinnice, to be foure moneths upon

any Voyage but some of the Companie have had this Disease.' He knows well its symptoms, including the joint lesions, the rashes, and the swollen gums. Exercise, he considers, is very necessary to avert the Scurvy—an important point in those days when the Atlantic was crossed in tiny ships,—and 'also it is a certaine and assured medicine against this Disease, to have such quantitie of Beere, brewed with Graynes & Long pepper, as in the morning twice everie weeke there may be given a good draught to a man But White Wine or Syder, boyled and brewed with Graynes & Long peper, in like quantitie is very singular good. And it is not fit to suffer the gums to abound with flesh: and therefore sometimes let them bleede, & cleere them withe strong Vinegar,' nor should the patients 'be suffered to eat any salt meates, if other may be had.'

II. A NOTE ON AN EARLY RECORD OF SLEEPING SICKNESS IN WEST AFRICA

The earliest mention of the Sleeping Sickness is usually considered to be in Winterbottom's 'Account of the Native Africans in the neighbourhood of Sierra Leone,' published in 1803 (Note 6). In 1734, however, on his return from a voyage to West Africa, John Atkins, a Naval Surgeon, gave a clear description of cases of the disease on the Guinea Coast. As Atkin's journey had been made in the year 1721, the observation must be referred to that date.

His description is to be found in the Appendix to his little volume 'The Navy Surgeon,' and runs as follows:—'The *Sleepy Distemper* (common among the Negroes) gives no other previous Notice, than a want of Appetite two or three days before; their sleeps are sound, and Sense & Feeling very little; for pulling drubbing or whipping will scarce stir up Sense and Power enough to move; and the Moment you cease beating the smart is forgot, and down they fall again into a state of Insensibility, drivling constantly from the Mouth as if in deep salivation; breathe slowly, but not unequally nor snort.

'Young People are more subject to it than the old; and the Judgment generally pronounced is Death, the Prognostick seldom failing. If now and then one of them recovers, he certainly loses the little Reason he had, and turns Ideot. . . .

‘In Searching for the Cause of this Distemper it will be necessary to repeat what I have observed, that the Bulk of Slave-Cargoes mostly consist of Country People, as distinguished from the Coast people, apparent if the principal Way of Supply be considered. At Whydah more Slaves are bought than on the whole Coast besides: & Why? The King of that Country, and his next neighbours, understand sovereignty better than others, and often make War (as they call it), to bring in whole villages of those more simple Creatures inland, to be sold at Market, and exchanged for the Tempting Commodities of *Europe*, that they are fond and mad after.

‘The immediate cause of this deadly Sleepiness in the Slaves is evidently a Super-abundance of Phlegm or Serum, extravasated in the Brain, which obstructs the Irradiation of the Nerves; but what the procatartick Causes are, that exert to this Production, eclipsing the Light of the Senses, is not so easily assigned. . . .

‘The cure is attempted by whatever rouses the Spirits; bleeding in the jugular, quick purges, Sternatories, Vesicatories, Acupuncture, Seton, Fontanels, and Sudden Plunges into the Sea; the latter is most effectual when the Distemper is new, and the Patient as yet not attended with a drivling at Mouth and Nose.’

John Atkins lived from 1685 to 1757. A short account of his career, mostly from his works, is given by Dr. Norman Moore in the Dictionary of National Biography. Atkins was a good observer, and a man of some learning and independent character, indeed too independent for success in the service he had chosen, for disputes and criticisms of his brother officers led to his failure to find re-employment in the Navy after his return from Africa. Little else is known of his life, nor have his published works attracted any considerable attention (Note 7).

III. AN EARLY ACCOUNT OF THE SAND-FLEA, *PULEX PENETRANS*

Some of the earliest settlers in the New World have left accounts of how their earthly sojourn was made burdensome by the ravages of the little sand-flea or chigoe. The first printed record is, perhaps, that of the Spanish writer Fernandez de Oviedo, who in 1547 mentions the insect in the West Indies (Note 8).

In 1558 there was published in Paris a book with the curious title 'Les Singularitez de la France Antarctique, Autrement nommée Amerique (sic!): et de plusieurs Terres & Isles decouvertes de

LES
SINGVLARIT^E
TEZ DE LA FRAN-
CE ANTARCTIQUE, AV-
trement nommée Amerique:& de
plusieurs Terres'& Isles de-
couuertes de nostre
temps.

Par F. André Theuet, natif d'Angoulesme.



A PARIS,
Chez les heritiers de Maurice de la Porte, au Clos
Bruneau, à l'enseigne S. Claude.

1558.

AVEC PRIVILEGE DV ROY.

Photo by Donald Macbeth, London

nostre temps, Par. F. André Theuet, natif d'Angoulesme.' The book was translated into English ten years later, and published in

London in black letter type as 'The new found worlde, or Antarticke, wherein is contained wonderful and strange things, as well of humaine creatures, as Beastes, Fishes, Foules, and Serpents, Trees, Plants, Pines of Gold, etc., etc.

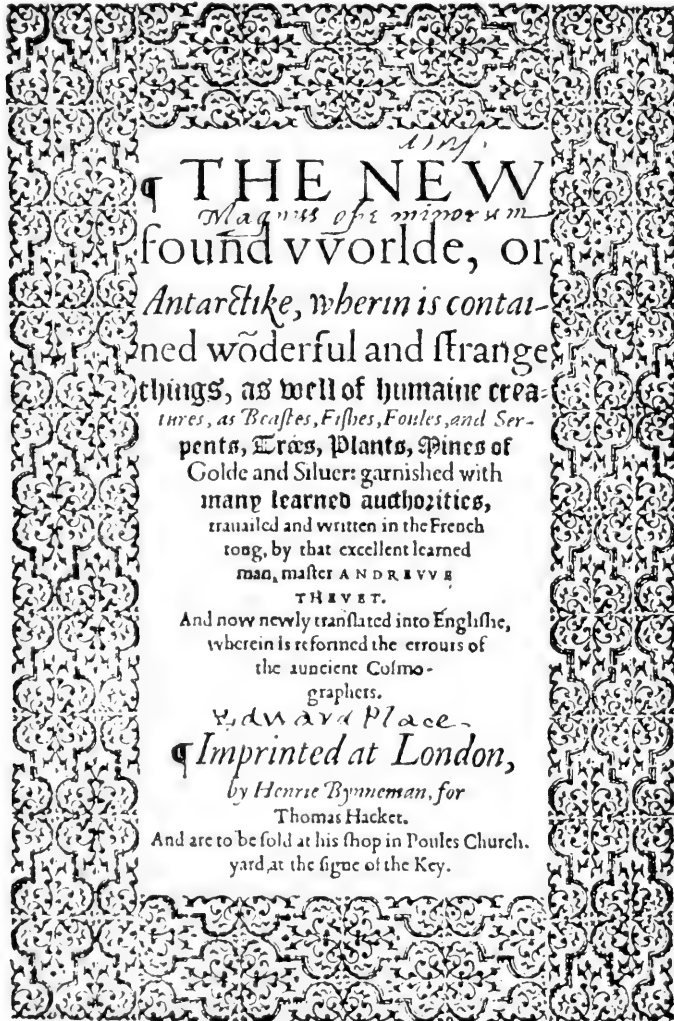


Photo by Donald Macbeth, London

travailed and written in the French tong by that excellent, learned man, Master Andrewe Thevet, and now newly translated into English.'

The following quotation, referring to the sand-flea, is made from the English translation, with a few corrections and additions from the original French where necessary:—

'I will not forget, that among their diseases they have one marvellous indisposition, which commeth by little wormes that enter into their feete, named in their language *TOM*, that are little ones [French 'lesquels ne sont gueres plus gros que cirons'—are scarcely bigger than acari (Note 10)] and I thinke that they ingender in their feet: for there will be sometimes such a number in one place that they will rayse a knob as big as a beane, with doloure and paine in that place, the which paine also chaunced to us, for being there, our feete and our handes were covered with little clothes [French 'bosettes' = bumps], in which when they were broken, was onely one white worme with filthe. And for to shunne this grieffe, the wilde men make a certain oyle of a fruite named *Hibonconhu*, like unto a date, the which is not good to eate; they preserve it in little vessels of fruites, named in their language *Caramenio*, and therewith they rub the places that are grieved, a thing very necessary [French 'propre' = suitable] as they doe affirme against these wormes.

'Also sometimes they anoint therewith all their bodies when they are weary. Besides this the oyle is proper for woundes and sores, as they have knowne by experience.'

The word *Hiboconhu* used here is probably the equivalent of *Ipecacuanha*, under which title were, and still are, known a considerable number of plants of Brazil and other parts of the New World. *Hiboconhu* is not, however, the *Ipecacuanha* of our official Pharmacopœia, the fruit of which bears no resemblance to a date. The drug *Ipecacuanha* itself did not reach Europe until the latter part of the seventeenth century.

André Theuet, the author of this work, lived from 1502 to 1590. He was a man of humble birth, who entered upon theological studies, but became indiscriminate in his wide reading. He was known in his day for his vast memory and for his restless and wandering habits. He travelled both in the Orient and in the New World, and wrote very credulous accounts of his journeys. Returning to France, he took orders and spent the declining years of his life in literary work.

NOTE 1. The relationship of literature and seacraft of this period, the reader will find interestingly discussed by Commander C. N. Robinson and Mr. John Leyland in Vol. IV of the 'Cambridge History of English Literature,' and by Sir Clements Markham in his 'Life of John Davis,' London, 1889.

NOTE 2. Those bitten by the Tarantula were supposed to be similarly affected by a desire to jump into the sea (*vide* Athanasius Kircher's 'Magnes sive de Arte Magnetica Opus,' Rome, 1641, p. 870), as were also, at times, the St. Vitus' dancers of the Middle Ages.

NOTE 3. Haeser, in his 'Historisch-pathologische Untersuchungen,' Dresden, 1841, part 2, p. 26, identifies the Tabardilla with Typhus. He considers, on the authority of the three writers, Vallesius, Toreus, and Coyttarus, that there was an epidemic of this disease in Spain in 1557. This disease can, however, scarcely be the one described by our author under the name Tabardilla, the symptoms of which hardly fit in with those of Typhus.

NOTE 4. By 'Casada' is doubtless meant Cassava or Mandioc, a plant extensively cultivated in the West Indies and in Tropical America. The fleshy, tuberous roots are used as food, and from them Tapioca is obtained. A cold compress could be made from them.

NOTE 5. Ventose: an obsolete word for a cupping glass.

NOTE 6. The first detailed account of the disease was probably that of Clarke in the 'London Medical Gazette,' September, 1840, p. 970, and later in the 'Edinburgh Monthly Journal of Medicine,' April, 1842, p. 32, and the 'Trans. of the Epidemiological Society,' I, 116.

NOTE 7. The publications of John Atkins are as follows:—

(1) 'The Navy Surgeon,' with the Appendix quoted above, London, 1734. 2nd Edition, London, 1737.

(2) 'A Voyage to Guinea, Brasil, and the West Indies; in His Majesty's Ships, the Swallow and Weymouth,' London, 1735. An account of this voyage is abstracted in Moore's 'Collection of Voyages and Travel,' London, no date (? 1740), and in 'A Collection of Voyages and Travels,' published in London anonymously in 1745. Some attention is given to these writings of Atkins in the 'Allgemeine Historie der Reisen' of J. J. Schwabe, Leipsig, 1748.

(3) 'A Treatise on Chirurgical Subjects,' London, 1736 (?). This volume contains some chapters of (1) re-printed, and among them the passages on 'the sleepy distemper.'

NOTE 8. Oviedo, 'Cronica de las Indias' 1547, fol. XXI, gives the animal the name of 'nigua.'

NOTE 9. The word 'ciron,' which I have here translated 'Acarus,' is a term which is of doubtful meaning in any work which appeared before the microscope came into use. The little *Acarus scabiei*, being on the limits of unaided vision, is in early writings repeatedly confused with the *Pediculus*, and although Saint Hildegard in the twelfth century, Guy de Chauliac in the fourteenth century, and Paracelsus in the succeeding century, had all definitely referred to the *acarus*, it is usually difficult to be sure of the identity of the organism, even in the works of these writers. In the present context, however, the reference to the *burrowing* habits of the chigoe, and the statement that it is scarcely larger than the 'ciron,' makes the translation 'acarus' a fairly safe one.

Addendum. Since writing the above the writer's attention has been drawn to a reference to Atkins' work by Dr. E. D. Whittle in the 'Malay Medical Journal' for April, 1911, and in the Bulletin of the Sleeping Sickness Bureau, for August, 1911, p. 329.

THE CULTIVATION OF *TRYPANOSOMA RHODESIENSE*

PRELIMINARY NOTE

BY

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While doing research under the Sir Edwin Durning-Lawrence fund it was suggested to me by Sir Ronald Ross, that I might attempt to cultivate trypanosomes pathogenic to man, especially the new species of Stephens and Fantham (1910), namely *T. rhodesiense*.

After many failures success was attained by the use of a modification of the Novy-McNeal-Nicolle medium. In the water of condensation of this medium *T. rhodesiense* has been successfully cultivated for two weeks by incubating at a temperature of 25° C. to 28° C., and subcultures of one generation have continued to develop for eleven days, and then all flagellate forms disappeared. In this preliminary note I shall give as concisely as possible, the changes which take place in the culture tubes. It was found that these cultures were most successful if young trypanosomes were inoculated into the tubes. By young trypanosomes, I mean trypanosomes taken from an infected rat about the second day after their appearance in the peripheral blood.

In some of the culture tubes the development was rapid, and when such occurred the culture died out much more quickly, i.e., in about seven days.

The most remarkable feature about the cultures is the striking changes which take place in the trypanosomes. The trypanosomes become larger, and forms are found which are very similar to those described by Sir David Bruce and his colleagues (1911) in the gut of tsetse-flies fed on animals infected with *T. gambiense*. When the development is rapid, about the third day, the trypanosomes are seen to have become much larger, and on the fourth day two distinct types can be distinguished which resemble the so-called sexual forms

of Professor Kleine and Dr. M. Taute (1911) found in tsetse-flies fed on animals infected with *T. gambiense*. In cultures which developed more slowly, it was found that trypanosomes disappeared about the third and fourth days, reappeared about the sixth day, and on the eighth day spirillar forms were seen to be splitting off. The differentiation into the so-called male and female forms takes place during the eighth, ninth and tenth days.

In cultures of *T. rhodesiense* long, thin forms are found with a posterior nucleus, the nucleus being close up to the blepharoplast, and not behind it. Stout forms of large size corresponding to the female forms of Kleine and Taute have been seen. Many of these stout forms have very large nuclei, and in some their nucleus is posterior. Kleine and Taute, in their study of the development of *T. gambiense* in the tsetse-fly, figure numerous trypanosomes, both thin and stout, with the nucleus behind the blepharoplast; and Sir David Bruce and colleagues (1911) found slender forms which were crithidial, but only a very small percentage of these occurred in the intestine of *Glossina palpalis*. So far I have not found definite crithidial forms. When long, thin forms and stout forms are found in the culture together, I have often seen what might possibly be conjugation occurring between the so-called males and females in fresh as well as in stained specimens. Another very constant feature in cultures of rapid growth is the early occurrence of a multiple longitudinal fission of the trypanosomes into numerous thin or spirillar forms, very similar to that described by Leishman (1905) in the cultivation of *Leishmania donovani*.

In cultures in which development was slower, and which lived for fourteen days, it was found that *T. rhodesiense* seemed to disappear from the water of condensation for two or three days, and then reappeared. On such reappearance trypanosomes were observed as well as large plasmodial forms which evidently split up into numerous brood trypanosomes, and again the two distinct types, the so-called sexual forms of Kleine and Taute found in tsetse-flies, were seen.

From a study of these cultures I think that we may have a sexual phase in the life cycle of trypanosomes, and that in all probability we have occurring in culture tubes just that which takes place in the cycle of development of trypanosomes in the alimentary tract of

tsetse-flies after feeding on an infected animal. My chief reason for so thinking is that the trypanosomes found in cultures appear in forms very similar to those described by Kleine and Taute in the intestinal tract of tsetse-flies. It is, however, most difficult at the present stage of this research to make a definite statement regarding these forms, or to say what exactly occurs after conjugation, for at present what has been described as occurring in tsetse-flies is the development of *T. gambiense*, and as I have only studied *T. rhodesiense* in cultures it is impossible to compare the two morphologically, but the general behaviour of *T. rhodesiense* in cultures shows changes which in many respects resemble the development of *T. gambiense* in tsetse-flies.

During periods in which flagellate trypanosomes are very scarce or absent altogether from cultures, distinct rounded bodies have often been found like those described by Fantham (1911).

In the cultures obtained, there was undoubted multiplication of trypanosomes, as evidenced by the fact that in two cultures the flagellate trypanosomes entirely disappeared from the water of condensation of the cultivating media for at least two days, and again reappeared showing active division and becoming very numerous.

Division of trypanosomes was well seen in living specimens, and stained specimens showed division of nucleus and blepharoplast. Another feature noted in the cultures was the complete or almost complete absence of a free flagellum, which seemed to stop short at the anterior end of the trypanosome or only to project slightly beyond it.

So far, inoculation of animals from the cultures has been unsuccessful, which may be accounted for by the fact that the inoculations were possibly made before complete development of the trypanosomes had taken place, since we know that tsetse-flies after feeding only became infective after a certain latent period.

I hope to publish at an early date a full account of these cultures of *T. rhodesiense*, with illustrations of the different forms which occur. The trypanosomes employed to obtain these cultures were undoubtedly *T. rhodesiense*, since posterior nuclear forms were found in the blood before inoculation, and I have been able to exclude an accidental contamination of *T. lewisi* by using tame rats,

which were carefully examined before inoculation. In addition to this, a careful comparison has been made by growing *T. lewisi* as a control, and it is found that the cultures of *T. rhodesiense* showed no similarity to those of *T. lewisi*.

Finally, the modification of the Novy-MacNeal-Nicolle medium used in the cultivation of *T. rhodesiense* consists in substituting for defibrinated rabbit's blood, citrated rat's blood heated to 45° C. for half an hour, and sea salt was substituted for ordinary sodium chloride.

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THE TRYPANOSOMES FOUND IN A HORSE NATURALLY INFECTED IN THE GAMBIA. A DOUBLE INFECTION

BY

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(Received for publication 24 April, 1912)

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I. CONCLUSION OF A PREVIOUS PAPER

A former paper (1911) dealt with the trypanosomes found in two horses naturally infected in the Gambia. These horses were referred to as *Horse A* and *Horse B*. The former, *Horse A*, contained in its peripheral blood two forms of parasite, one long the other short; the latter, *Horse B*, presented one form of parasite only. The conclusion of the paper was as follows:—

(1) We consider the trypanosome found in *Horse B* to be *T. dimorphon*, *sensu* Laveran and Mesnil.

(2) The long form in *Horse A* appears to us to be *T. vivax*.

(3) As regards the short form found in *Horse A*, we do not feel justified at the present stage in assigning its position. It may be a Dimorphon-like trypanosome of low pathogenicity, or simply a modification of the long parasite of *Horse A*.

This further work, an account of which is now presented, deals solely with the two forms of trypanosome found in *Horse A*, and was directed chiefly to deciding the point as to whether the short aflagellar trypanosome was a modification of the free flagellated long form, or belonged to a different species.

II. THE SEPARATION OF THE SHORT FORM FROM THE LONG FORM

The blood of *Horse A* contained a great preponderance of trypanosomes of long form, there being roughly speaking a thousand long forms to one short form. When the blood of *Horse A* was inoculated into laboratory animals, very varying results were obtained, according to the species of animal used. Thus, Goat 1485, inoculated directly from *Horse A* contained in its blood both long and short forms of trypanosome, and these were present in much the same proportion as in the blood of *Horse A*. On the other hand, rabbits, guinea-pigs, rats and mice inoculated with blood from *Horse A*, either failed to become infected or presented in their blood short, aflagellar trypanosomes only. There was one exception to this rule, namely, Rabbit 1467, referred to in the previous paper. In this animal both long and short forms of parasite were present in the blood during the course of the infection, the long forms predominating until the last day, when the short forms suddenly became numerous. Two mice and a rat inoculated from this rabbit became infected with short forms only. It was early apparent that, if the long form of parasite were to be maintained in animals, certain of the smaller laboratory animals (dogs, rabbits, guinea-pigs, rats, etc.) would prove of little service. Goats, however, showed themselves to be very susceptible to infection with the long form, and it has been found possible to preserve this type by passage from goat to goat. The short trypanosome was passed through the smaller laboratory animals, but proved to be, in the early stages, of remarkably low pathogenicity, especially as regards rats and mice.

The inoculation of the blood of *Horse A* into certain rabbits and guinea-pigs, resulted in infection with short forms only. It was thus possible to obtain an apparently pure infection with the short forms in certain species of animals, the long form not appearing in them.

As regards the separation of the short form from the long form, it was observed above that Goat 1485, inoculated from *Horse A*, presented both forms of parasite in its blood. A dog which was inoculated from this goat became infected, but no long forms of parasite ever appeared in its blood, short aflagellar trypanosomes only being present. A second goat, Experiment 1497, inoculated from *Horse A* presented long forms only in its blood, and it was found that a dog inoculated from it failed to become infected, either with long or short forms, as did also five rats and two mice. A goat, however, became infected on inoculation from Goat 1497, and in its blood long forms only were discovered. From this time on, the blood of the goats in which the long form has been maintained has never given rise on inoculation into the smaller animals to an infection with short forms of trypanosome.

III. THE EVIDENCE UPON WHICH THE DIAGNOSIS OF A DOUBLE INFECTION IS BASED

Apart from the distinctive differences in morphology and measurements which are dealt with below, the following experimental evidence is now adduced to prove that two separate species of trypanosome co-existed in *Horse A*.

(a) The long-form strain of trypanosomes which has been kept up by passage from goat to goat for a period of nearly a year, maintains its characteristic appearance. Short forms have never been seen in the blood of the goats since the first passage. Two monkeys, *C. callitrichus* and *Macacus rhoesus*, six dogs, two cats, seven rabbits, five guinea-pigs, one brown wild rat, eighteen tame rats, and eleven mice, have been inoculated from different goats at different times. Many of the animals were re-inoculated. In none of these animals did the short form of trypanosome ever appear, nor was their blood infective on sub-inoculation into other animals of the same species.

(b) The short form of trypanosome which appeared in a rabbit inoculated from *Horse A*, was carried down to mice, which became infected. From one of these mice a fresh horse was inoculated. This animal became infected, and had parasites visible in its blood for one day only, and the parasites were all of a short aflagellar type. The blood of this horse was highly infective for a goat,

dogs, rabbits, guinea-pigs, rats and mice. In all the animals inoculated from this horse short trypanosomes only were observed. The most careful search failed to reveal a parasite of long form. Therefore, neither in this experimentally infected horse, which was inoculated from a mouse showing short trypanosomes only in its blood, nor in a goat inoculated from this horse, were long forms of trypanosome discovered. When one considers the facts that in *Horse A* the long form of parasite was so greatly in excess of the short, and that goats have proved themselves very susceptible to infection with this long form, one would expect that if the short parasite were merely a variation of the long form, it would, on being brought back into horses and goats, assume the long form. This did not occur.

(c) Many laboratory animals, after having proved themselves quite refractory to the long form of parasite, even after repeated large and small inoculations, both subcutaneously and intraperitoneally, became readily infected on being inoculated with blood containing the short form of parasite. Table I shows the effect of inoculating short-form parasites into three dogs refractory to the long form.

(d) Four rabbits and two white rats intraperitoneally inoculated with blood from goats infected with the long form, became infected and parasites were found in their blood for brief periods (one to ten days). The parasites were all of the long type. Efforts to convey this infection to animals of the same species by intraperitoneal and subcutaneous injection have so far failed, though repeatedly made. These facts militate very strongly against the idea that the short form of parasite is simply a modification of the long form, and it appears certain that the short form is quite a distinct species from the long form, which is *T. vivax*.

IV. PATHOGENICITY OF *T. VIVAX* IN GOATS

In Table II are given the details regarding the pathogenicity of the long-form parasite, *T. vivax*, in a series of goats.

It will be seen that the average incubation period in a series of fourteen consecutive goats was nine days, and the average duration of the disease was thirty-one days. No goat which became infected has recovered, and no goat has failed to become infected

on inoculation with goat's blood containing *T. vivax*. The majority of the goats were observed to become paralysed in their hind legs a day or two before death and several of them had opacities of the cornea. It will be observed from this table that there is no marked diminution in the incubation period nor in the duration of the disease in the later goats, such as would indicate any definite increase in virulence of the strain. A considerable difference in this respect will be found in considering the short form.

V. MORPHOLOGY AND MEASUREMENTS OF THE LONG FORM IN GOATS

The long parasite is free-flagellated and monomorphic. The posterior portion is broad and the body tapers gradually towards the anterior end. The nucleus lies near the centre of the trypanosome, but anterior to the broadest portion of the body; it is, as a rule, well defined, but occasionally somewhat diffuse. The blepharoplast, round and distinct, is situated either laterally close to the posterior extremity or terminally; the membrane is simple and narrow. The average measurement of a thousand of the long forms taken from four goats on twenty-two days of the disease is 21.7μ .

VI. THE MOTILITY OF *T. VIVAX* IN GOATS

The great rapidity of movement of the trypanosome, as seen in fresh preparations of the blood, was the most striking feature observed by Ziemann, who first described it in the Cameroons, in 1903. The rapidity with which it darts across the field is most characteristic. It was observed that this *T. vivax* from the Gambia possessed this character most markedly. However, after it had been preserved for some time in goats, a distinct change was noticed as regards its motility. It gradually became slower and slower in its movements. In some cases its movement became absolutely sluggish, the parasite clinging to the corpuscles and remaining stationary, somewhat in the manner of the *pecorum* group. This change commenced about October, and the sluggishness persisted until March. During this period the motility of the parasite was very limited and by no means suggestive of the designation '*vivax*' applied to the trypanosome. Further, this slowness of movement did not appear to depend entirely upon the

laboratory temperature at which the films were usually examined as it was observed also even when the film was transferred as quickly as possible, and examined in a warm atmosphere. In March the movements became more rapid, and now, in April, the parasite has regained its former rapidity of movement and its habit of dashing across the field and out of sight.

VII. PATHOGENICITY OF THE SHORT-FORM TRYPANOSOME IN LABORATORY ANIMALS

The experimentally infected Horse 1608 mentioned above, which was inoculated from a mouse showing only short forms, and which in turn showed only short forms in its blood, is taken as the starting point in considering the pathogenicity of the short form of parasite in rats. The appearance of short forms only in this horse, was an indication that the short form was not a derivative of the long form, and from this time on two strains of the short form have been maintained, one in rats only, derived from this horse, and another, derived from the original *Horse A*, in rabbits. In Table III are given the results of carrying on these two strains of the short form, and in addition the results of inoculations made into various other animals. A very marked increase in virulence was observed in this trypanosome after its passage through Horse 1608. The increase of virulence was most noticeable in regard to those animals which had previously proved very refractory, namely, rats and mice. For example, in the mouse from which the horse was inoculated the incubation period was eighteen days, and death had not occurred on the fifty-seventh day, whereas in the six Mice 1761, after the parasite had passed through Horse 1608, the average incubation was four days and death occurred on the thirteenth day. Thus, this parasite, already resembling *T. dimorphon*, *sensu* Laveran and Mesnil, not only in its appearance both in fresh and stained preparations, but also in its measurements, has further approached it in regard to its animal reactions.

VIII. MORPHOLOGY AND MEASUREMENTS OF THE SHORT FORM IN RATS

This trypanosome is a short, non-free flagellated parasite. The protoplasm stains more deeply than that of *T. vivax*, the nucleus is central and well defined, the blepharoplast terminal or lateroterminal

and round. In dividing forms there is frequently noted, besides a considerable elongation of the parasite, the presence of a very small portion of flagellum which appears to be free. The average measurement of a thousand trypanosomes drawn from two white rats on ten days of the disease is 13.3μ . A curve showing the percentage distribution of the various lengths is in course of preparation and will be published later.

CONCLUSION

- (1) *Horse A*, naturally infected in the Gambia contained in its blood two distinct species of trypanosome.
- (2) The first trypanosome, the long form, is *T. vivax*.
- (3) It appears justifiable to classify the short trypanosome, taking into consideration its morphology, measurements, and the later developments of its animal reactions, as *T. dimorphon*, *sensu* Laveran and Mesnil.

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YORKE and BLACKLOCK (1911). 'The trypanosomes found in two horses naturally infected in the Gambia.' *Ann. Trop. Med. and Parasit.*, Vol. V, No. 3, p. 413.

TABLE I.—Showing the effects of inoculating the short form of parasite into dogs which had proved refractory to the long form.

No. of Experiment	LONG FORM		SHORT FORM		Remarks	
	Animal from which inoculated with long form	Result	Animal from which inoculated with short form	Incubation in days		
Dog, 1609 ...	Goat 1605 reinoculated once	Parasites never seen up to 44th day	Guinea-pig 1651 B	14	17	Short forms only seen
„ 1612 ...	Goat 1584	Parasites never seen up to 21st day	Horse 1608	9	28	„
„ 1661 B	„ 1584 reinoculated twice	Parasites never seen up to 33rd day	Dog 1725	8	—	„ Developed acute pneumonia; killed by chloroform on account of pain

TABLE II.—Pathogenicity of *T. vivax* in Goats

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
Goat 1497 ...	Horse A ...	10th	44th	Highest temperature recorded 107° F.
„ 1559 ...	Goat 1497 ...	11th	64th	
„ 1584 A...	„ 1605 ...	6th	29th	
„ 1605 ...	„ 1559 ...	9th	47th	
„ 1673 ...	„ 1605 ...	9th	22nd	
„ 1685 ...	„ 1584 ...	4th	17th	
„ 1733 ...	„ 1685 ...	7th	16th	
„ 1735 ...	„ 1685 ...	10th	28th	
„ 1774 ...	„ 1733 ...	9th	27th	
„ 1801 ...	„ 1774 ...	13th	43rd	
„ 1838 ...	„ 1801 ...	13th	14th	
„ 1858 ...	„ 1801 ...	11th	16th	
„ 1895 ...	„ 1838 ...	11th	35th	
„ 1933 ...	„ 1895 ...	11th	32nd	
	Average ...	9.6	31	

TABLE III.—The pathogenicity of the short-form trypanosome in laboratory animals

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
Rabbits 1494 ...	Horse A ...	19th	164th	
„ 1784 ...	Rabbit 1494 ...	23rd	56th	
„ 1877 ...	„ 1784 ...	22nd	—	Alive on 91st day
Rat 1650 A...	Horse 1608 ...	6th	78th	
„ 1650 B...	„ 1608 ...	6th	74th	
„ 1663 A...	Rat 1650 A...	9th	97th	
„ 1663 B...	„ 1650 A...	12th	51st	
„ 1663 C...	„ 1650 A...	7th	45th	
„ 1870 ...	„ 1663 A...	9th	16th	
„ 1727 A...	„ 1663 B...	9th	41st	
„ 1727 B...	„ 1663 B...	9th	45th	
„ 1909 ...	„ 1870 ...	7th	—	Alive 80th day
„ 1919 ...	„ 1909 ...	10th	—	Alive 73rd day
„ 1952 ...	„ 1919 ...	10th	22nd	
„ 1977 ...	„ 1919 ...	7th	15th	
„ 2004 ...	„ 1977 ...	4th	8th	
No. of Experiment	Pathogenicity in other animals			
Goat 1657 ...	Horse 1608 ...	10th	19th	Paralysed in hind legs for two days before death
Dog 1609.....	Guinea-pig 1651 B	14th	17th	Dog previously used for long-form experiment.
„ 1612 ...	Horse 1608 ...	9th	59th	
„ 1688 ...	Guinea-pig 1651 B	29th	42nd	
„ 1725 ...	„ „	12th	13th	
Rabbit 1651 A	Horse 1608 ...	12th	43rd	

TABLE III.—Continued

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
Guinea-pig 1651 B	Horse 1608 ...	22nd	34th	
„ 1651 C...	„ 1608 ...	9th	73rd	
Rat 1613 ...	Guinea-pig 1651 B	8th	43rd	
„ 1732 A...	„ 1651 B	6th	9th	
„ 1732 B...	„ 1651 B	5th	9th	
„ 1732 C...	„ 1651 B	6th	12th	
„ 1732 D	„ 1651 B	8th	13th	
„ 1749 ...	Rat 1732 A...	3rd	10th	
„ 1858 ...	Mouse 1764 C...	9th	20th	
„ 1823 ...	Dog 1688 ...	8th	12th	
„ 1860 A...	Mouse 1855 ...	7th	11th	
„ 1860 B...	„ 1855 ...	6th	9th	
Mouse 1756 A ...	Rat 1749 ...	17th	74th	
„ 1756 B ...	„ 1749 ...	9th	11th	
„ 1761 A (1)	„ 1732 D	4th	20th	
„ 1761 A (2)	„ 1732 D	3rd	6th	
„ 1761 B (1)	„ 1732 D	3rd	6th	
„ 1761 B (2)	„ 1732 D	3rd	11th	
„ 1761 C (1)	„ 1732 D	4th	22nd	
„ 1761 C (2)	„ 1732 D	7th	12th	
„ 1764 A ...	„ 1749 ...	—	7th	Parasites never seen
„ 1764 B ...	„ 1749 ...	13th	22nd	
„ 1764 C ...	„ 1749 ...	10th	22nd	
„ 1839 A ...	„ 1823 ...	10th	17th	
„ 1839 B ...	„ 1823 ...	8th	10th	
„ 1839 C ...	„ 1823 ...	9th	14th	
„ 1839 D	„ 1823 ...	9th	15th	
„ 1855 ...	Mouse 1839 C...	4th	6th	

PAROPISTHORCHIS CANINUS
THE LIVER-FLUKE OF THE INDIAN PARIAH
DOG

BY

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WALTER MYERS LECTURER IN TROPICAL MEDICINE, THE UNIVERSITY OF LIVERPOOL.

(*Received for publication 7 April, 1912*)

Among a collection of specimens presented in 1911 to the Museum of the Liverpool School of Tropical Medicine by Captain R. Markham Carter, I.M.S., there was a bottle containing two small flukes. They were got from the liver of a native dog at Kasauli, India. On examining them I found that they were spinose flukes resembling those originally described by J. F. P. McConnell (1876 and 1878), from two Mahommedans, post mortem, and by T. R. Lewis and D. Cunningham (1872), and T. R. Lewis (1888), from the liver of the pariah dog. Lewis had thought that the flukes in question were *Distomum conjunctum*, Cobbold, but, as the figures show, they were certainly not this species, and Braun (1903) re-named them *Opisthorchis noverca*.

As this fluke, then, had not been described or recorded from India since 1878, I determined to re-examine it. A preliminary examination of Captain Carter's specimens left me in considerable doubt as to the nature of certain appearances. I accordingly wrote to Major Christophers, I.M.S., at Kasauli, who very kindly sent me a fresh supply, and stated that he could procure as many as I wanted. I mention this as it is evidence of the extreme commonness of this fluke in the North-West provinces at least, as, indeed, has quite recently been shown by the data of Gaiger (1911), who records nineteen out of fifty dogs infected.

External appearance. The most striking feature of this fluke when examined with a pocket lens is a cylindrical process, about 1/2 mm. long, projecting from the anterior portion of the ventral surface, which on closer inspection is seen to bear on its summit the ventral sucker and opening of the common genital pore. In my original specimens this stalk, or pedicle, was not visible, as it was retracted, and I was at first much puzzled by the spherical structure surrounding the sucker.

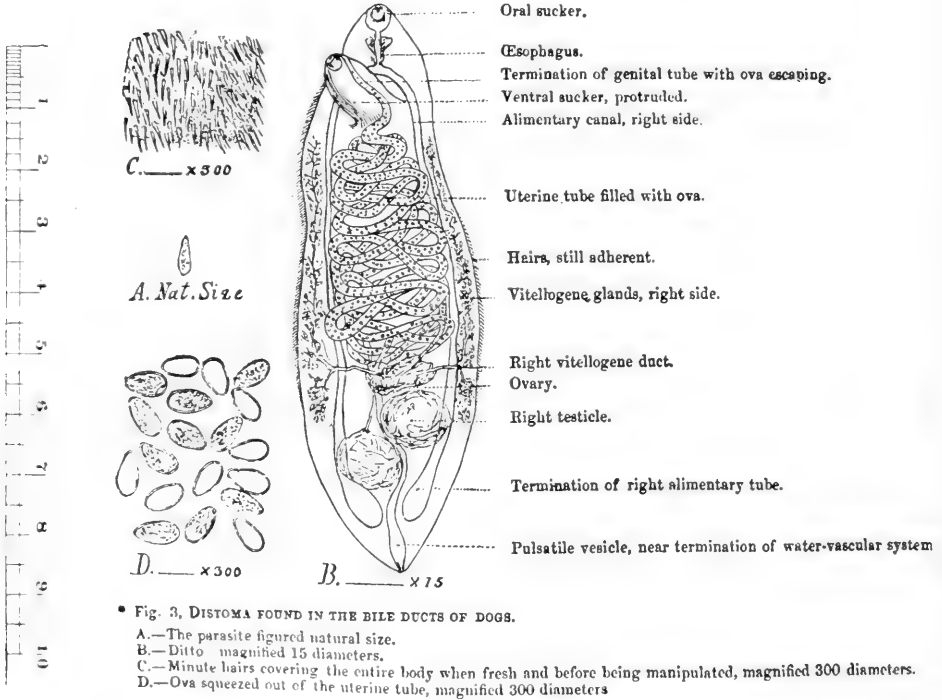
The length of the fluke is:—

Sublimate specimens (6) ...	average	3.6 m.m.,	maximum	5.5,	minimum	2.75
Formalin 10% specimens (6) ...	"	5.2	"	5.75,	"	4.75
Alcohol 70% specimens (6) ...	"	3.96	"	4.6,	"	3.5

The body is uniformly spinose, though, as a rule, spines are not present on the pedicle. In preserved specimens the body is slightly concavo-convex, the concavity being ventral.

Alimentary canal. The mouth is directed ventrally. The diameter of the oral sucker is 0.28 mm. The pharynx measures 0.224 by 0.184 mm., and is followed by a narrow oesophagus 0.04 mm. long.

The ventral sucker is 0.176 mm. in diameter.



Vitellaria. Consist of eight acini on each side, extending from slightly behind the base of the pedicle to the anterior border of the ovary or as far back as a line separating the posterior border of the ovary from the anterior border of the anterior testis. The arrangement of the acini is very constant, and in the examples I have examined I have found no appreciable variation. Inwardly

NOTE.—The figure on this page is a reproduction of Lewis and Cunningham's original figure (vide literature p. 123).

they encroach but little, if at all, on the gut caeca. The transverse vitelline ducts pass inwards and backwards from the level of the seventh acinus. They unite in rather an open **V** to form a receptacle dorsal to the anterior lobe of the ovary, at or slightly behind the level of the posterior vitelline acini. The junction of the radicles which go to form the transverse ducts on either side has the following arrangement. The duct from acini 1-6 is joined by that from acinus 7 and at the same point by the duct from acinus 8, but this arrangement, while commonest, is subject to some variation.

Testes. The anterior testis is 0.496 by 0.440 mm. The posterior testis is 0.520 by 0.480 mm. They are, as a rule, ovoid though they may also be to some extent regularly lobate, e.g., in one specimen examined the posterior testis was trilobed. One lies obliquely behind and adjacent to the other. The anterior testis is usually on the left side and the posterior testis on the right side. In some cases the organs are reversed, i.e., the anterior testis is on the right side and the posterior on the left side, with the seminal receptacle on the left side and commencing uterine duct on the right side.

The gut is slightly displaced by the anterior and more so by the posterior testis.

The left vas efferens passes forward parallel to the edge of the gut caecum. The right vas efferens runs a wavy course parallel to the right caecum, disappearing dorsally to the seminal vesicle.

The ovary is multilobular, the lobes (6-8) being irregular in size and shape. Not uncommonly the ovary shows three ovoid lobes ventrally.

Excretory vesicle. Curving forward between the testes, expands into a pyriform sac behind the ovary. The transverse excretory ducts enter this sac laterally forming a **Y**.

The right duct curves around the end of the seminal receptacle and crosses ventral and obliquely to the gut caecum; the left passes around the anterior border of the anterior testis. Anteriorly the longitudinal ducts can be traced as far forward as the bifurcation of the gut.

Shell gland. Extensive and diffuse, occupies an area between the ovary, seminal receptacle and the first uterine coil, and extending laterally beyond the left transverse vitelline duct. The area corresponds approximately to the loop of the vitelline glands.

Seminal receptacle. Banana-shaped, globular or pyriform, is situated to right of and dorsal to posterior lobe of ovary, may extend as a twisted sac as far as the gut caecum. It is distended with spermatozoa.

Laurel's canal. Arising from an ampulla at the end of the seminal receptacle, runs with a single curve medially and backwards, opening lateral to the anterior testis. In other cases after running backwards it bends forwards again and opens about the level of its point of origin. The opening is surrounded by an area, free from spines, in diameter about three times that of the opening.

Uterine coils. Commence at the level of the hindmost vitelline acini. They form rather loosely packed transverse loops, terminating slightly in front of the level of the first vitelline acini. From here the uterus passes forwards into the pedicle to the left and ventral to the seminal vesicle. The folds slightly overlap the caeca at some points, or displace them outwardly at others.

Seminal vesicle. Commences about the level of the first vitelline acini. The coils rapidly develop in extent, extending ventrally and displacing the uterine coil ventrally and to the left, they now form in cross-section 5-6 coils occupying practically the whole thickness of the fluke. As the vesicle passes forward to the base of the pedicle it is embraced on either side by the muscular (longitudinal) fibres which constitute the main bulk of the pedicle (until the sucker is reached) and serve to retract it as a whole. Further forward it diminishes in extent and now lies in its dorsal (anterior) side extending forward between the base of the sucker and the dorsal wall (fig. 2).

Common genital sinus. The pedicle now consists of sucker surrounded by muscles in which lie the two genital ducts on the dorsal side. Projecting beyond the sucker is a cuticular rim, or rather two rims, in a groove between which are a number of scales densely crowded together (fig. 2). It is into this groove that the genital ducts open. The opening of the genital sinus, as seen in transverse section, appears as a crescentic space which is densely crowded with a number of finger-like scales (fig. 7). The cavity of the sinus now becomes continuous with the general cavity embraced by the cuticular rims (fig. 8). At this point the cuticular rim is usually entirely covered with scales, so that when the two cavities

become one, scales line the whole single lumen. While scales are present at the opening of the sinus, the extent to which they embrace the whole of the circumference is a variable one, as in some cases they can only be detected around the sinus itself. The opening is thus strictly speaking into the sucker cavity, though not into the muscular portion. The exact position, however, varies according to the state of contraction of the pedicle and the resulting alteration in the relationship of the parts. If the pedicle and sucker are in a state of relaxation, the cuticular rim projects over the lumen and the sinus opens inwardly. If, on the contrary, the pedicle and sucker are contracted, the latter is dilated and projects or is on a level with the retracted rim; the sinus then appears to open externally, in any case it is anterior to the sucker corresponding thus to the position in the *Opisthorchinae*. The varying position of the circlet of scales in the cuticular rim can be well seen in total specimens where the pedicle is somewhat contracted so that the apex of the pedicle can be viewed end on. It is then seen that the circlet of scales in some cases clusters around the opening of the sucker, whereas in other cases, due presumably to the contraction of the fibrils seen in figs. 7 and 8, the circlet of scales, now less closely clustered, is not at the apex of the pedicle, but lower down on its external surface.

To consider now the identity of this fluke.

Lewis and Cunningham (1872), and Lewis, T. R. (1888), figure two flukes, one natural size, the other $\times 15$, from the liver of an Indian dog. I have measured these and found the dimensions to be:—(1) 6.5×2.0 mm.; and (2) 6.3×2.0 mm., respectively.

McConnell, J. F. P. (1876), figures five flukes, four natural size and one $\times 6$, about, from the liver of man. These have the following measurements:—(1) 8.25×2.75 mm.; (2) 9.25×2.75 ; (3) 9.25×3.0 ; (4) 8.25×3.0 ; (5) 9.8×3.1 . Mean 8.96×2.9 mm.

He himself gives the following dimensions. Average length $3/8$ in. (= 9.4 mm.), average breadth $1/10$ in. (= 2.5 mm.). He also states that only one or two specimens were found $1/4$ in. (= 6.25 mm.), and that these showed evidences of immaturity. Again a few were also found $1/2$ in. (= 12.5 mm.) in length (but the great majority exactly $3/8$ in. (= 9.4 mm.). In a second paper (1878) he makes the following statement: 'somewhat larger than the original entozoan discovered by Cobbold being fully $1/8$ in.

(9.4 mm.) in length, and several 1/2 in. (12.5 mm.) as against 1/4 in. (6.25 mm.) the average size of the latter.

Summing up these data we have:—

From McConnell's figures, average 8.96×2.9 mm., min. 8.25×2.75 , max. 9.8×3.1 mm.
 ,, ,, data, ,, 9.4×2.5 ,, ,, 6.25 ,, 12.5 mm.

Thus the length varies from 6.2-12.5 mm. Compared with Lewis's and Cunningham's fluke, McConnell's appears to be longer, though we have no evidence as to whether the respective measurements were made all in the fresh, or whether some were fresh, other fixed.

Again McConnell does not make any mention of or figure the pedicle bearing the sucker and genital opening as it is correctly figured by Lewis, but, on the contrary, states that the 'reproductive papilla or genital orifice (is) placed a little above and to one side of the former' (ventral sucker) and figures it in this position. These facts, more especially the latter, would lead us to suspect the existence of two different flukes.

Again, I have measured the eight eggs figured by McConnell with the following results:—Length 29.1μ , 29.1μ , 29.1μ , 28.3μ , 28.3μ , 26.0μ , 26.0μ , 26.0μ . Average, 27.7μ .

Comparing these data we get:—

			Size of Eggs
Lewis	... figures	...	$20.8-26.6 \mu$, mode 22.5μ
McConnell	... ,,	...	27.7μ
McConnell	... measurements	...	$33.0 \mu \times 18.7 \mu$
Author	... ,,	...	range $28-30 \mu \times 12-14 \mu$, mode $28 \times 12 \mu$

These figures, then, do not throw much light on the question. It should be noted, however, that according to McConnell's *statement* the egg of his fluke is longer than that of the fluke measured by myself. On the contrary, Lewis only *figures* eggs, and makes no statement as to size; it is quite possible, therefore, that the discrepancy is not a real one, but dependent on errors of reproduction.

I think, however, for the reasons previously stated, there can be little doubt that the fluke I have described, and that from the dog described by Lewis, are identical.

If this be so, the question of the name of this fluke comes up for consideration. Braun, as we have seen, assumed, that these two

flukes were identical. I believe the evidence I have brought forward is sufficient to negative this view and that we are dealing with two distinct species. As regards the name *noverca*, used by Braun, in 1903, on the assumption that the two species were identical, it may, I think, be retained for McConnell's fluke, as Braun uses the latter's figure to illustrate his description, but as I consider that the fluke I am describing is the same as Lewis's or at least not the same as McConnell's, I propose for it the name *indicus*.* Further, as regards the generic position of this fluke, I hold that the anatomical difference, viz.: the existence of a process or pedicle bearing on its summit the genital opening and ventral sucker is sufficient to separate this fluke from the genus *Opisthorchis*. I propose to place it in a new genus, *Paropisthorchis*.

My proposed name for this fluke of the *Indian dog* will, therefore, be *Paropisthorchis indicus*.*

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* Barker 1911 (?) also concludes that these two flukes are different. He names McConnell's fluke *Amphimerus noverca*, and for Lewis's and Cunningham's suggests the specific name *caninus*. I accordingly withdraw the name *indicus*, and my suggested name for this fluke will therefore be *Paropisthorchis caninus*. I have to thank Dr. Leiper for a copy of Professor Barker's paper.

EXPLANATION OF PLATES

PLATE X

Fig. 1. Total specimen, stained, cleared in creosote, from ventral side. $\times 40$.

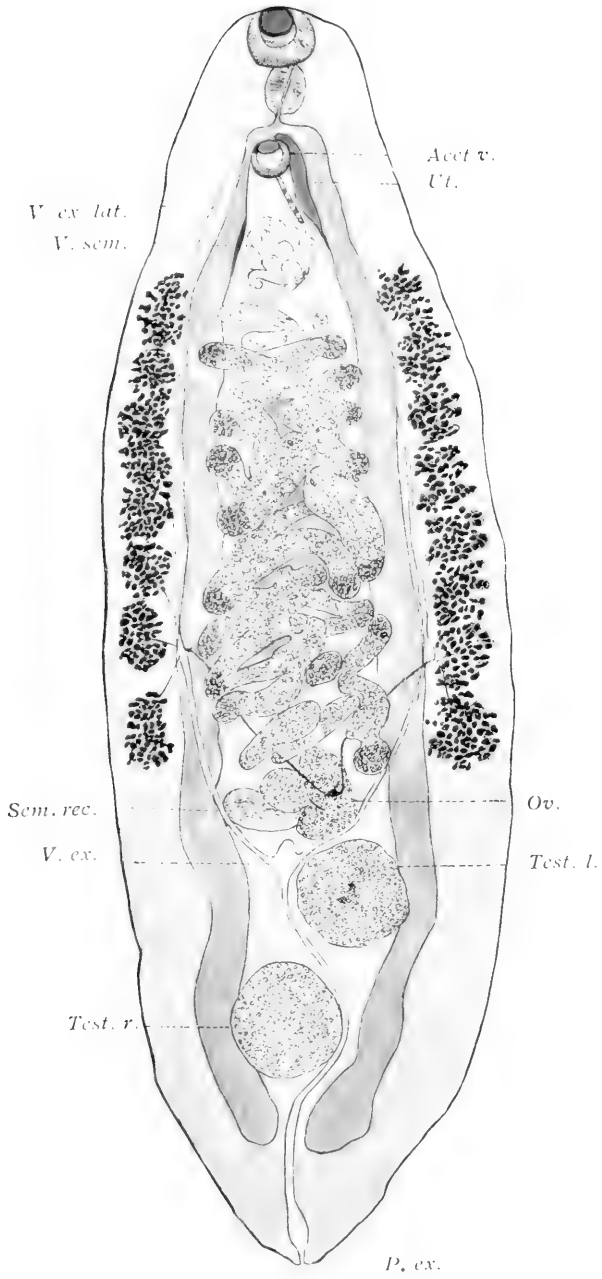


FIG. 1.



PLATE XI

- Fig. 2. Sagittal section; *b*, opening of seminal vesicle filled with scales. × 540.
- Fig. 3. Frontal (somewhat oblique) section; *a*, ventral sucker; *b*, genital sinus. × 450.
- Fig. 4. Frontal section, posterior to fig. 2, showing scales on right and left of the cuticular rim. × 450.
- Fig. 5. Frontal section, posterior to fig. 4, showing scales on posterior border of opening. × 450.

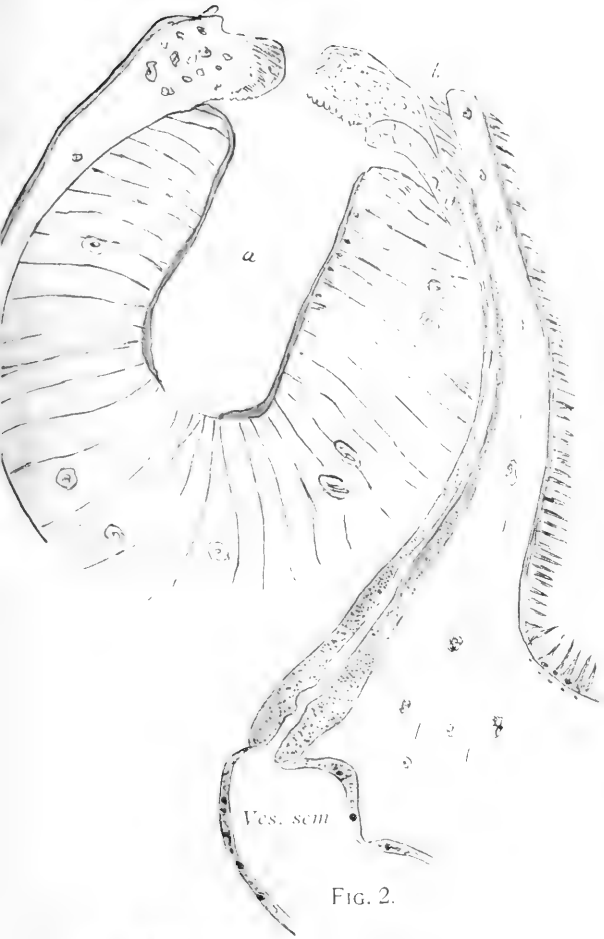


FIG. 2.



FIG. 5.

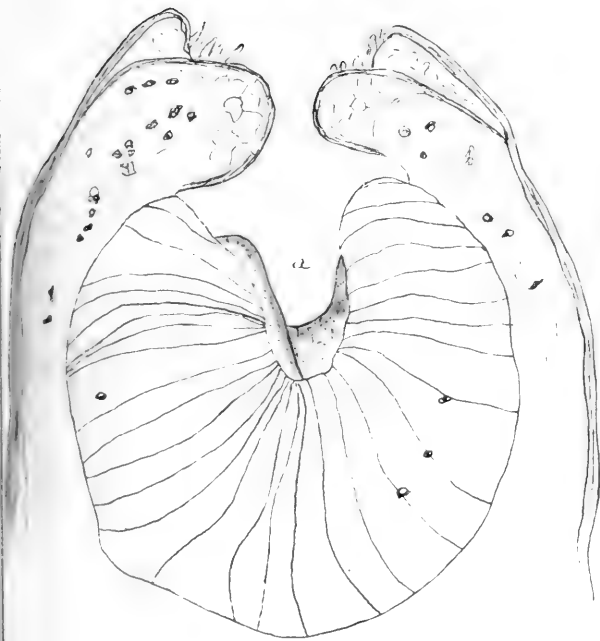


FIG. 4.

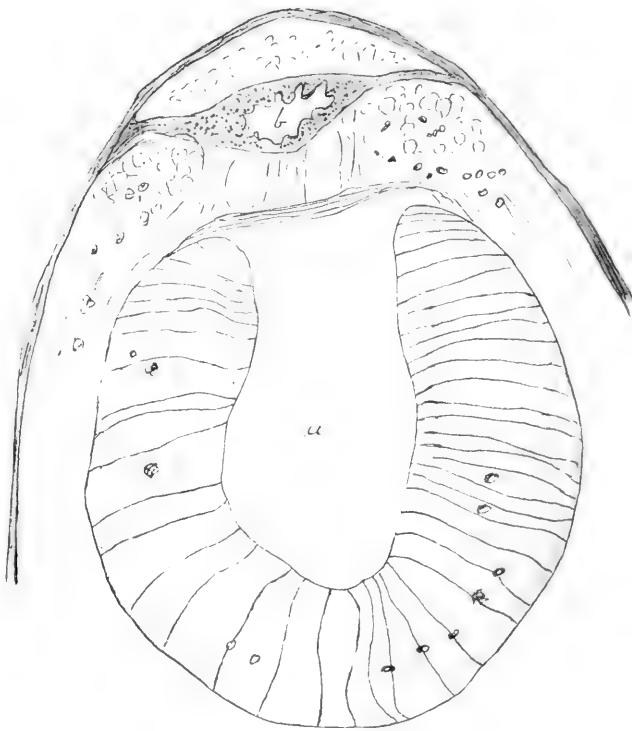


FIG. 3.



PLATE XII

- Fig. 6. Transverse section through pedicle; *a*, sucker, the lower portion is cuticular; *b*, genital sinus. × 450.
- Fig. 7. Transverse section through pedicle, nearer the apex than fig. 6; *a*, sucker, or margin thereof, now entirely cuticular; *b*, genital sinus crowded with scales, *c*, radial fibrils which first appear at this level. × 450.
- Fig. 8. Transverse section through pedicle, nearer the apex than fig. 7. The tubes *a* and *b* have now become confluent, and the whole cavity is surrounded by scales. × 450.

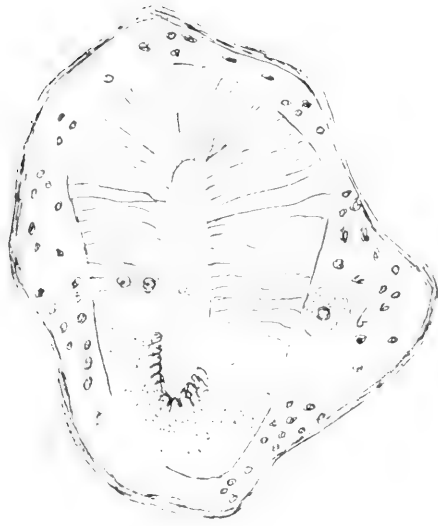


FIG. 6.



FIG. 7.



FIG. 8.



A NEW TSETSE-FLY FROM BRITISH EAST AFRICA

BY
PROFESSOR R. NEWSTEAD, F.R.S.

Glossina austeni, n. sp.

A small and somewhat slender species belonging to the palpalis group of tsetse-flies, and in addition to its slender form is distinguishable also by its bright ochraceous colour and relatively narrow head. In its general appearance it bears a somewhat close resemblance to a dwarfed specimen of *Glossina fusca*, Walker, but differs from this or any other member of this division of the Genus *Glossina* by the colour of the hind tarsi. Judging by its form alone, *G. austeni*, n. sp. seems more closely related to *G. tachinoides*, West., than any other species, but it is very clearly distinct and cannot be readily confused with any other member of the genus.

Female. Head buff relatively narrow; posterior surface grey, frontal stripe buff; ocellar spot dark brown; antennae and arista similar in form to those of *Gl. palpalis*, third segment with the apical two-thirds infuscated; proboscis bulb uniformly pale ochreous buff; palpi ochreous buff.

Thorax. Dusky buff, with dark brown markings similar to those in *G. fusca*, but with the dark lateral markings forming two rather indistinct, broad, stripes; pleurae dusky ochreous buff; apical scutellar bristles very short and spinelike.*

Abdomen. Dorsum bright yellowish buff or ochraceous; lateral margins broadly infuscated, the infuscations not extending to the distal margins, but they merge in the middle line on the last two segments, so that the latter appear almost uniformly dusky; these markings are, however, suggestive of interrupted bands.

* This is not likely to prove a constant character.

Legs pale ochreous buff, femora without streaks or infuscated patches; hairs to the front, middle, and hind coxae all black; front tarsi unicolorous; tips of the last two segments of the middle tarsi very narrowly darker; all the segments of the hind tarsi dark, but the last two are decidedly darker than the rest.

Wings without any marked infuscations. Length, 7 mm.

The type and only example before me is a female, and this is in an excellent state of preservation. It was captured by Mr. P. R. Filleul, Assistant District Commissioner, at Alexandra, Gorha, Jubaland, British East Africa, and forwarded under date March 17, 1912. We extend to Mr. Filleul our congratulations on the discovery of this interesting species of *Glossina*, and sincerely trust that he will be able to furnish us with further material, and also his observations on the habits and relative abundance of this insect.

THE MEASUREMENT OF *TRYPANOSOMA RHODESIENSE**

BY

J. W. W. STEPHENS, M.D. (CANTAB.), D.P.H.,

AND

H. B. FANTHAM, D.SC. (LOND.), B.A. (CANTAB.)

(Received for publication 28 March, 1912)

PLATE XIII

INTRODUCTION

The following paper contains the results of a biometric study of *Trypanosoma rhodesiense* (Stephens and Fantham).

This trypanosome, which is dimorphic, was described by us in July, 1910. It was considered to be a new species of trypanosome, producing Sleeping Sickness in man, since it could be distinguished morphologically by the fact that a certain percentage of short forms showed the nucleus either close to or even posterior to the blepharoplast, a feature which has never been recorded for *T. gambiense*, either before or since.

Otherwise, in *external* morphology *T. rhodesiense* closely resembles *T. gambiense*, for there are long, slender forms and short, stumpy forms, together with intermediate forms. These trypanosomes were figured by us (1910) in our original plate and are well shown in the accompanying coloured plate, for which we are greatly indebted to Lady Bruce.

METHODS

The blood-films used were quickly dried, fixed in absolute alcohol, and stained with a modified Romanowsky solution. Films of this nature contain trypanosomes most nearly approximating to the natural size. The flagellates suffer shrinkage in films fixed with sublimate-alcohol.

* Read before the Royal Society on May 2, 1912, and reprinted from Proc. Roy. Soc., B, Vol. LXXXV, pp. 223-234.

One thousand specimens of the trypanosome have been measured after the manner introduced by Sir David Bruce for the differentiation of various trypanosomes. In this method the length of the median longitudinal axis, including the free flagellum, is determined as accurately as possible. We found it advisable to modify Bruce's method in some respects:—

(1) Instead of drawing the trypanosomes with a camera lucida, it is much easier to project them on a screen, using a photomicrographic apparatus in a dark room, and then to trace them in outline with a finely pointed pencil. The magnification is verified by projecting a millimetre scale in the same manner. The magnification adopted was 2,500 diameters, using a 2 mm. apochromatic objective and an 8 compensating ocular. This method not only saves much eyestrain in drawing, but is also much quicker.

(2) A more important modification consists in the actual mode of measuring the trypanosomes drawn on paper. Sir David Bruce uses for this purpose a pair of compasses, set at a fixed distance of 2 mm., his trypanosomes being magnified 2,000 times. There are, however, two objections to this method:—

(a) It cannot and does not give an accurate measurement, because the compass makes a series of 'jumps' and theoretically and actually the measurements given are always less than the true ones.

We can illustrate our objections perhaps by supposing that we have to measure the outline made by the teeth of an old saw. If the teeth are equal and the distance between the compass-points is equal to the depth of a tooth, then the course can be measured. If the depths of the teeth are unequal, then it will be impossible to get an accurate measurement by the compass method, though this can be accurately done by the 'tangent line' method. Although the curves of a trypanosome do not change their direction so acutely as the outline of a saw, yet the curves often do change their direction to some extent and the principle of the objection remains. We therefore used the method which we call the 'tangent line' method.

The requirements are:—(1) a piece of tracing paper on which a straight line is drawn in ink, (2) a pin, (3) a millimetre scale. The tracing paper is placed over the drawing of the trypanosome, which is seen through it. When the tracing paper is fixed by slight

pressure of the pin placed on the ink line, the tracing paper can be rotated and the most tortuous curves followed with ease. One end of the ink line is placed on one end of the trypanosome. If the axis of the trypanosome curves, for example, at the nucleus, the pin is placed at this point and the paper is now rotated until the ink line coincides with the new direction of the axis. This is done as often as is necessary, and in fact the sharpest curves can be followed in this way, which is impossible by a compass, the points of which are at a fixed distance. Finally, the other end of the trypanosome is reached, the pin is placed there and the actual extent of the ink line traversed is measured by the millimetre scale. Further, the method has the advantage that it can equally well be applied to the measurement of any other curved line, for example, the axis of a spirochaete.

(b) Another objection to the compass method is that, if a start be made at the non-flagellar end of the trypanosome, it is uncertain that the finish will be exactly at the end of the flagellum. If not, there is always a portion of a compass distance which has to be guessed. With the tangent line method this is avoided, and the finish is exactly at the end.

The measurements could also be made by a self-registering rotameter ('map-measurer'), but we think that it is not quite such a convenient method for accurately following the curve.

It may be added that all the trypanosomes were outlined by one of us, and measured by the other.

MEASUREMENTS AND RESULTS

The following table gives the distribution, in respect to length, of 1,000 specimens of *T. rhodesiense* taken from various hosts, and measured in groups of 20 consecutive trypanosomes, neglecting only dividing forms.

TABLE I.—Distribution in respect to Length of 1000 Individuals of *Trypanosoma rhodesiense*

Animal.	In microns																			Average length									
	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		31	32	33	34	35	36	37	38	39
Man						1	2	2	3	4	2	1	1	1	3	1	1	1	1	—	—	—	—	—	—	—	—	—	—
"				1	3	1	2	2	3	4	1	2	1	1	2	1	1	1	—	—	—	—	—	—	—	—	—	—	—
"						3	3	2	2	2	5	3	2	2	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—
"			1	1	1	3	3	2	2	2	2	1	1	4	2	1	1	1	1	—	—	—	—	—	—	—	—	—	—
Monkey				1	1	1	2	2	3	1	3	—	2	2	3	—	2	1	1	—	—	—	—	—	—	—	—	—	—
"			1	1	1	1	2	1	1	2	3	2	3	2	2	1	4	1	6	—	—	—	—	—	—	—	—	—	—
Horse			1	1	1	1	1	1	3	—	—	—	1	3	1	1	1	4	—	—	—	—	—	—	—	—	—	—	—
"			1	1	2	4	2	2	4	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dog				2	2	—	3	3	9	1	1	1	1	1	1	1	2	—	—	—	—	—	—	—	—	—	—	—	—
"				—	2	—	2	1	4	1	2	4	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rabbit			1	6	2	3	5	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
"						1	1	1	—	—	3	1	—	2	2	1	5	1	3	—	—	—	—	—	—	—	—	—	—
Guinea-pig 25...						2	1	—	1	2	—	—	2	1	2	1	5	1	4	—	—	—	—	—	—	—	—	—	—
" 25...						2	1	—	1	1	—	—	2	1	1	1	5	1	4	—	—	—	—	—	—	—	—	—	—
" 21...						—	1	—	3	1	—	1	4	2	2	3	2	1	4	—	—	—	—	—	—	—	—	—	—
" 24...				1	3	—	3	2	2	1	—	1	4	2	4	2	2	1	—	—	—	—	—	—	—	—	—	—	—
" 24...						—	2	1	2	—	1	2	4	—	—	1	3	1	2	—	—	—	—	—	—	—	—	—	—
Mouse A						1	3	1	4	1	3	3	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
" B				1	1	1	1	3	4	1	5	1	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rat B 16				1	1	1	1	1	3	2	3	1	2	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
"				—	—	—	1	1	1	2	3	5	2	1	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—
"			1	2	2	3	—	—	1	1	1	—	—	—	—	—	—	3	2	4	1	2	—	—	—	—	—	—	—
"							2	3	3	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Animal.

In microns

Average length

"																												19.4
"																												25.5
"																												25.5
"																												23.9
"																												25.4
"																												23.1
"																												24.3
"																												19.0
"																												26.8
Rat B 40																												27.4
"																												26.8
Rat B 41																												27.9
"																												28.7
Rat B 42																												28.6
"																												29.1
"																												24.4
"																												25.5
"																												26.8
"																												26.3
"																												23.5
Rat B 46																												22.4
"																												22.2
"																												24.0
"																												22.9
"																												23.6
"																												21.8
Total	1	3	10	19	29	35	67	54	92	51	74	56	68	59	85	61	72	50	52	28	13	13	5	1	1	1	1	
Percentages	0.1	0.3	1.0	1.9	2.9	3.5	6.7	5.4	9.2	5.1	7.4	5.6	6.8	5.9	8.5	6.1	7.2	5.0	5.2	2.8	1.3	1.3	0.5	0.1	0.1	0.1	0.1	

In the following table the foregoing data are summarised to show the average, maximum and minimum lengths in the different hosts on various days of infection.

TABLE II.—Measurements of the Length of *Trypanosoma rhodesiense*

Animal	Day of infection	In microns			
		Average length	Maximum length	Minimum length	
Man	80 approx.	22.4	29.0	17.0	
"	117 "	20.4	28.0	13.0	
"	117 "	22.1	28.0	17.0	
"	123 "	19.8	30.0	12.0	
"	123 "	22.7	31.0	15.0	
Monkey	9	23.2	30.0	17.0	
"	10	21.7	27.0	15.0	
Horse.....	31	25.8	32.0	14.0	
"	32	22.4	28.0	14.0	
Dog	4	18.9	22.0	14.0	
"	7	20.9	28.0	16.0	
Rabbit	25	22.0	27.0	18.0	
"	38	16.8	20.0	14.0	
Guinea-pig 25...	18	26.2	31.0	17.0	
"	25...	25.2	30.0	17.0	
"	21...	24.4	31.0	18.0	
"	24...	21.7	30.0	15.0	
"	24...	24.2	30.0	18.0	
Mouse A	6	21.5	29.0	17.0	
"	B	7	20.4	25.0	14.0
Rat B 16	4	22.4	28.0	15.0	
"	5	22.0	27.0	17.0	
"	6	28.5	34.0	20.0	
"	7	17.2	22.0	13.0	
"	8	19.4	25.0	15.0	
"	9	25.5	31.0	19.0	
"	10	25.5	31.0	17.0	
"	11	23.9	30.0	16.0	
"	12	25.4	32.0	19.0	
"	12	23.1	29.0	18.0	
"	13	24.3	29.0	15.0	
"	13	19.0	27.0	13.0	
Rat B 40	3	26.8	33.0	21.0	
"	3	27.4	31.0	23.0	
Rat B 41	3	26.8	34.0	18.0	
"	3	27.9	33.0	22.0	
Rat B 42	7	28.7	33.0	22.0	
"	7	28.6	36.0	22.0	
"	7	29.1	34.0	23.0	
"	7	24.4	31.0	18.0	
Rat B 34	11	25.5	32.0	17.0	
"	11	26.8	39.0	18.0	
"	11	26.3	34.0	18.0	
"	11	23.5	31.0	16.0	
Rat B 46	12	22.4	28.0	17.0	
"	12	22.2	27.0	16.0	
"	12	24.0	30.0	17.0	
"	12	22.9	29.0	16.0	
"	12	23.6	29.0	18.0	
"	12	21.8	29.0	15.0	
		23.6	39.0	12.0	

On comparing these results with those obtained by Sir David Bruce for 1,000 *T. gambiense* and 1,000 *T. brucei* respectively, we get the following results:—

	In microns		
	Average length	Maximum length	Minimum length
<i>T. rhodesiense</i>	23·6	39	12
<i>T. gambiense</i>	22·1	33	13
<i>T. brucei</i>	23·2	38	13

From this table it is seen that the measurements of *T. rhodesiense* are practically the same as those of *T. brucei*, but differ from those of *T. gambiense*.

The average length of *T. rhodesiense* in man and other species of animals, summarised from Table I, is as follows:—

TABLE III

Animal	In microns		
	Average length	Maximum length	Minimum length
Man.....	21·5	31·0	12·0
Monkey	22·4	30·0	15·0
Horse	24·1	32·0	14·0
Dog	19·9	28·0	14·0
Rabbit	19·4	27·0	14·0
Guinea-pig	24·3	31·0	15·0
Mouse	21·0	29·0	14·0
Rat	24·5	39·0	13·0

On comparing figures obtained from Table III with those from similar hosts in the case of *T. gambiense*, measured by Bruce, we get the following results:—

TABLE IV

	Average length	Maximum length	Minimum length
	μ	μ	μ
Man—			
<i>T. gambiense</i>	24·3	33·0	15·0
<i>T. rhodesiense</i>	21·5	31·0	12·0
Monkey—			
<i>T. gambiense</i>	22·4	31·0	15·0
<i>T. rhodesiense</i>	22·4	30·0	15·0
Rat—			
<i>T. gambiense</i>	22·4	32·0	13·0
<i>T. rhodesiense</i>	24·5	39·0	13·0

This table also appears to indicate that there are some differences in size between *T. gambiense* and *T. rhodesiense*.

If now the 1,000 *T. rhodesiense* are divided according to length into three groups—(a) short and stumpy forms of 13 to 21 microns, (b) intermediate forms of 22 to 24 microns, and (c) long and slender forms of 25 microns and upwards (as has been done by Sir David Bruce in his researches on trypanosomes), and comparison of them with Bruce's results for *T. gambiense* and *T. brucei* be made, the following percentage distributions are obtained:—

TABLE V

	Short and stumpy, 13—21 μ	Intermediate, 22—24 μ	Long and slender, 25—39 μ
	per cent.	per cent.	per cent.
<i>T. gambiense</i>	51.2	23.1	25.7
<i>T. brucei</i>	32.8	25.5	41.7
<i>T. rhodesiense</i>	36.1	19.8	44.1

We note that *T. rhodesiense* is richest in long and slender forms and poorest in intermediate forms.

If the percentages in the three groups are calculated for (i) each of the hosts infected with *T. gambiense* recorded in Bruce's Table III, and for (ii) each of the hosts infected with *T. rhodesiense* recorded in our Table I, then large variations are found to occur. Thus, from a comparison of 1,000 *T. gambiense*, measured from seven species of animals by Bruce, on a variety of days, and 1,000 *T. rhodesiense*, measured by us from eight species of animals on a variety of days, the following results are obtained:—

TABLE VI

	<i>T. gambiense</i>	<i>T. rhodesiense</i>
μ	per cent.	per cent.
13—21.....	32.0 to 82.1	28.0 to 80
22—24.....	14.3 to 33.3	7.5 to 37.5
25—39.....	3.6 to 52.0	5.0 to 57.5

Also the following table summarises the variation in 240 *T. rhodesiense* from the same rat (Table I, Rat B 16) from the 4th to the 13th day of infection.

TABLE VII

T. rhodesiense (Rat B 16).

μ	per cent.
13—21.....	10 to 95
22—24.....	5 to 40
25—39.....	0 to 85

Thus it is clear that extreme variations in the length of the trypanosome are found in the different hosts, and on different days of infection in the same host, on examining the trypanosome in samples of 20.

If, again, a study of the distribution of 1,000 *T. gambiense*, 1,000 *T. rhodesiense*, and 1,000 *T. brucei** is made by the more usual method of quartiles or octiles, the following results are obtained:—

TABLE VIII

	125th	250th	375th	500th	625th	750th	875th
	μ	μ	μ	μ	μ	μ	μ
<i>T. gambiense</i>	18	19	20	21	23	25	27
<i>T. rhodesiense</i>	18	20	22	24	26	27	29
<i>T. brucei</i>	18	20	22	24	25	27	29

From this table it is seen that the measurements of *T. rhodesiense* and *T. brucei* are almost the same, but that they again differ from those of *T. gambiense*.

Our results are represented graphically in Chart 1:—

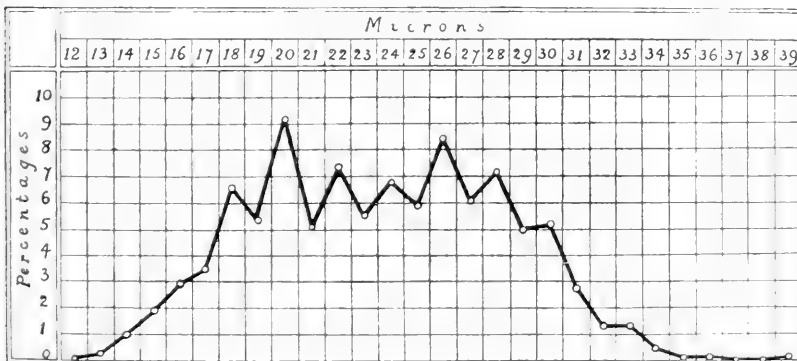


CHART 1.—Curve representing distribution, by percentages in respect to length, of 1000 specimens of *T. rhodesiense*, from various hosts.

* The figures for *T. brucei* have been deduced as accurately as possible from Bruce's curve (1911).

We also give a chart of 600 *T. rhodesiense* taken from the same species of host, namely, rats:—

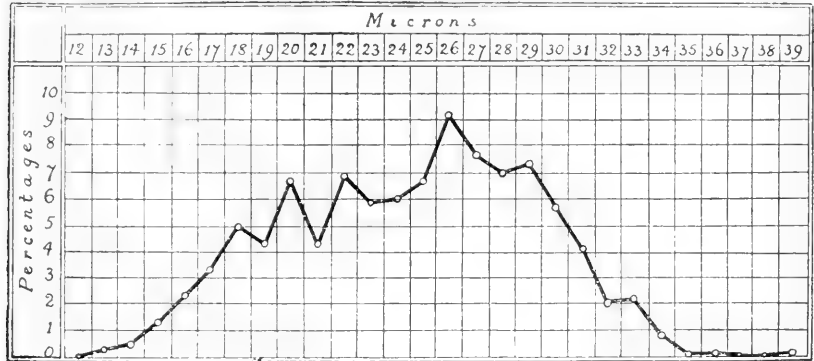


CHART 2.—Curve representing distribution, by percentages in respect to length, of 600 specimens of *T. rhodesiense* from rats. (See Addendum.)

If we now consider the graphic representation of our measurements of *T. rhodesiense*, as seen in Charts 1 and 2, and compare them with Bruce's curves of *T. gambiense* and *T. brucei*, we note the following points:—

While *T. gambiense* presents a curve with a single marked peak at 20μ , *T. rhodesiense* presents a series of small irregular peaks extending from 18μ to 28μ , with the highest peaks at 20μ and 26μ . In the case of *T. brucei* there is a slightly irregular curve extending from 18μ to 26μ , with a well-marked peak at 24μ .

Considering the three curves together, we note again that *T. rhodesiense* appears to be different from *T. gambiense*, but that the difference from *T. brucei* is slight.

DISCUSSION OF RESULTS

(1) We consider that a sample of 20 trypanosomes, at least in the case of dimorphic species like *T. rhodesiense*, from a particular slide on a particular day is too small, as the average length obtained in this way may vary in extreme cases between 24.4μ and 29.1μ (see Table I, Rat B 42).

(2) The day of infection on which the measurement is taken is very important, for, as we have seen in Table VII, on one day 10% of stumpy forms may be found, on another day 95%. This must, we think, be due to an actual change in the number of

trypanosomes of any particular length present, and not to an error of measurement.

(3) It is probable also that the host from which the trypanosome is taken is an important factor. It is difficult to be quite certain of this, because the variation may be due to the cause just stated, namely, the day of infection.

(4) However, giving these sources of error due weight, we think that the fact that there is a general resemblance between the curves representing the measurements of these three trypanosomes (*T. gambiense*, *T. rhodesiense*, *T. brucei*) shows that the method is a trustworthy one.

(5) The measurements of *T. rhodesiense* are much closer to those of *T. brucei* than to those of *T. gambiense*. We do not consider, however, that identity of measurement would necessarily imply identity of species. We still believe that the difference in internal morphology, namely, the presence of the posterior nucleus, is sufficient to separate *T. rhodesiense* both from *T. gambiense* and *T. brucei*.

(6) We think, however, that in the future, in order to get as accurate results as possible, it will be necessary on any particular day to measure larger samples than 20 trypanosomes. How large these samples must be, it is, at present, impossible to say, for we have not the requisite data. This is a point we propose shortly to investigate. At present we would suggest that, in order to eliminate unknown possible variations due to the use of different hosts, samples should always be taken from the *same* animal, and as we have shown that there are large variations on different days, samples should be taken on *every* day of the infection. Tame rats would appear to be the most suitable animals, as they are susceptible to the large majority of pathogenic trypanosomes. (See Addendum.)

Mr. Walter Stott, Honorary Statistician to the Liverpool School of Tropical Medicine, has kindly examined our figures and curves, and is of opinion that, on the whole, the data at present available are insufficient to enable statistical criticism to be applied, as there are no standard curves for comparison.

We propose, therefore, shortly to investigate the subject further from the various additional points of view that we have indicated.

Addendum, April 29, 1912.—Since writing the preceding we have completed a fresh series of measurements of *Trypanosoma rhodesiense* from a single rat, beginning with the first day of infection, and measuring 100 trypanosomes per day during 10 consecutive days of infection. We have thus obtained measurements of 1,000 trypanosomes from the same rat. On representing the results graphically, it was found that the curve resembled that of Chart 2 (for 600 trypanosomes from rats), rising with slight irregularities to a peak at 26μ (as does the curve of Chart 2), then falling rapidly and ending at 34μ .

Our remarks on pp. 140, 141 appear to be justified, but detailed discussion must be deferred.

REFERENCES

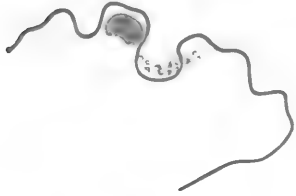
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EXPLANATION OF PLATE XIII

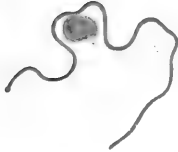
Various forms of *Trypanosoma rhodesiense*, drawn at a magnification of 2,000 diameters.

Note that some of the short and stumpy forms have the nucleus posterior.

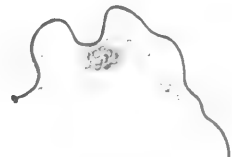
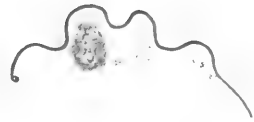
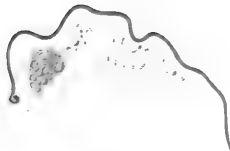
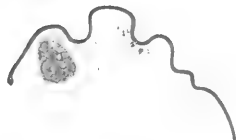
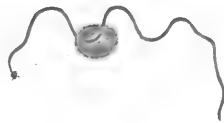
Stephens & Fitcham



Long & Slender



Intermediates



Short & Stumpy



M. Bruce, del.
M. P. Parker, lit.

Trypanosoma radfordi

TRYPANOSOMA RADFORDI



MICROSPORIDIOSIS, A PROTOZOAL DISEASE OF BEES DUE TO *NOSEMA APIS*, AND POPULARLY KNOWN AS ISLE OF WIGHT DISEASE

BY

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(Received for publication 16 May, 1912)

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I. INTRODUCTION

The year 1904 was rendered memorable to bee-keepers in the South of England by the sudden outbreak of a 'new' and 'mysterious' disease among adult bees. The centre of disease at first was the Isle of Wight, where great precautions were taken to prevent the spread to the mainland. Soon after this, a few cases were reported from Hampshire, but the Island remained the most stricken spot, huge numbers of bees succumbing to the malady.

Early in 1906, through the kindness of friends resident in the Isle of Wight, some dead and some infected, but still living, bees came into the possession of the present writers. A joint investigation was begun, and has continued up to the present time. We found that the dead and sickly bees received from our friends contained the minute Microsporidian parasite since named *Nosema*

apis. Some of the bees contained only young stages of the parasite, while in others, mature, very resistant forms of the parasite, the spores, were present. Experimental work to prove the pathogenicity or otherwise of the Microsporidian was at once commenced. The results will be given later (pp. 154-157), but it will suffice here to say that they fully confirmed the suspicions aroused by finding extensive destruction of the gut-epithelium of the bees due to the action of the parasites.

From the commencement we were hampered by the lack of suitable material, for fear of spreading disease to other districts was rampant among the owners of infected stocks; hence our experiments had to be undertaken on a somewhat small scale. This was not altogether a disadvantage in some respects. More detailed observations of the habits of infected insects can be made with a few insects, where the presence of a large number would cause distraction or distribution of attention, and so tend to confuse the issue. As a rule, spores of the parasite from one set of experiments had to be used at once for further cross-infection experiments.

In 1907, again we found *Nosema apis* in bees from another part of the Isle of Wight, and during 1908-10 found the organism in bees obtained from various districts in Surrey, Hampshire, Middlesex, Hertfordshire, Cambridgeshire, and Devon.

At the commencement of the spring of 1911, there was a great outcry among bee-keepers, who, on opening their hives, found dead bees only. Public comment in the press was a daily occurrence, and complaints of 'no bees, no fruit,' 'a fruitless year,' etc., appeared on all sides. Suggestions had been made by various investigators of the disease, under the Board of Agriculture, that in one case blood-poisoning, and in another a bacillus, was the cause of the trouble. Both cases had broken down. Having revised the accumulation of our experiments, examinations of bees, hives, combs, honey and excrement, we decided to bring forward our results. On April 4, 1911, therefore, microscopic preparations of *Nosema apis*, together with infected bees and combs, were exhibited by us before the Zoological Society of London, where an outline in brief of our results was announced and published. Since that time, owing to becoming members of the Board of Agriculture's Enquiry, we have

examined very many bees, but the result has been merely to confirm all our previous work, without adding any new facts regarding the parasite to our previous knowledge—a disappointing occurrence. On becoming members of the official Enquiry we delayed publishing our results *in extenso*, but we propose in these papers to give an account in full of our pioneer researches, confirmed, as they are, abundantly by subsequent investigations, both of our own and of our colleagues on the Enquiry under the auspices of the Board of Agriculture.

We would take the opportunity here of heartily thanking all those who have kindly helped us in obtaining material and have given us facilities for examining hives, surveying gardens and fields, and conducting general observations in the open country.

We may mention that our researches were begun in the Zoological Research Laboratory of University College, London, and continued in the Quick Laboratory, Cambridge, and in the Liverpool School of Tropical Medicine. We wish to thank the heads of these laboratories for the kindness and courtesy extended to us during our investigations.

We propose now to arrange our results in three papers, each complete in itself, the first dealing with the relation of *Nosema apis* to disease, the second concerned with the morphology and life history of *Nosema apis*, while the third relates to the methods of dissemination of the disease as we have observed them in the open—the only methods of practical value. The present paper, then, is concerned with the relation of *Nosema apis* to disease, together with such general observations on bee-structure and bee-life as are necessary for a ready understanding of the remainder.

II. OBSERVATIONS OF THE HABITS OF BEES INFECTED WITH *NOSEMA APIS*

In order, if possible, to determine definitely the symptoms of disease due to *Nosema apis*—we named the disease Microsporidiosis, as it was due to a Microsporidian parasite—we carefully noted all diseased bees sent to us, and all bees infected artificially, as well as bees belonging to dwindling colonies. In order to avoid errors of interpretation, detailed studies of normal bees were made at the same time.

Naturally, our observations on the subject coincide to some extent with those expressed either separately or in groups by bee-keepers, and can only be summarised here. The question of symptoms is rendered very difficult because the bees vary enormously among themselves, so that there seems, at present, no one great outstanding symptom common to all.

In most cases with which we were personally acquainted, examination of the ground and grass around the hives showed quite a number of bees feebly crawling about, and evidently distressed. If a finger were very gently placed in front of one of these crawling bees, it would usually climb on to the finger and remain there without making any attempt either to fly away or to sting. One of the present writers has collected some sixty such bees in the course of ten minutes without incurring the slightest trouble from stings. However, lack of desire to sting is not universal. Bee-keepers rarely commented on the loss of this power—but one is inclined to question as to how often they had the desire to obtain evidence on this point.

When collecting diseased bees, or handling them, we have noticed that the abdomens of a fair number were distended. Gentle touching of the abdomen with a blade of grass or the finger was sufficient to produce discharge of faeces which bespattered the area round about. Again, this symptom is not constant. When bees are unable to take cleansing flights the abdomen may also be distended with waste pollen, etc. But microscopical examination shows that in the excrement of the *Nosema* victim there may be thousands of tiny, oval, shining spores of the parasite mingled with undigested and indigestible pollen, while in the case of the bee suffering from lack of chance for cleansing flights, pollen alone is present.

Bees normally defaecate when on the wing, but *Nosema* infected bees are incapable of so acting. They defaecate for preference when stationary, and hence the soil, grass, or other low vegetation, the sides of the hive, the alighting board, the very combs and honey itself, all may show splashes of faecal matter, which microscopical examination shows to be highly infective owing to the presence of spores of the parasite.

The communal life has an enormous impress on the life of the

individual bee. Even though heavily infected, a bee will endeavour to perform its work as a forager, and while attempting to start its flight from the alighting board, it is not uncommon for it to fall from the board to the earth, there to crawl laboriously until death overtakes it. Similarly, foraging bees that have left the hive early in the day may have a rapid multiplication of this intestinal parasite occurring within them. They endeavour painfully to fill their pollen baskets, but, weakened both by their load and by the course of the disease, they collapse on reaching the alighting board, fall to the ground, and die there.

On one occasion we observed a number of bees fly very slowly from a hive towards a bed of Arabis. Some hours later none had returned, but in the Arabis bed were a number of fresh bee-corpses, in which various stages, but mainly the young, growing, multiplicative stages, of the parasite were found. In this case, the owner informed us that he had lost nine hives in all, but until then had never seen a dead bee. The dwindling of other colonies without the presence of dead bees being detected may be similarly explained in all probability.

While distended abdomens, dysenteric discharge, falling from alighting boards and crawling are among the most common features observed by us, other features are sometimes present, such as a sort of paralysis and dislocation of the wings.*

Again, bees infected with *Nosema* seem unable to preserve their spotless cleanliness, and become fouled with excrement. Healthy bees attempt to cleanse them, and in so doing ingest the spores of the parasite and themselves become infected.

On some occasions an infected colony seems quite unable to produce normal wax, and the honeycomb may be very rough, mingled with faeces and undigested pollen, or sometimes it rapidly darkens, so that the comb would be considered to be several years old, whereas as a matter of fact it was newly made. We have several such combs in our possession.

Owing to the death of the worker bees, it not infrequently happens that the colony comes to an end, owing to the chilling of the brood. Measures should at once be taken to prevent access of healthy (or infected) bees to such a hive, for there are grave

* Some bee-keepers lay stress on this symptom.

possibilities of the infection of both honey, pollen, and comb, with the result of the disease spreading to the robbers by means of their stolen goods.

III. THE ALIMENTARY CANAL OF THE BEE AND THE MODE OF FORMATION OF THE DIGESTIVE JUICES

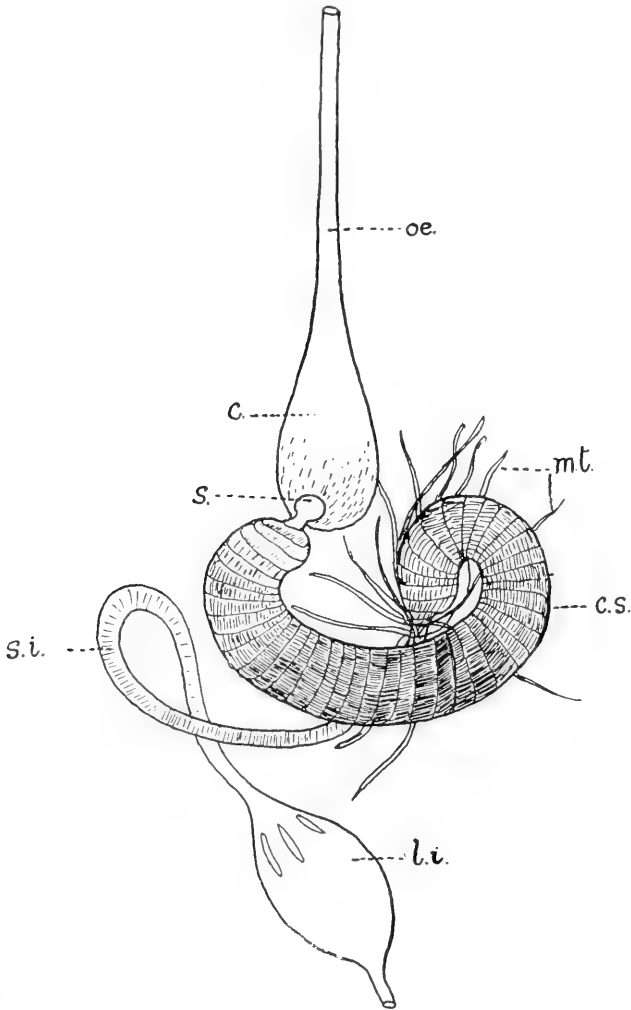
In this section it is not intended to give a detailed account of the well-known alimentary tract of the bee as such, but to indicate its structure and functions only in so far as they are related to disease due to *Nosema apis*.

The main features of the alimentary canal are shown in the accompanying text figure. A narrow oesophagus (*oe.*) passes from the mouth through the thoracic region, expanding as it enters the abdomen into the thin-walled crop (*c.*), or honey-sac as it is termed. This honey-sac serves as a storage place for pollen and nectar. The opening from the storing crop into the digestive region of the food canal is by a small aperture guarded by a complicated structure, the stopper (*s.*). The digestive region is the chyle stomach (*c.s.*), which in a normal bee is reddish in colour and somewhat firm. The inner wall is chitinous, but the chitin ridges allow access of digestive juices to pollen and nectar more readily here than in the crop. At the distal end, the chyle stomach narrows to the small intestine (*s.i.*) which, after a rather short course, widens again to a large intestine (*l.i.*). The dilated portion of the latter serves for storage of faeces, and can become greatly enlarged. It is sometimes called the colon. The actual rectal region is small. At the junction of the chyle stomach and small intestine a large number of very narrow Malpighian tubules (*m.t.*) open into the food tract, the actual perforations being in the small intestine. In the figure a few only of these are shown.

The parts of the food tract infested with *Nosema apis* are indicated in the figure by shading, and an attempt is made to indicate, by the depth of shading, the *average* intensity of infection, though this intensity may vary to a considerable extent.

Nosema apis seems to be a very specialised parasite, and at present almost exclusively an intestinal one, in the broader sense of the word. The method by which *N. apis* gains access to the organs is casual or contaminative, that is, by the mouth. The

spores, when swallowed with food or drink, pass into the oesophagus, where they become mixed with saliva from the bee's salivary glands, and then reach the crop. Here, softening of the



TEXT-FIG. I. Alimentary Canal of Bee. Infected areas shaded

spores, which have a tough coat or sporocyst around them, occurs. A few of the contained organisms, tiny amoebulae, are able to leave the softened sporocysts, and begin to creep about over the walls of the honey stomach; but this is not very common. After a period of softening in the crop, the majority of the spores pass

into the chyle stomach, where much more powerful digestive juices occur, and under the influence of these the majority of the spores lose their contents and the liberated parasites rapidly invade the walls of the chyle stomach and commence to multiply therein, ultimately giving rise to spores themselves. These spores, together with some of the original ones, pass onwards into the intestine. Some spores here lose their contents, which invade the cells of the gut, while others pass into the large intestine, which practically never is infected with parasites, and thence reach earth again.

The method of formation of the digestive juices found in the chyle stomach and small intestine is of some interest. In many animals, digestive secretions are produced by cells, which either discharge the secretion into some collecting channel, or else empty the fluid direct on to the surface where it is to be used. Such is not the case in the formation of the digestive fluids of the bees.

The secretory epithelium of the bee consists of somewhat columnar cells, many of which have large, rounded heads abutting on the lumen of the gut. Digestive juices are prepared by these cells, and are stored as droplets within them. When pollen or nectar passes into the chyle stomach from the crop, instead of the secretory cells discharging their contents, they themselves are set free into the gut, as entire cells, and after a period of shorter or greater duration they burst, or otherwise disintegrate, and set their contents free. Meanwhile, the epithelium, which has great powers of regeneration, has produced more digestive cells, so that large lesions are not obvious. Desquamated cells are quite common, however, in the gut contents, and young stages and even spores of the parasite may be found therein.

IV. THE EFFECT OF *NOSEMA APIS* ON THE ALIMENTARY CANAL

Examinations of numerous bees have shown that the oesophagus is usually empty, or contains only freshly ingested spores of the parasite. Its tissues remain unaffected. The walls of the crop are, in most cases, in a similar condition, but sometimes we have found the tiny germs or amoebulae, called planonts, that issue from the spore, creeping over the lining of the honey stomach, and penetrating some of its cells. The same occurrence is well seen in

the chyle stomach, where it is far more common than in the honey stomach. The amoebulae at first are very small, and creep over the surface or between the cells, and may begin to penetrate them. If one enters a cell it rapidly loses its irregular form, together with its power of movement, and becomes a more or less rounded, actively feeding individual, a trophozoite. While it is growing it lives at the expense of the protoplasm of the host cell, and gradually a clear space makes its appearance around the parasite. The organism soon becomes full-grown, and proceeds to multiply by several variations of binary fission. These dividing forms are known as meronts. Each meront, unlike its parent, is incapable of migrating to fresh cells, but it may proceed to divide actively, so that the entire epithelial cell may become crowded with several generations of meronts.

Also, more than one amoebula can penetrate the same cell, and as each produces a very large number of meronts, each of which ultimately forms a spore, the cells become crowded with spores.

But it is very remarkable how the distribution of *Nosema apis* among the cells of the chyle stomach varies. Cells in one part of the organ may be swarming with parasites, while the adjacent tissue may be quite uninfected. Also, while multiple infection of the cells of one part may be very common, the neighbouring areas contain only a few widely separated parasites.

When sections of the gut of the bee are examined, in certain areas the cells appear torn, while in the lumen in the vicinity crowds of spores occur. These lesions are the result of the pressure of the parasites on the cells, and an even more common condition in heavily infected areas is to find the secretory epithelium reduced to the condition of a pulp or sponge-like meshwork, enclosing large colonies of meronts and spores within its strands.

Again, when the epithelial cells are invaded by the *Nosema*, their natural secretory function becomes impeded by the presence of the parasite, and ultimately ceases. Consequently, derangements of the digestive processes follow, and the bee is unable to utilise such nourishment as would otherwise be obtainable from pollen or honey ingested by it. The result is that great weakness due to malnutrition sets in, and the exhausted bee falls a victim to the further multiplication of the parasite within.

Externally, when the parasites are able to reach the spore stage, the gut presents certain rather striking differences. It is much more fragile and brittle as a rule than is the gut of the normal bee, and paler in colour. When many spores are present in the chyle stomach, its reddish hue completely disappears, and is replaced by a chalky-white colour, due to the contained spores. The intestine also is much paler, and while the colour of the contents of the large intestine normally are a rather deep yellow, under the influence of the *Nosema* spores the colour changes to a paler tint, and in a few extreme cases it has been found to be a dirty white.

While the abdominal portions of the alimentary canal are the usual seats of the parasites, the diverticula of the canal seem singularly free from them. Up to the present we have found no stages of the parasite in the complicated series of salivary glands that open into the oesophagus, and on one occasion only have the Malpighian tubules been found to be infected.

But the youngest forms of *Nosema apis* are capable of adapting themselves to life in regions other than those of the alimentary tract. They may invade the haemocoelic fluid, and are able both to grow and multiply there to some extent. In some bees the haemocoelic fluid is more heavily parasitised than in others, while in many cases no parasites have been found in it. *Nosema apis* thus affords a study in progressive parasitism with increasing diffuse infiltration.

The effect of *Nosema apis* on the internal organs of the bee is given in greater detail in a subsequent paper (see p. 180).

V THE EXPERIMENTAL IDENTIFICATION OF *NOSEMA APIS* AS THE AGENT OF THE 'ISLE OF WIGHT DISEASE'

From 1906 onwards, when we first found *Nosema apis* in bees, we felt it was of the utmost importance to determine definitely whether the organism were pathogenic or not. Having made a number of observations of the behaviour of both normal and diseased bees, we devised a series of experiments, designed to follow on the lines of natural means of infection as far as possible. Control, healthy bees were kept constantly, and minute examination of all food supplied to the bees was made, for we had found that both infected combs and run honey containing spores of

Nosema apis had been bought by us in the open market. However, sources of infection other than spores supplied by us to the bees were excluded.

The experiments can be arranged in six groups. When we record that bees used by us died from the effects of *Nosema apis* during the infection experiments set forth, we mean in all cases that developing spores, or young stages, or both, of the parasite were found within the epithelium of the bees' digestive tracts—not merely that *Nosema* spores occurred in the lumen, and might only be passing through after being swallowed.

SERIES I. *Healthy bees were fed on honey contaminated with spores of Nosema taken from the guts of infected bees.* The bees were allowed to feed as they pleased on the infected food. Usually after two to four days they became quiet, showed a disinclination to move about, and their faeces contained spores of *Nosema apis*.

In June, 1906, twelve bees so infected all died within ten days, while control bees kept under similar conditions remained healthy.

In June, 1907, eight bees were fed on infected honey, and all had died by the end of eleven days. As before, the controls remained healthy.

June, 1910, an experiment using six bees resulted in the death of the bees in a week, the controls remaining alive for more than a month.

SERIES II. *Healthy bees were fed on honey or syrup contaminated with the faeces of bees infected with Nosema apis.* Controls were fed on pure honey or syrup. The results, summarised, were:

June, 1907. Three experiments, using ten bees each time. All died in eight days, ten days, and eleven days, respectively. Ten control bees were used each time, and remained healthy, living in the small experimental cages from twenty to twenty-nine days.

July, 1908. One experiment, using six bees for experimental feeding and six as controls. The experimental food was gross excrement in syrup, the controls being supplied with pure syrup. The infected bees all died between the eighth and tenth day, while the controls remained healthy for four weeks.

SERIES III. *Bees infected with Nosema apis, and in a weakened condition, were introduced into cages of healthy bees, and all were supplied with pure honey.*

Result: The healthy bees endeavoured to expel the sickly ones, who thereupon defaecated freely, fouling both their neighbours, the cage, and the food supply. Consequent on this, the hitherto healthy bees ingested *Nosema* spores, became infected, as shown by microscopical examination, and died. Control bees associated with bees dying of overwork lived the normal period.

June, 1908. Six sickly bees ('crawlers') were put with twenty-five healthy bees. Result:

5	bees	found	dead	on	3rd	day.
4	"	"	"	"	8th	"
6	"	"	"	"	10th	"
3	"	"	"	"	11th	"
4	"	"	"	"	12th	"
3	"	"	"	"	13th	"
2	"	"	"	"	15th	"

The remaining bees died within the month. It may be remarked that in order to distinguish the original sickly bees, they were marked with either zinc white or cinnabar powder, and so could be distinguished from their companions. A similar procedure was adopted in each of the preceding experiments. It was also found that sprinkling the bees with flour was a fairly efficacious way of marking them, for the very sickly bees made little attempt to clean themselves, and there was no risk of injury to the bees.

SERIES IV. These experiments were designed to determine whether the use of infected travelling boxes or hives would result in an outbreak among healthy bees placed therein.

Healthy bees were placed in a cage in which infected stock had travelled.

May, 1907. Eight healthy bees, caught on the wing, were placed in a soiled cage, and eight control bees, caught under similar conditions, were housed in a new cage. All the bees in the soiled cage were dead at the end of nine days, and *Nosema* spores were recovered from the corpses. The controls were all healthy for at least a fortnight, when they were killed. No parasites occurred in them. The only source from which *Nosema* spores could be obtained was the excrement in the soiled travelling box.

June, 1908. Twelve healthy bees, caught in the open country, were placed in an infected cage. Six were found dead on the

eighth day, four on the tenth, and one each on the eleventh and thirteenth day of infection. *Nosema* was found in all except the last two. Twelve controls all lived over a fortnight, except one that died on the tenth day, but no *Nosema* was found in any of the controls.

SERIES V. *Some healthy bees had infective excrement smeared or wiped on to their bodies, their controls being similarly treated with normal excrement.* Both sets of bees at once attempted to clean themselves. In so doing a certain amount of faeces was ingested. Experiments were performed in the months of May and June in 1907, 1908, 1909, 1910. The results may be summarised thus: Bees smeared with infected excrement died in from six to twelve days. The bees contaminated with normal excrement were observed from two to four weeks, during which time no deaths occurred. Our experiments confirmed the observation made in the case of sickly bees in a small observation hive. Here the excrement voided by the first set of sickly bees fouled some of their neighbours, and the latter died in a few days.

SERIES VI. *Honey in the comb from a 'dead' hive, of which no bee had survived, was put into a cage containing healthy bees.* Of these bees—twelve was a convenient number for detailed observation—ten were dead at the end of twelve days, and the remaining two had succumbed by the fifteenth day. Control bees, supplied with pure, run honey, remained apparently healthy, and continued so for three weeks. *Nosema* spores were present in the bodies of several of the bees that fed on the infected honey, and some young stages in all of them.

During the course of these experiments—sufficient to demonstrate the pathogenicity of the parasite to any thinking person—we made continuous field observations on the natural methods whereby bees can become infected. These results are set forth in detail elsewhere (pp. 197 et seq.).

Examination of bees obtained from many different localities since—often bees so long dead that they were useless for all except the gross structures, the spores—has shown that the conclusions we formed years ago are accurate. Experiments, using larger numbers of bees, have been made by others, and confirm fully our work. In this connection it may be of some interest to note that

bees suffering from 'Isle of Wight' disease, were sent to Zander* (1911, pp. 23-24), who first found *Nosema apis* spores in Bavarian bees, and that he reported that *Nosema apis* was not present in the English bees. Apparently, Zander diagnosed solely by the presence or absence of spores—the only thing possible in the case of bees long dead. Whether he received living or dead bees we know not, but it is certain that bees may die from the effects of young stages of the parasite, and so do not contain spores.

We may refer readers further interested in the details of the examination of diseased bee stocks to Tables I and II in the recent Report of the Board of Agriculture, wherein are summarised the results obtained from examining over 60 diseased stocks by Dr. Graham-Smith and the present writers in 1911.

VI. OTHER HOSTS OF NOSEMA APIS

In order to determine whether *Nosema apis* were a parasite solely of the hive bee, or whether it was present in other Hymenoptera, and whether these latter could act as carriers of the disease, we made as many observations as possible on insects caught at large, and also performed some cross-infection experiments. Owing to some difficulty in obtaining material, our experiments were restricted to cross-infection of mason bees (procured, again, with much difficulty) and wasps.

Mason Bees. These insects are not common in England. Those we used were obtained from a friend who had brought them from abroad. They were placed in a piece of old wall, which was screened for purposes of experiment. Twenty bees returning to the tunnels were captured to act as controls, and others were caught and examined to determine whether there were natural infection with *Nosema apis*. Such proved not to be the case. No Microsporidia were found. Bees dead of *Nosema apis* were then introduced into some of the tunnels in July, 1907. A fortnight after, no mason bees were to be seen. The wall was broken up, and some of the dead bees recovered from among the débris. Post-mortem examination of twenty of them showed that twelve contained spores of *Nosema apis*. Young stages were also found. The controls seemed perfectly healthy, and no form of the parasite was found in their bodies.

*Handbuch der Bienenkunde, Bd. II

Wasps were used in another experiment, as we had received complaints of honey losses due to wasps* robbing the hives.

A small nest of wasps that had been under detailed observation by one of us for other reasons for some time, was used for this experiment. Liberty to the wasps to fly at large was prevented by a muslin screen. After about a fortnight, no wasps left the nest, and when the latter was dug out, both adults and late brood were found to be dead. Examination was made of the wasps, and twenty worker wasps and the queen were found to contain some spores of *Nosema apis* in their alimentary tracts. Observations of young forms were precluded in most cases, as the wasps had been too long dead. When present, they were quite typical.

We have examined both mason bees and wasps from districts where bees have died 'mysteriously,' and have found *Nosema* spores in some cases, but in very few of the insects. Such spores fed to hive bees reproduced the disease. These field observations show that under some circumstances mason bees and wasps can act as carriers of *Nosema* spores. The morphology of *Nosema apis*, whether it be present in hive bees, mason bees, or wasps, seems to undergo no change whatever.

It may be of interest to add that Microsporidia have been found by us in several other British Hymenoptera. For example, we have found humble bees, chiefly *Bombus terrestris*, dying from heavy infection of a Microsporidian in the Malpighian tubules. This parasite resembles *Nosema apis*. Again, recent examination of leaf-cutting bees, belonging to the genus *Megachile*, shows that they are parasitised by another Microsporidian, both the Malpighian tubes and the digestive tract containing the parasite.

VII. PARASITE CARRIERS AND IMMUNITY

During the past six years we have made numerous examinations of hive bees from different parts of England. Many of these consisted of dead bees wherein spores only could be detected, if the parasite were present. In the early stages of our investigations every bee was examined individually, and it has been our experience sometimes to examine as many as 200 bees without finding any spores of *Nosema apis*. Then suddenly, a bee infected with spores was encountered, only to be followed by a long succession of bees without spores.

* Wasps have been observed carrying away bees dead of *Nosema* and then feeding their larvae on the corpses.

Similarly, we have examined large numbers of bees from healthy hives, in which no disease had appeared, and after many bees had been dissected and examined with negative results, one containing either young stages or spores of the parasite would occur. The infected living bees in each of such cases showed no marked difference from the other inmates of the hive, nor was any abdominal distension or weakness noticeable. They, themselves, seemed to have become immune to the disease. But such parasite-carriers may be very dangerous to the community of which they are members, should conditions alter and react unfavourably upon them. This is one of the dangers present in so-called 'recovered' stocks of bees. Some bees have survived attacks of disease, and have become partially immune to the parasite, but when unfavourable conditions set in, the parasite overcomes the resistance of its host, and the latter succumbs.

Judging from the remarks of certain Continental investigators, parasite-carriers seem to be more common abroad than in England, but the direct evidence on this point is lacking.

Regarding the acquisition of partial immunity by bees, there is some hope of an immune race arising, exactly as has happened in the case of silkworms infected with *Nosema bombycis*.

Bolle, investigating pébrine in Japanese silkworms, found that both the silkworm larvae, moths and eggs were heavily infected with *N. bombycis*, and yet the silkworms did not appear to suffer, nor was there much mortality among them. It is thought, therefore, that Japanese silkworms have greater immunity against *N. bombycis* than have European ones. It would be of interest, then, to ascertain what race of bees possesses the greatest immunity to *N. apis*.

Examination of stocks during 1911 by Dr. Graham-Smith and the present writers has shown that partial immunity existed in one special case, and we have information dating from 1907 of one hive that has remained alive and flourishing up to the present, though the hives on either side of it died out. It certainly seems that this hive has acquired some considerable degree of immunity, and it is to be hoped that more cases of the same kind will be reported as time goes on. At present the chief hope of the beekeeper seems to lie in destroying any stock that presents symptoms of disease in any form, and in ensuring complete absence of the parasite from the hive and food and from the soil in the immediate neighbourhood.

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THE MORPHOLOGY AND LIFE HISTORY
OF *NOSEMA APIS* AND THE SIGNI-
FICANCE OF ITS VARIOUS STAGES IN
THE SO-CALLED 'ISLE OF WIGHT'
DISEASE IN BEES (*MICROSPORIDIOSIS*)

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PLATES XIV, XV, XVI

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I. INTRODUCTION

The subject of this paper is the small, one-celled, parasitic organism, known as *Nosema apis*, which is responsible for a fatal disease among bees in Great Britain. The parasite was first discovered by the writers in 1906 in diseased bees from the Isle of Wight, where an epizootic was raging among the apiaries. From then onwards, as opportunity afforded, we have made a study of the parasite, a matter of much difficulty, as the young forms of the parasite are frail, and decompose very rapidly.

Nosema apis is a minute organism belonging to the Protozoa. One stage of the parasite takes the form of very resistant tough, oval bodies termed spores, which serve to infect new hosts. On this account the *Nosema* is put in that section of the Protozoa known as the *Sporozoa*. The spores are very small and have a somewhat complicated structure, a feature which places the parasite in the group known as the *Microsporidia*.

Among the *Microsporidia* are several organisms which are fatal to fishes, while others attack insects. One of the best known *Microsporidia* is a very near ally of *Nosema apis*, namely, *Nosema bombycis*, the organism that caused great mortality among the silkworms and enormous damage to the silk industry of France in the middle of last century. The genus *Nosema*, then, is associated with two fatal maladies of insects, as *Nosema bombycis* causes the pébrine of silkworms, while *Nosema apis* is a destructive agent among bees. In our paper of 1911, we named this disease of bees Microsporidiosis.

In this paper (which is based on our Report to the Board of Agriculture) care has been taken to explain all technical terms, and sometimes the explanation has been repeated. We trust that this procedure will be no offence to the scientific worker, and that it will be of service to those who have a keen interest in the bee folk without any special scientific leanings. Should the zoological terms, even under such conditions, be a worry, we would suggest that readers turn to our papers immediately preceding* and succeeding*, which are certainly in no way of a technical nature, but possess a distinct practical interest.

II. MATERIAL AND METHODS

During the early part of our research we were much hampered by paucity of material. As we could get very few infected bees—owing to the fear of the beekeepers that removal of bees would mean spread of disease—we used much of our first material for cross-infection experiments to decide whether or no *Nosema apis* really was pathogenic (pp. 155-7). The life history of the parasite was worked out to a large extent, with results which subsequent

* This Journal pp. 145-161 and 197-214

work has fully confirmed. It should be remarked that while an abundance of dead bees was sometimes available these were of use only for gross diagnosis, and that live bees were absolutely essential to get finer details and all the various young (but deadly) stages in the life history.

Much time was spent in the examination of fresh material. Bees were chloroformed rapidly and then dissected. Smears of the gut walls were prepared and examined fresh. Serial teased portions of the gut were made into hanging drops on slides, or kept in small paraffin-edged cells sealed under a cover-glass. *Intra vitam* staining with methylene blue was sometimes of service.

Detailed examination of teased and sectioned organs, other than those of the alimentary tract were also made (see Sect. V, p. 180). Eggs, larvae sealed and unsealed, adult workers, drones, and a few queens, have all been examined in detail.

Stained preparations were also made, and the guts of some bees, both normal and infected, sectioned serially. One important point was that detailed examination of normal bees was made, so that errors of interpretation of various structures have been excluded.

For fixatives, osmic acid vapour followed by absolute alcohol, corrosive-acetic-alcohol and Bouin's picro-formol-acetic fluid were employed. The stains that gave the best results were Giemsa, haematoxylin, modified Romanowsky and glycerin haematin.

Sections were usually very disappointing, partly because the infected epithelial cells were often shed into the lumen of the gut. In any case there was difficulty in differentiating young amoeboid forms from the tissues they had recently invaded. Spores were obvious enough wherever they occurred.

For the elucidation of certain features, such as a thread-like coiled structure in the spore known as a polar filament, special methods were used, and are briefly indicated in the part dealing with these structures (p. 174).

III. MORPHOLOGY AND LIFE HISTORY OF *NOSEMA APIS*

Several stages can be recognised in the life-history of *Nosema apis*. The first stage is that of the tiny, amoeboid germ (Pl. XIV, figs. 1-3) that issues from the resistant spore. This amoebula gives rise to uninucleate daughter forms, known as planonts because of

their migratory or wandering habits. Planonts can increase in numbers by division, and correspond roughly to the young, feeding and growing, or early trophozoite phase of other Sporozoa.

THE PLANONTS

Each amoebula as it issues from the spore, shows two nuclei as refractile spots (fig. 3). The planont moves by pseudopodia, one pseudopodium only being formed at one time, as a rule (fig. 3), and this generally at one part only of the organism. The small amoeboid body may behave in one of two ways:—

1. The two nuclei may fuse together—a process often known as autogamy, but strictly one of karyogamy—after which the parasite creeps slowly between the cells of the gut and ultimately penetrates them. Division of the uninucleate amoebula may occur while the parasite is free in the lumen of the gut of the host.

2. Protoplasm may collect around each of the nuclei of the amoebula, and then division occur, so that two daughter forms are produced. Each of these daughter forms may divide by binary or multiple fission, the latter giving rise to a colony of young planonts (fig. 1), each of which moves about over the epithelial surface of the gut, and finally penetrates between the cells or directly enters them (fig. 4) and becomes intracellular. Some of the planonts can also pass between the cells of the gut and reach the body cavity or haemocoel.

The small planonts ultimately become round or oval (figs. 4, 5), and are about $0.75\ \mu$ – $2.5\ \mu$ in diameter. When active, their movements are slow, and the pseudopodia, probably on account of the smallness of the organism, show little differentiation into ectoplasm and endoplasm, as a rule, though ectoplasm may be quite well seen occasionally in the pseudopodia. Small vacuoles are sometimes present. They are not contractile so far as could be observed. The nucleus is small and consists of a small chromatin mass or karyosome (figs. 2-6) suspended in a less dense substance, the nucleoplasm.

Free planonts on the surface of the gut epithelium, or detached, and so smeared with the rest of the gut contents, stain fairly well with Romanowsky stains. When they become intracellular they stain only moderately, and can be distinguished from the cell

contents only with some trouble. The method of penetration of a cell by a planont is most difficult of observation, though it has been seen in life on a few occasions.

A point of considerable importance is that more than one young planont can enter a single cell. In certain cases (fig. 6) there occurs inside a host cell a number of young parasites which are the original planonts and have not been produced by the division of any older growing form. Multiple penetration by planonts has been observed in life. Since each original individual, after a period of rest and growth, is capable of rapid division which is repeated again by the daughter forms produced, the ultimate result of the multiple infection on the cells invaded is to produce crowded masses of spores (Pl. XV, figs. 53-56, Pl. XVI, figs. 57, 69). These break up the intestinal lining into shred-like masses or a spongy pulp (Pl. XV, fig. 44). Rapid increase in the numbers of the parasites within the same host is ensured by colonies of planonts invading individual cells of the gut, as well as by the multiplication of the parasites later.

It may be mentioned that great precautions have been taken to avoid confusion of planonts with other organisms such as yeasts. The planonts can be distinguished from yeasts (1) by their movements, (2) by the stainability of their nucleus, (3) by chemical tests, of which that for fungus cellulose is the chief. Planonts have no fungus cellulose.

THE MERONTS

When the planonts have penetrated between the epithelial cells one of two courses may be adopted by them:—(i) They may reach the haemocoel or body cavity of the bee and remain there in a resting condition for some time. They lose their motility temporarily, become rounded or oval (Pl. XIV, fig. 2) and lie quiescent. After an interval their activity returns, and from the haemocoel they retreat between the cells to the epithelium of the gut, which they gradually penetrate. (ii) Other planonts are capable of penetrating the gut direct from the lumen or from between the epithelial cells. In either case, the active motile planont becomes passive, loses its pseudopodia and enters on a growing stage (fig. 5), which is

followed by multiplication after a short time. The intracellular parasite is now called a meront or dividing form (figs. 7, 8).

A meront at first is uninucleate (figs. 7-9), and in this condition resembles the planont. The structure of the meront at first only appears to differ from that of the planont in that the nucleus gradually becomes more chromatic and compact in nature. The organism, which has now entered upon its trophic or feeding phase, rapidly increases in size, and then begins to multiply by a process known as merogony (or schizogony). The method of multiplication, wherein meronts by division give rise to daughter meronts, may follow one of three main types:—

I. The simplest form of production of daughter meronts is by *binary fission*, but even here there is considerable variation in the forms produced, thus:—

A rounded meront usually has a round chromatic nucleus (figs. 7, 8). This nucleus becomes bowed and from an approximately central position becomes nearer the periphery. The chromatin concentrates into both ends of the bow, which is thus dumb-bell shaped (fig. 8). An indentation appears opposite the thin strand that connects the two heads of the dumb-bell, the constriction deepens, the chromatin masses separate completely (Pl. XIV, figs. 15-18), the invading ectoplasmic areas meet, and two daughter meronts are thus produced. These rapidly become either oval or rounded like their parent. The nucleus of such meronts may become rounded, or it may form an elongate rod of rather more scattered chromatin granules.

A second and common variation is seen when a somewhat elongate meront (figs. 10, 11) divides (figs. 12-14). The process is essentially the same as that previously described, but two meronts which are elongate are produced. The ectoplasm keeps the two in connection often for a considerable time (figs. 21, 22), but ultimately separation is effected.

Very rarely a meront is encountered in which cleavage of protoplasm has commenced before nuclear fission.

II. *Multiple binary fission* producing chains of meronts is found. While this method of multiplication is common in *Nosema bombycis* of the silkworm, it has been our experience to find it relatively uncommon in the *Nosema* of the bees we have examined,

but we may not have examined many bees at this particular stage of infection. The chain originates by binary fission, as previously described, but without separation of the units. The nuclear division is repeated, giving four or more parasites. Chains of three (fig. 30), five (fig. 43), and other odd numbers in a chain arise by irregularity in the sequence of division of individual daughter meronts. Sometimes oval or rounded meronts still slightly connected are encountered, and in each meront nuclear division is either proceeding, so that dumb-bell shaped masses of chromatin are present, or has been completed, giving two nuclei (fig. 26), which may be round or oval. Protoplasmic cleavage is often delayed in these and similar cases (fig. 25).

Again, it has been our experience to find a chain of several meronts in a fresh preparation, and to see it separate into its constituent individuals in the course of a few minutes. Rapid separation of the daughter meronts seems to be characteristic of *N. apis* as we have encountered it.

Pseudo-chains are sometimes produced by the rolling together and adhesion of originally distinct individuals. Great diversity of form in the nuclei enables one to distinguish a pseudo-chain in stained preparations.

III. Sometimes a meront grows relatively large before nuclear division occurs. Two courses are then open to it:—

In the first case, the large meronts show some tendency to be elongate (figs. 37-41). On the other hand, ovoid forms are encountered (figs. 33-36, 42). A special form of large ovoid meront is shown in figs. 33-35. Here, a large meront produces four nuclei (figs. 32, 35), marked cleavage of part of the protoplasm occurs, and a large amount of it concentrates around the four nuclei, giving thus four daughter meronts (figs. 33, 34) which lie within the remains of the parent form. Similar bodies are more common in *N. bombycis*, as described by Stempell, than we have encountered so far in *N. apis*.

Occasionally, a large multinucleate meront (fig. 38) appears to bud. There is then one daughter meront given off at a time terminally (fig. 39) instead of all the daughter forms being produced almost simultaneously (Pl. XV, figs. 46, 48, 52).

In the second case, other meronts show great delay in the

separation of the protoplasm, accompanied by rapid nuclear multiplication (figs. 46-48). The result is that a large body suggestive of the multinucleate schizont of a Schizogregarine or Coccidium is produced. Around each of the nuclei of the meront of *N. apis* a spore is ultimately formed. In *N. apis* we have found these large multinucleate bodies fairly often, but they are not found in all infected bees and, when present, they usually co-exist with ordinary meronts. These large meronts may be either intercellular or, as we have found more commonly, within cells of the epithelium (fig. 47). As the epithelial cells are shed naturally into the gut, the large meronts can be found free in the lumen of the gut. When the spores are formed, they lie within the cavity occupied by the original meront, and the whole structure finally forms a large, often circular, aggregation of spores (figs. 52-55). Stempell has described similar multinucleate bodies in *Nosema bombycis*, but is inclined to regard them as abnormal forms. At present, we consider the large structures of *N. apis* as merely variants in the method of growth and division, preceding spore formation, which variation seems to us to depend very largely on the factors of the space and nourishment available for the parasite.

In some cases a single cell may become parasitised by several planonts in succession, with the result that recently entered planonts, meronts of all ages, and even mature spores may be co-existing within the same cell (figs. 45, 49). We have found that some of the largest uninucleate meronts (figs. 50, 51) just about to become spores attain a length of 7.5μ , but there is much variation in size.

It is of interest and practical importance to note that many bees contain only the meront forms of *N. apis* at the time of their death. The tissue destruction due to these young forms of *N. apis* is very extensive, and produces weakness and exhaustion. In this respect the action of the meronts of *N. apis* may be well compared with those of the merozoites of *Eimeria avium* which are responsible for the death of young grouse, fowls, pheasants and pigeons.

SPORE FORMATION

Usually, after merogony has proceeded for some time, *Nosema apis* begins to prepare for life outside its present host by the

production of resting and resistant forms of the parasite known as spores. In other words, merogony is succeeded by a process of sporogony.

Two variations in the method of spore formation are found in *Nosema apis*, corresponding in the main to two of the different forms of merogony.

In the first case, a single oval or rounded uninucleate meront prepares to form a single spore (Pl. XV, figs. 50, 51). At this stage it represents the pansporoblast or sporont of other *Myxosporidia* and *Microsporidia*. The protoplasm is finely granular, the nucleus is distinct and at first single, though ultimately five nuclei are produced. The meront then becomes the sporoblast and the protoplasm contracts to a slight extent. A thin spore-wall or sporocyst is then secreted external to the cytoplasm, and thus from each uninucleate meront a single spore is produced. Figs. 53-56 represent groups of such spores. Great internal nuclear changes occur during the secretion of the sporocyst wall, but these are gradually obscured by the increasing opacity of the spore.

A second variation has been mentioned in that type of merogony wherein the original meront increases in size and undergoes repeated nuclear division, but separation of the protoplasm and nuclei as daughter meronts is prevented or delayed. Ultimately around each nucleus with its protoplasm areas of delimitation are produced (Pl. XV, figs. 46, 48) by the gradual concentration of the protoplasm, and then the secretion by it of spore walls (fig. 52). Such uninucleate masses before the secretion of the sporocysts are called sporoblasts. Nuclear changes are obscured in the living organism to a large extent, as in the previous case, but are of the same type and will be described in some detail later. When the sporocyst is fully formed, the sporoblast becomes the spore.

The internal structure of a mature spore is practically invisible in life and the general impression conveyed by microscopic examination of fresh preparations is that of a mass of spores, resembling rice grains, somewhat irregularly disposed lying within the remains of the host cell that was originally invaded by the single meront (Pl. XV, figs. 52-54, Pl. XVI, figs. 57, 69). When a large, multinucleate meront has been developed within a cavity

between cells, the appearance is that of a mass of spores with a slight haze or halo around them produced by the dead remains of the parent meront.

As before mentioned, multiple infection of single host cells by several planonts giving rise to meronts has been found (Pl. XV, figs. 45, 49). In these cases of multiple infection, the meronts when dividing may interfere mutually with the separation of the daughter meronts, causing temporary fusion of the various individuals. The result is that a fusion mass, or plasmodium, is produced. Each of the nuclei in such a plasmodium induces a concentration of protoplasm around itself, becomes a sporoblast, and finally a spore, as in the previous cases.

It is thus seen that the meront becomes successively the pansporoblast, the sporoblast and the single spore, and thus *Nosema apis* is placed in the group *Monosporogenea*, established by Pérez in 1905. The great power of merogony possessed by *N. apis* compensates for the production of one spore only from the pansporoblast.

SPORE STRUCTURE

The young spore is a somewhat elongate, usually oval body (Pl. XV, figs. 53-55, Pl. XVI, figs. 57, 69), sometimes more pointed at one end than at the other (Pl. XV, fig. 56, Pl. XVI, figs. 60, 62, 66), from $4\ \mu$ to $6\ \mu$ long by $2\ \mu$ to $4\ \mu$ broad. Very occasionally a spore may be $7\ \mu$ long.

When young, the contents of the spores are finely granular and a single nucleus can be seen within them in life (fig. 61), which nucleus stains well. The granular cytoplasm of the spore gradually concentrates towards one end which we may term anterior. This end may be somewhat pointed (figs. 61, 62). The other end then becomes filled with liquid and forms a large posterior vacuole, which is refractile in life. Soon tiny vacuoles form in the concentrated mass of protoplasm and these gradually fuse together to form an anterior vacuole which is smaller than the posterior one. This anterior vacuole is the polar capsule (figs. 58, 62, 63), and is so called because a long, spirally coiled thread termed the polar filament is ejected through it. In life the polar capsule seems far more difficult to detect than the larger posterior vacuole. The formation of the vacuoles completed, the cytoplasm

appears as a ring-shaped or girdle-like mass (figs. 63, 71, 77), with a vacuole at either end. At about this stage nuclear multiplication commences, and at the same time the chitinoïd sporocyst is secreted much more rapidly than hitherto. The nucleus first begins to elongate and becomes bowed. Gradually the chromatin concentrates into the ends, and the nucleus becomes dumb-bell like, and ultimately the ends separate. This division, then, results in a binucleate spore (figs. 63, 64). From one of the two nuclei a bud next arises. This separates from the parent nucleus and passes towards the anterior vacuole. The nucleus thus budded off is the polar capsule or capsulogenous nucleus. The second nucleus from the original division divides into two small nuclei (figs. 65-68), which at first are rounded and are embedded in the cytoplasm, or, as it is better called, the sporoplasm. These small nuclei gradually pass to the periphery, elongating and becoming thread-like as they do so (figs. 68, 72, 73). As they control the subsequent growth of the sporocyst, they may be termed the sporocyst nuclei, and are apparently comparable with the valvular or parietal cell nuclei of the Myxosporidia (pp. 176-8). The remaining nucleus of the sporoplasm also divides into two, so that the sporoplasm is binucleate and thus the full-grown spore contains five nuclei (figs. 65-67, 77). These nuclei, as will be gathered, are not always quite the same size and shape, and do not all persist for the same length of time, so that five nuclei are not always seen in the spore together. Some apparently ripe spores show only four nuclei (Pl. XVI, figs. 71-73, 74, 85). The polar capsule nucleus and the sporocyst nuclei after fulfilling their function may degenerate gradually.

By the time that the division of the nuclei is complete, the sporocyst has thickened enormously and the sporoplasm, which secretes the wall, tends to shrink away from the wall in some cases (Pl. XVI, figs. 72, 74, 85). The increasing density of the wall renders the details of the nuclear structures within very difficult of observation, and prolonged staining with or without a preliminary treatment with potash or creosote is necessary. The fully formed fresh spore is highly refractile, and a number of them seen with the microscope resembles a collection of polished grains of rice. Each spore is about one-thousandth the size of a rice-grain.

The quintinucleate character of the spore of *Nosema* is shown diagrammatically in the text figure on p. 177.

THE POLAR CAPSULE AND POLAR FILAMENT

Proceeding from the sporoplasm there is an elongate thread which is spirally coiled and lies usually in the vacuole at the posterior end of the spore. Unfortunately, in life, it is usually invisible within the spore, and even in stained preparations can only be seen with very great difficulty. On this account, Gurley called the *Microsporidia* the *Cryptocystes*. However, the polar filament can be made to extrude artificially by the aid of reagents, of which we have found iodine water and very dilute acetic acid the most useful. The polar filament extends from the extreme anterior end of the spore towards the posterior end. From the edge of the sporocyst it passes as a straight rod (fig. 73) backwards through the polar capsule, and after reaching beyond the greater mass of the sporoplasm it becomes spirally coiled on itself in the vacuole (figs. 72, 73). It measures about 60 μ in some of those that we have examined. Polar filaments are very difficult of observation under any circumstances. Figs. 75 and 76 represent the same spore before and after treatment with iodine water.

While the spore is at rest, the polar filament remains quiescent. Occasionally, as at times of rapid induced currents in the bee's gut, the spores thrust out their polar filaments (figs. 79-87), which hook into the cement between the epithelial cells and temporarily anchor the parasite. The gradual protrusion of the polar filament (figs. 79-80) can be seen at times in fresh preparations, and is more easily observed after the addition of iodine water or weak acetic acid.

Again, the polar filament is extruded when the amoebula is about to leave the spore. After being vigorously flung out, the attached end of the polar filament either snaps off or is forced out of the spore by the movements of the sporoplasm within, and thus a spore is seen with a separated polar filament in its vicinity (fig. 89). A spore whose polar filament has escaped (fig. 90) may show a very small pore marking the point of exit. The amoebula leaves the spore after the polar filament has been extruded, and moves actively in an amoeboid manner away from the spore. Spores devoid of amoebulae can be found, and then the sporocysts, sometimes with their two sporocyst nuclei, are recognised as empty by their decreased refractivity and by the minute pores whence the polar filaments escaped.

VARIATION IN THE APPEARANCE OF SPORES

While a very large proportion of spores show the features as detailed previously, yet there are certain well marked differences, in the form of young spores, more particularly, which are the results partly of the conditions of nutriment under which the parasites develop, and of the space available for their development.

(1) Young spores often are not perfectly oval, but are more pointed at one end, or somewhat egg-shaped (fig. 72). Others, on the contrary, become much more rounded (fig. 79). While the egg-shaped condition is fairly common, the rounded form is rarer.

(2) Young spores may not show the vacuole and polar capsule at once, but protoplasmic strands may cross either cavity, and so the appearance of several vacuoles is produced (fig. 70). Ultimately these vacuoles fuse, and the final result is the production of a single large posterior vacuole and a polar capsule.

(3) While a small but not very obvious thickening (figs. 79-81) was present at the attached end of every polar filament, in a very few cases this thickening appeared enlarged (fig. 85), but a much more common condition was to find the thickening very slight, in fact, on occasions it appeared to be absent altogether.

(4) Variations in the position of the sporoplasm are not very great as a rule. Some cases have been observed in which the sporoplasm receded far into the spore, the polar capsule being greatly enlarged. A small posterior vacuole is sometimes present (figs. 59, 63, 67), and the vacuole may be somewhat lateral (fig. 68). Spores in which the polar capsule was slight (figs. 58, 64) have also been found.

(5) There is much variation in the position of the nuclei contained in the spore. The most typical case of the quintinucleate spore is where the sporocyst nuclei are laterally placed, the capsulogenous nucleus is near the polar capsule and the sporoplasmic nuclei more or less central (figs. 67, 74, 77, 89). But the polar capsule nucleus not infrequently lies in the sporoplasm, and is only differentiated from the sporoplasmic nuclei by its somewhat smaller size and gradual disappearance. Similarly the sporocyst nuclei may be rounded (fig. 70) instead of elongate, and they may lie in almost any position in the spore instead of being lateral (figs. 70, 78, 90).

(6) Young spores may show wrinkles in their outer surface in stained preparations. In fresh specimens, such wrinkles are not visible, and they are to be regarded as artifacts due to the action of the fixatives.

We may note that the nuclei of the meronts and spores of *Nosema apis* seem to consist of chromatin, and that most variable (but 'fashionable' at the moment) substance, volutin, is absent.

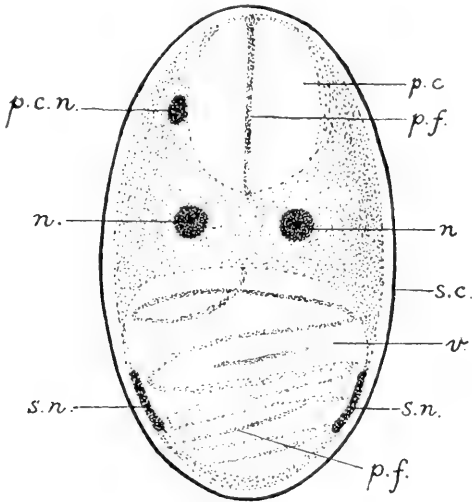
IV. THE HOMOLGY OF THE MICROSPORIDIAN SPORE AND ITS CONTENTS

The multinucleate condition of the sporont and spore of the *Microsporidia* is, at first sight, perplexing, and though many explanations seem to have been attempted, yet few are satisfactory, and they fail to reveal the undoubted affinity which exists between the *Microsporidia* and the *Myxosporidia*. The *Microsporidia*, e.g., *Nosema*, on account of their marked parasitism, have developed great reproductive power. In the case of *N. apis* this is seen in the great and varied power of merogony in the host, which compensates for the relatively limited capacity for sporogony, as seen in the production of only one spore (monosporogony) from a sporont or pansporoblast.

Many of the spores of the *Myxosporidia* contain two polar capsules, and two spores are usually formed in each pansporoblast. About ten nuclei are commonly found in such a pansporoblast. We could attempt directly the homology of the five nuclei of the *Microsporidian* pansporoblast with those of the *Myxosporidian* pansporoblast, but the comparison is, in our opinion, far more certain and convincing if we consider the annectant form, *Coccomyxa*, whose sporogony has been described by Léger and Hesse (1907).

Coccomyxa morovi, a parasite of the gall-bladder of the Sardine, is a monosporous *Myxosporidian*, showing many points of resemblance with the *Microsporidia*. The single, bivalved spore of *Coccomyxa* produced by each pansporoblast contains a clearly visible, single polar capsule. Within the young spore there is a binucleate sporoplasm, two nucleated parietal or valvular cells and one nucleated polar capsule cell. Thus we see that within a developing pansporoblast of *Coccomyxa* there are found five nuclei.

This fact is of the utmost interest and importance, for it reveals in the clearest possible manner, and in a way which apparently has not been clearly pointed out before, the affinity with the microsporidan spore, e.g., the spore of *Nosema apis* (cf. Text-fig.). Both spores contain a binucleate sporoplasm. The single oval sporocyst of *N. apis*, is, we believe, homologous with the bivalve 'shell' or spore coat of *Coccomyxa* and other *Myxosporidia*. In the formation of the spore of *N. apis*, we clearly see two sporocyst



TEXT-FIG. SPORE OF *NOSEMA APIS*

Slightly diagrammatic. As seen after long staining with Romanowsky solution.

- p. c.* polar capsule or anterior vacuole
- v.* posterior vacuole
- n.* sporoplasmic nuclei
- s. n.* sporocyst (valvular or parietal) nuclei
- p. c. n.* polar capsule (capsulogenous) nucleus
- p. f.* polar filament
- s. c.* sporocyst

nuclei which arrange themselves opposite one another at the periphery of the spore. The sporocyst of *N. apis* is not bivalved, and there is no sutural line, but it is of interest to note that long ago—before we thought of comparing the spores of *Nosema* and *Coccomyxa*—we noticed in a very few cases a more or less longitudinal 'line of weakness' (Pl. XVI, fig. 88) suggestive of the sutural line of the Myxosporidian spore. Lastly, there is a polar

capsule in the spores of *Coccomyxa* and *Nosema*, though in the latter the polar filament is not visible in life, and so is termed cryptocystic. The polar capsule in each case arises from a potential nucleate cell. These homologies throw an interesting light on the phylogeny of the *Microsporidia*.

Before concluding this section we should like to refer briefly to a recent paper by Crawley (1911) on *Sarcocystis rileyi*, parasitic in ducks. In the spore of *Sarcocystis rileyi*, Crawley found two vacuoles and two chromatin masses successively alternating with each other. The first (anterior) vacuole is probably homologous with the polar capsule of the *Cnidosporidia*, the first nucleus is compact and elongate and may be homologous with the compact parietal or sporocyst nuclei of the Microsporidian spore. The second vacuole may be homologised with the posterior vacuole of various Microsporidian spores. The second nucleus, which is vesicular, is probably homologous with the vesicular nuclei of the developing spores of *Myxobolus* or of *Paramyxa* and represents the nuclei of the sporoplasm, which may be germinal in character. These homologies have been discussed by us recently at somewhat greater length in another paper.* They are not without interest in considering the systematic position of the *Sarcosporidia*.

V. DISTRIBUTION OF PARASITE IN THE HOST.

Nosema apis is, at the present time, a specialised parasite, in that its parasitism is restricted almost entirely to the organs of the alimentary tract. In the oesophageal portions of the food canal spores of *Nosema* are occasionally found. These have been freshly ingested. The honey stomach of the bee also rarely contains stages other than the freshly absorbed, resistant spores, though on a few occasions we have found the small amoeboid planonts creeping over the lining of the crop and beginning to penetrate its cells. Beyond the crop is the small lock that leads into the more muscular mid-gut or chyle stomach. The walls of this organ are more easily attacked than those of the fore-gut, and the small amoebulae creep from the spores which are now softened by the digestive juice of the bee, and, after moving about for some time, penetrate the walls, round off, and become meronts. The amoebulae are found in all parts

* Proc. Camb. Philosoph. Soc., (1912), Vol. XVI, pp. 581-584

of the chyle stomach, but it is not uncommon to find one part of the organ swarming with parasites while a very short distance away the tissue is absolutely uninfected. Again, multiple infection of cells in one special area may be common, while a neighbouring portion contains very few, widely distributed parasites.

The chyle stomach narrows to form the small intestine, in which the same condition of distribution prevails. But here, some of the spores which are produced from parasites in the chyle stomach come to rest. Polar filaments are ejected and the spores anchored temporarily. The small, amoeboid germs emerge and proceed either with or without dividing to penetrate new cells of the intestine. This auto-infection of the bee with spores from its already contained parasites only goes on to a limited extent, but it is certainly present.

The hind-gut is rarely a seat of actual invasion by the *Nosema*, but its contents may be milk-white instead of yellowish on account of the *Nosema* spores which have been shed into it.

The chyle stomach is actively secretory in a normal bee. Recent work on the formation of the bees' digestive fluid seems to indicate that the large, secretory cells form the fluid within them in small drops. The drops are not expelled as such, but the cell in which they are formed is detached and falls into the lumen of the gut, where sooner or later its contained fluids are set free by its disintegration. These large, rounded secretory cells become invaded by *N. apis*, and consequently, free, floating in the gut contents, are found globular cells containing young stages of *Nosema*, multiplicative forms of various types and even fully formed spores. It may be mentioned that the bee is constantly renewing and replacing the secretory cells, and it is possible that an active shedding of infected cells may act as a clearance and cause the bee to become healthy for a short time, and so aid in explaining apparent recoveries from attacks of *Nosema apis*. Incidentally the young stages of the parasite are thus lost, and so are seldom seen in sections of the infected gut.

The colon and rectal regions of the gut are practically unattacked by the parasite.

Detailed examination has been made of organs other than those of the main digestive tract. The Malpighian tubules are numerous,

and as they pass off from the gut and have an excretory function, much attention was given to examining them. On one occasion only was any parasite found in the Malpighian tubules, and then meronts and a few spores were found in one tubule only of one bee.

Examination of the salivary glands and wax glands has, so far, proved negative, no form of parasite having been found therein.

The generative organs have been subjected to detailed examination whenever possible. The testes of drones, however, have so far proved negative, although the alimentary tracts of drones are frequently infected. Again, hereditary infection such as occurs in silkworms might be suspected. So far, the material available for examination prevents the expression of a definite opinion. Out of six queens examined by us, in one case only were there indications of *Nosema* in the ovaries, although three of the queens contained spores in the gut. This part of the investigation is of great interest, but much more material is needed before an authoritative opinion can be given. However, it must be noted that while ovarian infection in the queens is questionable at present, there is no doubt that queens can be heavily parasitised by *N. apis* in their alimentary canals, and queens can and do die of the same, though they may be the last in the hive to die. Owing to the infection of the digestive tracts of the queen, there is danger of the eggs becoming soiled with *Nosema* spores just after being laid.

Careful investigation has been made of the haemocoelic fluid of bees. In the first instance much work was done on the haemocoelic fluid of normal, healthy bees, so that the corpuscles of the fluid were well known. The fluid was obtained by snipping off legs from the bees and taking up the drops of fluid that exuded in a fine pipette or directly on to a slide. This fluid was either examined fresh, or after fixation and staining. A similar procedure was adopted with bees known to be unhealthy and suspected of containing *Nosema*—a suspicion that subsequent dissection fully confirmed. The result of examination of fresh preparations was that small, amoeboid bodies, certainly foreign to healthy bees, were found to be present. Examination of stained preparations showed that these bodies were identical in form and structure with the wandering planonts of *Nosema apis*. In

about six instances in which these small bodies were found in the haemocoelic fluid, the chyle stomach of the bees contained a far greater number of planonts, whose movements and appearance were carefully compared with the intruders. We were forced to conclude as a result of this that the planonts could and did pass through the intestinal walls, reach the haemocoelic fluid and remain there. Also, we have found the older stages, the meronts, and in one case one or two spores were found in the haemocoelic fluid. We know well that such development happens in the pébrine of the silkworm, and there seems little reason to suppose that *N. apis* may be more limited in its possible powers of migration than *N. bombycis*.

But *N. bombycis* is much more deadly than *N. apis*, as it already possesses the power of invading every tissue of the body and of destroying even the muscles of the body by its action. Should the power of diffuse infiltration possessed by *N. apis* increase with time, there is the probability of the virulence of the parasite increasing enormously.

Immature bee grubs of varying ages have been examined and found to contain *N. apis*, particularly in the meront stage, while occasionally a few spores were found in the cells of the mid-gut of the young hosts. Whether the larvae were infected by means of their food or hereditarily, there was not enough evidence to show. It seems that the contaminative method is more probable. It may be mentioned that grubs from one particular infected hive appeared somewhat smaller than normal bee larvae.

Muscular tissues of diseased bees also have been examined repeatedly. Except in a few instances where the parasites had developed near the muscles or against the sarcolemma of the muscles of the gut, the muscular system appeared free from the parasites. *N. apis* in this respect is very unlike *N. bombycis*, by whose action the muscle substance is destroyed and ultimately the sarcolemma becomes largely filled with masses of meronts instead of muscle substance.

To sum up, the main alimentary tract, particularly the chyle stomach and intestine, are the chief parts of the bee infected by the parasite. The gut diverticula appear to be free from parasites. But as the parasites may be found in the haemocoelic fluid, there is the

possibility of the extension of the *Nosema* into hitherto uninfected organs.

V. INTER-RELATION OF THE HOST AND THE PARASITE

Action and reaction are equal and opposite according to physical laws. We may then enquire as to what relationship or interaction exists between *N. apis* and its host. In examining fresh preparations of bee guts infected with *Nosema apis* we have often found the large intestinal cells containing one meront lying free in the gut lumen. Each meront appeared to be lying in a clear space and had the appearance of being surrounded by a halo. No space was seen around the meront which had only just become intracellular. The more mature the meront, the more marked was the space.

The production of the halo around the meront may be due to two causes:—

(1) Stempell, when working on *N. bombycis*, notes a similar space and ascribes it to the action of a ferment produced by the parasite. The same may be true in the case of *N. apis*. The said ferment decomposes the protoplasm, and the dissolved products serve as nourishment for the parasite. Support is given to this hypothesis by the gradual appearance of the space around the organism, suggesting, as it does, the progressive destruction of the surrounding tissue.

(2) The clear area may be due to the removal by simple absorption from the cytoplasm of the invaded cell of various granular constituents, used by the parasite as food. The organism would then exercise a sort of selective absorptive power, which would alter considerably the concentration of the liquids external to itself.

This view is supported by the fact that when the cells containing the parasites burst, and so discharge the parasites into the lumen of the gut, part of the 'halo' passes out as an investing sheath around the parasite, while a small portion remains behind. The alteration of the fluidity of the nearest cytoplasmic zone of the host-cell would allow of this occurrence. The 'halo' is not an integral part of the parasite, for it gradually disappears in water or normal salt solution, and the process can be hastened by warming.

The meront is then left with a clear-cut ectoplasmic outline which does not alter.

It might be suggested that the clear area or 'halo' is an attempt on the part of the host to shut off the parasite. We do not think such is the case, for the parasite certainly grows much more rapidly during the period when the halo is large than when it is smaller. This would hardly be the case were its nourishment being restricted in quantity or quality.

The tissues of the host undoubtedly offer some resistance to the parasite and the result is the production of intercellular multinucleate meronts and spores, as mentioned previously. But the parasite is, even in this condition, able to mature spores, and spore formation may be interpreted as due to the reaction of the host upon the parasite, causing the latter to shorten its active career in that particular host and to assume a form suitable for conveyance to a fresh bee.

The passage of the spores outwards into the lumen of the gut causes tears and gaps to appear in the intestinal wall. The bee, however, has great powers of re-forming cells, owing to the method by which its digestive juice is formed, and consequently the number of torn cells as seen in sections is not necessarily great. Injured cells are merely voided and new ones formed to replace them. When an intense infection is present, the bee seems to lose its power of re-forming cells, and, as a result, the digestive processes are far more deranged, the bee weakens rapidly and dies. The chyle stomach at this time may be full of *Nosema* spores when its normal reddish colour disappears, and it becomes chalk-white in colour.

The presence of the parasite in the haemocoelic fluid has an important bearing on its possible pathogenic effects. *Nosema apis*, at present, appears to be largely confined to the digestive tract, but, as it is able to live in the haemocoelic fluid, there are great possibilities of it becoming like *N. bombycis*, which is capable of attacking any and every tissue of the silkworm, and, therefore, is much more deadly. The deadliness of a parasite depends in part on its power of multiplication within one host. This power is very great in *N. apis*, and multiplication is brought about in many ways. The second factor influencing the fatal or non-fatal effect of a parasite is the extent of its distribution. When the

parasite can penetrate every tissue so that it is diffusely infiltrated throughout the host, the damaging capacity is enormously greater than when the parasite is local. As *Nosema apis* varies in its power of diffuse infiltration (as shown by its presence in the haemocoelic fluid, and, on one occasion, in the Malpighian tubules), its potentialities as a deadly organism may increase.

Much time and thought have been given to the consideration of the variation in virulence of *N. apis* in bees, but little definite evidence can be produced in explanation. However, it is known that in certain other protozoal diseases, such as the various forms of Trypanosomiasis, the newer the parasite is to the host, the more deadly is it found to be. Also, it has been shown that, after a time, the host acquires the power of tolerating the parasite to a greater or less extent. Arguing by analogy with *N. bombycis*, which is fatal to silkworms in practically all European cases, it would seem probable that *Nosema apis* has been a parasite of bees for a longer period than *N. bombycis* has of silkworms. If such be the case, there is, then, the possibility of an immune race of bees developing, and, indeed, certain experiments in cross-infection have shown that some bees may feed on honey containing thousands of *Nosema* spores and yet be unaffected by them. Such bees may be considered immune. Probably many Australian bees are immune to the effects of *N. apis*. Also, if a weak strain of bees is infected with *Nosema*, the disease could and would run riot among them, for any deadly parasite can easily overcome the resistance of a weak host. Immune bees are very dangerous, as they act as parasite-carriers.

Bees, in the early spring, are weaker after their hibernation than they are in the summer. Consequently, if they have harboured *Nosema* in their mid-guts during winter, or acquire it very soon after their awakening, the parasite may be expected to get the upper hand of the bees, and an outbreak of disease is reported. Bad seasons have a similar effect in lowering the vitality of the bees and so rendering them easier victims to microsporidiosis.

VII. SUMMARY OF THE LIFE CYCLE AND ITS SIGNIFICANCE

The most conspicuous stage in the life history of *Nosema apis* is the spore. This may form the starting point of the life cycle. When the spore is taken up mixed with honey or pollen from flowers or from comb in the hive, or sucked in by a bee engaged in cleansing another soiled or infected bee, the spore passes forwards into the chyle stomach of the bee before much change occurs in it, as a rule.

Within the chyle stomach, the spore wall is softened by the action of the digestive juice of the bee, which penetrates within to the contents. Stimulated by the juice, the sporoplasm apparently presses on the vacuole, with the result that the polar filament is forcibly ejected. It serves for a short time as an organ of attachment, fixing the spore to the gut wall. The sporoplasm concentrates and moves forwards, whereby the polar filament is forced still further outwards and becomes disconnected from the spore. The sporoplasm, retaining two of the nuclei, creeps out from the sporocyst, leaving the two sporocyst nuclei behind. The free sporoplasm becomes amoeboid, and the binucleate amoebula creeps about over the intestinal surface. The two nuclei may fuse, or more often, the amoebula proceeds to form more daughter amoebulae without previous fusion of nuclei. The final active amoebulae are small, roundish organisms, each with a single nucleus containing a karyosome. Each amoebula is capable of amoeboid movement. It penetrates the cells of the gut or passes between them, and, finally, either enters one of the cells or goes beyond and floats in the haemocoelic fluid.

Assuming that it enters an epithelial cell of the gut, it becomes rounded and passive therein, and, after a period of growth, during which time it is known as the trophozoite, it commences to multiply by several methods, resulting, usually, in a collection of several daughter individuals or meronts, or in chains of meronts. The chain condition is less common than the separate forms, in our experience, so far. Each meront is uninucleate. Alternatively, a meront may form a large multinucleate body, in which cleavage into daughter meronts does not occur at once. Such bodies may be intercellular or intracellular.

After a period of active growth and division, producing uninucleate meronts, spore formation begins. The organism is now called a pansporoblast. Active, amitotic,* nuclear division occurs, the result of which is ultimately to produce five nuclei. Two vacuoles form also in the young developing spore, a large one at one end, called the posterior vacuole, and a small one at the opposite end, termed the polar capsule. The living body substance or sporoplasm then forms a somewhat ring-shaped mass between the two vacuoles, and in it the five nuclei arising from the division of the original one are lodged. Two nuclei migrate to the sides. These become elongated, and may be termed the sporocyst nuclei. Of the other three nuclei, one controls for a time the polar capsule, and the other two the sporoplasm. The polar capsule gradually forms the spiral polar filament, which extends down into the vacuole. While these nuclear changes are taking place in the sporoblast or young spore, the latter is forming a coat around itself. This coat or sporocyst gradually thickens and obscures the nuclei beneath, and the final result is that, from one pansporoblast, a single spore, resembling a rice grain in shape, with a shining refractile envelope or sporocyst, is produced. In this condition the spore passes into the lumen of the gut, is voided with the faeces, and remains a source of infection for some time.

It will thus be realised that there are two distinct phases in the life of *Nosema apis* within the bee. This feature *Nosema* holds in common with other protozoal parasites, such as the *Coccidium* fatal to game-birds and poultry.

The first part of the life of the *Nosema* is occupied with growth and active division, so that the number of parasites within the host is enormously increased. This multiplicative stage, termed merogony, is the one that is most dangerous to the host. The young stages of the parasite, alone, are sufficient to kill the bee in many cases, and the parasite, as a result, many never reach the final stage of its development, the spore. Young grouse, similarly, are killed by the multiplying stages of *Eimeria* (*Coccidium*) *avium*, and cattle by similar forms of another *Eimeria*.

* The process of nuclear division may really be a sort of primitive mitosis, intermediate in character between mitosis and amitosis. The organism is too small to show further details of nuclear division.

When the power of the parasite to multiply further in the one host is exhausted, or when the bee can no longer supply it with food, it becomes necessary for the *Nosema* to leave its host and to renew its development in a fresh bee. Consequently, the parasite must protect itself in order to survive the period between leaving one host and entering the next. As a result of this need, it forms a hard, outer covering or spore coat, and becomes a spore. The spores are highly resistant to outside conditions, can live for some time without losing their infective power, and so can become new sources of infection for other bees.

In other words, the merogony of *N. apis* serves for the infection of, and has fatal effects on, a single host; sporogony is a means for the transference of the parasite to new hosts.

VIII. THE METHODS OF INFECTION

The method by which the *Nosema apis* enters the bee appears to be purely contaminative, that is, the parasites gain access to the host either with the food or drink of the bee, or by the bees licking one another or removing drops of infective excrement from their own bodies, or absorbing spores during cleansing operations within the hive.

As the methods of spreading *Nosema* spores are of great importance in combating disease, we have devoted a short paper to the subject (pp. 197-214), and merely give here a very brief outline of our results.

Bees, when healthy, rarely soil the combs or the interior of their hives. But, when *Nosema* is present, excrement is voided anywhere, and alighting boards, frames, combs and their contents, alike, may be bespattered, and hence infective. When food stores are thus contaminated, infection may continue over a long period.

Healthy bees frequently clean themselves and try to cleanse their neighbours, the tongue being largely used in this process. *Nosema* spores can thus be carried into the alimentary tracts of new hosts.

Infected drinking places are centres of infection for other bees, especially if the water be somewhat exposed and warm. Cold water is disliked, and warmer water, though less clean, is taken

in preference. Heavily infected drinking places have been examined by us.

Robber bees not only further deprive weak colonies of their food, but also carry *Nosema* spores into the new hive as well as the stolen honey. Weak or 'dead' hives are a source of danger to other communities so long as any stores remain in them, or as long as they can afford a refuge either to other bees or to other insects, such as ants.

Plants around infected hives may become heavily contaminated with faeces from the parasitised forager bees. Lilac, gooseberry, and apple blossoms, among others, have been found by us with the anthers encrusted with bee faeces containing spores of *N. apis*, and thus proving a potent source of disease to healthy bees visiting the blossoms.

Nosema spores are extraordinarily light, and are easily distributed by the wind. This method of distribution we have investigated on several occasions, and by detailed examination of the district around certain hives have traced the spores between one hive and another. Wind-borne spores, also, may be deposited in drinking places, where they are in a position for ready absorption by the bees.

Infected drones, by dropping excrement containing spores within their own hives and by visiting several hives in succession, as they commonly do, can also serve as agents in disseminating the spores of *Nosema apis*.

Ants and wax moths, both of which visit hives, can swallow *Nosema* spores but are unharmed by them. The spores pass unchanged through the alimentary tracts of these insects, and are ejected with their faeces in other places.

Human agency has added to the spread of the disease also. The use of old (and sometimes infected) comb and the recolonising of 'dead' hives without previous vigorous cleansing, preferably by fire, has sacrificed many new stocks of bees. The moving of bees to different parts of the country for the purpose of procuring more honey or honey of a particular kind, e.g. heather honey, has introduced parasite carriers into new areas and caused outbreaks. The sale of bees from infected districts also had added its quota to the spread of disease, and the best intentions underlying the sale—

such as the accounts that 'the bees did not like the pollen of this neighbourhood and were getting weak, so we sold them and sent them to X, where there is better pollen'—merely aided in the spreading of disease to new areas without saving the original sickening bees.

Moving of bees from one infected area to another is not open to such objections as bringing new healthy stock into an infected area. Bees that have survived attacks have acquired, to some extent, partial immunity, and so are more likely to succeed than are stocks in which the bee has not attained some degree of toleration for the parasite.

IX. CONCLUDING REMARKS

In conclusion, we may state that the complete life-cycle of *Nosema apis* in the adult hive bee, *Apis mellifica*, was set forth by us for the first time, and, from all we have seen, the cycle in the larval bee takes the same course as in the adults. Further, mason bees and wasps can become infected with *Nosema apis*. The parasite undergoes exactly the same development in their bodies as it does in the hive bee, a point of some interest, showing, as it does, the great powers of resistance to external influences possessed by the parasite, as well as its ability to live under the different conditions that prevail in the alimentary tracts of hive bees, mason bees and wasps. The problem of the existence of hereditary infection is now one that is claiming our serious attention, and the results of the past seem to be in process of confirmation, but much more material is necessary before this most difficult piece of work can be brought to a successful issue. This, we trust to achieve in the future.

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EXPLANATION OF PLATES XIV-XVI

All figures were outlined with the Abbé-Zeiss camera lucida, and 2 mm. apochromatic and $\frac{1}{12}$ " achromatic objectives (Zeiss) with compensating oculars 8 and 12 were used.

PLATE XIV

- Magnification of figures approximately 1,500 diameters, except Fig. 6, which is 2,150 diameters. Romanowsky stain in most cases.
- Fig. 1. Group of planonts of *Nosema apis* from the chyle stomach of *Apis mellifica*.
- Fig. 2. Group of planonts from the haemocoel of the bee.
- Fig. 3. Binucleate amoebula, showing pseudopodium. Fresh preparation.
- Fig. 4. Amoebula becoming round in an epithelial cell.
- Fig. 5. Meront with large nucleus.
- Fig. 6. Epithelial cell from the chyle stomach of the bee containing planonts and meronts. Haematoxylin.
- Fig. 7. Meront lying in a space in an epithelial cell.
- Fig. 8. Group of three epithelial cells containing growing meronts. One cell contains three meronts, and of these, one has its nucleus dividing. Nuclei of host cells are faint.
- Fig. 9. Three young growing meronts. Haematoxylin.
- Figs. 10, 11. Narrow meronts.
- Fig. 12. Meront in early stage of division. Nucleus bowed.
- Fig. 13. Dumb-bell stage in division of nucleus of meront.
- Fig. 14. Broader meront, showing complete separation of daughter nuclei.
- Figs. 15-18. Dividing, ovoid meronts.
- Fig. 19. Narrow, oval meront with elongate nuclei.
- Figs. 20, 21. Narrow meronts.
- Fig. 22. Two daughter meronts still united by their ectoplasm.
- Fig. 23. Meront with large nuclei.
- Fig. 24. Meront with nuclei dividing into two.
- Fig. 25. Meront in process of multiple nuclear division.
- Fig. 26. Two meronts from one division remaining attached, and each dividing again.
- Figs. 27-29. Meronts with three nuclei.
- Fig. 30. Chain of three meronts. Haematoxylin.
- Fig. 31. Meront with nucleus dividing to form four daughter nuclei. Haematoxylin.
- Fig. 32. Quadrinucleate meront showing four vacuoles. This is not common.
- Figs. 33, 34. Four daughter meronts enclosed within the remains of their parents.
- Fig. 35. Large epithelial cell with quadrinucleate meront.
- Fig. 36. Meront with six nuclei. Haematoxylin.
- Fig. 37. Somewhat irregular meront from between epithelial cells, destined ultimately to produce five spores.
- Fig. 38. Long meront with six nuclei.
- Fig. 39. Long meront from which a daughter meront is budding terminally.
- Figs. 40-42. Multinucleate meronts of various forms.
- Fig. 43. A chain of five daughter meronts which have not yet separated from one another.



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NOSEMA APIS



PLATE XV

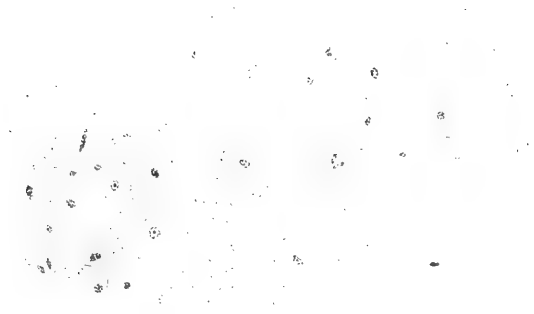
Magnification approximately 1,500 diameters throughout.

- Fig. 44. Piece of tissue from the mid-gut of a bee infected with *Nosema apis*, showing two colonies of meronts lying in spaces and four isolated meronts that have recently penetrated the tissue.
- Fig. 45. Single epithelial cell, as shed into the lumen of the gut, containing three dividing meronts, one uninucleate meront, and a practically ripe spore.
- Fig. 46. Large meront with daughter meronts differentiating within it.
- Fig. 47. Tissue, showing large, multinucleate meronts, in various stages of division.
- Fig. 48. A large meront similar to those shown in Fig. 47, but set free into the lumen of the gut.
- Fig. 49. Epithelial cell, showing the successive penetration of three planonts producing meronts.
- Figs. 50, 51. Meronts about to become spores.
- Fig. 52. Large meront with ten daughter meronts within it.
- Figs. 53-56. Host cells containing spores in varying numbers.
- Figs. 53, 54. Cells filled with spores of *N. apis* shed into the lumen of the gut. From fresh preparations.
- Fig. 55. A similar cell containing meronts and young pansporoblasts.
- Fig. 56. An epithelial cell bursting and liberating meronts and pansporoblasts into the gut.

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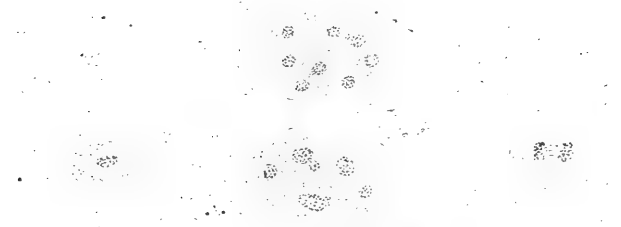
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PLATE XVI

Magnification approximately 1,500 diameters, except where otherwise stated.

Figs. 57-69. Various stages in the development of spores.

Fig. 57. Piece of epithelium showing two nests of sporoblasts and young spores of *Nosema apis*. The tissue between the colonies shows four recently entered meronts. Haematoxylin.

Fig. 58. Small, uninucleate sporoblast, showing two vacuoles, the posterior of which has not assumed its final position.

Fig. 59. Uninucleate, pear-shaped sporoblast, with small vacuole.

Fig. 60. Young spore with large, anterior polar capsule.

Figs. 61, 62, show the formation of the polar capsule and posterior vacuole.

Figs. 63, 64. Spores showing two nuclei, those of Fig. 64 being about to divide again.

Figs. 65-67. Spores showing the full number of nuclei (five). Fig. 66 shows base of polar filament. Giemsa. $\times 2150$.

Fig. 68. Spore with vacuole to one side. Haematoxylin. $\times 2,150$.

Fig. 69. Host cell containing colony of mature spores. Fresh preparation.

Figs. 70-78. Different appearances shown by spores after treatment with various reagents. $\times 2,150$.

Fig. 70. Spore with four nuclei and small posterior vacuole. Haematoxylin, after creosote.

Fig. 71. Spore showing four nuclei and girdle-like sporoplasm.

Fig. 72. Somewhat oval spore, showing three distinct nuclei, girdle-like sporoplasm and indications of a coiled polar filament. A fourth nucleus, that of the polar capsule, shows faintly. Long staining with modified Romanowsky after treatment with creosote.

Fig. 73. Larger spore, similar to 72, but showing the base of the polar filament at the broad end. Giemsa, after creosote.

Fig. 74. Spore showing rounded sporoplasmic nuclei and typical, elongate, sporocyst nuclei.

Fig. 75. Oval spore, as seen fresh in water.

Fig. 76. The same spore as 75, but swollen slightly and with polar filament extruded after treatment with iodine water.

Fig. 77. Good specimen of quintinucleate spore containing two rounded sporoplasmic nuclei, two elongate, curved sporocyst nuclei and the less distinct, degenerating polar capsule nucleus.

Figs. 78-80. Spores showing gradual protrusion of polar filaments. Spores treated with dilute acetic acid, with or without staining.



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NOSEMA APIS

From the collection of the author



- Figs. 81-83. Spores with polar filaments extruded. Iodine water.
- Figs. 84, 86. Fresh spores with polar filaments extruded. Free in the gut of the host. Physiological saline.
- Fig. 85. Spore showing polar filament with thickened base, binucleate sporoplasm, two sporocyst nuclei. Giemsa, after creosote. $\times 2,150$.
- Fig. 87. Fresh spore with polar filament extruded. From bee larva.
- Fig. 88. Small spore from epithelial cell of the gut of the bee larva, showing line of weakness.
- Fig. 89. Spore with polar filament just escaped, and lying near the spore. Binucleate sporoplasm and two sporocyst nuclei present, with remains of pole capsule nucleus. $\times 2,150$.
- Figs. 90. Spore that has lost its polar filament, showing minute pore through which the filament escaped. $\times 2,150$.



THE DISSEMINATION OF *NOSEMA APIS*

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I. INTRODUCTION

Nosema apis was shown experimentally by us to be the pathogenic agent of the so-called Isle of Wight disease (Microsporidiosis) among bees in 1906. That having been done, it was of great importance to determine the ways by which the bees became infected in Nature, and from 1906 onwards we have made many detailed observations and examinations on this subject. These observations are of considerable interest and practical importance. Naturally the work was tedious and much care was necessary in handling the bees and in obtaining information without causing spread of disease.

II. THE DISSEMINATION OF *NOSEMA APIS* IN NATURE

The method of acquiring Microsporidiosis is by the bees swallowing the spores of *Nosema apis*, but the conditions that render

this ingestion of spores possible, and the means whereby spores of the parasite are scattered from place to place, are extremely varied. They may now be set forth in some detail.

(a) DISSEMINATION OF DISEASE AS A RESULT OF THE
HABITS OF BEES

Bees are well known to be most cleanly in their habits. Under normal circumstances, fouling of the hive by excrement does not occur, for the bees defaecate in mid-air, when on the wing. When the unfortunate victims of *Nosema apis* are within the hive, however, soiling of the hive may occur. The abdomen of an infected bee is often very distended, and the slightest touch from another bee, or contact with the comb or frames, is sufficient to produce a discharge of faeces rich in spores of *Nosema apis*. Contamination of honey, pollen and wax then occurs, and other bees, feeding on such contaminated stores, ingest the parasites with their food, and themselves become infected. We have seen such soiling of the hive occur among infected bees kept under observation. Also we have made detailed examination of honey, comb and pollen stores from hives in which disease has appeared, and have found spores of *Nosema* in all of them. Further, scrapings of excrement from combs, frames and alighting boards have shown the spores when examined microscopically. Abdominal distension, without marked soiling, sometimes occurs, and has been termed 'dry dysentery.'

As already mentioned, bees, naturally, are very cleanly. We have placed diseased bees with healthy ones, and when the sickly ones have contaminated their healthy companions by faecal discharge, we have observed the healthy ones cleaning both themselves and their neighbours, and, resultant on this cleaning, themselves dying of *Nosema apis* in a very short time (cf. pp. 155-7). The original infected bees were usually marked either with zinc white or cinnabar coloured powder so that they were easily distinguished from the originally healthy ones.

Again, we have observed healthy bees suck up freshly voided faeces from obviously sickly neighbours, although an abundance of liquid honey had been provided for them. Possibly the warmer temperature of the faecal discharge rendered the latter attractive to the bees, but we cannot say with certainty why they preferred the

excrement to the fresh honey. Examination of the excrement showed the usual masses of semi-digested pollen, together with numbers of *Nosema* spores, and the same kind of substance was found in the fore-gut of some of the bees dissected immediately after they had absorbed the dejecta of their neighbours. There is no doubt that *Nosema apis* can be spread from bee to bee by the ingestion of excrement, either accidentally with food or drink or deliberately by the bees themselves.

Bees infected with *Nosema* are a source of danger even when dead. The bees dislike the presence of corpses in the hive, and dead bees and also dying ones are expelled or removed as soon as possible. The dead bee is often rolled over towards the exit, and the bees engaged in the removal become bespattered with tiny drops of faeces ejected from the body of the infected bee. Cleansing of themselves follows the removal of the dead, and the cleansing is fatal to its follower, for the ingestion of *Nosema* spores once accomplished, it is rare that the victim ever recovers.

(b) INFECTION DUE TO CONTAMINATED FOOD

Contaminated food and drink are fruitful sources of infection among bees. Dealing first with our results connected with the food-supply of the bees as the source of disease, we cite the following experiences. On several occasions friends have supplied us with frames from hives whose inmates were known to be infected with *Nosema apis*. Some frames contained brood, others pollen with both sealed and unsealed honey. Excrement was present on the frames and on the comb in some instances. When excrement was present and could be recognised as such, it was gently scraped up and mixed with water, and subjected to microscopical examination. In every case *Nosema* spores were found.

Portions of the wax, that so far as naked eye inspection went, were unsoiled, were washed with cold water and the washings examined. Old (and sometimes used) comb was similarly treated, or we varied the procedure by rubbing up some of the comb in a mortar with water, straining the mixture through butter muslin, and examining the slightly cloudy liquid so obtained. Some thirty sets of comb* have been examined by us in detail, and found to be infected, while about the same number were free from spores of the

* New 'foundations,' obtained direct from dealers, have been found by us to contain unaltered *Nosema* spores. We are informed that these foundations are made from old material by melting it down at 120° F. We think that this material should be sterilised at a higher temperature than 120° F.

parasite. When badly soiled comb was provided to one set of healthy bees, we observed that they began to cleanse the comb. Similarly the bees in a glass observation hive have been observed cleansing the comb of excrement voided by the drones. If the drones happened to be infected, the workers could, of course, acquire the spores of *Nosema apis* when engaged in comb cleaning.

While the wax that forms the combs may be a source of danger, the contents of the cells themselves are more often a source of disease. Considering the honey obtained direct from combs, we have found that in many cases it has contained spores of *Nosema*. The honey has been taken from both sealed or 'capped,' and unsealed ('uncapped') cells. The sealed cells contain honey, which, unless man intervenes, is destined for the use of the community in colder seasons; there is, then, little immediate danger if spores are found in capped honey early in the season. But the use of such infected honey accounts for the death of bees in the autumn and the discovery of a 'dead' hive when the latter is opened in the following spring, at any rate, in some cases.

Uncapped honey has also been shown to be infected with *Nosema* spores, and such honey has been found to have pollen grains in various stages of digestion mixed with it. Evidently it had become contaminated with excrement from some sickly bee, and was now serving as a source of infection to such healthy bees as might feed on the honey.

Pollen stores of bees have been examined at different seasons of the year, and in a number of cases have been found infected. In examining a comb it is a matter of some difficulty to say whether it is infected or not, unless every cell is sampled. Some pollen cells have been quite free from *Nosema* spores, while others in the same frame have contained large numbers of spores, and so were highly dangerous. The same applies equally to frames; one frame in a hive may have many of its cells contaminated, while the others may be apparently quite free.

Robber bees are a powerful source of trouble, not only from their attacks on the food of weaker colonies, but from their action in robbing infected hives, and so spreading the disease. In 1909-10, we had the opportunity of watching a hive, all of whose bees had died. It had been left unoccupied, and the owner refused for some

months to allow it to be opened. In the neighbourhood there were other hives, and we watched bees visiting the empty hive, and going away from it. Their honey stomachs were full of honey obtained from the hive, and in this honey, on several occasions, *Nosema* spores were found. Further, some of these robber bees, captured and kept in captivity, showed young stages of *N. apis* in the walls of their chyle stomachs after three to five days, and in the majority of cases died from the effect of these young forms. Later, we heard that several more hives in the village had become extinct. It is probable that this was the result of robbing the hive that we had observed, for it was agreed in the village that the said hive was the first case in which disease had appeared, and that there had been no disease until large numbers of the bees had been robbing the original 'dead' hive. This is but one example of many observations, but it is clear that 'dead' hives should be cleared of all stores to prevent robbing by other bees.

(c) INFECTION ACQUIRED AT DRINKING PLACES

At certain seasons bees require a great deal of water, and particularly is this so in spring, before there is an abundance of nectar obtainable from flowers. But bees are somewhat conservative as to the places where they will drink, and although there may be several sources of water in the neighbourhood of their hives, they prefer one spot to another, and satisfy their needs usually at the one place only. Such a place is known as a bees' drinking place. Bees prefer water that is slightly warm to that which is cooler, and we have noted bees drinking the stagnant but warm water in discarded, once ornamental urns in preference to the colder, cleaner water, obtainable from an adjacent spring.

In one locality we were able to make a three years' observation of a bees' drinking place. This was a number of stones around a water tap set in the midst of a mass of gooseberry and currant bushes. Ordinarily, there was little evidence of the bees' visits left on the stones, but when disease appeared in the neighbourhood—the result of importing some fresh stock—the sides of the tap and stones soon showed yellowish splashes of excrement, and the tiny pools in the hollows of the stones, at which the bees drank, contained bee faeces also. Some of the excrement and the water were collected and

examined. Both contained spores of *Nosema apis*, and so were a source of infection to any bees drinking at this spot.

In the same neighbourhood we have examined dew from low growing plants from time to time. On five occasions we found spores of *Nosema apis* in the dew.

(d) THE RELATION OF PLANTS TO ISLE OF WIGHT DISEASE

One question frequently asked is, 'What have the plants to do with the disease'? However, certain assumptions underlie this question. In the first place, the unfortunate bee, like the human race, may be subject to a relatively large number of diseases. In the second place, plants have not, of necessity, any connection whatever with any disease of bees. With regard to *Nosema apis*, the only share that the plants have is an indirect one:—

Healthy bees, it is well known, defaecate when on the wing. Bees infected with *Nosema*, on the contrary, defaecate when more or less stationary for preference. When suffering from *Nosema*, the hind-gut may become highly distended, and the slightest contact of the abdomen with foliage, flower, or fruit is sufficient to cause discharge of faeces. We have collected such faeces from foliage and flowers in the neighbourhood of a hive, of which but two or three bees remained alive. The others had died from the effects of *Nosema apis*, and many of their bodies contained a few spores.

On one occasion a bee keeper informed us that his bees always died in larger numbers than usual after visiting certain lilac bushes near the hives. He based his observations on the larger number found crawling near the hives a few days after they had been noticed among the lilacs. We examined the bushes, collected excrement, dust, and a little water from among some clusters of leaves, and, as it was inconvenient at the time to scrape excrement from the blossoms, obtained a liquid by washing some of the flowers. In each case, the subsequent microscopical examination showed the presence of *Nosema* spores, which, acquired by the bees, could restart the infective process. Some spores were recovered from practically every species of plant in both the flower and vegetable garden in this particular case.

In wet weather, bees venture out for water between the showers, and, rather than fly far in search of water, will drink from any pool

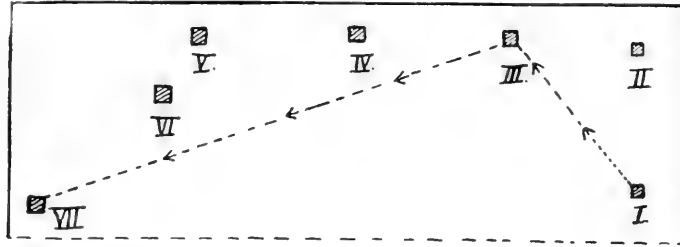
or puddle near to the hive. Now, the soil around the hive is often heavily contaminated with faeces from infected bees, at any rate, in a disease area. The spores are present in the rain pools, and are easily absorbed by the bees when the latter drink at such pools in times of wet or as emergency arises.

(e) PARASITE CARRIERS

The communal life of bees has made them a highly specialised race of insects. Consequent on the social habit of bees, disease can break out and spread in an amazingly short time, and the difficulty of tracing the origin of the outbreak is increased enormously. Many cases of 'Isle of Wight' disease suddenly appearing in an apiary or in a district have been reported, and for some time the origin of the trouble could not be traced. However, detailed examination of a large number of bees from swarms reported as 'perfectly healthy' by their owners, showed that, among these bees, a very few contained, not only the young stages of *Nosema apis*, but also occasionally, large numbers of the spores. These infected bees seemed quite as healthy as their uninfected fellows, and apparently the parasite had no ill-effects upon them. Some of these parasite carriers were detected by the greater fluidity of the faeces (though this is not always a feature of *N. apis*), and isolated. Uninfected bees were also kept isolated. Both lived about the same length of time. These parasite carriers had become immune, at any rate to some extent, to the presence of *N. apis*, but as the parasite continued to flourish in their bodies, and to produce the infective spores which were expelled with the faeces, they were a menace to the community in which they dwelt, and the cause of sudden outbreaks of disease where before none was known.

Drones can become infected as well as the working bees, and as they rove from hive to hive, and remain in various hives for different periods, they may introduce *Nosema* spores into the hive in which they defaecate freely. While many observers have noted that drones visit several hives other than their own, there seem to be no records as to the time spent by the drones in these other hives. From the point of view of the spreading of disease, this matter must be of some importance. One case noted by us among several may be of interest. We marked two drones with red cinnabar powder, and

set them at liberty. In the neighbourhood were seven hives arranged roughly on three sides of a rectangle. (Shown in plan in Text-fig. 1.) The drones both entered the first hive. The visit was short, and twenty minutes later they emerged together with other bees. After a brief halt outside Hive I, they entered Hive III. Two hours later, one drone emerged and flew across to Hive VII, which it entered. It had not left Hive VII at sunset, nor had the



TEXT-FIG. I.

Diagram of arrangement of hives showing order of the visits of drones.

companion drone emerged from Hive III. Watch was kept diligently next morning, and the exit of both drones observed. One had spent $20\frac{1}{2}$ hours in Hive VII, the other $22\frac{1}{2}$ hours in Hive III. In both cases, ample time for the scattering of hundreds of *Nosema* spores had elapsed. Attention then must be given to the drones of the colony as well as to the worker bees, both of which may have members that are veritable reservoirs of disease.

(f) WIND AS A DISTRIBUTING AGENT OF *NOSEMA APIS*

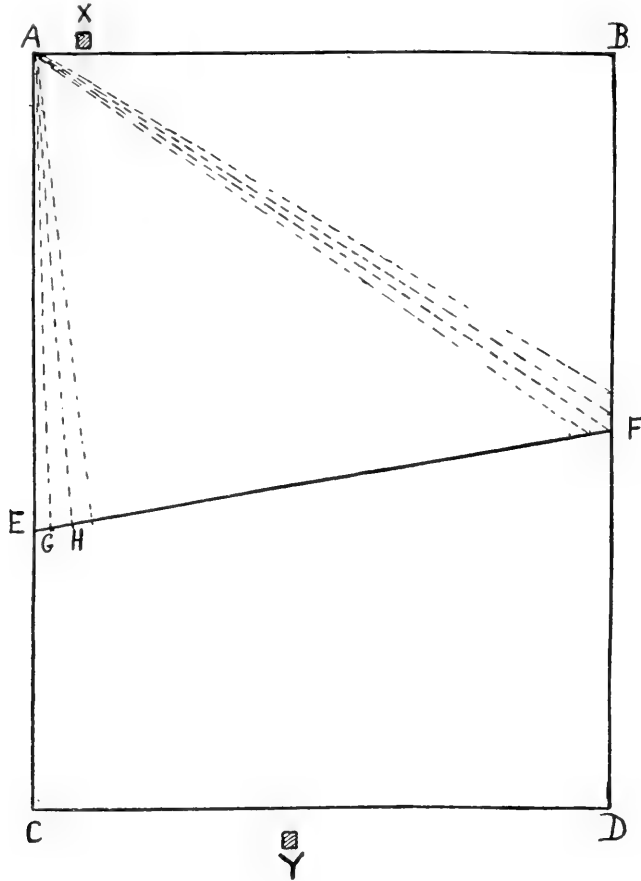
Previous researches in connection with the ways by which the oöcysts of *Coccidia* are distributed had shown that in restricted areas at any rate, distribution by the wind was of importance. The spores of *Nosema apis* are far smaller than those of any *Coccidium*. Magnified one thousand times, *Nosema* spores are only as large as a small grain of rice, while a *Coccidian* oöcyst at the same magnification, would be about ten times as large as a wheat grain. The weight of the *Nosema* spores is extremely small, and, owing to their lightness, they could be carried very considerable distances on the wind, and perhaps deposited on flowers or in water used by bees. One example of several observed in which the wind acted as a distributing agent for

Nosema spores may be quoted. It occurred in a district where one hive only was infected. As it was a new stock, the owner was loath to destroy the hive. The next nearest hive was four hundred yards away, and a field with a very low hedge, consisting of sloe and hawthorn, and one stunted crab apple, intervened. There was a drop in level between the two hives, the infected one being at the higher level. The soil was chalky, and a strong S.W. wind often deposited a fine, perceptible dust on the lower hive. On several visits, dust was collected from the hedge and from the plants in all parts of the field, and was examined microscopically. A few *Nosema* spores occurred in widely distributed samples. The owner of the second hive was warned to look out for crawling bees unable to fly. This was done, and later we received six sickly bees, picked from the ground outside the second lower hive. Four of these bees contained young stages of *Nosema apis*, one had spores, while one seemed uninfected. From that time onwards, crawling bees were found daily, and were destroyed by the owner as soon as taken. After a short time the infected stock was burned.

Meanwhile we examined the plants in the neighbourhood between and around the two hives. The method employed may be of interest and can be best illustrated by reference to the accompanying plan (Text-fig. II):—A B C D represents the field, crossed by the low hedge, E F. The first infected hive is shown at X, the second lower hive at Y.

A was made the centre for operations. At A, a stout stake was fixed in position, and the end of a ball of strong string marked in yard lengths was attached to it. The ball was then carried to G, the first position on E F, and the taut string fastened to a small stake stuck in the ground at G. Samples of water and dust from the foliage of plants in the triangle A E G were then collected, working systematically from A towards E G. Each sample was labelled fully as soon as made. A second string was then placed in position from A to H, and the new triangle A G H was investigated. The procedure was continued on these lines, moving alternately each string onwards, so as to form a series of triangles, until F was reached. Examination of a similar series of triangles made by strings stretching from A to points along F B completed the investigation of the area A E F B.

A similar method working from E as centre was adopted for the second part of the field, beyond which the lower hive was situated. Both sides of the low hedge were thus included in the examination. By the application of the method outlined above, though the work



TEXT-FIG. II.

Plan of Field illustrating method used in examining fairly large areas for *Nosema* spores

was tedious (for the base-lines of the triangles should not be more than two or three yards), we ensured investigation of the entire area, and did not waste time by going over any part twice. The simplicity of the arrangement and ease of manipulation were decidedly advantageous, especially as many hundreds of samples were collected in one day. Systematic examination of the samples

was made, and it was found that for a considerable distance *Nosema* spores were mingled with the dust on the plants. When the dust was washed down by rain, it could prove a powerful spreader of disease, if taken by bees as drink.

It is not possible to state with certainty that the second hive Y became infected by means of wind borne spores alone, since the bees might have acquired the disease at their drinking places or from drones that might have been received among them. But there certainly seems some evidence here, as in certain other cases, that the action of the wind led to subsequent infection, while there was no doubt whatever as to the wind distributing the dust containing *Nosema* spores.

It was of interest to note that bees from the original infected hive were busy among the blossoms of the crab-apple, and that many of the flowers were bedaubed with excrement, not only on the petals, but also forming irregular yellowish lumps on the anthers. Healthy bees visiting the blossoms could easily acquire *Nosema* spores with the pollen or nectar or by contact with moist faeces from which they would clean themselves later.

(g) THE AGENCY OF OTHER INSECTS FREQUENTING HIVES
IN THE DISSEMINATION OF *NOSEMA APIS*

When investigating the means by which the spores of *Nosema apis* are spread, we considered carefully the possible effect of other insects* which visit hives, and so could acquire the spores of the parasite when stealing honey. Wax moths and ants are the causes of much trouble to bee keepers in some parts of the country, though in certain districts it has been quite difficult to obtain wax moths at all. The result is that we have examined relatively few wax moths, but from the examination of the specimens of the greater wax moth, *Galleria melonella*, we concluded that *Nosema apis* was not present as a parasite in the body of the wax moth. No young stages or other forms were found in the intestinal wall. But on five occasions, small numbers of spores were found in the alimentary tracts of moths as well as in their faeces. We may, then, conclude that wax moths are occasional agents in the dispersal of *Nosema* spores, and so in the spread of the disease. Wax moths are well known to visit hives successively, and so can act as carriers of disease.

* The rôle of wasps as agents in dispersal is discussed elsewhere (p. 159).

Ants are often extremely active in and around hives, and examinations of large numbers of both black and tawny ants have been made by us. On one occasion, a frame from a hive in which all the bees had died, a few long dead bees and a number of living ants from the hive were sent to us. The bees contained a few spores of *Nosema apis*; the honey and pollen in the frame were also infected. Rapid examination was made of the ants, with the somewhat surprising result that some sixty per cent. of them were found to contain free spores of *Nosema apis*. No developmental stages of the parasite were found. Spores were also present in the excrement of the ants. Examination of ants from several other districts where disease among the bees was reported, has been made, and the result has been to show the presence of *Nosema* spores in the guts of some of the ants in almost every case.

As ants will visit any hive available for the purpose of getting honey, and as they ingest spores of *Nosema apis*—which, so far, does not seem to attack them—and void the spores intact with their excrement, they must be added to the list of means whereby infection may be spread from hive to hive.

Small pupiparous flies, *Braula coeca*, were present on some of the bees sent for examination. These little pests cause irritation to the bees, and the dwindling of some stocks in one or two localities was ascribed to 'sweating due to lousy bees.' Though we examined a large number of these small flies, no stage of *Nosema apis* was found in them.

A few Ichneumons, also parasites of bees, were examined, but as the results were negative and the numbers dissected very few, we can say nothing with regard to any possible rôle of Ichneumons in the spread of *Nosema apis*.

(h) THE PROBLEM OF HEREDITARY INFECTION

Pébrine, in France and other European countries, is well known to be present in the ovaries and eggs of the silkworm moth, *Bombyx mori*. The organism, *Nosema bombycis*, is very nearly related to *Nosema apis*, the parasite of bees. Hence, it was considered possible that it might have the same power as the silkworm parasite has, and, like it, give rise to hereditary infection of the bees. Unfortunately, we received very few living queens, and, although spores of *Nosema*

apis have been found in queens, the question as to whether the eggs are infected with the parasite remains uncertain. We hope to continue the work later, if suitable material is available.

However, we may note here that we have examined very young bee larvae, still unsealed, sealed larvae, and young bees taken just as they issued from the comb, all of which were infected with *Nosema apis*. The young stages of the parasite, as well as some of the mature spores, were present, lodged in the epithelium of the gut. Whether the grubs and young bees had been infected as eggs, or whether they had obtained *Nosema* spores from their food or from the nursing bees, could not be determined; but they had acquired the parasite, which had developed and multiplied within them. In one case only was any difference noted between infected and normal larvae, and in this case, the infected grubs and young bees were smaller than the healthy ones.

III. HUMAN AGENCY IN DISSEMINATION. PREVENTIVE MEASURES

Finally, a word must be said as to the human agency at work in spreading disease. Perhaps we cannot do better than repeat what we printed more than a year ago, that diseased bees, whether they had died as a direct result of *Nosema*, or had been sulphured as a preventive measure, should be *burnt*, and the hives occupied by them destroyed or thoroughly disinfected. The latter process seems to us unsatisfactory even when most thoroughly done, for we found that such strong reagents as creosote and pure lysol merely had the effect of dissolving the outer layers of the spore, and did not seem to affect the contents within.

During the course of our six years' investigation of bee diseases, we have found numerous cases where old comb is supplied to bees constantly. In one or two cases, the sale of old comb by one bee-keeper to another resulted in an outbreak of disease among the new possessors of the comb. As before mentioned, we have examined many old combs, and found them contaminated with *Nosema* spores, and so capable of infecting bees.

The re-stocking of hives, in which all the bees have died, again has been encountered, and in some cases, no attempt at cleansing had been made. New swarms introduced into such hives rarely

succeeded, and in some cases six successive re-stockings all failed. The use of a painter's lamp over all the woodwork of the hive, and burning of the top soil around the hive, followed by liming of the soil for some distance around is necessary before an infected hive can be considered safe for new stock.

Another mistake made is the uniting of two or more weak sets of bees. Though one set of brood may not get chilled for a somewhat longer period than would otherwise be the case, owing to the importation of more 'nurses,' yet the result to the colony is as bad in the end, for the *Nosema*, after a short time, seems to break out with even greater severity than before.

On one occasion, two sets of bees from greatly dwindled colonies (partly due to foul brood) were united together in a new hive. Both sets were black bees. After a few weeks, the swarm had increased greatly in size, while a hive of yellow bees belonging to another owner had almost disappeared. The large hive was quietened by smoke, and the frames examined. They had on them clusters of black and of yellow bees. The latter hive had been suffering from *Nosema apis*. Shortly after the discovery of the attraction of the yellow bees into the black's hive, some bees were found crawling up grass stems, and falling from the alighting board, blacks and yellows alike, and in the course of a fortnight the entire hive had died out. The danger of the mingling of weak stocks and of the passing over of bees from one hive to another is clear.

In certain places, it has been the custom for many years to send hives of bees away to other districts for collecting honey. We know personally of cases where heather honey is desired, and the bees are sent away in the one case to Yorkshire, in another to a heathery tract in Sussex, during the time the heather is in flower. In each case some weak stocks, or stocks containing parasite carriers, have been sent away, and *Nosema apis* has developed to an alarming extent in the districts where the stocks were lodged.

In one or two cases we have received bitter complaints from bee-owners, whose bees 'seemed poorly.' On enquiry, the usual reason given was that 'the bees did not like the pollen they could get' in the district, and so had become sickly. In one case, acting on this belief, bees found afterwards to be infected with *Nosema apis* had been sold and distributed from a village in an eastern county to

villages in Wales, Cheshire, Cornwall, and Scotland. In this case, the original vendor heard that each hive had died out shortly after its arrival, and his remaining stocks died also. We were able to examine some of the dead bees, and the *Nosema* spores present soon showed the cause of the trouble. It is very unwise to send stocks from place to place, especially from a disease area to a place that has hitherto been uninfected.

Nosema apis is known to be widely distributed among bees on the Continent, particularly in Germany. Importation of foreign bees always involves the risk of importing parasite carriers, and under new conditions, the parasite carriers may become the centre of bad outbreaks of disease. In one case known to us, some seemingly healthy bees were brought from a village in the Thuringian forest to a southern district in England, in which, prior to the introduction of the foreigners, disease had never been known. In less than a year, every apiary in the district was extinct. Some of these bees were among the earliest in which we found *Nosema apis*. Undoubtedly the disease appeared here as a direct result of the importation of foreign bees.

Honey from hives in which disease has appeared should not be used for feeding other bees. Nor is it wise to sell such honey. On several occasions we have bought run honey in the open market place, and subsequent microscopic examination has shown it to contain spores of *Nosema apis*. If such were used for feeding bees, outbreaks of disease would almost certainly follow. Its use for human consumption is, we consider, not to be commended, though opinions vary on this point.

Success in combating the disease seems to lie in preventive measures rather than in treatment. It is better by far to sacrifice the first stock showing signs of disease than to lose the whole apiary as a result of sparing the first set of weaklings. A supply of pure water in early spring, and generous treatment over winter food stores aid greatly in maintaining the vitality of the colonies, always a great factor in the combating of disease.

GENERAL SUMMARY

The three preceding papers, recording our researches on Microsporidiosis in bees, may be briefly summarised thus:—

(1) It was first established by us that *Nosema apis* was the cause of a fatal disease in bees in Great Britain. The malady is popularly known by the name of the Isle of Wight bee disease, though it exists in many parts of the British Isles, on the Continent, in America and Australia. In April, 1911, we proposed the name Microsporidiosis for the disease.

(2) *Nosema apis* is, in the main, a parasite of the digestive tract of the bees. Queens, drones and worker bees alike can be infected. There are two well marked phases in its life-cycle:— (i) A multiplicative phase, termed merogony, which occurs in the epithelium of the chyle stomach and intestine of the bee. During this phase, the numbers of the parasites within the host are greatly increased, with concomitant destruction of the bee's secretory epithelial cells. (ii) A second phase, termed sporogony, leads to the formation of minute resistant, resting spores, which are shed in the faeces of the bee, and foul the hives, the alighting boards, food, drinking places and general surroundings of the hives. The spores are the cross-infective stages, producing infection of fresh bees when swallowed in food or drink.

(3) The symptoms of Microsporidiosis in bees are varied. Inability to fly, crawling, dislocation of the wings, and distension of the abdomen, followed by an early death may be mentioned; also 'dry dysentery' in some cases. Dwindling of stock and death of the bees often occurs, especially during wet and cold seasons. In warm, bright weather there is abundance of nectar and pollen, and the health of the bees is at its highest. But in wet or very damp weather, when the vitality of the bees is lower and they are less capable of resistance, it has been found that severe outbreaks of disease occur. *Nosema apis* flourishes in bees enfeebled by unfavourable conditions, such as weakness following hibernation.

(4) Some bees develop the power of adapting themselves to some extent to the presence of *Nosema*, and so are able to live for a relatively long time. The parasites ultimately produce

numerous spores, while the bees seem almost unaffected. Such bees are known as parasite-carriers. The excrement of such bees is infective to other bees, and the parasite-carriers serve as reservoirs of disease.

(5) *Nosema apis* was shown to have fatal effects by feeding healthy hive bees, mason bees and wasps on food infected with *Nosema* spores, derived either from the food canal of infected bees or from their excrement, or from naturally contaminated honey; also by the direct contamination of healthy bees by smearing them with infected excrement and allowing them to clean themselves, and by housing them in cases in which diseased stock had travelled. Spores after keeping a long time did not infect so quickly.

(6) In Nature the method of infection is contaminative. We have examined hives, comb, pollen from combs, and honey, and found spores in or on all of them. Examination of bees' drinking places has proved the presence of spores in the water, which has become fouled by the excrement of the bees. Flowers and water collected from foliage in the neighbourhood of infected hives, and the soil around the hives, have also contained spores. In some cases the wind undoubtedly acts as a distributor of spores, which can be tracked from one place to another in the dust on foliage. Robber bees and visiting drones also act as carriers of disease.

(7) Examination by us of all the insects that we could find in association with the bees or hives showed that ants and wax moths could swallow *Nosema* spores and pass them through their own bodies uninjured. These insects visit any hives indiscriminately and void faeces within them, hence, should they have ingested spores, they act as mechanical carriers. Ichneumons and small pupiparous flies ('Bee mites,' i.e., *Braula caeca*) so far have given negative results, but more material is necessary before a final conclusion can be made. Naturally infected mason bees and wasps have been found. It is possible that they became infected by robbing hives.

(8) Preventive measures seem of most service in combating the disease. The only certain destructive agent for Microsporidian spores is fire. All dead bees should be burned. A painter's lamp should be used over the woodwork of infected hives before new stocks are placed in them. The soil around infected hives should

be removed to a depth of several inches and burned, the surface soil being then limed fairly heavily. The importance of cleanliness in the hive and its surroundings cannot be too strongly emphasised. Union of weak stocks is a mistake. Infected bees should not be brought into uninfected areas, though bees from one infected area taken to another may succeed, as they may have acquired a partial immunity. It seems advisable to destroy all diseased stocks. The provision of an abundance of pure honey and uncontaminated water for drinking is of undoubted service. Importation of bees from the Continent should be carefully regulated, for much disease occurs among bees there, particularly in Germany.

I. FURTHER OBSERVATIONS ON THE VARIATIONS IN THE NUMBER OF LEUCOCYTES AND CRESCENTS IN MALARIA

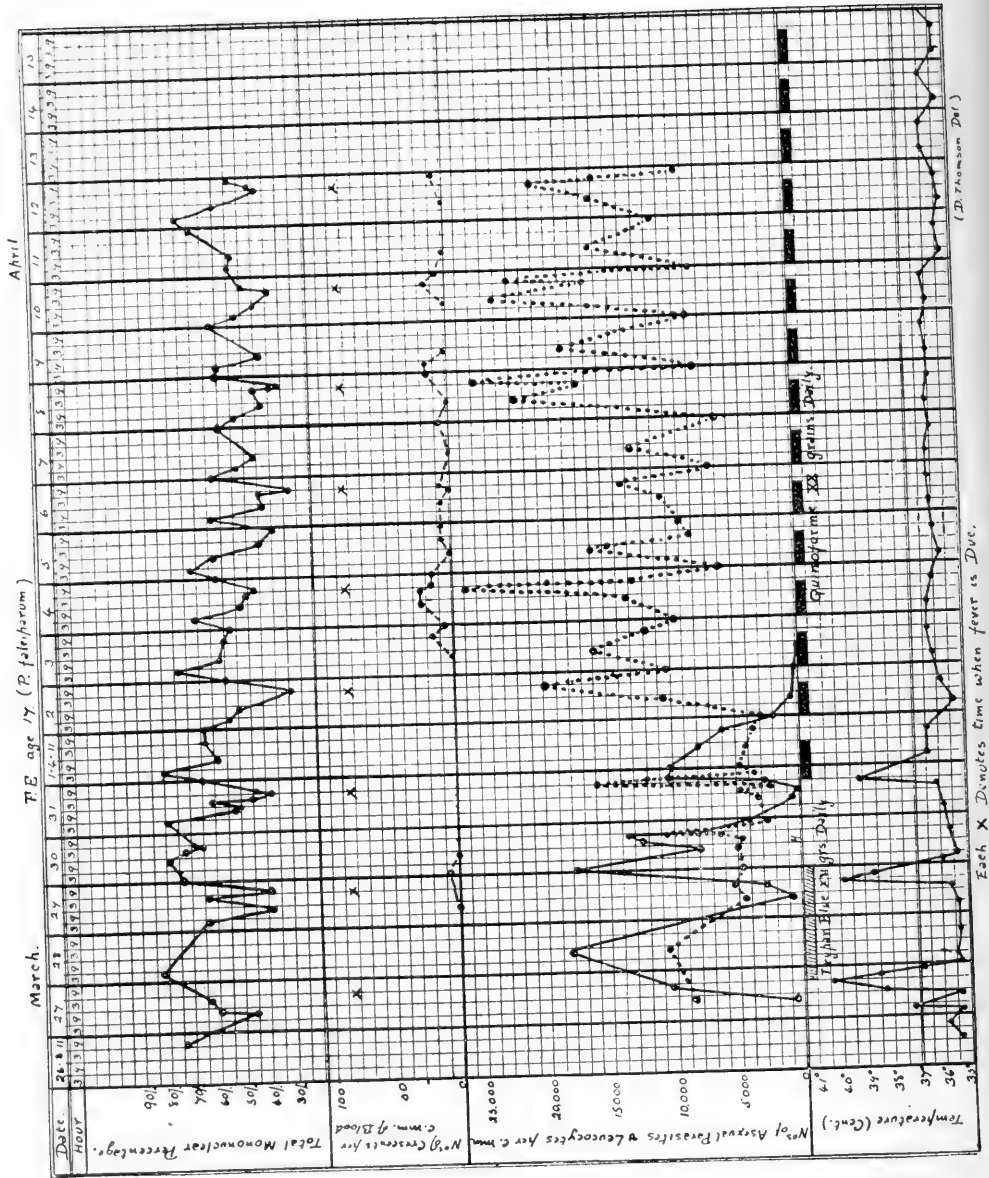
BY

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

(Received for publication 22 May, 1912)

(a) VARIATIONS IN THE NUMBER OF LEUCOCYTES IN CASES OF APPARENTLY CURED MALARIA

In a previous paper (Thomson, 1911) it was pointed out that, after a case of malaria had been apparently cured by quinine, the leucocytes began to vary in number in a remarkable manner. This phenomenon begins to show itself about one to two weeks after the disappearance of the parasites. In some cases it occurs earlier, in others later. About the time of the day at which the fever paroxysm had formerly occurred, the leucocytes showed a marked but transient increase, the polymorphs playing the greater part in this increase. I have now some slight reason to believe that this phenomenon is due to a small number of parasites, too scarce to be detected, sporulating about the same time as when they were numerous. On first thought, therefore, one would expect that this late leucocytic variation would be quotidian, tertian, or irregular in type, according as the fever was before it. I have found, however, that it is much more often quotidian and irregular in type than tertian. In fact, the case F. E. (*P. falciparum*), see chart, is about the only one I have noted so far which shows a tertian tendency in this leucocyte swing. When one considers, however, that an absolutely pure case of tertian infection is practically never found, it is not surprising that one seldom gets a case in which the leucocytes show a pure tertian swing. Cases in which the temperature curve shows a pure tertian type are common enough, but in these cases, if one examines the blood carefully, one finds that a few parasites sporulate between times, and these are sufficient to produce a variation in the total mononuclear percentage, although



(D. Thomson 24)

not sufficient in number to produce a temperature. I think that this is why cases which are apparently purely tertian from the temperature curve, show a quotidian variation in the number of leucocytes later on.

In order to support my belief that a large number of malarial parasites on sporulating cause a leucopenia, whereas a small number on sporulating cause a leucocytosis, I have tried the effect of hypodermic injections of dead malarial parasites on the leucocytes, on twelve occasions. My technique was as follows:— Having ascertained the number of parasites per c.mm. of blood in a case of malaria during a paroxysm, I drew off several cubic centimetres of blood from a vein into an equal volume of citrate solution in a sterile syringe. This was transferred to a sterile vessel and carbolic acid added so as to make a 1% strength. This was left in the cold for a few days, so as to ensure the death of the parasites. By injecting hypodermically a given volume of this material into a person, one was able to watch the effect on the leucocytes of a given number of dead parasites.* A leucocyte count was made immediately before the injection, and two to three more counts made at half-hour intervals afterwards.

In three of these twelve experiments I injected three, four and five millions of malignant tertian parasites, respectively, and got a slight increase in the number of leucocytes in one half to one hour afterwards.

In three more I injected doses of thirty to forty millions, and obtained a slight decrease in the number of leucocytes.

On five occasions I injected from ten to twenty millions, and obtained a marked increase of leucocytes in three of these in one half to one hour after the injection.

The remaining experiment was an injection of five million benign tertian parasites, taken during a rigor. This resulted in a slight increase of leucocytes in half an hour.

It would therefore appear that it requires an injection of ten to twenty million malignant tertian parasites to cause a leucocytosis, whereas numbers from thirty to forty million and upwards cause a leucopenic effect (as in a malarial paroxysm). On one

* The material was mixed with sterile water before injection, so as to haemolyse the corpuscles so that the poisonous effect of the parasites, might be more rapid.

occasion the latter number caused a slight rigor, but no increase of temperature. During a malarial paroxysm there is as a rule an accompanying leucopenia; some observers, however (Stephens and Christophers, 1908), have described a transient leucocytosis lasting about twenty minutes just at the very commencement of the rigor. I have noticed this on two occasions only, and think that it may be explained by the supposition that a very few parasites sporulate before the majority of them, and these few cause the temporary leucocytosis, which soon becomes a leucopenia when the majority sporulate.

In cases of comatose malaria (malig. tert.) one frequently finds a marked leucocytosis during the fever paroxysm. I am quite unable to explain this exception. Recently I observed a leucocytosis of 80,000 per c.mm. in a case of uncomplicated comatose malaria four hours before death.*

(b) FURTHER REMARKS

A. Scherschmidt (1912) has partially confirmed the occurrence of a periodic post-malarial leucocytosis in nine out of twenty-three cases of malaria, or only in about one-third. His work has evidently been very thorough, as he took blood counts almost continuously at intervals of one hour, but in the majority of his twenty-three cases these were taken between 10 a.m. and 4 p.m., and only on a few isolated days did he examine as late as 8 p.m. He therefore only observed the leucocytes during a six-hours period of the day. I feel sure that he would have got better results by examining the blood as I stated, every four to six hours. I examined the blood of my cases at such intervals between 10 a.m. and 12 midnight, that is to say, I examined over a fourteen-hour period of the day as against the six-hour period of Scherschmidt.

On the first day of observation one should make a blood count at, say, 10 a.m., 2 p.m., 6 p.m., and 12 midnight. Next day make counts at 9 a.m., 12 mid-day, 4 p.m., and 8 p.m. By varying the times daily in this way, one hits upon the time at which the leucocytosis occurs. When this is found, three counts daily should enable one to bring out the variation on subsequent days. I think

* The post mortem did not reveal any complication.

that Scherschmidt has unfortunately mistaken my meaning, as he examined the blood of his cases every hour for a period of four to six hours, instead of at intervals of four to six hours. It is not necessary to examine the blood every hour, as the leucocytosis as a rule lasts for several hours. In a few of his cases, Scherschmidt commenced his blood counts too soon after the disappearance of the parasites. It is best to wait for seven to fourteen days after the parasites have apparently gone, due to the influence of the quinine treatment. Again, in two of his cases there was a more or less continuous leucocytosis throughout his daily four to six hours period of examination. It is quite probable that this period was the time of the post-malarial leucocytosis, and had he made a count at 12 midnight, it might have revealed the fall to normal. Scherschmidt used the Thoma Zeiss method of counting. The method I adopted was much more suitable for this work, as by means of a special pipette (Thomson, 1911) I was able to take samples of blood rapidly and leave over the counting of leucocytes in these samples till convenient. A differential count could also be made on these samples. Continued observations as above are, however, very trying, whatever method may be used, and I think that Scherschmidt deserves much credit for his laborious work.

Before proceeding to the consideration of the crescents, I would like to call special attention to chart of Case F. E. (*P. falciparum*), as it shows two points of importance very clearly.

(1) It shows how the asexual parasites leave the peripheral circulation before the sporulation and rise of temperature. This peculiarity of malignant tertian was first pointed out by Marchiafava and Bignami.

(2) It shows how the total mononuclear leucocyte percentage varies inversely with the temperature.

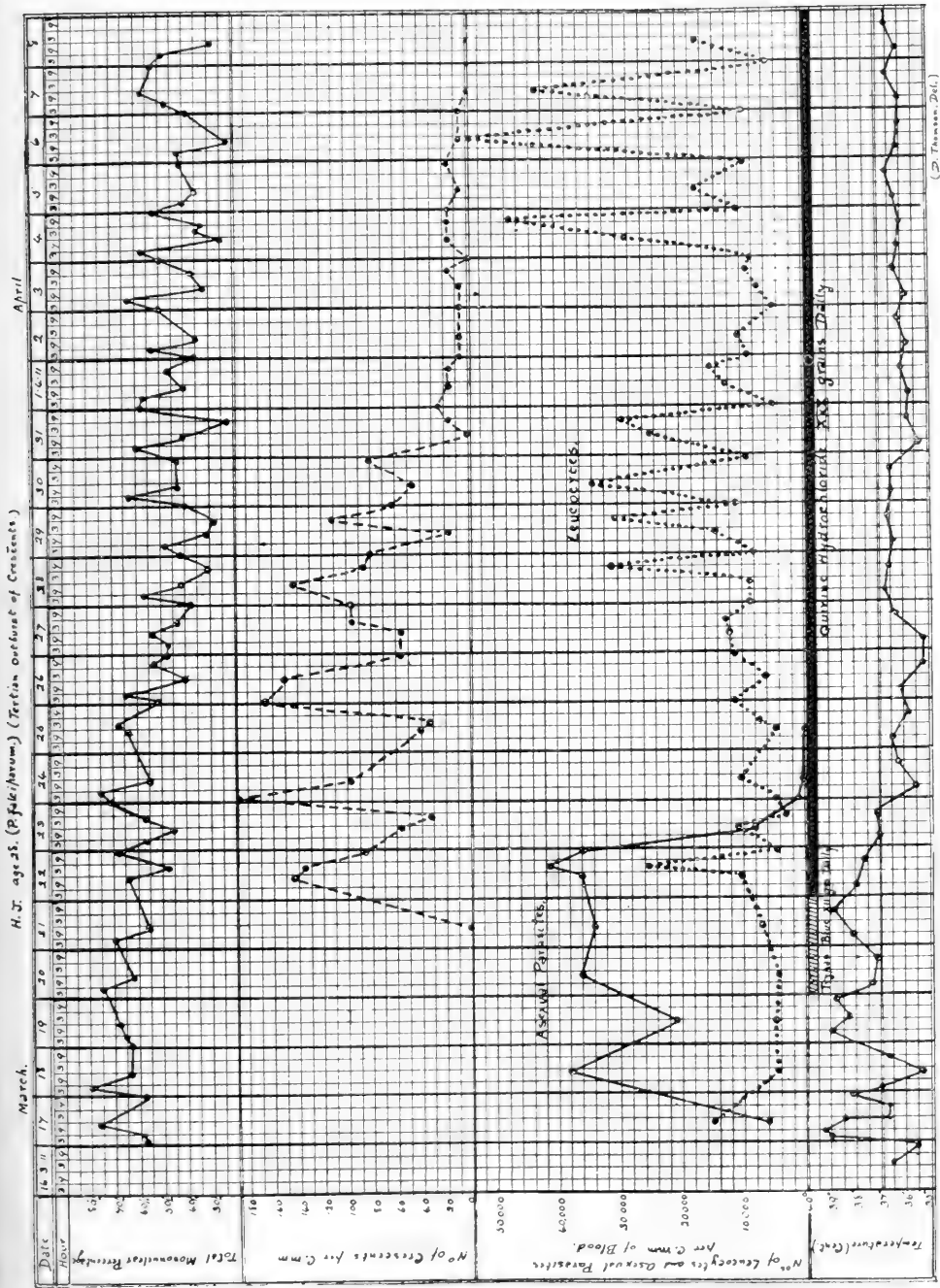
Further, it will be noticed that trypan blue in doses of twelve grains daily had little or no curative effect, whereas Quinoforme, a basic formate of quinine (French preparation) proved very satisfactory.

(c) VARIATIONS IN THE NUMBER OF CRESCENTS

In a previous paper (Thomson, 1911) it was stated that the crescents come into the peripheral circulation in outbursts from the inner organs (spleen and bone marrow), daily, every other day, or irregularly, according as the fever ten days previously had been quotidian, tertian, or irregular. At that time I had only observed this phenomenon occurring daily and irregularly; in fact, it is most commonly irregular, as malignant tertian malaria in the majority of cases is irregular in type. Classical tertian or quotidian cases are much more rare. I am now able to record a case which showed tertian outbursts of crescents following upon a tertian case of *P. falciparum* (see accompanying chart H. J.). This chart shows four distinct tertian outbursts of crescents. It also shows how quinine (thirty grains daily) very gradually reduces the number of crescents till they can no longer be detected.

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(Dr. Thomas, M.D.)



II. THE DESTRUCTION OF CRESCENTS: CONCLUSIONS REGARDING THE PRE- VENTION OF MALARIA BY THE ADMINISTRATION OF QUININE

BY

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

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(A) THE DESTRUCTION OF CRESCENTS

I am still able to adhere to my previous statement that quinine in doses of twenty to thirty grains daily will reduce the crescents to numbers below one per c.mm. within a period of three weeks (Thomson, 1911). One may find a case in which they are not reduced to this negligible quantity in three weeks (Ross and Thomson, 1912), but this is altogether an exception, and indeed in the majority of cases the crescents usually disappear altogether before this three weeks' treatment is over. It was pointed out that the quinine did not destroy the crescents directly, but only indirectly by destroying the source of supply, viz., the asexual parasites. It was also stated that methylene blue, in doses of twelve grains daily, appeared to have some direct destructive action upon the crescents, but I now feel inclined to withdraw this latter statement, as further experiments have shown that the crescents under this latter treatment do not always disappear any more quickly than with quinine, and so probably the methylene blue acts only indirectly also, by destroying the asexual source of supply.

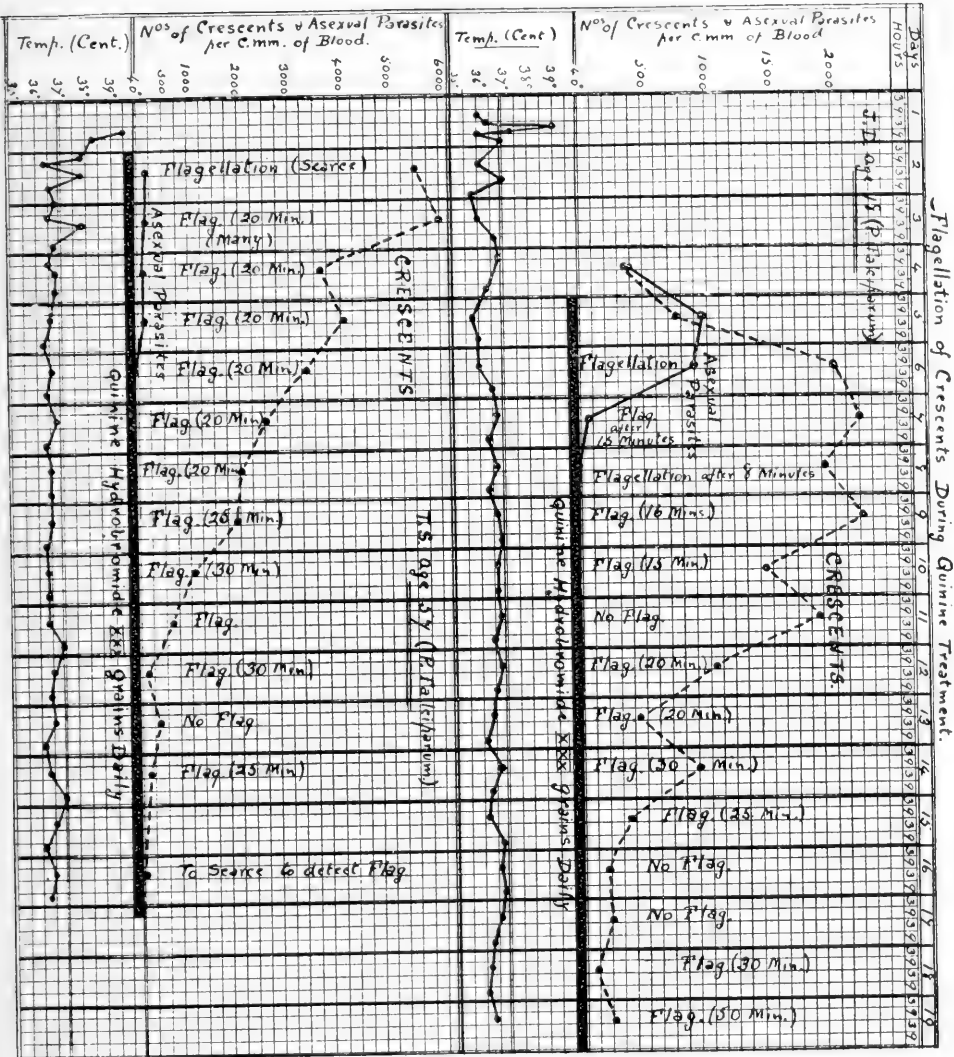
It has been stated that picric acid, in doses of six grains daily, causes crescents to disappear (Surveyor, 1910). I have unfortunately not been able to confirm its efficiency in this respect, as on five different occasions I tried this treatment, but was unable to note that it had any appreciable destructive power either upon the asexual parasites or on the crescents. On two of these occasions the picric acid was given by mouth in doses of three grains twice daily; in the remaining three, picrate of ammonia was given in similar doses.

On one occasion I tested the effect of Soamine injections, and found no reduction in the number of crescents, and there was a true fever relapse during the treatment, so that its effect on the asexual parasites could not compare with that of quinine. Again, the effect of X-Rays was tested, a patient receiving fifteen minutes' exposure over the spleen, but no reduction of crescents resulted. Also many observers have noted that '606' has little or no destructive effect on crescents. It would appear, therefore, that so far no drug or other means has been found which will directly destroy them.

Professor M. Hartmann (1911) thinks it would be possible to induce parthenogenesis in crescents, artificially, thereby transforming them into asexual parasites, which could then be destroyed by quinine. This, however, is a very theoretical statement, and even though it could be carried into practice, I think it would be a very bad method of crescent destruction, and one which might be followed by another crop of crescents about ten days after the induced parthenogenesis.

Ziemann (1906) discusses the destruction of crescents, and thinks that the macrogametes are more resistant to quinine than the microgametes, so that after some days of quinine treatment only macrogametes remain, and that these resistant macrogametes by parthenogenesis give rise to relapses. This again, however, is a more or less theoretical discussion, and lacks proof by practical experiment. Sir Ronald Ross suggested that I should try to throw more light on this point, and in consequence I made very careful observations on two cases with numerous crescents, during continuous administration of quinine, thirty grains daily. The results are shown graphically on Chart 3, which proves that during quinine treatment, so long as crescents could be found in the peripheral blood, flagellating forms could also be found. Each day a droplet of blood was placed on a glass slide, and after breathing on the slide, a cover-glass was superimposed and the film carefully searched for flagellating forms. I was able to get active flagellating forms onwards till the crescents became too few in number to be found, even after a long search. In one case flagellation was still found after fifteen days, and in the other after thirteen days continuous administration of quinine in doses

CHART III.



of thirty grains daily (given by mouth in liquid form). In the first case it would appear that the flagellation was more delayed late on in the treatment than at the commencement. This may have been due to the fact that late in the treatment the crescents were becoming so scarce that it took a long time to find one, though I am inclined to suspect that it was due to the cold weather which set in at the time. In Liverpool, flagellation does not as a rule occur till about twenty minutes' exposure, but I note that in the many specimens sent to me by Dr. James from Panama that his crescents flagellated in ten minutes, and often as early as four minutes after exposure. This point, however, requires further observation.

It would appear to me, therefore, that the male crescents are quite as resistant to quinine as the female variety; and that no drug or other method, so far, has been found which can directly destroy crescents, that the only known method of destroying them is by indirect means, in other words, we can only destroy crescents by destroying the asexual parasites, which are the crescent producers. When these are destroyed the source of crescent supply is cut off, and the crescents remaining, live their natural term of life and die a natural death. This occurs within three weeks after the attack on the asexual forms, and, as the best agents for destroying the latter are quinine and methylene blue, hence these drugs are the best drugs for cleansing the system of crescents. I do not think it is to be greatly regretted that we have no specific drug for crescent destruction, because every case of malaria should have a prolonged course of thorough quinine treatment for at least a month to destroy the asexual parasites, and this length of treatment also destroys the crescents indirectly at the same time. I cannot see my way to believe that relapses are due to surviving crescents undergoing parthenogenesis. Relapses can be explained just as readily by the supposition that they are due to the successive sporulations and consequent increase of surviving asexual forms, for although in the vast majority of cases, quinine rapidly reduces the number of asexual parasites to below the detectable limit, yet all of them are not necessarily destroyed after a severe treatment. A case published by Ross and Thomson (1912) shows that the asexual parasites may show great resistance to quinine. Again,

though the occurrence of parthenogenesis in malaria is quite possible. yet it must indeed be very rare and unusual, as very few observers have seen it in crescents, at any rate.

(B) CONCLUSIONS REGARDING MALARIAL PROPHYLAXIS

There has always been a considerable controversy regarding the respective merits of mosquito destruction and quininisation of the population as a means of reducing the incidence of malaria. I think, as a general rule, the former method is the better, because, as Sir Ronald Ross has pointed out, mosquitos are pests causing much inconvenience quite apart from malaria, and they carry several other diseases, so that their destruction is desired for more than one reason. In some cases, however, the quinine method may be more practicable in places where the population is sparse and scattered, and where swamps abound, but there is no reason why both methods should not be carried out simultaneously, for in rendering a malarious locality healthy it is obvious that the two means of prevention complement each other and are better than one alone. The mosquito method alone, destroys one stage of the parasite with its host, and hence prevents the spread of the disease, but the human population already infected, remain so, often for months and sometimes for years. To relieve these, quinine must be given, and, if the amount given is adequate, not only are they relieved of their sickness, but their crescents are destroyed, so that another link in the perpetuation of the disease is broken. I think all are agreed on this point. But there still remains to be discussed, the question as to the best procedure, and best method of administration of quinine to a population to cleanse it of malaria. In the light of recent researches on the subject, I am going to bring forward ideal methods, which, though impossible to carry into practice, as stated, may yet serve as guides, so that those practical forces which are available, namely, money, legislature and medical staff, may be directed in the most profitable manner.

The Ideal Methods of Quinisation of a population, showing the demands of science necessary for the certain and rapid extinction of malaria by quinine alone.

In British colonies where malaria is rampant the ideal aim of experts is to get every person to take five grains of quinine daily, whether ill or not. Those who are ill with malaria, of course, would take more than this amount daily, till better, and then proceed as before. In carrying out this ideal, therefore, each adult would take 1,825 grains of quinine per annum, if well during the year, and more, say 2,000 grains per annum, if ill at some period of that year. Though this method is undoubtedly excellent, yet I think that the following method would be much more scientific and efficacious, and shall call it *Ideal Method A*:—‘That every adult person in the population (children in proportion according to age) should take twenty grains of quinine daily and simultaneously for a period of three weeks, quarterly, i.e., four times a year.’ This amounts to 1,680 grains per adult per annum, if well during the year, and probably 1,800 grains, if ill at some period in the year. This, in the aggregate, is less quinine than in the former method.

The faults of the 5 grains daily method:—

(1) Five grains of quinine daily is insufficient to prevent infection from mosquitos. I have known several cases of fever contracted during this daily dosage.

(2) Five grains of quinine daily will take a long time to eradicate malaria from the system. In many cases it will not eradicate it. The fact that a person can take an acute malarial attack during such dosage, proves that it is an insufficient amount to render the blood uninhabitable to the parasite.

(3) This amount of quinine makes the blood less suitable for the parasites, and, hence, tends to keep the disease latent in the system without properly curing it. A few parasites may be present in the blood not sufficient to cause fever, and the person may feel comparatively well and congratulate himself that he is free from the disease, yet he may be harbouring numerous crescents for long periods of time, since those latent chronic cases are often the most fertile producers of crescents (Thomson, 1911).

Points in favour of 'Ideal Method A.'

(1) The administration of quinine in doses of twenty grains daily for three weeks is almost certain to destroy both the asexual and sexual parasites.* After this period, the person will be non-infective to mosquitos, and, in the great majority of cases, will be cured of the disease. Very few patients have a tendency to relapse after this treatment. By this method, therefore, every person would be non-infective to mosquitos, and freed from a tendency to relapse, four times a year.

(2) Infective mosquitos must necessarily become much fewer in number.

(3) The amount of quinine taken is less in the end, and none is taken during nine months of the year.

(4) After the third day the majority of people feel very little inconvenience when taking 20 grains of quinine daily. I have satisfied myself on this point over and over again with regard to adult males, whilst in hospital and doing light indoor work.†

(5) It is quite as practicable a method as the five grain daily method. It would be as easy to make a population take 20 grains daily for 3 weeks, quarterly, as to make it take 5 grains daily for a year.

As an alternative to the above, I will now put forward '*Ideal Method B,*' which embodies the same scientific principles as '*A,*' but which is more scientific and less tyrannical in its administration. This Method B differs from A in that a quarterly census of the blood of the population would be taken before administering the quarterly quinine. Only those who had parasites in their blood would require to take the 3 weeks' course, and those with crescents would, if possible, be isolated in mosquito-proof hospitals till non-infective. Further, immigrants would not be allowed to enter the area until their blood had been examined and pronounced non-infective. Infective immigrants would require the 3 weeks' period of treatment, with isolation, before being admitted to the population. With regard to the blood census, the quickest and surest method of examining the blood would be obtained by Ross's '*Thick Film Method*' (James, 1911). The blood of several

* Exceptions to this occur. See Ross and Thomson (1912).

† It may not be so with those who have to do outdoor work during this treatment.

individuals could be placed on the same slide and numbered. This would reduce the number of slides required. No skill is required in smearing the blood, and as the blood by this method is concentrated about 20 times more than in ordinary smears, a two minutes' microscopic search is sufficient to decide the presence or absence of parasites. To carry out this blood census it would require one medical man with several assistants per 1,000 of the population, during the time of the census. It might be sufficient to make a blood census only of the children up to a certain age, and in places where malaria was not excessive, it might be sufficient to confine this method to those who were ill, especially with regard to children.

Before concluding I would like to state once more that the methods put forward above are ideal, and though they cannot be carried out to the letter in practice, yet they must form the ideal basis of malarial prevention by quinine, and they must, therefore, be considered in attempts to form systems of state medicine and sanitation in tropical countries, and it should be remembered that the carrying out of these methods in part only must always do some good. It is better to understand what is really required by science, and, looking at the great difficulties of giving an effectual quinine prophylaxis, a simultaneous though partial mosquito reduction will probably be always required.

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ON HAEMOLYSIS IN MALARIAL FEVER

BY

G. C. SIMPSON, M.B., B.Sc.

PRELIMINARY NOTE

(Received for publication 29 May, 1912)

In a previous paper* it was shown, (by estimation of the excretion of blood pigments,) that in all probability there is a greater or less degree of haemolysis in all cases of malaria, and that the great haemolysis of blackwater fever was probably only a more marked phase of such general haemolysis.

In the last eighteen months fifty cases of malaria in the Royal Southern Hospital, Liverpool, have been examined at various stages of the paroxysm, and in the intervals, to discover if at any period a haemolytic principle could be discovered in the serum.

The blood was drawn into citrate solution and centrifuged in the pipettes, and the serum was separated from the corpuscles: the corpuscles were washed with normal saline, re-centrifuged, and made into a 5% emulsion. Non-malarial blood was similarly treated.

Mixtures were made each in one or two dilutions, incubated at 37° C., and examined after half an hour, one hour, and longer intervals.

The following mixtures were used:—

	Malarial corpuscles	and malarial serum.
	„	„ „ normal serum.
	„	„ „ „ and malarial serum.
Normal	„	„ „ „ „ „ „ „
	„	„ „ malarial serum.
	„	„ „ „

In no case did the control serum haemolyse the control corpuscles. In only one case did malarial corpuscles appear to be dissolved by control serum, and then only to a very slight extent.

* *Annals of Trop. Med. & Parasit.*, Vol. IV, No. 3, p 313, 1910.

In no case did addition of control serum to malarial serum appear to modify the effect of malarial serum alone. (Heated malarial sera were not tried.)

Only seven times out of one hundred observations did the malarial serum exert any haemolytic action, and in two of these it was extremely slight: in the other five cases definite haemolysis took place.

I. C., age 32. Simple tertian fever: No quinine, first attack. Onset eleven days before admission. Shivering at 11.30 a.m. Paroxysm came on later: 9,200 parasites per c.mm., young and sporulating, found in blood at 11 a.m. 11.30 a.m., serum markedly haemolytic to control corpuscles. More rapid haemolysis to patient's corpuscles. Patient's corpuscles appeared slightly dissolved in control serum. 2.30 p.m., fever present. No haemolysis. Next day, no fever, no haemolysis.

II. T., age 18. Simple tertian: No quinine. 11.30 a.m., preliminary rigor. Serum markedly haemolytic to control corpuscles and to patient's corpuscles. 3 p.m., T. 101.6° F. No haemolysis. Quinine given. No further attacks.

III. F., age 30. Simple tertian: No previous quinine. Probably five months' infection. 4,000 parasites per c.mm., 9.30 a.m., preliminary rigor. No haemolysis. 11 a.m., T. 103.2° F. Serum haemolytic to control corpuscles and to patient's corpuscles. Later, in quiescent stage. No haemolysis.

IIIA. Relapse on discontinuing quinine treatment after discharge: double tertian fever. A very slight shiver before onset of paroxysm. Serum very slightly haemolytic to control corpuscles.

IV. L. Malignant tertian fever: No fever for twelve months till day before observation. T. 103° F. Parasites 7,200 per c.mm. Later, parasites 17,000. Serum haemolytic to control corpuscles and patient's corpuscles.

V. D. Malaria, blackwater fever: Onset at 12.30 a.m. 1 a.m., serum gave very slight haemolysis with normal corpuscles. 3 a.m., 5 a.m., 11 a.m., serum not haemolytic to normal corpuscles; serum not haemolytic to normal corpuscles in presence of control serum.

It will be seen that four of the five positive results were obtained in simple tertian fever, only one in malignant tertian, though most

of the cases examined were of the latter type. A very slight result was obtained in the case of blackwater fever.

In three of the four positive results obtained with simple tertian fever, the positive reaction was obtained in the premonitory stage, and disappeared shortly after when the fever was established. In the other case it was absent in the premonitory stage, and appeared shortly after. None of the cases were under quinine treatment.

The results appeared to be of sufficient importance to communicate, in the hope that further work may be done in places where the material is more abundant.

Positive results appear most likely to be obtained in simple tertian cases at the onset of the paroxysm, when the young sporulating forms are set free in the blood.

They are less easy to obtain in malignant tertian cases where the sporulation is spread over a longer period of time. It is possible that the haemolysis is due to the direct mechanical action of the young forms entering corpuscles, but this would scarcely give such complete haemolysis as occurred in Cases I and II, and it is probably due to a specific haemolysin.

In conclusion, my thanks are due to Dr. D. Thomson, Malarial Research Assistant in the Tropical School, for his assistance and for the particulars of the cases. The funds for the research were allotted to the Liverpool School of Tropical Medicine by the Advisory Committee for the Tropical Diseases Research Fund (Colonial Office).



THE ANTI-NEURITIC BASES OF VEGETABLE ORIGIN IN RELATIONSHIP TO BERI-BERI, WITH A METHOD OF ISOLATION OF TORULIN, THE ANTI-NEURITIC BASE OF YEAST

BY

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In a recent paper¹ from these laboratories an account was given of the chief investigations into the causation of Beri-beri up to 1910. This account was largely an abstract of the masterly monograph in which Schaumann² described the work of other investigators, added much important work of his own, and brought evidence to suggest that other diseases might have a similar etiology to Beri-beri.

Some of our earlier results were briefly mentioned in that paper, and the account of our further researches will be prefaced by a brief summary of some of the numerous papers on this subject which have appeared in the interval.

One of the most interesting papers gives an account of the later researches of Fraser and Stanton³ who have already contributed so largely to the knowledge of this subject.

They demonstrate that the active anti-neuritic substance of rice meal is soluble in water and in alcohol, show that it is stable in acid, unstable in alkaline solution (p. 74), and that its thermostability varies with varying physical factors. They show that it is not a phytinbody or a fat, and that it probably is not a protein nor does it contain phosphorus.

They further confirm the fact that the phosphorus content of a rice is an indicator of its safety as an article of diet, and, with a view to the prevention of Beri-beri, recommend administrative measures to prevent the sale in the Malay States of rice with less than 0.4 % phosphorus pentoxide.

Eijkman⁴ recapitulates his earlier work on the subject, and all must regret that he and his colleagues were prevented from continuing their work in Batavia and Java *where, in the years 1889 to 1897, they had so far advanced the study of Beri-beri and Polyneuritis on experimental lines, and had demonstrated that Beri-beri could be cured and its occurrence prevented by the use of hand-milled rice.*

His further researches to isolate the active substance from rice meal are recorded. Cure of polyneuritis in fowls was effected by two or three doses of extracts of rice meal; less than five grams of extract containing 0.085 % P_2O_5 and 0.012 % N, sufficed to restore to activity birds severely lamed. He strongly opposes Schaumann's theory (1910) that the active substance is a phosphorus-containing compound, though awaiting fuller accounts of his later communications⁵.

Shiga and Kusama⁶ report extensive investigations disproving the bacterial and toxic theories of Beri-beri, and confirm much of Eijkman's, and Fraser and Stanton's work.

Kilbourne⁷ shows that the potassium content of rice meal is of almost equal value to the phosphorus content as an indicator of its safety.

Hight⁸ in Siam, and Aron⁹ and Heiser¹⁰ in the Philippines, have shown that Beri-beri followed the introduction into those countries of steam milled rice, and that the disease disappeared from institutions (jails, etc.) on the re-introduction of hand-milled rice. They have further demonstrated that rice can be milled by machine, without impairment of keeping powers and palatability, and with the retention of the valuable outer layers, which render it free from the risk of inducing Beri-beri.

Their work is most interesting and important from the administrative point of view, and has already led to the diminution of Beri-beri in their districts. A Bill was introduced into the Philippine Legislature to discourage the use of polished rice, and

prevent Beri-beri, by putting a duty on rice with a phosphorus content less than 0.4 % P_2O_5 —the same limit that Fraser and Stanton have suggested in the Malay States. Such rice is already prohibited in Government Institutions by administrative orders.

Many of these papers were read at a Meeting of the Far Eastern Association of Tropical Medicine (Philippines, 1910), and a resolution was adopted affirming strongly that Beri-beri is a disease due to the continuous consumption of white polished rice.

Chamberlain and Vedder¹¹ following Fraser and Stanton, showed that an extract of rice meal in 70 % alcohol, concentrated at a low temperature till alcohol free, maintains its activity to cure polyneuritis. They further showed that the active substance is able to dialyze through parchment. A daily dose of these extracts, containing 0.16 mg. P_2O_5 and 4.06 mg. Nitrogen, cured, in a few days, fowls severely lamed.

In another contribution¹² they confirm these results, having kept fowls for 100 days on polished rice and the extract without development of neuritis. They find that the sucrose and ash in the extract are inactive, and so exclude 0.91 of the 1.34 % solids in the extract.

In addition, they find that the active substance is absorbed by bone-black, and are now proceeding to attempt to isolate and analyse it.

Funk¹³ has isolated from rice meal a crystalline nitrate of an organic base which is extremely active in reviving pigeons with polyneuritis from feeding on polished rice.

The necessary dose contains about 4 mgs. of Nitrogen, corresponding to 0.05 gram. of the nitrate of the base, to which he allots the provisional formula $C_{17}H_{18}O_4N(HNO_3)$.

The crystals were in the form of microscopic needles, melting at 233° C., insoluble in cold water or alcohol, soluble, with difficulty, in hot water. They were free from ash and from chlorine and sulphuric acid.

This is the first record of the isolation and analysis of an active substance, and the method by which it was obtained will be briefly described. One and a half kilograms of rice meal was extracted with four litres of acid alcohol; separation of the filtrate was completed by the hydraulic press; about three and a half litres of extract were obtained, and evaporated in vacuo at 30°, leaving a fat-like residue. This was melted and treated with water, and filtered while warm. The aqueous part was treated with ether to remove all fatty substances; it cured pigeons in doses corresponding to 20 grams of the original polishings.

The total aqueous extracts from 54 kilos rice meal amounted to 17 litres, which was treated with sulphuric acid and phosphotungstic acid throwing down 900 grams of precipitate. The precipitate was dried, washed with 5 per cent. H_2SO_4 , ground with baryta and shaken three hours with water. The precipitate was filtered off, the filtrate smelt of ammonia and methylamine. The baryta was precipitated with sulphuric acid, and the filtrate neutralised with hydrochloric acid, and evaporated in vacuo at room temperature. The residue was extracted with alcohol, and the alcoholic solution was active in doses = 40 grams of rice polishings. The solution was free from proteins, phosphorus, and carbohydrates.

The alcoholic solution gave a crystalline precipitate with mercuric chloride, which was separated, washed, and recrystallised from water; this consisted mostly of cholin, but some active substance was also present. Active substance was present in both the alcoholic and aqueous filtrates.

Aqueous filtrate. The mercury was removed, and the filtrate evaporated and taken up in alcohol was treated with platinic chloride to remove cholin. After removing the platinum from the filtrate it was treated with phosphotungstic acid, giving a crystalline precipitate which yielded an active substance when freed from phosphotungstate with baryta and carbon dioxide.

Alcoholic filtrate evaporated and dissolved in water: mercury removed by sulphuretted hydrogen; chlorine, &c., were removed by successive treatment with silver sulphate, sulphuretted hydrogen, and baryta. The alkaline solution was precipitated with silver nitrate and baryta, the precipitate decomposed with sulphuretted hydrogen and freed from silver and barium. It proved active, and, after evaporation in vacuo, crystals were with difficulty obtained from alcohol, with the composition, &c., given above.

ACTIVITY OF VARIOUS FOOD STUFFS

We have continued the attempt, by the use of various additions to rice diet, to prevent or delay the onset of polyneuritis or to cure animals already suffering from it.

In the earlier experiments phytin, nucleins, caseins, lecithins, etc., were used, in part from commercial sources and in part isolated by us (using care to avoid the use of strong reagents).

Addition of commercial nuclein and lecithin to the diet of polished rice had no apparent influence either on the time of onset of incapacity or death or on the loss of weight. Addition of casein seemed to postpone for a few days (five) the onset of incapacity and death, and the birds lost on the average considerably less weight. A mixture of casein, nuclein and lecithin also proved an ineffective addition.

The addition of an acid extract of rice meal, neutralised by bicarbonate of soda and taken to dryness on a water bath, led to a very rapid loss of weight; in the light of further results this extract would appear to have been 'denaturised' by excess of alkali or overheating.

Phytin extracted from rice meal, but not re-purified, prolonged the life of the birds distinctly, and lessened the wasting to a fair extent: this may have been due to the adhesion of a small amount of the active material to the phytin.

Casein, separated from milk and not re-purified, again seemed to postpone the onset of lameness and to lessen the loss of weight. All four birds were alive after thirty days on this diet—one decidedly lame, two slightly lame, the other not affected. With non-protective substances some birds would certainly have died earlier than this.

Lecithin was prepared from egg yolk: part was separated from solution by cooling apart by acetone precipitation. This also proved inactive.

In these experiments casein and phytin alone seemed to contain any of the active principle. We also thought that certain sapogluco-sides (saponin and hederin) had had a slightly favourable influence, but the experiments had to be abandoned owing to severe diarrhoea at an early period.

BIO-CHEMISTRY OF EXTRACTS OF RICE MEAL AND YEAST

Among other points, we find that nearly twice as much of the phosphorus of rice meal goes into solution in water after denaturisation at 120° C. Of the soluble phosphorus of rice meal nearly five-sixths dialyses: of the soluble phosphorus of denaturated rice meal only two-thirds dialyses. The protective properties of the fractions separated were not tried, as the quantities were insufficient for continued feeding experiments.

More than twice as much of the phosphorus of dried yeast, after denaturisation at 120° C., appeared as 'phosphatide phosphorus.'

Alcoholic (90 per cent.) extract of rice meal was found to be active (as stated by Fraser and Stanton) in protecting birds from the onset of neuritis, and in curing them, but concentration on the water bath rendered the extracts inactive.

The extracts were concentrated under a fan at room temperature to small bulk till all smell of alcohol had disappeared. These extracts preserved some activity (cp. Chamberlain and Vedder, loc. cit.). Four birds, which were very weak and disabled with polyneuritis, were each given the extract from 25 grams of rice meal, daily: in the first week they showed a decided improvement; became more active, three gained 5%, 15% and 8% in weight, respectively, in one week (loss previous to treatment, 32%, 30% and 36%). The other, though it became more active at first, lost

a further 2 % in seven days, and 14 % in ten days, being then 47 % below its original weight. It died on the 15th day of feeding, 7 % further loss, but for the last week had been receiving small doses of Hederin daily.

Of the other three birds, two fell in weight (5 % and 2 %) between the seventh and tenth days, the other one gained a further 2 %; two fell again in weight, one losing a further 5 %, the other falling 5 % more to nearly its weight at the commencement of treatment. The last, on Hederin, just maintained its increase. All were now extremely weak again, and could scarcely survive more than a day. They were now put on yeast, and rapidly improved, walking and flying well in a few days, and gaining, respectively, 9.6 and 6 % in the week.

Attempts were made to precipitate the active principle from these extracts by the lead acetate method. Neither the normal or basic lead acetate precipitates proved active: the filtrate, however, was active: the precipitate from this by phosphotungstic acid did not, however, prove active.

Funk's method was now tried direct on the original extract of the meal. *A strong odour of ammonia and methylamine was noticed in the treatment with baryta.* In spite of continued treatment with this extract, the birds died in a few days. (? Cholin poisoning, C. & F.).

We considered that other foodstuffs might give more favourable results, and so, for the present, abandoned the investigation of rice meal.

On account of the resemblance of the active substance in solubility, etc., to some of the peculiar lecithins and bases described by Winterstein in wheat meal, we tried the lead acetate precipitable portions of Katjang beans, but found them inactive. Feeding with fresh brain also failed to preserve birds from death when incapacitated with neuritis.

An attempt to isolate the active substance from Katjang beans proved unsuccessful, neither the lead acetate filtrate nor precipitate proving active. Further experiments will be made.

Natural yeast had been previously found to possess marked preventive and curative properties, and extracts from yeast were next investigated.

INVESTIGATION OF YEAST EXTRACTS

After various experiments¹⁶ the following method was adopted: Twenty pounds of commercial fresh pressed yeast were extracted in the cold with successive quantities of methylated spirit, using in all about twenty litres of spirit; the yeast was filtered through thick calico, and the alcoholic filtrate was freed from alcohol at room temperature by means of an electric fan. There remained about seven litres of watery fluid, dark yellow in colour, smelling strongly of beer, and with an intense bitter taste.

This water extract was mixed with sufficient plaster of Paris to make it 'set.' The plaster matrix, after standing overnight, was ground to a fine powder, and extracted in the shaking machine with successive small quantities of methylated spirit made faintly acid with hydrochloric acid. These extracts were freed from alcohol, as before, and the watery fluid obtained, amounting to 3-4 litres, was precipitated with excess of basic lead acetate. The lead precipitate, having previously been found to be inactive, was discarded. The filtrate was freed from lead with sulphuretted hydrogen, and then concentrated to a syrup in vacuo at 38° C. This syrup was treated with absolute alcohol, and the sticky hygroscopic yellow precipitate (creatinin, etc.) was filtered off. The alcoholic filtrate was again freed from alcohol and then precipitated with baryta and silver nitrate. This precipitate was decomposed with sulphuretted hydrogen, filtered, excess of sulphuretted hydrogen removed by the fan, and then taken to dryness in vacuo at 38° C. A small quantity of a brown, sticky, hygroscopic mass was obtained in this way, easily soluble in cold water, and intensely active.

A dose of 0.006 gram administered to a bird with severe convulsions and lameness, improved the convulsions in four hours: the bird was flying strongly in twenty hours, and the lameness disappeared in forty-eight hours. Two further doses of 0.003 gram were given on the third and eighth day; the bird appeared normal, and gained weight on polished rice diet, but died on the fifteenth day without return of lameness or convulsions. Similar results were obtained with other birds, but there was not sufficient substance to make prolonged experiments to see if the substance would restore the birds to their original weight and condition, etc.

The substance was further purified by treatment with alcohol: it was insoluble in ether and acetone, and on standing yielded feathery crystals identical with those found in Experiment X.¹⁶

The ash consisted principally of barium nitrate and a small amount of phosphate, which may not prove to be merely impurity.

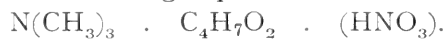
Pending further investigations into the exact nature of the ash and its relationship to the organic compound, it is assumed to consist entirely of impurity, and the composition of the residue is approximately—

$$\begin{array}{r} \text{C} = 40\cdot5 \\ \text{H} = 8\cdot07 \\ \text{N} = 13\cdot32 \\ \text{O} = 38\cdot11 \\ \hline \end{array}$$

$$\begin{array}{r} 100\cdot00 \\ \hline \end{array}$$

This corresponds to the formula $\text{C}_7\text{H}_{17}\text{N}_2\text{O}_5$ or $\text{C}_7\text{H}_{16}\text{NO}_2(\text{HNO}_3)$.

As the action of baryta splits off trimethylamine we may assume the presence further of this group, and write it:—



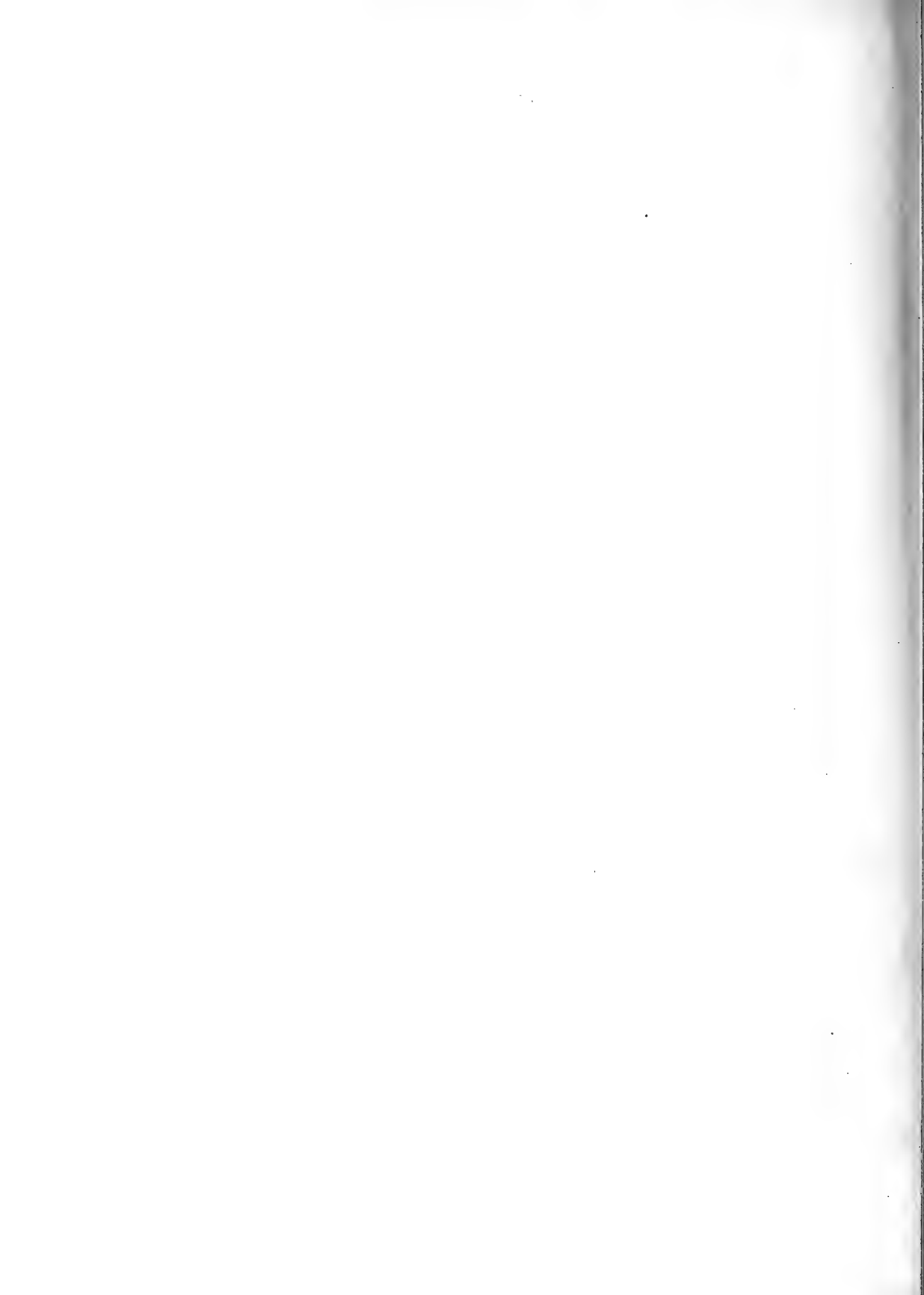
The substance isolated by us from yeast gives an oily precipitate with gold chloride (resembling Kutscher's bases), but we have not been able to obtain, as yet, enough of the gold salt for identification.

Attempts are being made to isolate larger amounts of the active principle, and to work out more completely its constitution and its influence on metabolism. It is also important to determine if the antineuritic bases of rice, wheat, and other foodstuffs are the same as that of yeast or different (as Funk's results indicate), and also whether they are breakdown products of more complex substances present in the foodstuffs.

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SOME OBSERVATIONS ON THE
MORPHOLOGY AND BIOLOGY
OF *PROWAZEKIA URINARIA*
(*BODO URINARIUS*, HASSALI.)

BY

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PLATES XVII, XVIII

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I. INTRODUCTION

The funds for this research, which was carried out in the Liverpool School of Tropical Medicine, were provided by a grant from the Post-graduate Research Fund of the Queen's University, of Belfast. I have much pleasure in thanking Sir Ronald Ross for permission to work in his laboratories and for his kindly interest in the work. Also, I wish to thank Dr. J. W. W. Stephens, Dr. H. B. Fantham, Dr. D. Thomson and Dr. J. G. Thomson for valuable help and suggestions during this investigation.

II. HISTORICAL

The flagellate used in this investigation is a typical member of the genus *Prowazekia* (Hartmann and Chagas, 1910). This genus was separated from the genus *Bodo* because it was found, by the more exact cytological methods of recent years, that under the term *Bodo* was included two distinct types of flagellates which differed in their nuclear structure and flagellar attachments.

The first reliable account of the cytological details of a member of the genus *Bodo* was the description given by Prowazek (1904) of *Bodo lacertae* (Grassi, 1881). This species does not possess a kinetonucleus. Hartmann and Chagas (1910) described a flagellate *B. cruzi* which, while answering to Stein's definition of the genus *Bodo*, possessed both a principal nucleus and a kinetonucleus. These authors, therefore, thought a new genus should be created to cover this discrepancy, and, as a different nuclear and flagellar apparatus had been described in the only member of this genus (*B. lacertae*) which had been thoroughly worked out, they made a new genus *Prowazekia* to include the Bodo-like flagellates possessing a kinetonucleus, and they named their new species *Prowazekia cruzi*. The name of the new genus was in honour of Prowazek, who, in 1903, figured a structure in *Bodo sp.*, which he called the 'giesselsackchen,' and which was really the kinetonucleus.

This new genus has not been generally accepted yet, as some authorities, notably Alexeieff (1910, 1911a, 1911b) think that the term *Bodo* should be used as the generic title of the genus which Hartmann and Chagas have called *Prowazekia*, and that the Bodo-like protozoa of the type *B. lacertae*, which does not possess a kinetonucleus, should be included under the genus *Heteromita*, which was the old generic name of *B. lacertae*.

The earliest reference to the occurrence of Bodo-like flagellates in the urine was that of Hassall (1859). This observer found flagellates in 50 samples of urine from a number of different patients, some of whom were suffering from albuminuria and some from cholera. Weakness and debility was a feature of all the cases which showed flagellates in the urine. The reaction of the urine was alkaline or only feebly acid, and the flagellates only appeared after the urine had stood several days. Hassall named

this organism *Bodo urinarius*, and gave a very good description of it. The flagellate measured 14μ by 8μ , and was round or oval in shape, sometimes with one end of the body enlarged. These forms had 'one, usually two, and sometimes three lashes or cilia,' and showed very active motility.

In his drawing of these flagellates, Hassall depicts the round or oval forms, the 'sausage-shaped' forms and the 'carrot-shaped' forms described later on in this article. Cultures were obtained by Hassall on a mixture of an alkaline solution with albumen, and it was noted that the cultural forms were usually smaller than the original flagellates. In 1868, Salisbury* described a similar flagellate in the urine under the name *Trichomonas irregularis*. This parasite was again described by Kunstler (1883) under the name *B. urinarius* (*Cystomonas urinaria*, Blanchard, 1885, *Plagiomonas urinaria*, Braun, 1895). The form which he described was found in a mass of pus in the freshly passed urine of a case of pyelitis. This flagellate was 10 to 15μ long by 4 to 5μ broad, with a broad anterior end and a pointed posterior end. The anterior end showed a depression and a beak-like process from which the flagella originated. This form is similar to the 'carrot-shaped' form described in this article. (Text fig. 3.)

From the descriptions given by Hassall and the above authors I believe the flagellate described in this article to be identical with *Bodo urinarius*, but, because it possesses a kinetonucleus, it must now be placed in the genus *Prowazekia*, and be called *Prowazekia urinaria*. Ultimately, this flagellate may be found to be identical with some of the numerous free-living species of *Prowazekia*.

Recently, several members of the genus *Prowazekia* have been described as occurring in the human faeces, both in diseased and healthy persons in various parts of the tropics. Castellani and Chalmers (1909) described a flagellate *B. asiaticus* in the faeces of a case of ankylostomiasis in Ceylon. This species has been investigated further by Whitmore (1911) under the name *Pr. asiatica*, and they believe it to be a free-living form. A somewhat similar flagellate *Pr. cruzi* was isolated by Hartmann and Chagas (1910) on an agar plate which had been inoculated from

* Quoted by Blanchard, p. 78, 1889.

the faeces of patients in Brazil. (This species was also thought to be a free-living form.) In the same year, Mathis and Leger, in Indo-China, described a form *Pr. weinbergi* in the stools of patients with diarrhoea and also of patients with no intestinal trouble. This species was found to occur in the faeces even when obtained with aseptic precautions, and, therefore, these authors believe that it is an intestinal inhabitant, but with no pathogenic rôle. On account of the ubiquitous distribution in air, dust, water, etc., of the genus *Bodo*, in the old sense, as was pointed out by Alexeieff (1911*b*), I think further evidence is necessary to establish the fact that these flagellates were actually present in the fresh faeces and were not the result of subsequent contamination.

A free-living form *Pr. parva* has been described by Nägler (1910) in the slime on the stones at the biological station at Lunz.

III. OCCURRENCE OF THE FLAGELLATE

The flagellate with which this research was conducted was first observed by me in March, 1912, in the deposit, after centrifugation, of a 24-hour old specimen of urine from a Mexican sailor in the Royal Southern Hospital, Liverpool. This patient was suffering from malignant tertian malaria, and had a history of an attack of blackwater fever a few days prior to admission. The same flagellate was again seen in the deposit of a 6-hour old specimen from the same patient a few days later. In the first case, the reaction of the urine was alkaline, and in the second case, neutral. In both cases the urine contained albumen and abundant casts. Neither of the above specimens was taken with aseptic precautions, so the possibility of contamination could not be excluded.

As the old descriptions of the flagellates found in urine were rather vague, and because of the increasing prominence of the genus *Prowazekia* since the discovery in the human faeces of several species, it seemed to me that the flagellate was worth investigating further.

At first it appeared as if the organism had been passed in the urine, and this view was supported by the following facts:—

(1) It had been found on two different occasions in the urine of the

same patient. (2) It could not be found either by direct examination or cultural methods in the urine, taken under the same conditions, of any of the other patients in the same ward, although nearly thirty were examined. (3) Similar examinations of the patients' faeces were negative. (4) The examination of the water supply also gave negative results. (5) Unsuccessful attempts were made to find it in a vessel of water exposed to the air as recommended by Alexeieff (1911*b*) for obtaining *Prowazekia caudata* (*Bodo caudatus*).

These observations are rather negatived by the facts that later specimens taken with aseptic precautions did not show the flagellate, and that a temperature of 37° C rapidly kills the cultures. I am, therefore, inclined to think, from the evidence as a whole, that this flagellate was probably not an inhabitant of the urinary tract, but was an accidental contamination.

IV. TECHNIQUE

(1) *Isolation of 'pure' cultures.*

In examining cultures of protozoa, obtained from the faeces or urine, or in fact any protozoal cultures, it is very important for the investigator to make sure that his culture, which apparently contains only one species, does not contain several closely allied species or members of several closely allied genera. The flagellate used in this investigation was isolated in 'pure' culture by the method recommended by Sellards (1911) for the isolation and culture of amoebae.

A little of the infected material was well diluted with sterile water. Then a number of minute drops of dilute serum (1 in 25 pleural fluid) were placed on a series of sterile cover-glasses. These drops were of such a size that with a 1/2 inch objective the whole drop could be seen at once. A straight platinum wire was then dipped into the diluted culture, and each of the drops was touched with it. These drops were then examined with the 1/2 inch objective until one was found which contained a single flagellate. To this drop a little more dilute serum was carefully added, and the drop was spread out slightly, so that in future examination it would not be too deep for all parts to be seen with a 1/12 inch

objective. This coverslip was then inverted over the cell of a hanging-drop slide and ringed with vaseline, and the drop was again examined very carefully, with both $1/2$ inch and $1/6$ inch objectives, to make sure that it contained only one flagellate, and also that no cysts were present.

It may be noted here that in the early stages of multiplication the daughter forms produced by the first one or two divisions tend to remain near the spot where the parent cell was. This fact is of importance in the examination of these hanging-drop cultures, because, if two collections of six or eight cells are found at opposite sides of the drop, while the intermediate space is free from cells, it is almost certain that these collections have originated from separate parent cells and that, therefore, the resulting culture may not be 'pure.' By this method it is not only possible to obtain a culture from a single flagellate, but at the same time the rate of multiplication and the mode of formation of division rosettes may be noted.

Ordinary cultures may be obtained from these hanging-drop cultures by removing the coverslip, and inoculating suitable media from it with a platinum loop.

(2) *Examination of Fresh Preparations.*

In studying the biology of this flagellate, fresh cover-glass preparations were found to be more useful than hanging-drop preparations. In these cover-glass preparations the very active motility of the organisms had usually slowed down sufficiently in $1/2$ to 1 hour to permit the flagellar movements, the ingestion of food, etc., to be observed. A truer idea of the characters of the motility could be observed in hanging drops.

(3) *Examination of Fixed Preparations.*

Another method of making preparations is to place a drop of the culture on a slide and expose it to the vapour of osmic acid (4 %) for fifteen to thirty seconds, and then place a cover-glass on this drop. By this method the morphology can be carefully studied, and measurements can be made more accurately than in stained preparations, because there is not the same liability to shrinkage and distortion as so often occurs in stained smears. The

examination of these preparations is greatly facilitated by adding a little dilute haematoxylin solution to the drop before covering it. This method shows up the two nuclei very well, and in some cases even the origin of the flagella from the basal granules can be made out.

(4) *Staining.*

The stains used were iron-haematoxylin, glycerine haematin, Delafield's haematoxylin, Giemsa's stain, Leishman's stain, and Romanowsky's stain. Heidenhain's original iron-haematoxylin method was found to give better results than Rosenbusch's modification of this method. In both cases it was found that the flagellates on films fixed with osmic acid vapour for thirty to forty-five seconds kept their natural shape better than those fixed in hot sublimate alcohol (Schaudinn's fluid).

To obtain good results with Giemsa's or Leishman's stain the following method of preparation and fixation of the films was found to be the best.

(1) A drop of fresh serum was mixed on a slide with a drop of the culture.

(2) This drop was fixed in osmic acid vapour for thirty to forty-five seconds and a film was then made of it.

(3) This smear was again exposed to osmic acid vapour for the same time and it was then ready for fixation and staining. In staining with Giemsa stain the film, made as above, was

(4) Fixed in methyl alcohol twenty minutes.

(5) Stained with dilute Giemsa's stain (one drop to 1 c.cm. of distilled water) for one to three hours.

(6) Wash off the stain with tap water. Place a cover-glass on the wet film and examine with the $1/6$ inch objective, and if the staining is too deep, repeat the washing until the correct colour is obtained. A rapid rinse in orange-G-tannin solution will greatly shorten this process in deeply stained films, but the results are more uncertain.

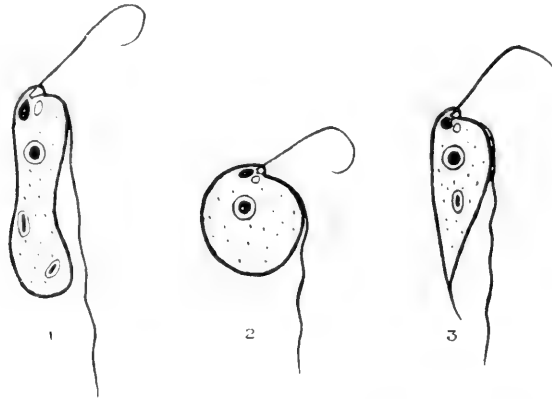
(7) Blot almost dry, clear in xylol, and mount in acid-free Canada balsam.

The films may be stained with Leishman's stain in the ordinary way, but it is better to allow the diluted solution to act on the

film for at least thirty minutes, and to regulate the amount of washing afterwards by frequent microscopic examinations. It was found that the Giemsa method showed up the flagellar attachments better than the Leishman method did, whereas the latter demonstrated the nuclear karyosome better. (Plate XVIII, figs. 12 and 14.)

V. MORPHOLOGY

This protozoon is a typical specimen of the genus *Prowazekia* (Hartmann and Chagas, 1910), formerly included in the genus *Bodo*. It possesses two flagella (an anterior and a lateral), and two nuclei (a principal nucleus and a kinetonucleus or blepharoplast). Morphologically, it bears a very close resemblance to some members of the genus *Trypanoplasma*, especially those with cytophagous habits, and appears to differ from these forms chiefly in the fact that the lateral flagellum is not joined to the body by an undulating membrane.



TEXT-FIGS. 1-3.—Types of *Prowazekia urinaria*

- 1.—'Sausage-shaped' form
- 2.—Round form
- 3.—'Carrot-shaped form

For the purpose of description in this article, the side of the body along which the lateral flagellum lies will be termed the flagellar side, while the opposite side will be called the aflagellar side.

(1) *Shape and Size*.—In cultures, three types are found (*a*) the

sausage-shaped' type; (b) the round or oval type; and (c) the 'carrot-shaped' type.

(a) The 'sausage-shaped' type (Text fig. 1, Plate XVII, figs. 5-8) is long and cylindrical with rounded ends which are usually somewhat thicker than the rest of the body. This type is often slightly curved, with its concavity towards the flagellar side. Its average length is about 18μ by 4μ , but forms varying from 10μ by 2.5μ to 25μ by 6μ may be seen.

(b) The round or oval type (Text fig. 2, Plate XVII, figs. 1-4) varies in size from small round forms 4μ in diameter to large oval forms 15μ by 10μ , with sizes varying between these limits. These forms occur most commonly in old cultures and on the surface of solid media.

(c) The 'carrot-shaped' type. These forms (Text fig. 3, Plate XVII, fig. 11) are rounded anteriorly, and taper away to a point posteriorly, the aflagellar side of the body being usually flattened. In transverse section this form is not circular like the two previous types, but is flattened. It shows great variations in size, from small forms 6μ by 3μ to large forms 25μ by 4μ . The external morphology of this form closely resembles *Bodo urinarius*, Kunstler (1883).

At first, it might be thought that these three forms represented different species, but this was disproved by the fact that the 'sausage-shaped' forms upon division may give rise to two daughter cells, one round and the other 'carrot-shaped.' (Text fig. 9.) This was, later, confirmed by isolating a single protozoon, and growing it in a hanging-drop culture after the manner recommended by Sellards (1911). In this culture all three types were found to develop from a single flagellate. In all these forms the aflagellar side is carried forward to form a rounded, beak-like projection or rostrum, which overhangs a V-shaped depression on the anterior edge—the cytostome.

(2) *Protoplasm*. When examined in fresh preparations, the protoplasm is seen to contain a large number of small, highly refractile granules, which are more numerous towards the posterior end. The rostrum, being free from granules, appears homogeneous. In most cases, but more especially in specimens taken from cultures on solid media, numerous vacuoles may be seen containing bacteria.

The body is enclosed by a thin periplast, which permits of amoeboid movements of the protoplasm, although no true pseudopodial processes are developed except in dividing forms. When the parasite is leaving a cyst or squeezing through a narrow opening between two masses of bacteria, the body may become distorted, and false pseudopodia may be formed (Text fig. 16, Plate XVIII, fig. 27), which must not be confused with true pseudopodia, because these false pseudopodia are merely the results of pressure, and are not spontaneous outgrowths. These false pseudopodia disappear quickly when the parasite becomes freed. (Text fig. 17.)

In preparations stained with Giemsa's stain the protoplasm appears of a blue colour, the rostrum staining a lighter colour than the rest of the body, while vacuoles containing bacteria in various stages of digestion may be seen, especially towards the posterior end (figs. 5-7 and 16). Sometimes a few rather small vacuoles may also be seen which do not contain any remains of digested matter (figs. 6 and 15) and some of the flagellates may show a few fine red-staining granules scattered through the protoplasm (figs. 1-3, 11 and 12). Most of the flagellates have a well-marked contractile vacuole when examined in fresh preparations, but this cannot be seen in stained specimens. It is situated very close to the base of the cytostome on its aflagellar side, and does not show any iodophile reaction. (Text figs. 1-3.)

(3) *Principal nucleus (Trophonucleus)*. In the elongated forms it is situated in the anterior half of the body, and is usually eccentrically placed, being nearer the aflagellar margin. When seen in fresh preparations the nucleus is round or oval in shape with a well-marked karyosome, which has a clear area around it, bounded by a distinct nuclear membrane. The size of the nucleus does not vary much in the trophic forms, being usually 2.5μ to 3.5μ in diameter, while the karyosome is about 1.5μ to 2μ .

The karyosome is well seen in specimens stained with Leishman's stain. It takes up a bluish colour while the extra-karyosomal chromatin shows up as fine reddish granules (figs. 12 and 14). In Giemsa preparations the nucleus is reddish purple in colour, and appears granular, and in most cases it is usually impossible to distinguish the karyosome clearly.

Using Heidenhain's iron-haematoxylin stain, the karyosome stains black with a clear area around it, and it usually takes on lighter colour with this stain than the kintonucleus does. If a drop of fresh material was fixed in osmic acid vapour and mixed with a little dilute haematoxylin stain, it was found that the karyosome took up the stain much more rapidly than the kintonucleus.

(4) *The kintonucleus* or *blepharoplast* is situated close to the base of the rostrum, on the aflagellar side of the body, usually being in contact with the periplast. In fresh preparations it may not be so easily seen as the principal nucleus. It is a relatively large body, usually pear-shaped with the apex pointing forward (fig. 15), but sometimes, especially in forms about to divide, it is larger and more distinct, and is round or oval in shape (fig. 16).

In specimens stained with any of the Romanowsky stains it appears as a homogeneous body of a deep reddish purple colour, and is always darker than the principal nucleus. The kintonucleus measures 2μ to 3.5μ in its greatest diameter. The relatively large kintonucleus of this protozoon resembles very closely the kintonucleus or blepharoplast of some Trypanoplasms.

(5) *The Flagella* are two in number, an anterior and a lateral. The anterior flagellum, which is the shorter of the two, is directed forwards and is thicker and more motile than the lateral flagellum, being always in continuous motion, coiling and uncoiling rapidly. This motion chiefly affects the anterior two-thirds of the flagellum, the basal portion being more rigid, and the coiling movements are always towards the flagellar side. The lateral flagellum, which is usually twice as long as the anterior one, is directed backwards and lies in close apposition with the body for the first part of its course, the remainder trailing freely behind the organism. It is thinner and more wavy than the other flagellum. The anterior flagellum measures from 8μ to 12μ in length, while the lateral varies from 15μ to 25μ . There is no constant relation between the size of the body and the length of the flagella, but the flagella are usually longer in the smaller forms in proportion to the size of the body (figs. 1-3 and 8.)

The third flagellum described by Hassall in some individuals was probably the long, thin, pointed end of the 'carrot-shaped' forms. (Text fig. 3.)

In well-stained Giemsa preparations the flagella stain red, and can be seen to arise from two small red-staining bodies, the basal granules ('diplosome' of Prowazek); these are often so close together as to appear as a single body. They are situated very near the apex of the kintonucleus, and in some cases appear to be actually in contact with it (fig. 6). A very fine thread joining these granules to the kintonucleus has been observed once or twice in well-stained specimens (fig. 17). This thread probably corresponds to the 'rhizoplast' observed by Hartmann and Chagas (1910) in *Pr. cruzi*, and by Whitmore (1911) in *Pr. asiatica*. The anterior flagellum runs directly forward and leaves the body at the apex of the rostrum. The lateral flagellum runs transversely outward along the anterior margin and leaves the body as a free flagellum near the junction of the anterior and lateral margins, at which point it turns directly backwards.

The origin of the flagella and the course taken by them show a very close resemblance to similar structures in *Trypanoplasma ranae* (Walker 1910), which resemblance is still further accentuated by the presence in this trypanoplasma of a slight depression at the anterior end, which Walker suggests may represent a cytostome.

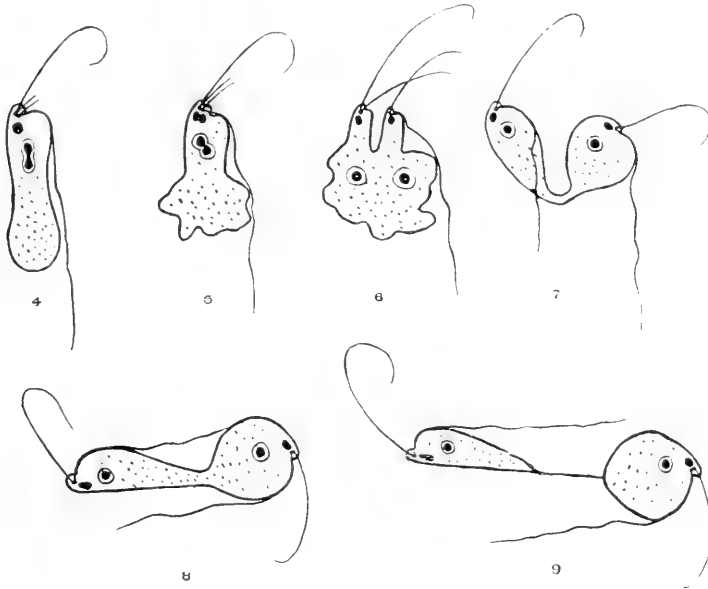
VI. MOVEMENTS

All the trophic forms are very actively motile, the elongated forms being especially so. The movement is of a jerking character and is seen to be produced by the coiling and uncoiling of the anterior flagellum or tractellum, while the lateral flagellum or gubernaculum acts as a rudder. In hanging-drop and fresh preparations the flagellate may be seen to move actively about among the masses of bacteria, and every now and then it attacks one of these masses. While the organism is 'rooting about' among the bacteria it is anchored to the mass by the lateral flagellum. If a flagellate, during its wanderings, gets into a channel between the bacteria which is too small to permit further progress, it must turn around before it can get out, because the organism is only capable of motion with the anterior end forward, thus differing from members of the genus *Trypanosoma*, which can move with either end forwards. This turning movement is rendered possible by the amoeboid character of the protoplasm.

Before division or encystment, the movements become very sluggish, and forms taken from the surface of solid media are not very motile at first, but on being transferred to a fluid medium, very soon become active again.

VII. MULTIPLICATION

By isolating and cultivating a single organism in a hanging-drop preparation by the method of Sellards (1911), it was found that at room temperature this single cell divided into two, in times varying from twelve to twenty-four hours, but that as soon as division had once taken place it occurred again every four hours, so that twenty-four hours later it was possible to count approximately one hundred and twenty-eight cells in the drop, after which time it was impossible to enumerate them.



TEXT-FIGS. 4-9.—Showing method of division

In fresh preparations, if the elongated forms be examined, the first sign of multiplication is the appearance of two new flagella, growing out from the apex of the rostrum. (Text figs. 4-5, Plate XVIII, fig. 20.) These flagella at first are short and thick, but they soon increase in length and become thinner. At the same

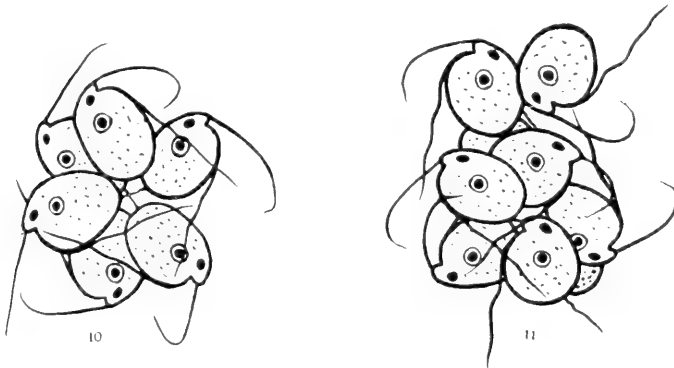
time, the movements of the cell become very sluggish and all the food vacuoles disappear. The posterior end of the body gradually accumulates a large number of small refractile granules, and becomes very amoeboid, even showing true pseudopodia of small size. (Text figs. 5-6.) This amoeboid movement slowly extends forward until the whole body is involved. A split appears at the anterior end between the two new flagella and extends gradually backwards, until we get a form like **Y**. (Text figs. 6-7.) The very amoeboid character of the cell at this stage gives rise to forms, which, if the act of division had not been carefully followed from the start might be wrongly interpreted. Thus, when division has proceeded a little further, the two limbs of the **Y** may straighten out, giving rise to a form which is apparently undergoing transverse division. (Text fig. 8.) At another time when the active movements of the two daughter cells have stretched the connecting protoplasm between them to a narrowed band, if the movements cease, the two cells are drawn together and partly fuse again, an appearance, which, if seen first at this stage might be mistaken for conjugation, especially if the conditions of examination are unfavourable to the cell and complete division does not occur before death takes place. The occurrence of well-marked pseudopodial processes at this stage gives rise to very curious forms.

The two daughter-cells, by their exertions, gradually stretch the connecting link until it becomes a very fine, highly refractile, elastic thread, often 60 or 70 μ long (Text fig. 9), this soon breaks, liberating the two cells. Immediately after division, although the movements of their flagella may be active, the movements of the daughter cells are slight, being apparently hampered by the relatively long flagella, but active motility is soon attained. The daughter cells may be equal or unequal in size, and may conform to different morphological types (Text fig. 9). In the division of a rounded form the whole body becomes amoeboid, and a clear line of demarcation appears across it.

The principal nucleus may divide before the kintonucleus, but the reverse may occur in other cases. The division of the principal nucleus appears to take place in two ways. In one case the equatorial plate is formed by the karyosomal chromatin, while in the other method it is the extra-karyosomal chromatin which

forms the equatorial plate. The kintonucleus when dividing becomes elongated, the central portion becomes narrower and narrower, and gradually the kintonucleus divides into two. The phases of nuclear division, which I have observed, seem to be similar to those figured by Alexeieff (1911a) in *Prowazekia caudata* (*Bodo caudatus*).

In stained preparations the new flagella appear to grow out from the two basal granules (fig. 20), and not to be formed by the splitting of the original flagella. In some cases where division is very active the daughter cells divide again before they are completely separated, giving rise to division rosettes composed of four to eight small individuals, with their anterior flagella pointing outwards. (Text fig. 10.) These should not be confused with the



TEXT-FIG. 10.—Division rosette

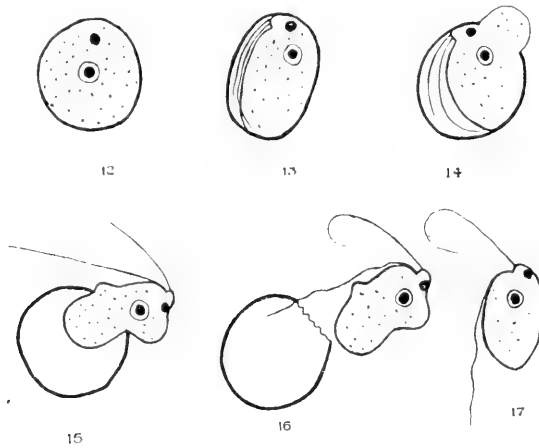
TEXT-FIG. 11.—Mass of agglomerated flagellates

agglomerated masses which are sometimes seen, but which usually contain a larger number of cells, and in which the flagella may point in any direction. (Text fig. 11.) These agglomeration masses seem to be formed by the sticking together of very young forms. These very young forms when they come in contact with other young forms tend to adhere, whereas the older forms do not. It is possible that some of these division rosettes may be formed from the large vacuolated amoeboid masses, about 18μ by 10μ with multiple flagella, which are sometimes observed.

VIII. ENCYSTMENT

In old cultures very numerous round or oval cysts 5μ to 7μ in diameter may be seen. In fresh preparations these cysts are seen to be enclosed by a very thin cyst wall. They are highly refractile bodies with numerous granules inside them, and in some cases a nucleus may be seen (Text fig. 12). Before encystment the cell becomes almost motionless, the movements of the flagella are very sluggish, then, apparently, the thin cyst wall is secreted around the cell, and the flagella are withdrawn inside this cyst.

In good preparations stained with Giemsa the cyst shows a dark blue protoplasm with a nucleus and a kinetonucleus, as well as two folded-up flagella, and the delicate cyst wall can usually be made out, staining a reddish colour (figs. 22 and 23).



TEXT-FIGS. 12-17. Flagellate emerging from cyst

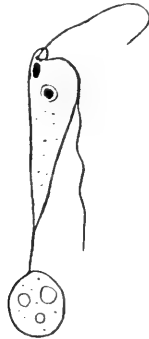
If cysts are placed in a fresh culture medium the enclosed organisms can soon be seen to emerge from them. The first indication of this is that the granules begin to move and the parasite can be seen to be slowly turning round inside the cyst with spasmodic jerks. A little later it is possible to see the two flagella working actively, and at the same time the flagellate makes violent efforts to rupture the thin cyst wall, causing it to bulge in places (Text fig. 13). Finally, the wall bursts and the anterior end of the organism with the two flagella becomes protruded

(Text figs. 14-15, and Plate XVIII, figs. 24-26). After a short quiescent period the flagellate begins to make renewed attempts to free itself, and gradually it emerges, leaving the very thin, almost invisible cyst wall behind (Text fig. 16).

Immediately after leaving the cyst the flagellate may be deformed (Text fig. 16 and Plate XVIII, fig. 27), but its normal shape is soon regained. At first, the movements are sluggish, but this is only for a very short time.

Nägler (1910) and Hartmann and Chagas (1910) describe cysts containing several nuclei in the forms observed by them, but neither in fresh nor in stained specimens have I been able to see any indication of two or more cells being contained in the same cyst.

In examining preparations containing cysts, great care must be taken not to confuse the cysts of various fungi, which may contain a number of spores, with the cysts of the flagellate. Alexieff (1911c) has pointed out that this mistake has probably occurred in



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TEXT-FIG. 18.—Separation of vacuolated mass from 'carrot-shaped' flagellate

the investigation of *Trichomonas intestinalis*. Once or twice, a rounded vacuolated mass of protoplasm, 4 to 5 μ in diameter, was noticed to separate off from the posterior end of some of the elongated forms (Text fig. 18 and Plate XVII, fig. 9), the exact significance of which was not clear.

IX. BIOLOGY

(1) *Cultivation*. This protozoon was found to multiply freely in urine, forming a scum on the surface, but dying out on the ninth day. Sub-cultures were made on ordinary salt agar, nutrient agar,

serum agar, blood agar, peptone salt solution, nutrient broth, and diluted blood serum. In all these media an abundant growth was found to take place in symbiosis with bacteria. One of the best media was found to be ordinary agar, to the condensation water of which a little blood serum had been added; on this media, motile forms and cysts can be found for at least eight weeks at room temperature (10-20° C). In cultures made on solid media the forms in the water of condensation tend to be elongated, while round and oval forms will be found living among the colonies of bacteria on the surface of the media. On the surface of fluid culture media a scum forms, and upon examination is found to consist of countless numbers of these organisms showing very active motility. This protozoon dies inside twelve hours in anaerobic cultures. Its love of oxygen is also shown by the fact that in fresh cover-glass preparations they tend to congregate around air bubbles or at the edge of the coverslip if it is not ringed with vaseline, and by the fact that in cultures of liquid media made in deep tubes, the protozoon is only present on the surface and not in the depth of the culture.

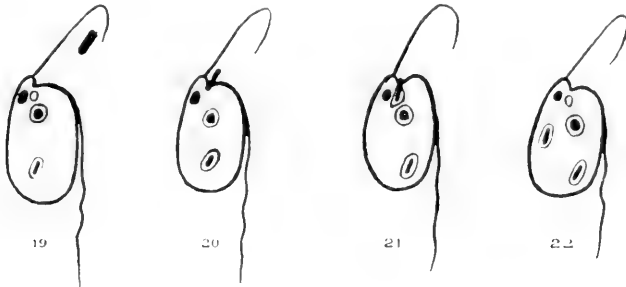
The best temperature for growth seems to be about 20° C, but growth occurs at 30° C. Higher temperatures rapidly kill the cultures, a temperature of 37° C killing them inside one or two hours.

All attempts at growing this flagellate in the absence of bacteria were unsuccessful.

In none of the articles on the cultivation of *Prowazekia* from the faeces have I been able to find any reference to the temperature at which the cultures were grown, nor whether the organism found was aerobic or anaerobic. These points seem to me to be of considerable importance in determining whether these organisms were actually living in the intestine or were merely accidental contaminations after the faeces were passed.

(2) *Capture and Ingestion of Food.* Although the very thin free end of the anterior flagellum, which also acts as a feeler, is capable of grasping bacteria, it is usually by the coiling movements of this flagellum that they are propelled towards the cytostome (Text fig. 19). The food enters the cytostome and, by the aid of well-marked infolding movements of the lips of the cytostome,

is received into a vacuole which forms at the bottom of the cytostome (Text figs. 20-21 and Plate XVII, fig. 1). The vacuole quickly travels towards the posterior end of the body, and in its passage takes a definite course, passing between the principal nucleus and the kinetonucleus along the aflagellar side of the body (Text fig. 22). The cytostome is capable of being greatly distended, as was noticed when flagellates were seen trying to swallow red blood cells, and also when trying to swallow large masses of bacteria.



TEXT-FIGS. 19-22.—Stages in ingestion of bacillus as food

All the bacteria which are propelled towards the cytostome by the anterior flagellum are not ingested, but the organism seems to have a special liking for certain bacteria. This was well illustrated in one case where a small oval flagellate attempted to swallow a strepto-bacillus which was longer than itself, but finding, after most of the bacillus was swallowed, that it was impossible to swallow all of it, the organism disgorged the bacillus again. This same bacillus was immediately attacked by another organism with a similar result, and the same performance was gone through by at least twelve separate protozoa, all of which made unsuccessful attempts to swallow it. Sometimes two flagellates started at opposite ends of this bacillus at the same time and tried to swallow it. This preference for special bacteria is also shown by the way these flagellates in fresh preparations tend to accumulate around certain clumps of bacteria, while other clumps are untouched.

In stained preparations the ingested bacteria are seen to be contained in vacuoles, and to be in various stages of digestion.

(3) *Excretion.* When living specimens are examined, the food vacuoles are seen to accumulate at the posterior end of the body.

After a time, these vacuoles approach the surface of this part and burst, discharging any undigested contents. No distinct cytophyge could be made out.

The contractile vacuole is best seen in the larger flagellates, and measures about 1μ to 3μ in diameter when in diastole. It is situated very close to the base of the cytostome, and is intimately connected with it, seeming to be joined to it by a short canal. The formation of this vacuole is usually rapid, and contraction takes place every fifteen to thirty seconds at a temperature of about 20°C , but at lower temperatures and under adverse conditions it may only occur every two to three minutes.

It was thought at first, from its intimate connection with the cytostome, and because it disappeared when a food vacuole was formed, that the contractile vacuole was really the distended end of the cytostome, but later it was found that, when material containing living flagellates was mixed with some Indian ink, none of the particles appear in the vacuole. From these observations it seems improbable that the contractile vacuole is formed by the dilatation of the fundus of the cytostome.

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EXPLANATION OF PLATES XVII, XVIII.

The figures were drawn with an Abbé camera lucida, using ocular No. 4 and a Leitz 1/12 inch oil-immersion objective. All the figures were stained with Giemsa, except fig. 12 and fig. 14, which were stained with Leishman. Magnification 1,650.

PLATE XVII.

Fig. 1. Round form with bacillus in process of being ingested.

Figs. 2-3. Round forms.

Fig. 4. Oval form.

Figs. 5-8. 'Sausage-shaped' forms, with numerous ingested bacteria.

Figs. 9-10. Intermediate forms between the 'sausage-shaped' forms and the 'carrot-shaped' forms.

Fig. 11. 'Carrot-shaped' form.

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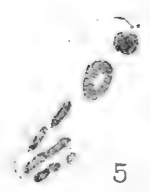
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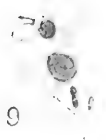
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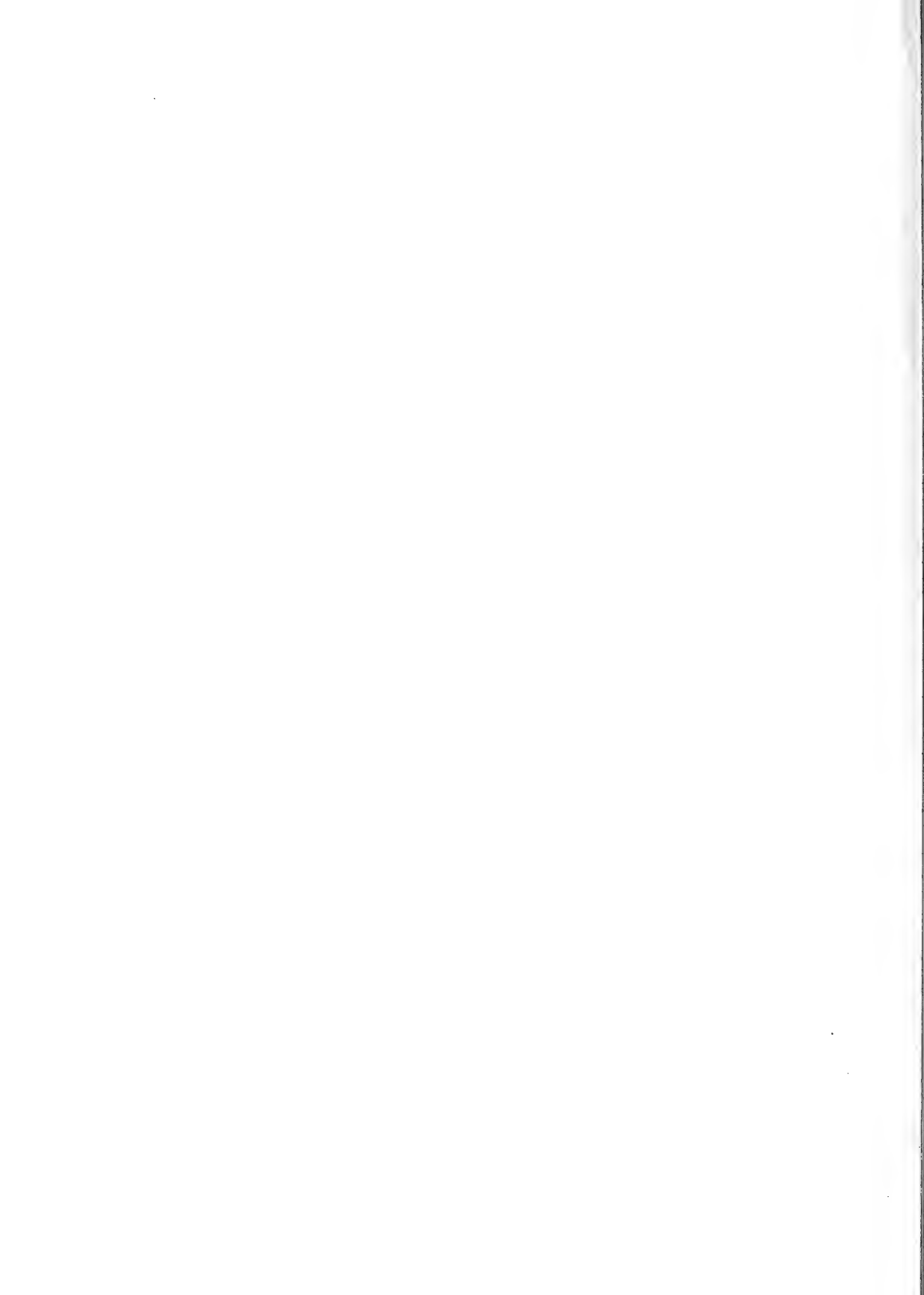


PLATE XVIII.

- Figs. 12-14. Show the nuclear karyosome and extra-karyosomal chromatin.
- Fig. 15. Round form with a large pear-shaped kintonucleus.
- Fig. 16. 'Sausage-shaped' form with a very large round kintonucleus.
- Fig. 17. Shows the 'rhizoplast' connecting the basal granules with the kintonucleus.
- Figs. 18-19. Forms with dividing nuclei, each showing two karyosomes.
- Fig. 20. In this form the principal nucleus has divided, and two new flagella have grown out from the basal granules.
- Fig. 21. Amoeboid dividing form, with two principal nuclei and two kintonuclei.
- Figs. 22-23. Cysts showing folded-up flagella.
- Figs. 24-26. Flagellate emerging from cyst.
- Fig. 27. Flagellate immediately after leaving a cyst, showing a 'false pseudopodium.'

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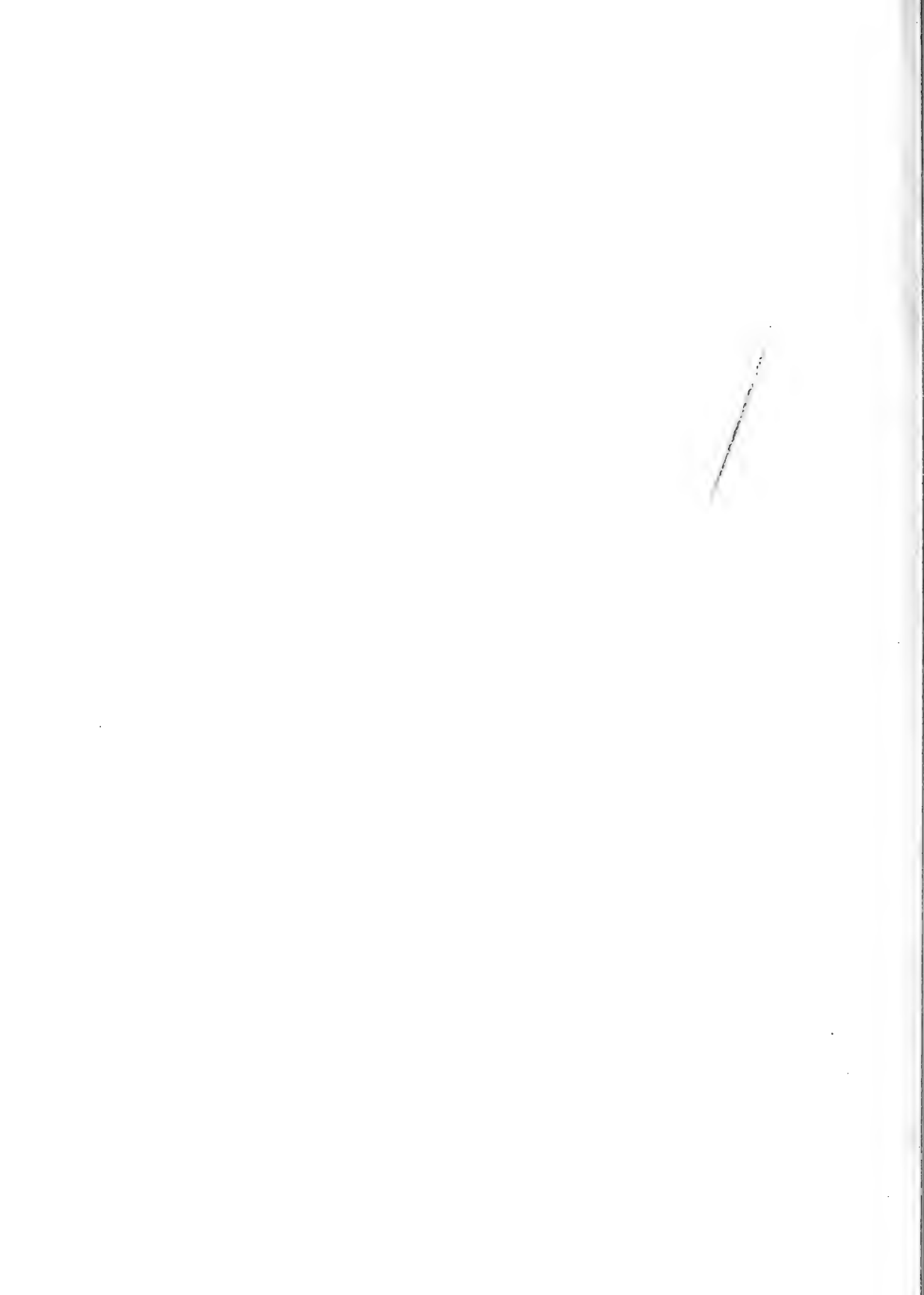
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A FURTHER REPORT ON THE TRANSMISSION OF HUMAN TRYPANOSOMES BY *GLOSSINA MORSITANS*, WESTW.

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(Second interim report of the Luangwa Sleeping Sickness Commission, British South Africa Company)

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I. INTRODUCTION

In an earlier report,* details were given of a number of experiments on the transmission of *Trypanosoma rhodesiense* (Stephens and Fantham) by laboratory-bred and 'wild' *Glossina morsitans* (Westw.), on the transmission of an identical organism by naturally-infected tsetse flies of that species, and on the occurrence, in game, of a trypanosome indistinguishable from the human parasite. As a result of these experiments, the following conclusions were drawn:—

1. The human trypanosome, in the Luangwa Valley, is transmitted by *Glossina morsitans* (Westw.).
2. *Glossina morsitans*, in nature, has been found to transmit the human trypanosome.

* Kinghorn and Yorke, *Annals Trop. Med. and Parasitol.*, VI, pp. 1-23, March, 1912.

3. Certain species of game have been found to be infected with the human trypanosome.

Since writing that paper, additional work has been done along similar lines, and the results obtained have served to strengthen the validity of the conclusions at which we had previously arrived. These experiments are given below, and bring the investigations on the human trypanosome up to the date on which the Commission left the Luangwa Valley for the Congo-Zambesi watershed.

As the methods pursued have been precisely similar to those we have already described, it will be unnecessary to make further reference to them.

It may be noted that the earlier transmission experiments were made during the dry and during the commencement of the wet seasons, while those now given were carried out during the rains proper. The two series serve, therefore, to demonstrate that *Glossina morsitans* is capable of transmitting the human trypanosome during the whole year. A brief summary of the chief meteorological observations is given in Table 1.

TABLE 1.—Meteorological observations at Nawalia, N. Rhodesia, $12^{\circ} 25' S.$, $32^{\circ} 2' E.$, altitude 2,100 feet (approximate).

1911-1912.	External shade temperature mean	Laboratory temperature mean	Relative humidity %	Rainfall inches	Number days on which rain fell
June.....	67.2	—	48.6	0	—
July	68.7	67.4	45.7	0	—
August	73.3	71.2	35.8	0	—
September ..	77.5	71.5	*31.5	0	—
October	86.1	84.5	*31.8	0.26	2
November ...	87.1	84.6	41.1	1.61	8
December ...	82.3	79.6	69.1	8.54	20
January	80.6	78.4	77.7	14.97	16
February ...	79.2	77.1	73.8	5.55	16
March	79.0	72.0	62.5	5.10	6
April (to 9th)	—	—	—	0.01	1
				36.04	69

* Approximate.

II. TRANSMISSION OF THE TRYPANOSOME

A. BY LABORATORY-BRED *Glossina morsitans*

Experiment 1. Commenced December 29th, 1911, with twenty laboratory-bred flies.

These flies were infected directly from a patient in whose peripheral blood three trypanosomes per field (Zeiss Oc. 4, Obj. DD) were seen. They were afterwards fed daily for sixty-five days on a series of healthy monkeys, none of which became infected. From the sixty-seventh to seventieth day of the experiment, the seven flies then alive were fed on a guinea-pig heavily infected with the human trypanosome, and were then fed for a further period of thirty days on a clean monkey. This did not become infected.

Experiment 2. Commenced January 12th, 1912, with twenty-three laboratory-bred flies.

These were fed for four days on a patient showing, on an average, one trypanosome to three fields in the peripheral blood, and afterwards on healthy monkeys, as indicated in Table 2.

TABLE 2.—Showing transmission of human trypanosome by laboratory-bred *Glossina morsitans*.

Day	Animal	Number flies fed	Result	Remarks
0—3rd	Patient	23	—	
4th	—	—	—	Flies starved
5th—8th	Monkey No. 237	22	Negative	
9th—12th	„ „ 238	22	„	
13th—16th	„ „ 240	22	„	
17th—20th	„ „ 254	20	Infection	
21st—23rd	„ „ 237	18	—	Died on 24th day
24th	„ „ 240	17	Infection	
25th—29th	„ „ 260	9	Negative	} Flies divided into two groups
	„ „ 261	9	Infection	
30th—60th	„ „ 272	16-0	Negative	Infected fly did not feed

On February 20th, the twenty-ninth day of the experiment, the fly numbered D 18 died, and on dissection was found to show a massive intestinal infection of trypanosomes. Unfortunately the fly had been dead for some hours before it was examined, and it was found impossible to dissect out the salivary glands. The whole abdominal contents, therefore (gut and glands), were crushed up in normal saline solution and inoculated into a healthy monkey, which became infected five days later. The disease ran a typical course.

None of the other flies—dissected as they died—were found to harbour trypanosomes in the proboscis, gut, or salivary glands.

In this instance the time which elapsed from the date of the *first* infective meal until the date on which the fly became capable of transmitting the trypanosome (allowing five days for the incubation period in the monkey) was nineteen days.

B. BY 'WILD' *Glossina morsitans*

Experiment 3. Commenced January 12th, 1912, with forty-two freshly-caught flies.

After being fed for one day on a monkey infected with the human trypanosome, and showing numerous parasites in the peripheral blood, the flies were fed on a clean monkey for nine days. They were then starved for one day, and subsequently allowed to feed on clean monkeys and rats from the eleventh to the thirty-third day. None of these animals became infected. The flies were dissected as they died, and while trypanosomes were found in the gut and proboscis of several, in no instance was an infection of the salivary glands observed.

Experiment 4. Commenced January 12th, 1912, with forty-two freshly-caught flies.

The details of this experiment are exactly similar to those of Experiment 3, with the exception that from the first to the ninth day the flies were fed on a native fowl instead of on a monkey. They were starved on the tenth day, as before, and afterwards fed on clean monkeys and rats from the eleventh to the thirty-eighth day. None of these animals became infected. Trypanosomes were found in the proboscis and gut of several of the flies, when dissected, but in no case were the salivary glands implicated.

Experiment 5. Commenced February 14th, 1912, with one hundred and four freshly-caught flies.

On the 13th of February, the flies were fed on a healthy monkey which did not become infected, thus excluding the possibility that they were already infected with the trypanosome. On the four succeeding days they were fed on a guinea-pig infected with the human trypanosome, and showing numerous parasites in the peripheral blood, and afterwards on clean monkeys, as indicated in Table 3.

TABLE 3.—Showing transmission of the human trypanosome by freshly-caught *Glossina morsitans*.

Day	Animal	Number flies fed	Result	Remarks
4th				Flies starved
5th—10th	Monkey No. 269	98	Negative	
11th				Flies starved
12th	" " 269	64	"	
13th—27th	" " 280	41	"	} Died on 28th day Flies divided into two groups, A and B
13th—29th	" " 281	47	Infection	
28th—29th	" " 286	33	Negative	Group A, only, fed
30th	" " 269	17	Infection	Group B, only, fed
30th—38th	" " 300	10	Negative	} Flies of group A divided into 3 sub-groups, A1, A2, and A3
30th—40th	" " 301	10	"	
30th—40th	" " 302	12	"	
31st—33rd	" " 303	12	"	} Flies in group B divided into 3 sub-groups, B1, B2, and B3
31st—37th	" " 304	11	Infection	
31st—34th	" " 305	12		Monkey escaped on 35th day
34th—39th	" " 310	11	Negative	Sub-group B1 fed
35th—52nd	" " 315	12	"	Sub-group B3 fed
39th—52nd	" " 300	15	"	Sub-groups A1 and B2 fed
41st—52nd	" " 301	28	"	Sub-groups A2, A3, and B1 fed

The insects were dissected as they died, but only in one, the infective fly, was an infection of the salivary glands observed, though in a considerable number an infection of the proboscis and gut was found.

The duration of the developmental cycle of the trypanosomes in the fly would appear to be twenty-five days in this experiment. The flies were fed for the *first* time on the infected guinea-pig on February 14th, and the first monkey became infected on March 15th, thirty days later. The average incubation period of the disease in monkeys is five days, so that the cycle took twenty-five days to complete.

It may be pointed out, however, that all our estimations of the latent periods of the trypanosomes in the flies represent the probable durations only. Although the average incubation period in monkeys is five days, this has been found to vary from three to eight days, and it is possible, therefore, that the cycle may have been slightly shorter, or longer, in any one instance.

Moreover, a further source of error is introduced in those experiments in which the flies were fed on an infected animal for more than a single day. It has yet to be determined whether only a definite percentage of flies is inherently capable of transmitting the disease, or whether *any* fly will do so, provided that it has an opportunity of feeding on an infected animal at some particular time during its existence. If the latter alternative be correct, the peculiar factors governing their infectability have still to be ascertained. Assuming the first view to be correct, then the latent period of the trypanosomes in the flies must date from the *first* occasion on which the insects were fed on the infected animal, while, if the second be correct, the latent period may date from *any* of the meals on the infected animal.

In our earlier paper, the latent periods of the parasites in the flies were given as eleven, thirteen, and fifteen days, while in the present series they are nineteen and twenty-five days.

A synopsis of all the transmission experiments reveals some interesting features.

As will be seen, all (three in number) the experiments made during the dry, and commencement of the rainy seasons, were successful, while only two of the five carried out during the rains proper were

positive. Further, in the rainy season, only 3 of 231 flies proved to be transmitting the trypanosome, a percentage of 1·29, as against at least 4, and probably 8, in the dry season. The larger figure depends on the number of salivary gland infections observed.

Although some hundreds of *Glossina morsitans* have been dissected, a salivary infection has been found only in those flies which proved to be transmitting the human trypanosome.

Superficially, therefore, the meteorological conditions would appear to have a considerable influence on the development of the human trypanosome in *Glossina morsitans*, but while we consider these findings of sufficient importance to be emphasised, we cannot definitely state that such is the case, as our experiments are too few in number, and have been carried out during a single dry and wet season only.

TABLE 4.—Synopsis of transmission experiments with laboratory-bred and 'wild' *Glossina morsitans*.

Experiment	Date on which started	Season	Number flies used	Variety of flies used	Result
1	20/8/11	Dry	26	Laboratory-bred	Infection
2	14/11/11	Comm't rains	16	"	"
3	14/11/11	"	57	'Wild'	"
4	29/12/11	Rainy	20	Laboratory-bred	Negative
5	12/1/12	"	42	'Wild'	"
6	12/1/12	"	42	"	"
7	12/1/12	"	23	Laboratory-bred	Infection
8	12/2/12	"	104	'Wild'	"

Amongst the bred flies only, 3 of 85 transmitted the parasite, a percentage of 3·52, as compared with 4·76, the figure given in our former report.

In none of the transmission experiments have we observed an instance of 'late infection,' although the majority of them were continued for periods varying from forty to seventy days. Both sexes have been found to be capable of transmitting the trypanosome.

As mentioned above, an infection of the salivary glands was found only in those flies which had transmitted the parasite, and the limited data at our disposal would indicate that the development of *Trypanosoma rhodesiense* in *Glossina morsitans* is strictly comparable to that of *Trypanosoma gambiense* in *Glossina palpalis*, that is, the trypanosomes, on being ingested by the flies, very quickly lose their virulence and do not regain it for a variable period, after which they are found both in the gut and salivary glands. In one instance only, have parasites been seen in the proboscis of a bred *Glossina morsitans*, and this in a fly which was not transmitting the organism. They were few in number and were not collected in the rosettes usually found in the proboscis of tsetse flies infected, in nature, with other varieties of trypanosomes. The evidence which we possess would indicate that the infection of the proboscis of this fly was fortuitous and not particularly related to the regular transmission of the human trypanosome.

III. TRANSMISSION OF THE TRYPANOSOME, IN NATURE, BY *GLOSSINA MORSITANS*

From day to day varying numbers of 'wild' tsetse flies were fed on clean monkeys and in certain cases (5), the animals became infected with a trypanosome indistinguishable from the human one. The identity of the parasites isolated in this manner was checked by a careful study of the morphology and of the pathogenicity.

TABLE 5.—Showing result of feeding naturally-infected *Glossina morsitans* on healthy monkeys.

Date	Animal	Number flies fed	Result
October 30—31, 1911	Monkey No. 96	82	Infection, human trypanosome
January 7—12, 1912	" " 210	269	" "
" 16—18, 1912	" " 217	200	" "
March 20, 1912	" " 316	101	" "
" 28, 1912	" " 333	74	" "

In all, 3,202 freshly-caught *Glossina morsitans* have been fed on healthy monkeys, and the human trypanosome has been isolated in 5 out of 28 experiments. Assuming that only one fly was transmitting the parasite in each instance, the ratio of flies infected, in nature, is 1 to 640, or 0.15 per cent., as compared with 3.5 per cent. amongst the bred flies which were fed on infected animals.

IV. OCCURRENCE OF THE TRYPANOSOME IN GAME

A few additional head of game have been examined, and the results are shown in Table 6.

TABLE 6.—Result of the examination of game for trypanosomes.

Animal	Number examined	No. in which trypanosomes were found in buck's blood	Number inoculations made	No. positive inoculations in which parasites were seen in buck's blood	No. positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculation
Zebra	2	0	1	0	0	0
Roan	3	0	1	0	0	0
Hartebeest	4	0	0	0	0	0
Waterbuck	2	1	0	0	0	1
Mpala	11	0	2	0	0	0
Bushbuck	2	0	2	0	1	1
Bushpig	2	0	0	0	0	0
Warthog	3	0	0	0	0	0
Totals, 1st report ...	29	1	6	0	1	2
	98	25	50	8	6	31
	127	26	56	8	7	33

The percentage of the local game harbouring trypanosomes may be estimated most correctly by considering only the number from which inoculations were made, namely 56. This number

includes only those subinoculated animals which lived for a sufficiently long period to determine the infectability of the blood inoculated. An analysis of the inoculations reveals the following details:—

Total number of inoculations	56
Number of successful inoculations in which parasites were found in buck's peripheral blood	8
Number of successful inoculations in which <i>no</i> parasites were found in buck's peripheral blood	7
Number of unsuccessful inoculations in which parasites were found in buck's peripheral blood	5
Total number of buck found infected by direct examination and by inoculation	20

We have already pointed out that in this vicinity *Trypanosoma vivax* and *Trypanosoma nanum* are found, to both of which monkeys and rats are insusceptible. As these were the animals used for our game inoculations, it is, therefore, impossible to give an absolutely correct estimate of the percentage of game infected, but from the data given above, it is evident that the minimum is 35·7, and it is highly probable that the actual percentage is much greater. We base this opinion chiefly on the fact that infections with *Trypanosoma vivax* and *Trypanosoma nanum* appear to be of frequent occurrence in various species of game.

Each of four local goats which were examined, was found to be infected with one or other of these trypanosomes, and as goats more closely resemble game than any other variety of domestic stock in their reaction to infection, it appears justifiable to take the course of infection in them as an indication of the course pursued by similar infections in game. These goats have been under continuous observation for long periods, and, as a rule, trypanosomes were found in the peripheral blood at rare intervals only, in some cases as much as two months apart. It will be seen, therefore, that a casual examination might easily fail to reveal the presence of trypanosomes, and that inoculations into

monkeys and rats would meet with no greater success. This is probably what occurs in connection with game. If sheep and goats had been available for inoculation the correct percentage of infection in game could be calculated, but this, unfortunately, has been an impossibility at Nawalia.

In addition to the seven buck and one warthog mentioned in our former report, one bushbuck has been found to harbour the human trypanosome. This parasite, therefore, has been isolated from 16 per cent. of the local game, the species implicated being waterbuck (4), hartebeest (1), mpala (2), bushbuck (1), and warthog (1).

V. COMPARISON OF THE MORPHOLOGY AND PATHOGENICITY OF THE 'HUMAN,' 'GAME,' AND 'FLY' STRAINS OF *TRYPANOSOMA RHODESIENSE*

We concluded that one of the trypanosomes isolated from game, and from naturally-infected tsetse flies was identical with the 'human strain' of *Trypanosoma rhodesiense*, as it exhibited precisely the same morphology and pathogenicity. Additional observations have strengthened this conclusion.

(1) *Morphology.*

In fresh preparations, all three strains show the same mixture of short, slowly-moving, and long, active forms, the relative numbers of which vary in the peripheral blood of any animal from day to day.

In stained preparations, it is sufficient to say that it is impossible to distinguish any one of the three strains from the others. Short forms in which the macronucleus lies actually posterior to the blepharoplast have been observed in each of the three strains.

The measurements of the three strains also show an extremely close agreement. Eleven hundred individuals of each have been measured, and the results are given in Tables 7, 8, and 9. The total number of parasites drawn from each variety of laboratory animal is the same in the case of each strain, and only twenty-five have been measured from any one preparation, as it has been found that the average length varies within wide limits, from day to day, in any given animal.

TABLE 7.—Giving details of measurement of 1,100 individuals of the 'human' strain.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Monkey	5 ... 6th	25	21·03	27·75	15·5
"	5 ... 11th	25	19·5	26·19	13·27
"	6 ... 8th	25	19·41	28	13·5
"	6 ... 15th	25	26·3	31·5	19·67
"	20 ... 9th	25	22·3	30·3	17·1
"	20 ... 13th	25	19·97	26·25	13·25
"	25 ... 9th	25	21·28	28·25	18
"	25 ... 13th	25	19·57	29·75	15·25
"	33 ... 8th	25	24·2	28·75	16·75
"	33 ... 9th	25	20·59	29·75	15·25
"	87 ... 11th	25	22·81	31·5	18·25
"	87 ... 21st	25	22·41	29·25	18·5
"	87 ... 22nd	25	19·95	27	17
Dog	244 ... 6th	25	22·26	29·25	18·75
"	244 ... 8th	25	20·16	24·5	17·5
"	244 ... 13th	25	21·72	31·25	18·25
"	244 ... 14th	25	19·7	22·5	17·25
Rabbit	13 ... 4th	25	23·5	30·5	14·5
"	13 ... 22nd	25	18·11	24·75	15·5
"	13 ... 24th	25	19·52	39·25	14·5
"	86 ... 13th	25	21·91	29	16·75
Guinea-pig	14 ... 14th	25	21·09	30·25	17·5
"	14 ... 20th	25	22·03	31·75	16
"	14 ... 22nd	25	22·21	33·25	14·5
"	139 ... 25th	25	20·66	27·25	15·75
"	139 ... 36th	25	18·2	26·5	14
"	139 ... 52nd	25	18·4	28	13·75
Rat	15 ... 22nd	25	20·03	25·5	15·75
"	15 ... 26th	25	21·08	28·25	16·75
"	16 ... 15th	25	22·98	33·25	15·75
"	183 ... 12th	25	22·44	30·75	18·75
"	184 ... 12th	25	22·12	31·25	18
"	184 ... 14th	25	19·64	31	14·25
"	184 ... 20th	25	22·17	27·5	17
"	184 ... 28th	25	20·59	30	16·75
"	208 ... 10th	25	19·32	23·25	16·75
"	208 ... 20th	25	19·69	24·5	17
"	212 ... 6th	25	23·33	31	18·75
"	212 ... 7th	25	20·88	32·5	15·5
"	212 ... 16th	25	18·66	22·5	13·75
Mouse	27 ... 12th	25	20·95	26·75	18·5
"	28 ... 6th	25	19·94	23	17·25
"	91 ... 6th	25	23·94	27·25	18·5
"	91 ... 10th	25	28·65	33	21·5
		1,100	21·25	39·25	13·25

TABLE 8.—Giving details of measurement of 1,100 individuals of 'game' strain.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Monkey	71 ...	7th	25	24.79	32.9	17
"	71 ...	9th	25	19.84	23.8	15.3
"	99 ...	38th	25	26.36	34.25	19
"	120 ...	8th	25	20.02	23.5	18
"	120 ...	11th	25	21.9	29.25	17.25
"	120 ...	13th	25	17.4	20	15
"	130 ...	8th	25	25.97	35.5	19
"	130 ...	11th	25	22.05	30.5	16.25
"	199 ...	5th	25	22.47	25.75	15.75
"	199 ...	7th	25	23.6	32.25	16.75
"	201 ...	7th	25	23.4	31	17.75
"	201 ...	8th	25	21.62	25.5	17.5
"	201 ...	9th	25	19.58	21.75	17.25
Dog	Native ...	?	25	19.1	26	15.5
"	262 ...	5th	25	21.69	25.75	18.5
"	262 ...	7th	25	19.13	23.5	13.5
"	262 ...	11th	25	18.34	22.5	16.25
Rabbit	79 ...	11th	25	20.02	29	15.2
"	249 ...	9th	25	16.18	19.5	13.75
"	249 ...	13th	25	22.29	32	15.25
"	249 ...	13th	25	20.91	28.5	15.75
Guinea-pig	251 ...	10th	25	20.87	33.25	15.25
"	251 ...	11th	25	22.87	34.5	15.75
"	251 ...	13th	25	23.11	33.75	15
"	251 ...	15th	25	23.5	32.25	14.75
"	251 ...	17th	25	24.09	34.25	13.75
"	251 ...	21st	25	21.67	29.75	14.5
Rat	81 ...	14th	25	21.05	31.5	16
"	128 ...	20th	25	20.25	21.75	17.5
"	128 ...	22nd	25	20.3	23.75	16
"	129 ...	?	25	20.9	28	16.25
"	157 ...	21st	25	25.65	30.5	14.5
"	157 ...	42nd	25	19.27	21.5	16.75
"	157 ...	49th	25	22.8	32.5	16.25
"	195 ...	26th	25	21.8	35	16.5
"	195 ...	36th	25	19	24.5	17
"	213 ...	17th	25	17.38	19	14.5
"	213 ...	26th	25	22.31	34.25	17.5
"	221 ...	7th	25	18.91	23	16.5
"	221 ...	14th	25	21.91	35.5	11.75
Mouse	176 ...	9th	25	20.13	26.5	17.5
"	176 ...	14th	25	20.99	26.5	16.75
"	178 ...	6th	25	22.89	29.5	17.25
"	178 ...	7th	25	21.6	27	16.5
			1,100	21.38	35.5	11.75

TABLE 9.—Giving details of measurement of 1,100 individuals of 'fly' strain.

Animal		Day of disease	Number measured	Length in microns			
				Average	Maximum	Minimum	
Monkey	96	...	7th	25	25·7	32	16
"	96	...	8th	25	24·8	33·5	16·5
"	96	...	9th	25	25·6	36·25	16
"	96	...	10th	25	23·3	30·75	15·75
"	96	...	11th	25	22·6	31	15·25
"	96	...	14th	25	20·3	23·5	16·5
"	114	...	27th	25	22	28	15·25
"	114	...	32nd	25	20·9	25·25	18
"	114	...	41st	25	20·8	30·75	15·25
"	210	...	8th	25	24·66	30·5	17·5
"	210	...	10th	25	20·29	23·25	18·25
"	217	...	9th	25	26·03	30·75	22
"	316	...	9th	25	24·69	32·25	17·5
Dog	235	...	5th	25	26·7	33	19
"	235	...	7th	25	21·4	28	19
"	235	...	9th	25	20	28	18·25
"	235	...	13th	25	20	21·25	18·5
Rabbit	245	...	7th	25	23·5	29·5	16·5
"	245	...	8th	25	20	28	14·5
"	245	...	9th	25	18·75	27·75	16·25
"	245	...	13th	25	22·84	30	17
Guinea-pig	246	...	13th	25	19·87	23·5	16·7
"	246	...	15th	25	20·88	26	16·25
"	246	...	18th	25	17·63	21·5	13
"	246	...	19th	25	19	27·25	16·5
"	246	...	20th	25	18·95	25·25	14·25
"	246	...	21st	25	21	27·5	15·25
Rat	103	...	4th	25	24·1	30	17
"	103	...	5th	25	20·3	30	16·5
"	103	...	8th	25	18·8	30·75	14·5
"	218	...	6th	25	19·47	24·75	16·5
"	218	...	9th	25	19·3	29	14·5
"	218	...	14th	25	20·1	22·5	17
"	218	...	16th	25	19·41	26·75	16
"	218	...	18th	25	22	30·5	18
"	229	...	6th	25	24·55	29·5	19
"	229	...	8th	25	21·09	29·25	17
"	229	...	9th	25	19·5	21·75	17·5
"	229	...	13th	25	22·3	29·5	18·5
"	229	...	15th	25	20·31	22·75	17
Mouse	247	...	4th	25	23·1	29·5	19·5
"	247	...	6th	25	23·66	29·5	19·25
"	247	...	9th	25	22·6	34	18·75
"	247	...	14th	25	20·91	25·25	17·25
				1,100	21·67	36·25	13

TABLE 10.—Comparison of the measurements of the 'human,' 'game,' and 'fly' strains.

Strain	Length in microns		
	Average	Maximum	Minimum
'Human'	21.25	39	13.25
'Game'	21.38	35.5	11.75
'Fly'	21.67	36.25	13

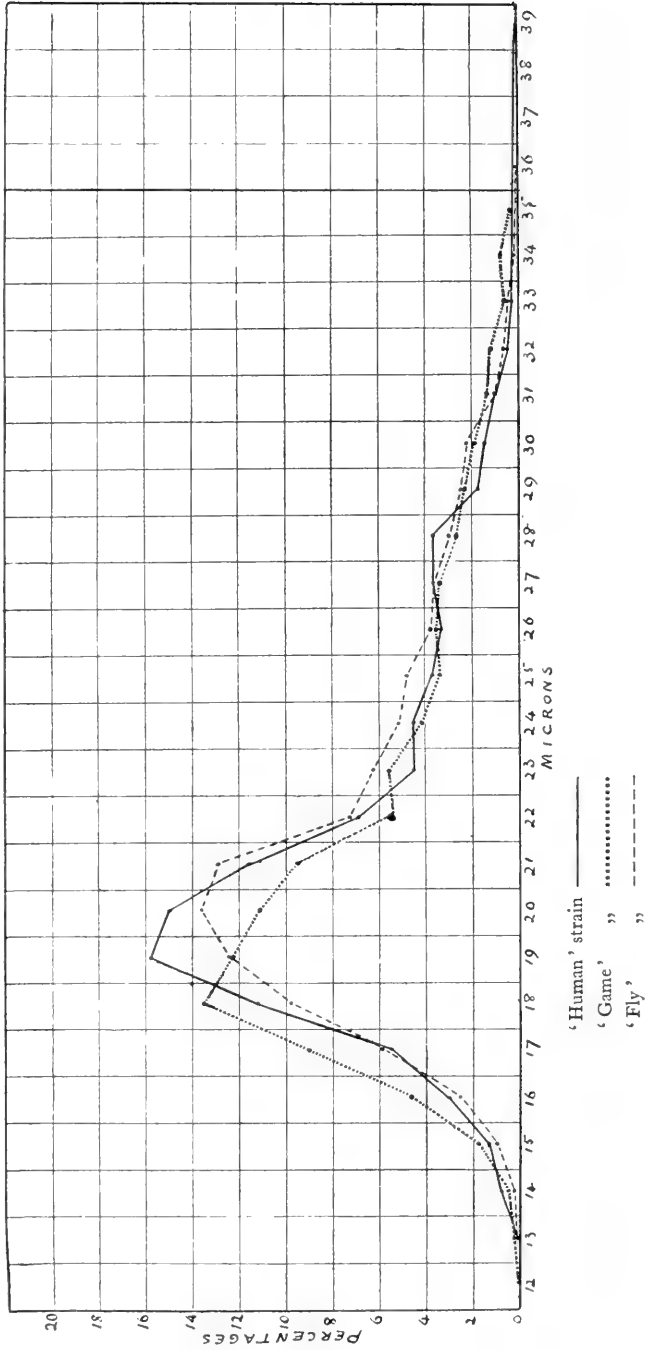
The similarity in the measurements is, perhaps, best appreciated by a glance at the curves obtained by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total numbers measured.

A comparison of the percentages of 'short and stumpy,' 'intermediate' and 'long' forms is also of interest.

TABLE 11.—Comparison of percentages of 'short and stumpy,' 'intermediate,' and 'long' forms of the 'human,' 'game,' and 'fly' strains.

Strain	Short and stumpy forms 11-21 μ	Intermediate forms 22-24 μ	Long forms 25-39 μ
'Human'	64.78	15.98	19.14
'Game'	62.87	15.34	21.56
'Fly'	58.68	18.81	22.41

CHART I. Comparison of curves of the 'human,' 'game,' and 'fly' strains obtained by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total numbers measured (1,100 of each strain).



(2) *Pathogenicity.*

The pathogenicity of the three strains is synopsised in Table 12.

TABLE 12.—Comparison of pathogenicity of the 'human,' 'game,' and 'fly' strains.

Animal	'Human' strain			'Game' strain			'Fly' strain		
	No.	Incubation days	Duration days	No.	Incubation days	Duration days	No.	Incubation days	Duration days
Monkey ...	12	2-7	4-42	14	4-11	7-40	6	4-6	9-54
Dog ...	1	5	26	1	5	25	1	5	26
Rabbit ...	3	4	16-61	1	4	30	1	6	19
Guinea-pig	2	12-19	65-81	2	10-11	53 one alive after 66 days	1	11	alive after 72 days
Rat ...	16	2-8	15-82	10	3-6	11-43	4	3-5	24-48
Mouse ...	4	4	15-63	2	4-5	48-51	1	4	alive after 72 days

As a result of these observations, we are forced to conclude that the 'game' and 'fly' strains are identical with the human trypanosome.

VI. SUMMARY

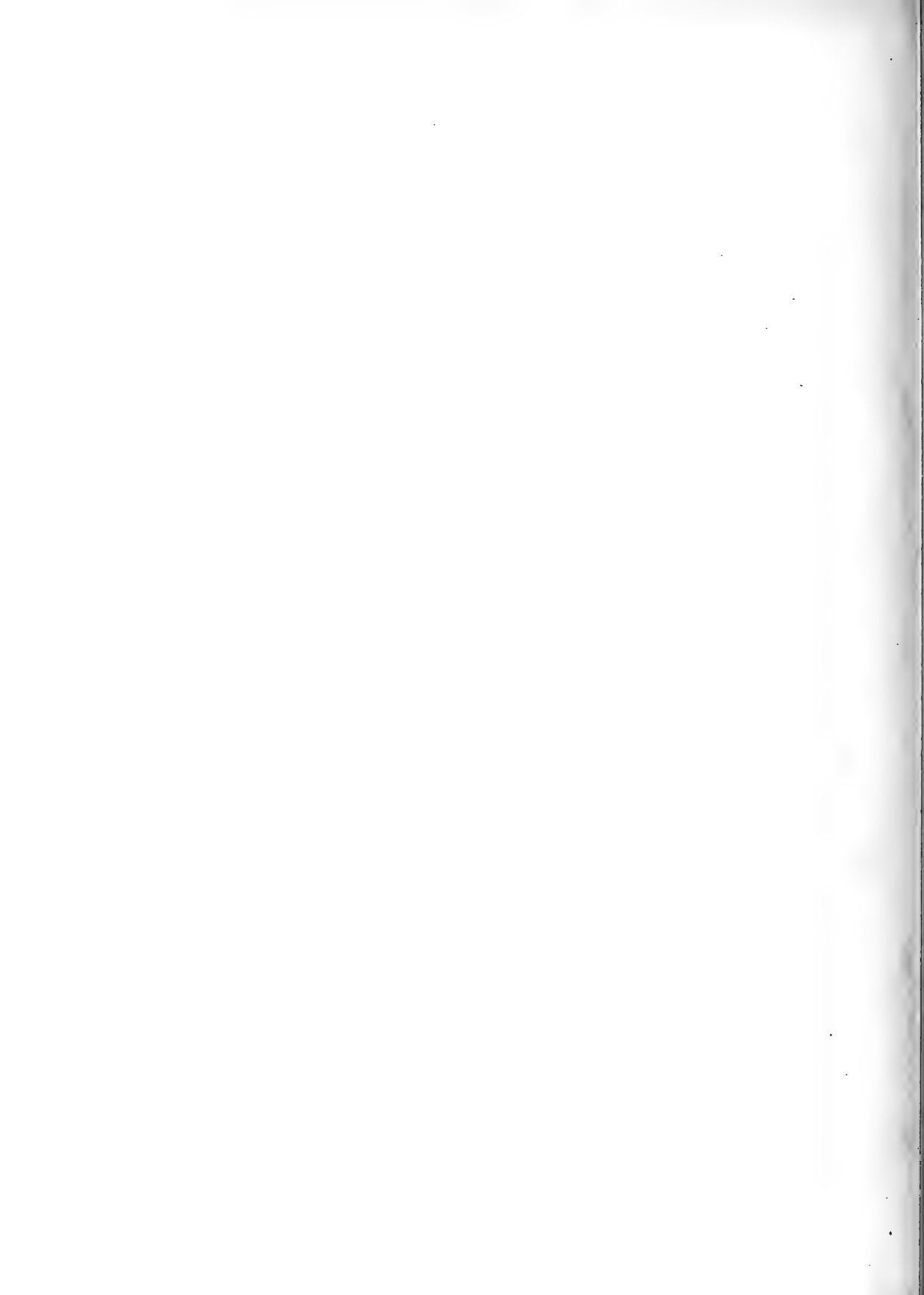
(1) *Trypanosoma rhodesiense* is transmitted by *Glossina morsitans*.

(2) *Glossina morsitans* transmits *Trypanosoma rhodesiense* in nature.

(3) A considerable percentage of the local game (16) is infected with *Trypanosoma rhodesiense*.

NAWALIA, N. RHODESIA

April 12, 1912



THE MEASUREMENTS OF A THOUSAND EXAMPLES OF A SHORT FORM OF TRYPANOSOME FROM A DOUBLE INFECTION

BY

B. BLACKLOCK, M.D.

(From the Runcorn Research Laboratories)

(Received for publication 15 June, 1912)

STRAIN OF TRYPANOSOMES

The trypanosomes, an account of the measurements of which is given below, were derived from a horse naturally infected in the Gambia. This horse suffered from a double infection with two distinct species of trypanosome. An account of the animal reactions of these two species has already been published, and also the measurements of one of the species *T. vivax*, Blacklock (1912).

SPECIES OF ANIMAL HOST

The measurements were made from the parasites as they appeared in white rats. Two of these animals were chosen in which the disease ran an acute course, the parasites increasing from the first day of their appearance and becoming numerous in the blood at the time of the animal's death.

PLAN OF MEASUREMENT

A hundred parasites were drawn, in groups of twenty, on each of five days of the disease, in each rat. The number of days represented between the two rats is, therefore, ten, and the number of parasites drawn from each rat is five hundred.

METHOD OF FIXING, STAINING, DRAWING AND MEASURING

Thin films, made from the blood of the ear, were dried, fixed for five minutes in absolute alcohol, and stained with Giemsa's stain for twenty minutes. Non-dividing parasites (taken in order as they were found) were drawn in clear outline, with the help of the Abbé camera lucida, using No. 18 Zeiss compensating ocular with a 2 mm. apochromatic objective. The measuring was done by Stephens' method.

CONSIDERATION OF THE RESULTS OBTAINED

From Table I, where an analysis of the 1,000 trypanosomes is given, it will be seen that the average measurement of the 1,000 dealt with is 13.3μ , the maximum trypanosome measuring 19.5μ , and the minimum 9μ . Between the averages of the two sets of 500 each there is a difference of only 0.6μ , the first set averaging 13.0μ and the second 13.6μ .

In Table II the parasites are tabulated according to their percentage in microns under three heads, viz., those measuring less than 12μ , those between 12μ and 15μ , and those measuring 15μ and over.

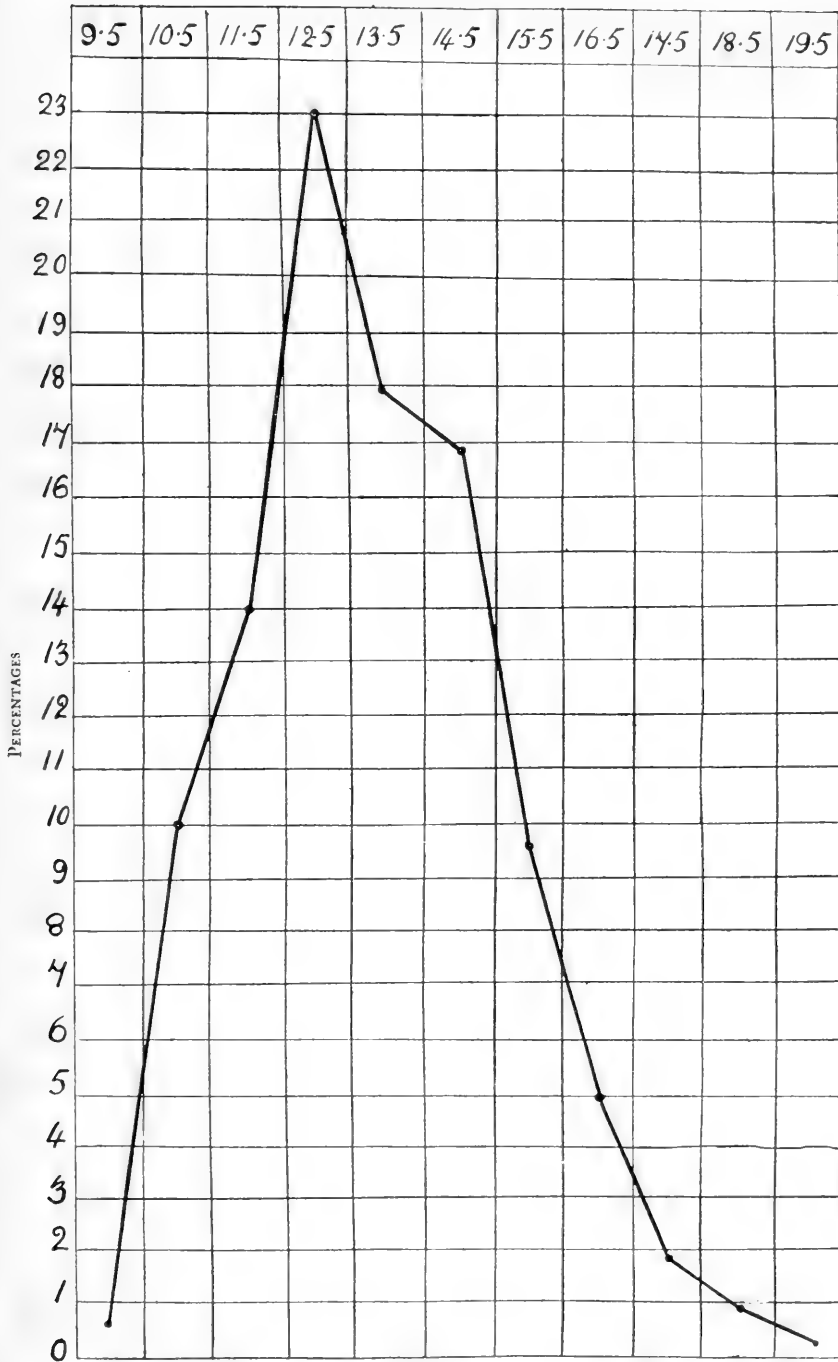
From this Table it will be seen that in each group, the 1,000 and the component 500's, the largest number of trypanosomes lies between 12μ and 15μ . In Charts I and II, which give for the groups of the short form a graphic representation of the percentages in length, the same fact is clearly shown.

It must be noted, however, on coming to smaller numbers than 500, and taking individual hundreds, that in one case of a hundred in the first rat, the largest number of trypanosomes measure less than 12μ , and in one case of a hundred in the second rat, the largest number measures more than 15μ . It would appear, therefore, that the measurement of small numbers of trypanosomes of this species might give somewhat less reliable results than in the case of *T. vivax*.

In Chart III the curves of the two trypanosomes are given side by side. It will be seen that the two curves lie almost completely apart, and that their apices are separated by 10μ .

CHART I.

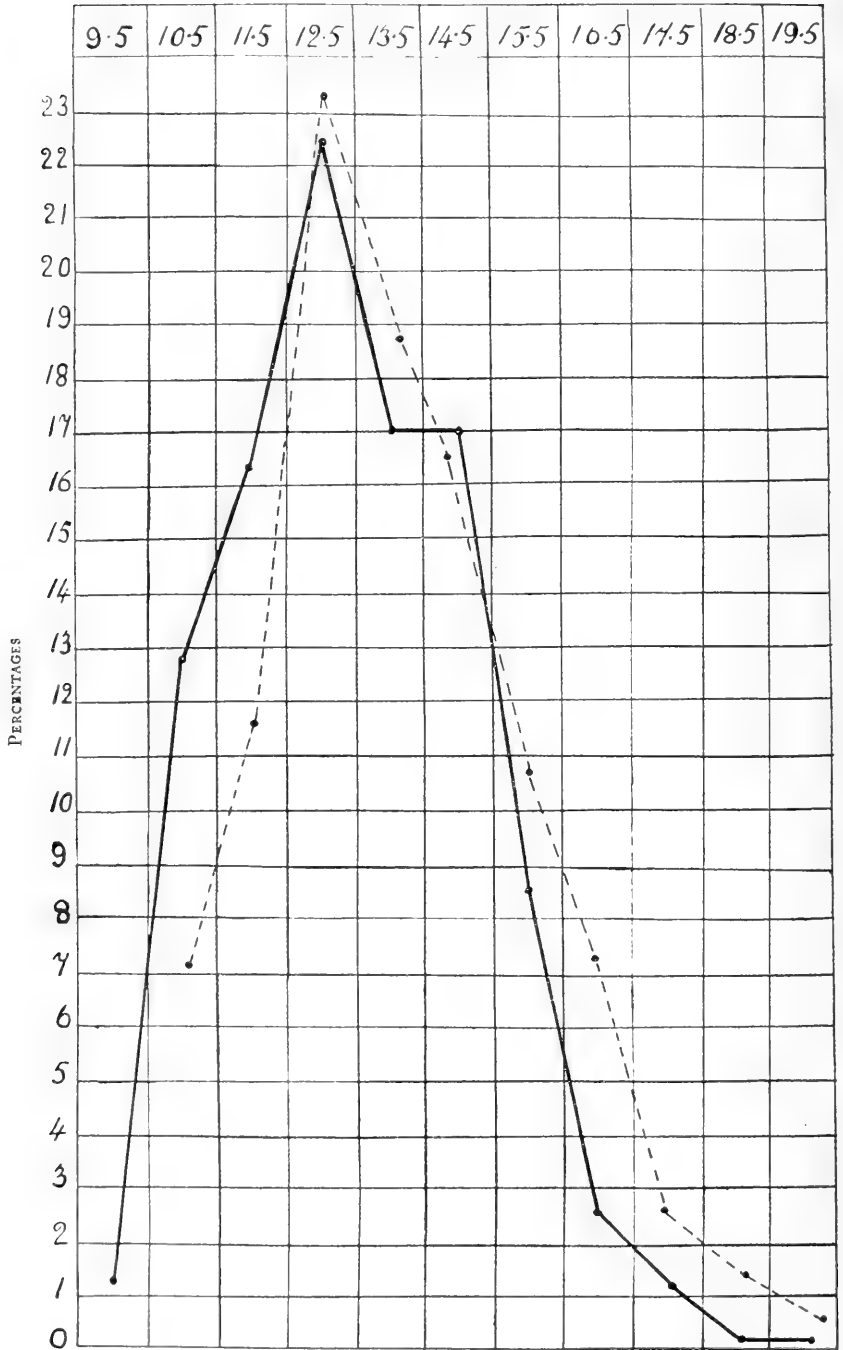
Length in microns



1,000 trypanosomes of the short form measured on 10 days from two white rats

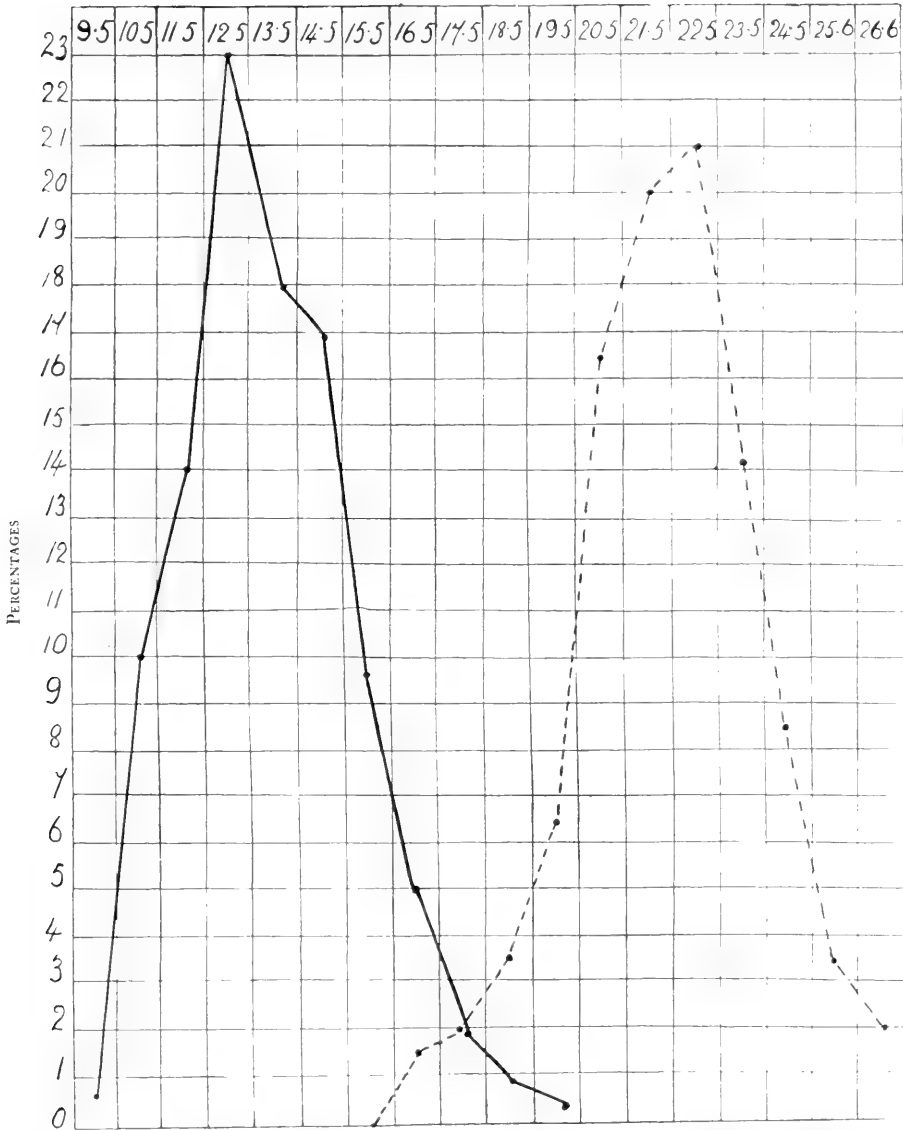
CHART II.

Length in microns



— 500 trypanosomes of the short form measured on 5 days from one white rat (1)
 - - - 500 " " " " " " " " (2)

CHART III.
Length in microns



--- A thousand trypanosomes of the long form *T. vivax* on 22 days in 4 goats
 — " " " " short " " " " 2 white rats

TABLE I. Analysis of 1,500 examples of the short trypanosome in white rats drawn and measured.

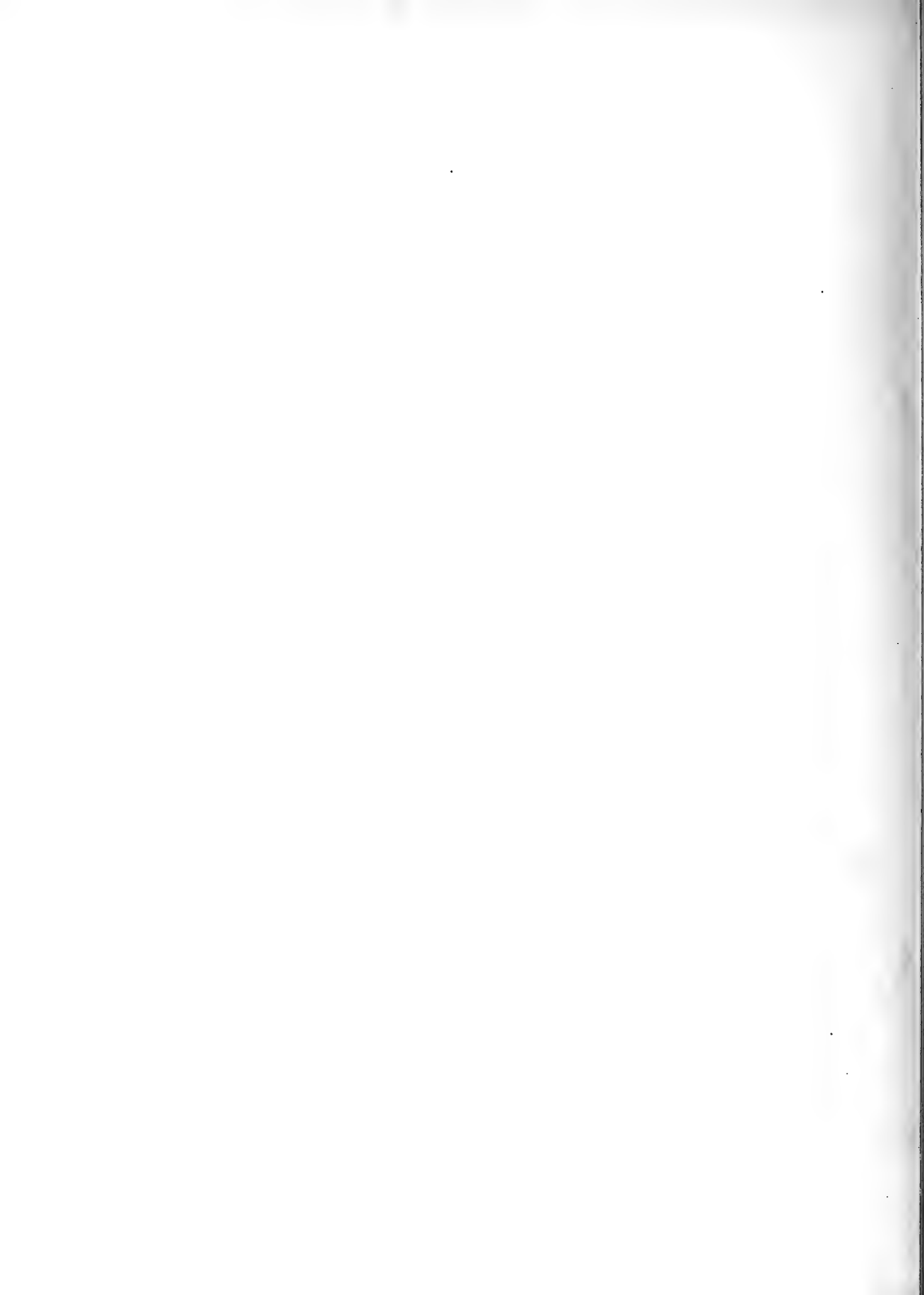
Number of trypanosomes measured	Number of animals represented	Number of days represented	Maximum trypanosome measured	Minimum trypanosome measured	Average measurement of total number drawn	In microns				The longest minimum trypanosome in any group of 20	The shortest maximum trypanosome in any group of 20
						Highest average in any group of 20	Lowest average in any group of 20	Highest average in any group of 20	Lowest average in any group of 20		
1,000 composed as under	2	10	19.5	9	13.3	16.4	11.7	14.2	14.2	14.2	14.2
500	1	5	19	9	13	15.2	11.7	13.3	14.2	14.2	13.3
500	1	5	19.5	10	13.6	16.4	12.4	14.2	14.2	14.2	14.2

TABLE II. Showing percentage incidence according to length in microns of 1,000 examples of the short trypanosome, and of the groups composing the total

Number of trypanosomes	Number of rats from which drawn	Number of days represented	Percentage of		
			(1) Trypanosomes measuring less than 12.4	(2) Trypanosomes measuring between 12.4 and 15.4	(3) Trypanosomes measuring 15.4 and over
1,000 composed as under	2	10	24.7	57.6	17.7
500	1	5	30.6	56.6	12.8
500	1	5	18.8	58.6	22.6

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Ann. Trop. Med. & Parasitol., Vol. V, No. 4, Feb., pp. 521-538.



LEISHMANIASIS AND BABESIASIS IN YUCATAN

BY

HARALD SEIDELIN, M.D.

(Received for publication 25 June, 1912)

Yucatán is one of the Southern States of the Mexican Republic. It forms, together with the neighbouring State of Campeche and the Territory of Quintana Roo, the Peninsula of Yucatán. The northern coast-line of the peninsula and a considerable portion of the interior are well cultivated and densely populated, whilst in the southern and eastern parts there are wild forests. In Mérida, the capital of Yucatán, is a modern hospital, to which patients are brought from all parts of the peninsula. Here, I had my headquarters during my recent Yellow Fever Expedition for the Liverpool School of Tropical Medicine, and thus had, besides the yellow fever work, the opportunity of making several observations, of which the following may be of interest for readers of the 'Annals.'

LEISHMANIA TROPICA

I had, for a long time, been on the look-out for the various forms of tropical ulcers, but failed to find any of a specific nature, until Mr. Th. Maler, an Austrian Archaeologist, who has lived for nearly fifty years in Yucatán, told me about a peculiar affection which he had observed with great frequency during his travels in the interior. He said that the 'chicleros,' Indian labourers who collect chicle (the raw material of chewing-gum), suffered severely from a certain kind of ulcer, which always started on the external ear, often destroying it completely, and invading the face to a large extent. The natives did not, as a rule, apply for treatment, and, even if they did, favourable results were but seldom obtained. Mr. Maler, however, claimed to obtain a perfect cure in a short time by a single application of pure liquid carbolic acid, by means of a brush.

On making inquiries among the local physicians, I found that this disease was, apparently, very little known, but after a short time, Dr. L. Guzman was able to send me two cases. Both were young natives, *chicleros*, and in both cases extensive ulcers were present on the upper part of the helix of one ear, with somewhat elevated, irregular borders, considerable destruction of the tissues and beginning deformation due to cicatricial retraction. In one case, there was also a non-ulcerated nodule on the cheek. When these cases were demonstrated, several colleagues, who had practised in the country districts, declared they knew the disease quite well; in fact, one practitioner, Dr. M. Canto, remembered to have seen, years ago, about 150 cases in the course of a few years. The disease is evidently met with in all parts of the peninsula, both in the interior of Yucatán, in Campeche and in Quintana Roo. Also many laymen knew the affection as a typical one, the 'ear ulcer of the *chicleros*.'

Later on, four other cases, evidently of the same nature, came under my observation. In all cases ulcers were present on the ear, and in two of them additional ulcers, in one, on the forearm, and in the other, on the leg. In the former of these two cases, the patient explained that he used to rest his head on his arm when sleeping in his hammock, in such a way that the ulcerated portion of the ear would come in contact with that particular place of the forearm where the ulcer had formed secondarily.

In all cases, smears were made from the ulcers, and, in the first case, from the nodule on the cheek. In four cases *Leishmaniae* were found, mostly in small numbers, but in the liquid which was obtained from the closed nodule, the parasites were fairly numerous. In one case a first examination proved negative, but a few days later, parasites were found. In the two negative cases, I had no opportunity of repeating the examination, but, in view of the clinical features, I must consider the ulcers in these two cases identical with those in which parasites were found.

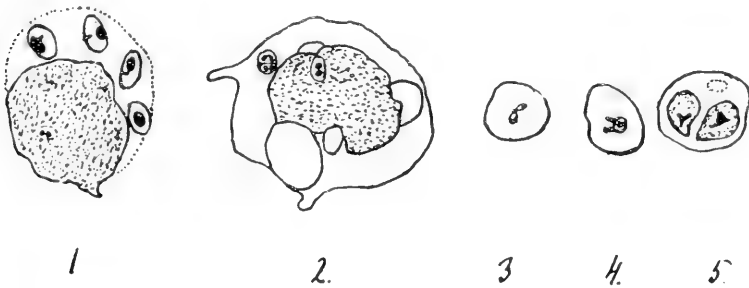
In addition to these cases, I saw one in which the diagnosis remained doubtful. The patient was an elderly man, who had been working in the woods, and had suffered for some time from an ulcer on the ear, which had, however, healed almost completely when I saw him. At that time he had an ulcerated nodule on the

back of the wrist. This nodule was punctured, and smears prepared, but no parasites could be found.

The affection had lasted, in the six cases, from two to eight months, but in severe cases the ulcers were said to persist for about two years.

It is a common belief in Yucatán that the disease is transmitted from one individual to another by means of some insect; in one part of the country a 'white mosquito' is said to act as transmitter, in another district a black fly is suspected, and several laymen suggested to me the possibility of indirect transmission. They believed that a parasite of flies, probably a *Trombidium*, might infect itself on a patient, and then be transported to a healthy individual.

The treatment with pure carbolic acid, as recommended by Mr. Maler, was applied in several cases; these patients improved rapidly, though the parasites did not disappear; on the contrary, in one case, the parasites were very scarce at the first examination, but fairly numerous two or three weeks later, though at that time the ulcer had a much cleaner aspect than when seen for the first time.



The parasites observed had the morphological characters of *Leishmania tropica* (fig. 1); they were mostly situated inside large mononuclear cells, from two or three to about twenty in a single cell.

A peculiar feature was that in the four cases in which the *Leishmania* was found, a characteristic diplococcus was also observed. In two cases secondary infection was evident, many different bacteria being present in the smears, but in the other

two cases the diplococcus was the only associated microbe. One of the latter cases was that of the young man with a non-ulcerated nodule on his cheek. The skin over this nodule was apparently intact, and nothing suggested that a secondary infection might have occurred. I feel inclined, therefore, to regard this coccus as primarily associated with the *Leishmania*, in which case it would probably be of pathogenic importance.

The diplococcus (fig. 2) was free, or enclosed in polymorphonuclear leucocytes, or in large mononuclear cells; it showed considerable resemblance to the gonococcus, morphologically, but retained the colour by the Gram-process. No culture was obtained on ordinary media.

Dermal *leishmaniasis* has repeatedly been observed in the New World. From Brazil it has been described by Lindenberg (1909), Carini and Paranhos (1909), Splendore (1911) (mucous membranes), and Werner (1911); from French Guiana by Nattan-Larrier, Touin, and Heckenroth (1909); from Dutch Guiana (Surinam) by Flu (1911); and from Panama by Darling (1910, 1911) and Darling and Connor (1911). The case described by Darling and Connor is the only one in which the same localisation on the external ear was observed, which seems to be so characteristic for the affection, as seen in Yucatán. In fact, the photographs given by these authors show very much the same characters which I have observed in the cases here reported. The other peculiar feature which I found in my cases, the association of the *Leishmania tropica* with a characteristic diplococcus, is not mentioned in the descriptions referred to.

BABESIA BIGEMINA AND B. CANIS

In an earlier paper (1912) I mentioned an acute, febrile disease, which is observed in Yucatán in imported cattle, and known by the name of 'yellow fever.' The symptoms are similar to those of Texas fever. High febrile temperatures, albuminuria, haemoglobinuria, oliguria, anuria, haematemesis and melaena are observed. The disease causes considerable losses: according to an estimate of a cattle-importer, it would kill in three months cattle valued at about \$70,000 Mex. (£7,000). Very little or nothing is done to combat the disease.

Native cattle are said to suffer from the disease in a mild form, especially the calves.

I saw seven cases of this disease. In six cases I found parasites which showed ring-forms, double pyriform bodies (fig. 3), and also the characteristic division forms (fig. 4), described by Nuttall and Graham-Smith (1908). I have not been able to follow the whole development of the parasites, but I think there is no doubt about the diagnosis of *Babesia bigemina*. In several cases the parasites were extremely scarce, at a time when the temperature was very high, and in one case, in which salvarsan was applied without any final result, they continued so until the fourth day of the disease; in this case the cow died on the fifth day, and only on this day the parasites were somewhat numerous in the peripheral blood.

In the one case in which no parasites were found, the blood was examined on the first day only, of an attack of fever, which did not prove fatal, but during which typical symptoms were observed. It is probable that a repeated examination would have shown the presence of parasites.

In a dog, I found a number of very small *Babesia*-like parasites and, after searching several smears, the typical double form shown in fig. 5. This dog did not suffer from any acute, febrile disease, nor was red urine observed. It suffered from a chronic cachexia.

Coloured drawings, corresponding to the diagrammatic figures here given, will be published in the Expedition Report (*Yell. Fev. Bur. Bull*, II, 2, 1912), where also some more details will be found, as well as a map of Yucatán.

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TRYPANOSOMES INFECTING GAME AND DOMESTIC STOCK IN THE LUANGWA VALLEY, NORTH EASTERN RHODESIA

BY
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AND
WARRINGTON YORKE

(Third interim report of the Luangwa Sleeping Sickness
Commission, British South Africa Company)

(Received for publication 22 July, 1912)

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I. INTRODUCTION

In two previous reports (1912), brief references have been made to various trypanosomes found in game and domestic animals in the vicinity of Nawalia, Northern Rhodesia, but as these papers dealt exclusively with the human parasite (*Trypanosoma rhodesiense*, Stephens and Fantham), no details were given concerning the other organisms encountered. It is proposed to deal with these at greater length in the present communication.

Within the confines of the Luangwa Valley a numerous and varied selection of game is found, despite the fact that *Glossina morsitans* is everywhere abundant, but, on the contrary, due doubtless to the presence of these insects, domestic stock is extremely scarce, and in many districts, non-existent. A few goats are occasionally found, but some evidence exists to show that these animals are not so insusceptible to trypanosomiasis, under natural conditions, as is locally supposed. Dogs are very seldom

seen, and the natives, themselves, recognise the impossibility of keeping them in the midst of 'fly.' Cattle were found in one village only.

II. METHODS

In our experience, trypanosomes are more readily detected in a buck's blood by the examination of thin, stained smears, than by that of fresh preparations. Except under exceptional circumstances, several hours elapsed after an animal had been shot before its heart reached the laboratory, and this, together with the great heat, had a very deleterious effect on the parasites, destroying their motility, and permitting degenerative changes to occur. In several instances in which fresh preparations, made under these conditions, were examined, as well as blood smears made in the field and afterwards stained, no trypanosomes were found in the fresh blood, whereas they were in the permanent preparations.

In making smears, it was found advisable to cut the animal's throat immediately it had been shot, and to obtain the blood from one of the arteries. It is claimed that such films have the following advantages:—

1. Clean, uncontaminated preparations are obtained.
2. The trypanosomes have had no opportunity of degenerating, and thus stain more sharply.
3. The preparations are permanent.
4. The parasites can be identified more easily in stained, than in fresh preparations. In this particular, the examination of thin films has an obvious advantage over that of thick films.

The preparations were dried in the air, fixed in absolute alcohol for ten to fifteen minutes, and stained with Giemsa.

Owing to the impossibility of obtaining 'clean' sheep and goats, all the inoculations from game were made into monkeys and rats, the amount of blood used varying from 1-10 c.cm. It is recommended that, when possible, sheep and goats be used as well, since they are susceptible to most of the pathogenic trypanosomes, whereas animals such as dogs, monkeys and rats, are not. This is an important consideration when dealing with such parasites as *Tryp. vivax* and *Tryp. nanum*.

III. EXAMINATION OF GAME

A total of 127 head of game, comprising nineteen genera, was examined at Nawalia, and trypanosomes were found by direct examination, by inoculation, or by both methods, in thirty-three. Details are given in Table I.

TABLE I.—Results of examination of game for trypanosomes

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculation
1. Elephant	1	0	1	0	0	0
2. Rhinoceros... ..	1	0	1	0	0	0
3. Hippopotamus ...	1	0	0	0	0	0
4. Zebra	5	0	3	0	0	0
5. Roan	8	1	2	0	0	1
6. Wildebeest... ..	2	0	1	0	0	0
7. Kudu	7	3	3	1	1	4
8. Hartebeest	6	0	1	0	1	1
9. Waterbuck	28	16	14	5	1	17
10. Puku	10	1	6	0	0	1
11. Mpala	29	1	13	1	1	2
12. Bushbuck	9	4	6	1	2	6
13. Bushpig	4	0	1	0	0	0
14. Warthog	9	0	3	0	1	1
15. Lion	2	0	0	0	0	0
16. Hunting Dog	1	0	1	0	0	0
17. Giant Rat	1	0	0	0	0	0
18. Genet	2	0	0	0	0	0
19. Squirrel	1	0	0	0	0	0
	127	26	56	8	7	33

It will be seen that parasites were found by direct examination in twenty-six cases, a percentage of 20·4. This is a high figure for single observations, and it is probable that had several preparations from each buck been searched, the percentage of successes would have been much greater. In several instances, only a single trypanosome was found in a film covering the greater part of a slide, and this, after a very careful examination extending over two hours.

A more accurate estimate of the percentage of animals harbouring trypanosomes is afforded by considering only those from which inoculations were made. An analysis of these gives the following figures:—

Number of inoculations made	56
Number of positive inoculations in which parasites were found in buck's blood.....	8
Number of positive inoculations in which no parasites were found in buck's blood.....	7
Number of negative inoculations in which parasites were found in buck's blood.....	6
Total number found infected	21

These figures show that at least 37·5 % of the local fauna were infected with trypanosomes. Both *Tryp. vivax* and *Tryp. nanum* have been found in game, and to both these species monkeys and rats are refractory, so that no conclusions can be drawn regarding the presence or absence of these trypanosomes in animals in which parasites were not found in the blood smears. Had sheep and goats been available for inoculation, it is probable that many more buck would have been shown to harbour the two organisms in question. As a conservative estimate, the percentage of game actually infected with trypanosomes in the vicinity of Nawalia might be placed at fifty.

A further point, which is brought out in the Table, is that different species of buck appear to vary widely in their susceptibility. Amongst the commoner species trypanosomes were never found, either by direct examination or by inoculation, in zebra, wildebeest and bushpig, and only rarely in roan, hartebeest, puku, mpala and warthog. Waterbuck, bushbuck and kudu were the species found to be most heavily infected.

TABLE 2.—Percentages of various species of game found infected with trypanosomes

Animal	Number examined	Percentage harbouring trypanosomes
Bushbuck	9	66.6
Waterbuck	28	60.7
Kudu	7	57.1
Hartebeest	6	16.6
Roan	8	12.5
Warthog	9	11.1
Puku	10	10.0
Mpala	29	6.9

To a certain extent, perhaps, these differences may be accounted for by the habitats affected by the various species of game. Kudu and bushbuck, and waterbuck to a lesser extent, are usually found in thick cover, from which they seldom emerge, and where they are more constantly exposed to the bites of tsetse flies. Mpala, puku, and wildebeest are usually found in open country, frequently remaining for the greater part of the day on wide, bare plains, and here the flies are less noticeable than in the bush. Specific differences in the amount of immunity enjoyed by buck are probably, however, of much greater importance.

In Table 3 are given the species of trypanosomes occurring in each animal in which parasites were found. In compiling the table, information obtained from the result of inoculations, where these were made, has been utilised. This enables a differentiation to be made between such parasites as *Tryp. pecorum* and *Tryp. nanum*, which are, morphologically, indistinguishable. *Trypanosoma vivax* has a characteristic morphology, and can thus be identified in blood smears without difficulty.

As would be expected, double infections are not uncommon amongst game, and several instances of this are recorded in the table.

No data exist as to the ultimate effect of infection on game.

All the animals which were shot appeared to be in perfect condition, and presented no objective signs of disease. Whether or no buck succumb to trypanosomiasis it is impossible to say, but they have increased steadily since rinderpest swept through the country, and so it may be assumed that their tolerance to trypanosomes is very great.

TABLE 3.—Trypanosomes found in game

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Bushbuck	1 Negative	<i>T. pecorum</i>	<i>T. pecorum</i>
"	2 <i>T. pecorum</i> or <i>T. nanum</i>	Negative	<i>T. nanum</i>
"	3 <i>T. sp.</i>	<i>T. sp.</i>	<i>T. sp.</i>
"	4 <i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
"	5 <i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
"	6 Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
Waterbuck	1 <i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i>	<i>T. pecorum</i>
"	2 <i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>
"	3 <i>T. pecorum</i> or <i>T. nanum</i> and <i>T. vivax</i>	Negative	<i>T. nanum</i> and <i>T. vivax</i>
"	4 <i>T. pecorum</i> or <i>T. nanum</i>	Negative	<i>T. nanum</i>
"	5 Negative	<i>T. pecorum</i>	<i>T. pecorum</i>
"	6 <i>T. vivax</i>	Negative	<i>T. vivax</i>
"	7 <i>T. vivax</i>	Negative	<i>T. vivax</i>
"	8 <i>T. rhodesiense</i>	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
"	9 <i>T. pecorum</i> or <i>T. nanum</i> and <i>T. vivax</i>	Negative	<i>T. nanum</i> and <i>T. vivax</i>
"	10 <i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
"	11 ? <i>T. rhodesiense</i>	Animal died day after inoculation	? <i>T. rhodesiense</i>
"	12 <i>T. rhodesiense</i>	<i>T. rhodesiense</i> and <i>T. pecorum</i>	<i>T. rhodesiense</i> and <i>T. pecorum</i>
"	13 <i>T. vivax</i>	No inoculation	<i>T. vivax</i>
"	14 <i>T. rhodesiense</i> and <i>T. vivax</i>	<i>T. rhodesiense</i>	<i>T. rhodesiense</i> and <i>T. vivax</i>
"	15 <i>T. vivax</i>	No inoculation	<i>T. vivax</i>
"	16 ? <i>T. rhodesiense</i> , and <i>T. vivax</i>	No inoculation	? <i>T. rhodesiense</i> , and <i>T. vivax</i>
"	17 ? <i>T. rhodesiense</i>	No inoculation	? <i>T. rhodesiense</i>
Kudu	1 Negative	<i>T. pecorum</i>	<i>T. pecorum</i>
"	2 <i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
"	3 <i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i>	<i>T. pecorum</i>
"	4 <i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
Roan	1 <i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
Warthog	1 Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
Puku	1 <i>T. vivax</i>	No inoculation	<i>T. vivax</i>
Mpala	1 Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
"	2 <i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>
Hartebeest	1 Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>

IV. EXAMINATION OF DOMESTIC STOCK

The domestic animals examined, and the species of trypanosomes found in them, are given in Table 4.

TABLE 4.—Examination of Domestic Stock for Trypanosomes

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Cow	<i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
..	<i>T. pecorum</i> or <i>T. nanum</i>	Result not yet known	<i>T. pecorum</i> or <i>T. nanum</i>
Goat No. 39	<i>T. vivax</i>	Negative	<i>T. vivax</i>
.. .. 94	<i>T. vivax</i> and <i>T. nanum</i> or <i>T. pecorum</i>	Negative	<i>T. vivax</i> and <i>T. nanum</i>
.. .. 202	<i>T. pecorum</i> or <i>T. nanum</i>	Negative	<i>T. nanum</i>
.. .. 258	<i>T. vivax</i>	Negative	<i>T. vivax</i>
Dog	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
..	<i>T. pecorum</i>	<i>T. pecorum</i>	<i>T. pecorum</i>
..	<i>T. pecorum</i>	No inoculation	<i>T. pecorum</i>
..	<i>T. pecorum</i>	No inoculation	<i>T. pecorum</i>
..	<i>T. sp.</i> (? <i>montgomeryi</i>)	Negative	<i>T. sp.</i> (? <i>montgomeryi</i>)

The only native village in which cattle were found was Kambwiri's, some forty miles south-east of Nawalia. At present there are only three head, all that are left of a big herd which existed there some four or five years ago. Two of the three appeared to be in good condition when seen, but the headman of the village fully expected to lose them within a few months. The third beast was obviously ill. The cow in which trypanosomes were found, at Fort Jameson, was bred on the Government Farm, and had never been beyond the limits of the township. Tsetse flies have never been seen within some miles of the place, but *Stomoxys sp.* is abundant in the kraals, and, at certain seasons of the year, various species of *Tabanidae* are common.

In several of the villages on the main road from Nawalia to Fort Jameson, a number of goats were found at the end of August, 1911, and again at the beginning of April, 1912, but at

the end of that month not a single animal was alive. *Glossina morsitans* was found around all these villages. The four goats mentioned in Table 4 were under observation at Nawalia for a considerable length of time. During this period, parasites were found in the peripheral blood only at rare intervals. Two were rather thin, but not markedly so, and, apart from this, there were no signs of disease. Goat No. 258 was examined at frequent intervals for two months before parasites were first found, while in the others, trypanosomes were seen on the first occasion. Nos. 39 and 258, after having been under observation for nine and four months, respectively, died on the road when the Commission left Nawalia, most probably from being overdriven. The other two are still alive, seven and four months after the diagnosis was made.

The dog in which *Tryp. rhodesiense* was found, came from a village just on the Nyasaland border. The natives said that it had not been out of the village for over a year previously. As the disease runs an extremely acute course in these animals, there can be no doubt that the dog was infected locally.

V. DESCRIPTION OF TRYPANOSOMES

1. *Trypanosoma rhodesiense*.

This parasite has been fully dealt with in the previous reports, and requires, therefore, no further description. It was isolated from four waterbuck, two mpala, one hartebeest, one bushbuck and one warthog—16% of the game from which inoculations were made—and from one native dog. Parasites resembling *Tryp. rhodesiense* were found in blood films made from three other waterbuck, from which no sub-inoculations were made.

2. *Trypanosoma vivax*.

This organism was found in eight waterbuck, one puku, and three goats.

Morphology

(a) In fresh preparations it appears as a club-shaped organism, characterised chiefly by the extraordinary rapidity with which it moves across the field.

(b) In stained preparations it is seen to be more or less club-shaped, with a long, free flagellum. The greatest width is posterior to the nucleus, which is situated about the middle of the body. The blepharoplast is large and rounded, and lies close to the posterior extremity of the parasite. The undulating membrane is, as a rule, very feebly developed, or absent.

The mean length of 125 trypanosomes was 23.3μ , the maximum 28.25μ , and the minimum 19.75μ . The greatest width varied between 2 and 4.25μ , average 3.2μ .

Pathogenicity

Inoculations were made into the following animals:—

4 Monkeys	all remained negative
1 Rabbit	„ „
2 Rats	both „ „

Transmission

The vector is probably *Glossina morsitans*. Experiments are now in progress.

3. *Trypanosoma nanum*.

Found in the following animals:—one bushbuck, three waterbuck, and two goats. Possibly it was also present in two other bushbuck, one other waterbuck, two kudu, a roan, and two cattle, but as no sub-inoculations were made, it was impossible to differentiate it from *Trypanosoma pecorum*.

Morphology

(a) In fresh preparations it appears as a short, sluggish organism. As a rule, it does not progress.

(b) In stained preparations it is found to be short, with a more or less rounded posterior extremity from which the body tapers forwards to the acute anterior end. The nucleus is placed at the centre of the body. The blepharoplast is small, and is situated near the posterior extremity. There is no free flagellum, and the undulating membrane is absent, or, at most, very slightly developed. The protoplasm is in general free from vacuoles and granules.

The mean length of 175 individuals was 14.4μ , the maximum 19μ , and the minimum 10μ . The breadth, at the level of the nucleus, varied from 1 to 2.25μ , the average being 1.5μ .

Pathogenicity

The following animals were inoculated:—

3 Monkeys	all remained negative
1 Rabbit	” ”
3 Rats	” ” ”

Transmission

The vector has not yet been determined with certainty, but some evidence exists to show that *Glossina morsitans* is the principal one. Experiments are now in progress.

4. *Trypanosoma pecorum*.

Found in the following animals:—one bushbuck, one mpala, four waterbuck, two kudu, three dogs, and one wild rat. It was possibly present in two other bushbuck, a fifth waterbuck, two other kudu, a roan, and two cattle, but as mentioned before, it could not be distinguished from *Trypanosoma nanum*, in the absence of sub-inoculations.

Morphology

(a) In fresh preparations, this parasite resembles *Trypanosoma nanum* very closely. It is a short, sluggish organism, showing no degree of translatory power.

(b) In stained preparations it appears as a short organism, with an obtuse or rounded posterior extremity from which it tapers to the attenuated anterior end. The nucleus is oval or rounded, situated near the middle of the body. The blepharoplast is small and rounded, and lies close to the posterior extremity. The undulating membrane, if present, is very feebly marked, and there is no free flagellum. The general protoplasm stains uniformly, and is devoid of granules and vacuoles.

The mean length of 400 individuals was 13.6μ , the maximum 19μ , and the minimum 9.5μ . The breadth, at the level of the nucleus, varied from 1 to 2.5μ , the average being 1.5μ .

Pathogenicity

A synopsis of the pathogenicity is given in Table 5.

TABLE 5.—Pathogenicity of *Trypanosoma pecorum*

Animal	No. inoculated	Incubation period days	Duration days
Monkey	9	8-14, average 11	28-225 (still alive)
Rabbit	1	10	215
Guinea-pig	1	5	39
Rat	11	3-11, average 5½	6-32
Mouse	2	5	27-75

Transmission

Trypanosoma pecorum was obtained in one of twenty-eight experiments in which freshly-caught *Glossina morsitans* were fed on clean monkeys. While, therefore, this is sufficient to demonstrate the ability of this fly to transmit the parasite, there is a mass of circumstantial evidence which indicates that it is not the only vector, and that possibly it is not the most important one. In the vicinity of Nawalia alone, *Trypanosoma pecorum* was found in 7.94% of the game examined, and in three of five dogs. In addition to these, it was found in one wild rat which had been kept in the laboratory for some weeks in a wide-meshed wire cage. Various species of Tabanids were seen entering the cage, but it is impossible to say whether the rat was infected by one of these. In all, 3,202 freshly-caught *Glossina morsitans* were fed on clean monkeys, and while *Trypanosoma rhodesiense* was obtained in five of the experiments, *Trypanosoma pecorum*, which is of frequent occurrence in game, was only recovered in one.

In a recent number of the Sleeping Sickness Bulletin (1912) the information regarding the rôle of insects, other than tsetse flies, in transmitting trypanosomes is reviewed, and shows that there is an increasing amount of circumstantial evidence that various species of *Tabanidae*, more particularly, may do so. Within the past four years there have been three outbreaks of trypanosomiasis

amongst cattle on farms near Fort Jameson, and also on the Government Farm there. Here, tsetse flies may be absolutely excluded. *Stomoxys* and *Lyperosia* are constantly present in the kraals, and during the rains various species of *Tabanus*, *Pangonia*, and *Haematopota* are common. It appears probable, therefore, that some of these species may be important factors in the spread of trypanosomes, but in the absence of definite experiments to determine the point, no conclusions can be drawn.

5. *Trypanosoma* sp.

This parasite, which has not yet been fully worked out, was isolated from one bushbuck.

Morphology

(a) In fresh preparations, the trypanosome is seen to be markedly polymorphic, resembling, in this particular, *Trypanosoma rhodesiense*, *Trypanosoma gambiense*, and *Trypanosoma brucei*. Short, sluggishly-moving forms are seen, as well as long, free-flagellated, active ones. As a rule, the short varieties are more or less stationary, while the long ones progress fairly rapidly. The relative number of each of the forms varies widely, from day to day, in any one animal.

(b) In stained preparations, every gradation between extremely short, aflagellar forms, indistinguishable from *Tryp. pecorum*, and long, slender, free-flagellated parasites are seen. In general, the trypanosome morphologically closely resembles *Tryp. gambiense* or *Tryp. brucei*, except for the presence of occasional *pecorum*-like forms.

The mean length of 525 individuals was 20μ , the maximum 33.25μ , and the minimum 10.75μ . The curve representing the distribution of the various lengths of the trypanosomes, expressed in percentages of the total number measured (525), differs from that of *Tryp. gambiense* in that the apex occurs at 17μ .

Pathogenicity

The reaction of laboratory animals to the trypanosome is given in Table 6.

TABLE 6.—Pathogenicity of *Trypanosoma* sp.

Animal		Incubation period days	Duration, days
Monkey	No. 166 ...	8	94
"	" 312 ...	10	Alive after 66
"	" 360 ...	7	" 43
Rabbit	" 370 ...	6	" 31
Guinea-pig	" 371 ...	Negative	
Rat	" 219 ...	7	104
"	" 253 ...	12	Alive after 107
"	" 361 ...	10	" 43

Transmission

Unknown.

6. *Trypanosoma* sp. (?*Montgomeryi*.)

Found in one dog, which was obtained from a village in the hills on the Nyasaland border.

Morphology

(a) In fresh preparations, the parasite appears as a broad, stumpy organism, which shows no marked degree of translatory power.

(b) In stained preparations, the trypanosome resembles, at first sight, *Tryp. pecorum*, but on closer examination it can be readily distinguished from this parasite by its great breadth. The ratio of the breadth to the length is 1:4.8. The posterior extremity is sub-acute or rounded, the anterior attenuated. The greatest width lies at the level of the nucleus, which is situated at the middle of the body, or posterior to it. The blepharoplast is very large and

rounded, and is situated near the posterior extremity. Frequently it lies at one edge of the trypanosome, and projects laterally as a small excrescence. The undulating membrane is, as a rule, absent, and when present is simple, and feebly developed. Occasionally, a short, free flagellum, $1-2\mu$ in length, is to be seen, but this is generally absent. The cytoplasm often contains coarse granules and vacuoles. The latter are most commonly seen in the posterior portion of the body, whereas the granules may be scattered generally throughout the protoplasm.

The average length of 200 individuals was $15\cdot88\mu$, the maximum 20μ , and the minimum 10μ . The breadth, at the level of the nucleus, varied from $1\cdot25$ to $6\cdot5\mu$, the average being $3\cdot29\mu$.

Pathogenicity

One rat was sub-inoculated from the dog, and had not become infected up to the thirteenth day afterwards, when it was accidentally killed. Unfortunately, the dog died some days previously, so that the strain was lost.

Transmission

Unknown.

Diagnosis

It will be seen that, morphologically, this parasite differs widely from *Tryp. pecorum*. The average length is greater, $15\cdot88\mu$ as compared with $13\cdot6\mu$. The most characteristic difference, however, is the great breadth of the organism, which is, on an average, 2·2 times that of *Tryp. pecorum*; average breadth $3\cdot29\mu$ as compared with $1\cdot5\mu$. It appears to resemble most closely the parasite described by Montgomery and Kinghorn (1909) as the Ninamwenda strain, and for which the name *Trypanosoma montgomeryi* was proposed by Laveran. But, in view of the fact that we were able to examine material from one dog only, we cannot regard its identity with this parasite as established.

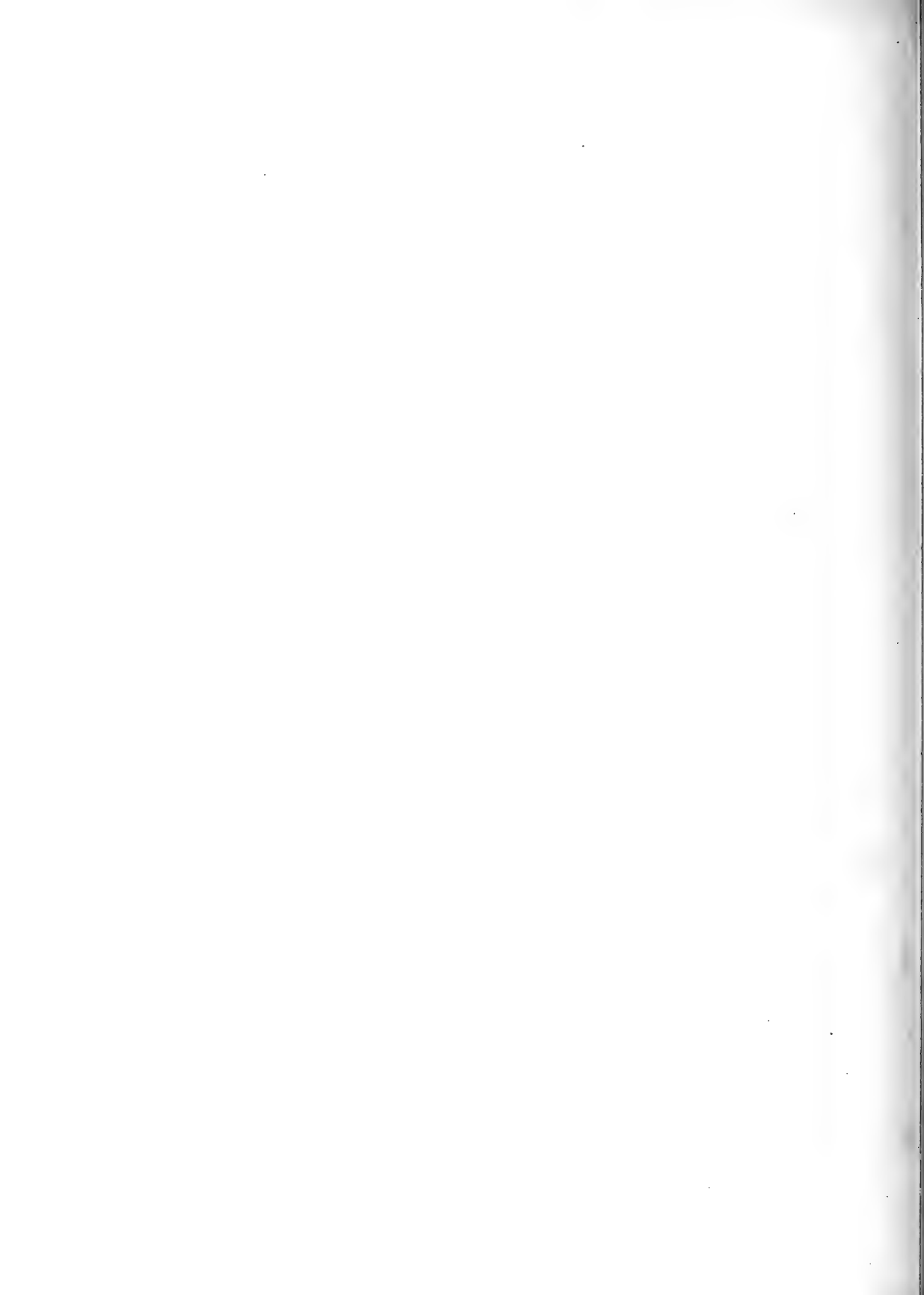
VI. SUMMARY

1. Trypanosomes are of frequent occurrence in game and domestic stock in the Luangwa Valley. At least 37.5 % of the buck harbour parasites.
2. Six species of trypanosomes were found, viz.: *Tryp. rhodesiense*, *Tryp. vivax*, *Tryp. nanum*, *Tryp. pecorum*, and two others, of which, one was possibly *Tryp. montgomeryi*.
3. *Glossina morsitans*, in nature, transmits two of these trypanosomes, viz.: *Tryp. rhodesiense* and *Tryp. pecorum*, and probably also transmits at least two others, namely, *Tryp. vivax* and *Tryp. nanum*.
4. Circumstantial evidence exists to show that *Tryp. pecorum* may be transmitted by biting insects other than tsetse flies.

NGOA, N. RHODESIA,
May 28, 1912.

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TRYPANOSOMES OBTAINED BY FEEDING WILD *GLOSSINA MORSITANS* ON MONKEYS IN THE LUANGWA VALLEY, NORTHERN RHODESIA

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I. INTRODUCTION

During the sojourn of the Commission at Nawalia, experiments were undertaken with the object of ascertaining the species of trypanosomes transmitted, in nature, by *Glossina morsitans*, Westw. The flies, as they were brought to the laboratory, were fed on clean monkeys, which were the only animals available for the purpose. Unfortunately, owing to the lack of healthy goats and sheep, no definite conclusions can be drawn as to whether the fly was infected with such species as *Trypanosoma vivax* and *Trypanosoma nanum*, both of which are of common occurrence in game and domestic stock. A certain amount of evidence exists,

however, to show that both parasites are transmitted, in nature, by *Glossina morsitans*. Experiments are now in progress to determine this point.

II. RESULTS OF FEEDING WILD *GLOSSINA MORSITANS* ON HEALTHY MONKEYS

From day to day, freshly-caught *Glossina morsitans* were fed on healthy monkeys. In all, 3,410 flies were fed in batches, as they were brought to the laboratory, on 33 monkeys, but as five of the latter died within two or three days, inferences can only be drawn as to the infectivity of the 3,008 flies fed on the remaining 28 animals. Details of the experiments are given in Table I.

TABLE I.—Results of feeding freshly-caught *Glossina morsitans* on healthy monkeys

No. of Experiment	Date	No. of flies fed	Result	Trypanosome isolated
1	21.6.11	18	Negative	
4	6.7.11	100	Infection	<i>Tryp. ignotum</i> , sp. nov.
7	19.7.11	97	Negative	
22	28.7.11	160	Infection	<i>Tryp. ignotum</i> , sp. nov.
35	7.8.11	193	"	<i>Tryp. ignotum</i> , sp. nov.
95	30.10.11	90	Negative	
96	30.10.11	82	Infection	<i>Tryp. rhodesiense</i>
121	14.11.11	98	Negative	
143	4.12.11	100	"	
100	6.12.11	105	Infection	(?) <i>Tryp. rhodesiense</i> ; animal died day after becoming infected
215	13.1.12	41	"	<i>Tryp. ignotum</i> , sp. nov.
210	7-12.1.12	269	"	<i>Tryp. rhodesiense</i>
217	16.1.12	200	"	<i>Tryp. rhodesiense</i>
224	23.1.12	28	Negative	
259	13.2.12	104	"	
316	20.3.12	101	Infection	<i>Tryp. rhodesiense</i>
317	21.3.12	112	"	<i>Tryp. pecorum</i>
326	25.3.12	93	Negative	
329	26.3.12	130	Infection	<i>Tryp. ignotum</i> , sp. nov.
330	27.3.12	137	"	<i>Tryp. ignotum</i> , sp. nov.
333	28.3.12	74	"	<i>Tryp. rhodesiense</i>
334	29.3.12	67	Negative	
336	30.3.12	109	Infection	<i>Tryp. ignotum</i> , sp. nov.
340	1.4.12	108	"	<i>Tryp. ignotum</i> , sp. nov.
342	2.4.12	52	Negative	
343	3.4.12	85	Infection	<i>Tryp. rhodesiense</i> and <i>Tryp. ignotum</i> , sp. nov.
348	4.4.12	90	Negative	
353	6.4.12	137	Infection	<i>Tryp. pecorum</i> and <i>Tryp. ignotum</i> , sp. nov.
*94	15.9.11	194	—	Goat, subinoculated monkeys and rats did not become infected

* As yet we are uncertain whether goats are susceptible to *Tryp. ignotum*, hence the flies fed on this animal have not been considered in estimating the proportion infected with this parasite.

It will be seen from the table that three species of trypanosomes were isolated, namely *Trypanosoma rhodesiense*, *Trypanosoma pecorum*, and a third, hitherto undescribed parasite, for which we propose the name *Trypanosoma ignotum*.

An analysis shows that of the 3,202 flies used, at least 19 were capable of infecting monkeys. This figure is based on the assumption that, with the exception of Experiments Nos. 343 and 353, each batch contained but a single infective fly. In each of these experiments it is highly probable that at least two infective flies were present, as in the former both *Trypanosoma rhodesiense* and *Trypanosoma ignotum*, sp. nov., were found in the monkey's blood, and in the latter both *Trypanosoma pecorum* and *Trypanosoma ignotum*, sp. nov. The proportion of flies infected with each of the three trypanosomes is given in tabular form.

TABLE 2.—Proportion of wild *Glossina morsitans* infected with *T. rhodesiense*, *T. pecorum*, and *T. ignotum*, sp. nov.

Species	No. of flies fed	No. of infections	Ratio of infected to non-infected flies
<i>T. ignotum</i>	3008	10	1 : 300
<i>T. rhodesiense</i>	3202	6*	1 : 534
<i>T. pecorum</i>	3202	2	1 : 1600

* In our second interim report, one experiment in which *T. rhodesiense* was obtained, was inadvertently omitted, so that this parasite was isolated in 6, instead of 5 instances. In addition, Monkey No. 100 became infected four days after the flies had been fed on it. As the animal died the same day we were unable to decide the species of trypanosome present, but from the short incubation period it is highly probable that the parasite was *T. rhodesiense*. On this assumption the ratio of infected to non-infected flies would be 1 to 455.

III. DESCRIPTION OF TRYPANOSOMES

1. *Trypanosoma rhodesiense*
2. *Trypanosoma pecorum*

In a previous report, 1912 (a), it was stated that *Trypanosoma pecorum* had been isolated from one group of flies only. This statement now requires to be qualified, inasmuch as in Experiment No. 353 both *Trypanosoma pecorum* and *Trypanosoma ignotum*, sp. nov., were obtained. In stained preparations of the blood of

Pathogenicity

In monkeys, the virulence of the trypanosome was found to be very great. The disease is of a fulminating character, the parasites increasing rapidly in number until the animal's death. The trypanosome is of equal virulence in those animals infected directly by the bites of the tsetse flies and in those cases where the strain was passed from monkey to monkey. The incubation period varied from three to ten days, the average being seven, and death occurred two or three days after the parasites appeared in the peripheral blood.

It is interesting to note that on reaching the plateau three monkeys failed to become infected when inoculated with the Valley strain of the parasite. At Nawalia a failure was not recorded, and it is difficult to understand those mentioned, more particularly as the parasite is of frequent occurrence in the plateau flies and monkeys infected with this strain react in the same manner as those infected by the Valley flies.

A rabbit was successfully infected with the strain by subinoculation from a monkey. The incubation period was 22 days, and the duration of the disease 66.

One guinea-pig, five rats, and four mice were found to be refractory. Moreover, negative results were obtained by feeding infective flies on rats.

TABLE 4.—Pathogenicity of *Trypanosoma ignotum*, sp. nov.

Animal	Number used	Incubation period days	Duration days
Monkey	20	3-10, average 7	5-14, average 10
Rabbit	1	22	66
Guinea-pig	1	Negative	
Rat	5	„	
Mouse	4	„	

Diagnosis

Morphologically the parasite appears to be distinct from any hitherto described species. The graph showing the distribution of the trypanosomes in respect of length resembles very closely that of *Trypanosoma uniforme*, but the parasites are at once distinguished by the fact that whereas *Trypanosoma uniforme* is invariably furnished with a free flagellum this, as mentioned above, is of rare occurrence in *Trypanosoma ignotum*, sp. nov. Moreover, the difference between the two trypanosomes is clearly demonstrated by the reaction of subinoculated animals. According to the Royal Society's Commission (1911), *Trypanosoma uniforme* is innocuous to monkeys, an observation which has been confirmed by Fraser and Duke (1912), who record that they were unable to infect these animals by subinoculation from game harbouring the parasite, although goats were readily infected. Whether or not the trypanosome with which we are dealing will infect cattle and goats we are, at present, not in a position to state, but the fact that a large number of monkeys, and one rabbit, quickly succumbed to the disease indicates clearly that the two parasites are not identical.

No information is at present available regarding the original host of the trypanosome. Although the parasite has been isolated from wild *Glossina morsitans* much more frequently than any other trypanosome, it has never been found in game or domestic stock. Nothing resembling it has been seen in the peripheral blood of any animal examined in the Luangwa Valley and on the Congo-Zambesi watershed. These include 174 wild animals (elephant, rhinoceros, hippopotamus, buffalo, eland, zebra, wildebeest, roan, kudu, hartebeest, true waterbuck, Crawshay's waterbuck, puku, mpala, bushbuck, duiker, klipspringer, bushpig, warthog, lion, hunting dog, caracal, galago, squirrel, genet, giant rat and rabbit), 35 domestic animals (cattle, goats, and dogs), 203 monkeys, 128 wild rats, and 15 wild mice—making a total of 555. Fifty-seven monkeys have been subinoculated from game and domestic animals, and in no instance has an infection with this trypanosome been observed.

In view of the fact that we have been unable to find the vertebrate host, we propose to name the parasite *Trypanosoma ignotum*.

Pathogenicity

In monkeys, the virulence of the trypanosome was found to be very great. The disease is of a fulminating character, the parasites increasing rapidly in number until the animal's death. The trypanosome is of equal virulence in those animals infected directly by the bites of the tsetse flies and in those cases where the strain was passed from monkey to monkey. The incubation period varied from three to ten days, the average being seven, and death occurred two or three days after the parasites appeared in the peripheral blood.

It is interesting to note that on reaching the plateau three monkeys failed to become infected when inoculated with the Valley strain of the parasite. At Nawalia a failure was not recorded, and it is difficult to understand those mentioned, more particularly as the parasite is of frequent occurrence in the plateau flies and monkeys infected with this strain react in the same manner as those infected by the Valley flies.

A rabbit was successfully infected with the strain by subinoculation from a monkey. The incubation period was 22 days, and the duration of the disease 66.

One guinea-pig, five rats, and four mice were found to be refractory. Moreover, negative results were obtained by feeding infective flies on rats.

TABLE 4.—Pathogenicity of *Trypanosoma ignotum*, sp. nov.

Animal	Number used	Incubation period days	Duration days
Monkey	20	3-10, average 7	5-14, average 10
Rabbit	1	22	66
Guinea-pig	1	Negative	
Rat	5	"	
Mouse	4	"	

Diagnosis

Morphologically the parasite appears to be distinct from any hitherto described species. The graph showing the distribution of the trypanosomes in respect of length resembles very closely that of *Trypanosoma uniforme*, but the parasites are at once distinguished by the fact that whereas *Trypanosoma uniforme* is invariably furnished with a free flagellum this, as mentioned above, is of rare occurrence in *Trypanosoma ignotum*, sp. nov. Moreover, the difference between the two trypanosomes is clearly demonstrated by the reaction of subinoculated animals. According to the Royal Society's Commission (1911), *Trypanosoma uniforme* is innocuous to monkeys, an observation which has been confirmed by Fraser and Duke (1912), who record that they were unable to infect these animals by subinoculation from game harbouring the parasite, although goats were readily infected. Whether or not the trypanosome with which we are dealing will infect cattle and goats we are, at present, not in a position to state, but the fact that a large number of monkeys, and one rabbit, quickly succumbed to the disease indicates clearly that the two parasites are not identical.

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In view of the fact that we have been unable to find the vertebrate host, we propose to name the parasite *Trypanosoma ignotum*.

Transmission

In one experiment the infective fly was determined to be one of a group of ten. These were then killed and dissected. Nine of the flies were found to show no trypanosomes in the gut, proboscis, salivary glands, or sucking stomach, whereas in the tenth a heavy infection of the proboscis was encountered. The gut, salivary glands, and sucking stomach were negative. This observation would indicate that the development of the trypanosome occurs in the proboscis.

**IV. TRANSMISSION OF *TRYPANOSOMA VIVAX* AND
*TRYPANOSOMA NANUM***

We are unable, at present, to make any definite statement on this subject, but might record that the goats on which the flies used for breeding purposes were fed became infected with both these trypanosomes. Unfortunately, we cannot exclude, with certainty, the possibility of the goats having been bitten by flies other than *Glossina*. More exact experiments to determine the point are now in progress.

V. CONCLUSIONS

The following trypanosomes, *T. rhodesiense*, *T. pecorum*, *T. ignotum*, sp. nov., and probably also *T. vivax* and *T. nanum*, are transmitted, in nature, by *Glossina morsitans* in the Luangwa Valley.

NGOA, N. RHODESIA,
June 20, 1912.

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EXPERIMENTS WITH CRUDE CARBOLIC ACID AS A LARVICIDE IN BRITISH GUIANA

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CRUDE CARBOLIC ACID AS A LARVICIDE

The question of the choice of a larvicide in British Guiana is somewhat difficult. Paraffin, either in its pure or crude state, and even heavy petroleum oil, is useless, except in a few isolated cases. Most of the ponds and small temporary areas of water must be treated with a soluble form of larvicide, as owing to the strong wind always blowing, also the absolutely flat open nature of the ground, anything floating on the surface, such as oil is quickly blown away to one side and evaporated before its asphyxiating properties come into play.

In the case of large areas of water such as drainage trenches, the breeding of larvae is kept within bounds by keeping them well stocked with the small fishes known locally as 'Cockerbellies,' and clearing away all floating vegetation as far as possible. But these fish are in many cases unable to gain access to the small depressions in the land holding temporary water, and places such as recently dug pits for earth to burn, footprints of cows, etc.; it is in these cases that we have now adopted crude carbolic acid as combining the most important features of cheapness and efficiency.

It was employed with complete success during the camping out of the Local Forces. A few days before the camp was pitched the ground was thoroughly surveyed especially to windward of the camp site. All puddles were, as far as possible, connected by narrow

drains into deepish pits and treated with crude carbolic acid, isolated areas were treated separately, and the large trenches to windward especially cleared from vegetation and well stocked with fish.

In most of the small pits, when treated with carbolic acid, the larvae were seen to die in about an hour, but in the case of pupae the time was much longer (frequently as much as twenty-four hours), but usually when examined carefully the following day after treatment no larvae or pupae were seen alive.

We are inclined to the belief that, in the case of a temporary small collection of water, if crude carbolic acid is applied it does not evaporate to any large extent but concentrates as the puddle dries up, and further, that, having dried up, the ground is sufficiently impregnated with the carbolic acid to render water deposited later by a shower of rain fatal to larvae. Sufficient data have, however, not yet been collected to make an authoritative statement on this point. As will be seen by the laboratory experiments herewith, a dilution of 1 in 20,000 is efficient as regards all larvae inside two hours, but in the case of pupae a much longer time is required—probably owing to the fact that pupae possesses a thick chitinous shell and have no alimentary canal into which to take the poison.

For use we calculate one teaspoonful to every two cubic feet of water or one ounce to 16 cubic feet; this gives a dilution of about 1 in 16,000, and allows a fair margin of safety to cover errors in calculation.

The crude carbolic acid containing all its impurities, such as cresol, rosolic acid, oily and tarry substances, is much more efficient than the purified, more highly soluble product, possibly owing to its sticky nature making it more adherent to the larvae and pupae, also no doubt in a few cases blocking up the syphon tubes.

Experiment II bears out this point. In this series of experiments the crude carbolic acid was first freed from its oily and tarry substances by filtration before use. As will be seen in this form, the toxic effects are developed more quickly, but it is not efficient in such high dilutions; as in a dilution of 1 in 20,000 half the larvae and all the pupae were alive at the expiration of three hours; whereas in the case of crude carbolic acid it was fatal to all the larvae in one and a quarter hours.

With regard to the effects on animals, the dilution used would not be fatal, as $12\frac{1}{2}$ gallons would only contain one drachm (60 minims) of carbolic acid—not a very serious thing for an animal capable of drinking $12\frac{1}{2}$ gallons at one drink.

The water treated in this manner has a distinctly tarry odour, and animals do not drink it at all readily on this account; and, further, the water treated in this way acquires a blackish colour.

We have not, however, found crude carbolic acid useful for spraying large areas with a Mackenzie spray, owing to the fact that some of its constituents are not soluble in water; but by constantly stirring the mixture in a bucket during the process a fairly satisfactory distribution can be obtained. This, however, requires supervision. When a large area of ground, containing a number of small holes, such as footprints, has to be treated it has been found more satisfactory to use preparations more freely miscible with water, such as Cyllin, Chloro-Naphtholeum or Sanitas Okol, although the expense is much greater.

EXPERIMENT NO. 1.—Crude Carbolic Acid

No.	Dilution	Larvae		Pupae	
		Condition of larvae	Exposure time	Condition of pupae	Exposure time
1	1 in 250	All dead	$\frac{3}{4}$ hour	All dead	$\frac{3}{4}$ hour
2	1 in 500	"	"	"	"
3	1 in 750	"	"	"	"
4	1 in 1000	"	"	"	"
5	1 in 1500	"	"	"	"
6	1 in 2000	"	1 hour	"	10 hours
7	1 in 4000	"	"	"	12 hours
8	1 in 6000	"	"	"	"
9	1 in 8000	"	"	"	"
10	1 in 10000	"	"	"	"
11	1 in 12000	"	"	"	17 hours
12	1 in 14000	"	"	"	"
13	1 in 16000	"	$1\frac{1}{4}$ hours	"	"
14	1 in 20000	"	"	"	"
15	1 in 25000	"	24 hours	1 alive only	24 hours
16	1 in 30000	6 alive	"	2 alive	"
17	1 in 40000	All alive	"	All alive	"
18	1 in 50000	"	"	"	"
19	1 in 60000	"	"	"	"

THE MORPHOLOGY OF *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* IN CULTURES: AND A COMPARISON WITH THE DEVELOPMENTAL FORMS DESCRIBED IN *GLOSSINA PALPALIS*

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I. INTRODUCTION

This research was undertaken at the suggestion of Sir Ronald Ross under funds allotted by Sir Edwin Durning-Lawrence (Bart.), in the case of one of us (J.G.T.), and under a grant from the Post-

Graduate Research Fund of the Queen's University of Belfast in the case of the other (J.A.S.). We wish to acknowledge our indebtedness to Sir Edwin Durning-Lawrence, Bart., and to the Queen's University of Belfast for their generous help, and to thank Sir Ronald Ross, Dr. J. W. W. Stephens, and Dr. H. B. Fantham for their kind interest and the valuable aid given to us.

The results recorded in this paper have been obtained from experiments conducted in the Liverpool School of Tropical Medicine during a period of over six months. We have directed our attention to the cultivation of trypanosomes pathogenic to man, namely, *Trypanosoma gambiense* (Dutton) and *Trypanosoma rhodesiense* (Stephens and Fantham).

The first experiments were made only with *Trypanosoma rhodesiense*, and a preliminary note by one of us (J.G.T.) was published in the Annals of Tropical Medicine and Parasitology, in May, 1912, giving a short account of the cultivation of this trypanosome.

It was noted that this trypanosome in culture assumed morphological characters which strongly suggested that we were obtaining in culture tubes a development similar to that which takes place in the intestinal tract of tsetse flies. On this account it was decided to attempt the cultivation of *T. gambiense* in order to compare its cultural morphology with the developmental forms of this trypanosome found by Sir David Bruce and his colleagues (Captains Hamerton, Bateman and Mackie) (1911 b) in *Glossina palpalis*. Unfortunately there is at present no description of the developmental characters of *T. rhodesiense* in the tsetse-fly, so that in this paper it is only possible to compare the cultural characters of this trypanosome with those of *T. gambiense*. We await, therefore, with great interest, a description of the morphology of *T. rhodesiense* in the fly, so that a comparison may be made with the forms found in culture.

II. HISTORICAL

Numerous attempts have been made, with more or less success, to cultivate pathogenic trypanosomes ever since MacNeal and Novy (1903) found that it was possible to cultivate *T. lewisi*, for apparently an indefinite period, on an artificial medium composed of a mixture

of nutrient agar and defibrinated blood. Novy and MacNeal (1903-1904) succeeded in cultivating *T. brucei*, but their results were not so successful as in the case of *T. lewisi*, although they managed to get sub-cultures and to infect animals from these. The cultural characters of this trypanosome were found by them to differ essentially from those of *T. lewisi*.

Novy, MacNeal and Hare (1904) were also successful in cultivating *T. evansi*, but were unable to obtain sub-cultures, and inoculation experiments gave negative results. The results of Novy and MacNeal with *T. lewisi* and *T. brucei* were confirmed by Smedley (1905) in most of the important details, but this observer was unable to cause infection by inoculating cultures of *T. brucei*.

The first record of any attempt to cultivate the trypanosomes pathogenic to man was that of Thomas and Breinl (1905). These observers succeeded in keeping *T. gambiense* alive ('by transference from tube to tube') for sixty-eight days on a blood agar medium composed of blood and nutrient agar made up with veal or chicken meat infusion. In the next year Gray and Tulloch (1906) using a blood agar medium made with dog's blood which had not been defibrinated, succeeded in keeping cultures of *T. gambiense* alive until the twentieth day, when the cultures were overrun by a growth of cocci. Sub-cultures were unsuccessful. These observers noted the resemblance of the cultural forms to those found in the tsetse-fly after feeding on animals infected with *T. gambiense*. They found on the fifteenth day very large forms sometimes 54μ in length. Laveran and Mesnil attempted to cultivate *T. gambiense* in blood agar tubes, and found motile trypanosomes 35 to 40μ long twenty days after.

Various other trypanosomes have been successfully cultivated, and an excellent epitome of this subject will be found in the Sleeping Sickness Bulletin (1909).

III. TECHNIQUE

(a) *Culture Media.* Many experiments have been made during this investigation in the endeavour to obtain a suitable medium. Fluid media such as nutrient broth and horse serum to which haemoglobin had been added proved unsuccessful. The following fluid medium, however, was found to be of great use. Human pleuritic fluid

was rendered complement free by heating to 45°C. for one hour. This was added to small sterile test tubes, about 1 c.c. to each tube, and into each was introduced about one-third volume of citrated human blood. These were then placed in a water bath at 45°C. for half an hour. In this culture fluid it was found that both *T. rhodesiense* and *T. gambiense* developed, and in one tube we found *T. rhodesiense* continued to exist for twenty days at room temperature. This is most interesting when we take into consideration the fact that we were experimenting with human blood, and the trypanosomes used were those pathogenic to man. From this medium sub-cultures were successfully obtained in the modified Novy-MacNeal-Nicolle medium described below.

In a preliminary note on the cultivation of *T. rhodesiense* (1912), it was announced that this trypanosome had been grown on a modification of the Novy-MacNeal-Nicolle medium (Nicolle, 1908), made by substituting for defibrinated rabbit's blood, citrated rat's blood heated to 45°C. for half an hour, and by using sea salt for ordinary sodium chloride. Since writing this, however, it has been found that pure sodium chloride is probably just as efficient in making up the medium. Most of our experiments have been made with this modification of the Novy-MacNeal-Nicolle medium, and we have so far found it the most suitable for cultures and sub-cultures of both *T. rhodesiense* and *T. gambiense*. A full description of the technique used in its preparation is given below. The agar medium is made up as follows:—Agar, 14 grams; sodium chloride (pure), 6 grams; and distilled water, 900 c.c. The agar must previously be washed thoroughly to remove, as far as possible, all impurities. This is done by allowing it to soak for about thirty-six hours in distilled water, which should be frequently changed. This agar medium was then added in quantities of 3 c.c. to sterile test tubes, and sterilised by heating to 100° C. for twenty minutes on three successive days.

A sterile mixture of rat's blood and sodium citrate solution, 1%, is now prepared by the following method. Into a sterile syringe draw up 2 c.c. of a 1% solution of sodium citrate, and then kill a normal rat. Sear the surface of the right ventricle, insert the needle of the syringe into the heart and draw up the blood into the syringe.

About 4 c.c. of blood may be procured from a small rat, but of

course the amount varies according to the size of the animal used. Thus a mixture of rat's blood and 1% sodium citrate solution in a proportion of about 2 to 1 is obtained. The melted agar is cooled to a temperature of 45°C., and at least an equal quantity of citrated blood is added to each tube. Here it is of interest to note that Novy and MacNeal (1904) when cultivating *T. brucei* also found that it was necessary to add at least an equal quantity of blood to the agar in order to obtain a successful culture. These tubes are kept in a water bath at a temperature of 45°C. for half an hour, in order, as far as possible, to destroy the complement without making any appreciable change in the character of the blood. The tubes are sloped, and when cool are protected with rubber caps to prevent evaporation. They are then placed in an incubator at 25°C. for two days to allow the water of condensation to collect, and also to permit of any contamination being observed. In our experience this has proved the most satisfactory medium, but we find that citrated human blood treated in the same way is also good. The fact that in our experiments citrated blood was more satisfactory than defibrinated blood is of interest because Plimmer and Bradford (1899) found that the addition of sodium citrate solution to blood containing *T. brucei* prolonged the vitality of that trypanosome, and also because Rogers (1904) was successful in growing *Leishmania donovani* in citrate solution.

Since experimenting with these media, Dr. Row, of Bombay, has very kindly sent to us a paper (1912) on the preparation of a fluid medium for the cultivation of Protozoa, and we think it might be most suitable for the growing of trypanosomes, but so far we have been unable, through lack of time, to give it a proper trial.

It seems that one of the essential factors for the development of trypanosomes is the presence of a certain amount of fresh free haemoglobin, and under such conditions it is natural that forms similar to those found in the gut of the tsetse-fly will develop, because whenever a fly sucks blood the process of digestion soon breaks up the corpuscles, thus setting free the haemoglobin necessary for the further development of the trypanosomes.

Sir David Bruce and his colleagues (1909b) found that about 5% to 8% of *Glossina palpalis* became infective after feeding on a case of sleeping sickness, and suggested that this was comparable

to that which occurs in cultivation where only a few tubes owing to some unknown reason develop. Novy and MacNeal (1904) in growing *T. brucei* failed twenty-five times in succession to get cultures, and in another series of experiments only four out of twenty-five attempts were successful. We have found the same difficulties in our experiments, and many tubes have been inoculated unsuccessfully without any apparent reason. This difficulty of always obtaining a culture shows that it is necessary to inoculate a large number of tubes in order that at least one or two successful growths may be obtained, and the same applies to sub-cultures which should be made in batches. If this is not done we are almost certain, except when very lucky, to have disappointments, and this uncertainty of obtaining a culture shows that a medium might easily be rejected as being of no use simply because a sufficient number of trials had not been made. The same difficulty makes it uncertain what the best formula for a medium really is, but we think that the modification of Novy-MacNeal-Nicolle medium described in this paper, if carefully tried, will be found to be successful. Strict asepsis must be observed in all cases, because it was found if the cultures became contaminated the trypanosomes died out very rapidly.

(b) *Inoculation of media.* The blood used to inoculate tubes was obtained, with aseptic precautions, from the heart of an infected animal which had just been killed. We find, like Smedley (1905), that a rat is the most suitable animal from which to obtain this blood. It is advisable not to add too much blood, and it seems also best to inoculate trypanosomes when there are many of the stout forms present in the blood. It is most difficult, however, to make any definite statement regarding the most suitable time to inoculate from an animal. At first it was thought that it was best to inoculate the tubes early during an infection and so introduce young trypanosomes, but we have found since then that trypanosomes inoculated later, after infection had lasted for a considerable period, also developed well in culture tubes. After many experiments we have concluded that only about three or four drops of blood ought to be added to 1 c.c. of condensation water, and we also think that probably the number of stout forms present in the blood plays an important rôle in development. This may or may not be the case, since it is so difficult to exclude the uncertainty of the medium.

(c) *Incubation of cultures.* Our cultures were usually incubated at a temperature of 22°C. to 24°C. It was found that higher temperatures make the trypanosomes develop more rapidly, but on the other hand they more quickly disappear from the culture fluid. Cultures grown at a temperature of 28°C., like those described in the preliminary note on *T. rhodesiense* (1912), only showed flagellates for seven days. This rapid growth is a disadvantage in that it does not admit of sufficient time to make complete observations of the cultures. The effect of temperature on the cultures has been pointed out by Novy and MacNeal (1904), who found that the higher temperatures not only produced a more rapid developmental change in the trypanosomes themselves, but also more quickly altered the medium. Roubaud (1909) stated that a temperature of 28°C. caused *T. gambiense* to disappear more rapidly from the gut of tsetse flies than lower temperatures did.

(d) *Sub-cultures.* Two or three loopfuls from a successful culture are transferred to a new tube by means of a sterile platinum wire, and in a culture grown at a temperature of 22°C. to 24°C. it is found that the sixth or seventh day is usually the most suitable for making such an inoculation. Novy and MacNeal (1904) in cultivating *T. brucei* also found this to be the most suitable time. The success of sub-cultures made at this time appears to be due to the predominance of 'healthy developing forms.'

(e) *Examination of cultures. Fresh specimens.* The cultures were examined in fresh preparations every twenty-four hours, and we also kept culture fluid under observation for twenty-four hours by using a hollow slide and cover-glass sealed with vaseline. By these methods the cultures were examined when the trypanosomes were alive, and thus movements, division, and other phenomena could be carefully observed.

Stained specimens. These cultures were most difficult to stain on account of salts and free haemoglobin, and most disappointing results were often obtained, but the following method carried out carefully gives very good slides. To a perfectly clean slide convey a platinum loop full of culture fluid. Shake up the culture fluid thoroughly before doing so. Expose this drop to the vapour of a 4% osmic acid for about 30 seconds, and then with the edge of a piece of cigarette paper make a smear. Expose this smear again

to the osmic acid vapour for a few seconds, and then fix the slide in pure methyl alcohol for twenty minutes. Dry, using no heat. Now take a piece of cigarette paper, cover the film with it, and carefully pour on it some Giemsa stain (1 drop to 1 c.c. of distilled water). Allow this to remain on the slide for two or three hours, the cigarette paper acting as a filter and preventing deposit. Wash the slide thoroughly in tap water, and examine before drying under the low and high powers of the microscope so as to determine the depth of staining, which may be too faint. If the stain is too faint pour on some fresh Giemsa for five or ten minutes, and then again wash thoroughly. It is important that the stain used has not deteriorated as so often happens with Giemsa stain if kept for any length of time. If it should happen that the slide is badly stained and looks too blue, it may be restained successfully by decolorising with methyl alcohol and staining several hours film downwards in Giemsa.

IV. CULTURAL CHARACTERS

(a) *Morphology of T. gambiense and T. rhodesiense in Cultures.* As the behaviour of these two species of trypanosomes in cultures is very similar, it is proposed to discuss their cultural characters together and afterwards to describe any points of difference we have observed in these characters. After a period of twenty-four hours it is found on examining some of the culture fluid that many of the trypanosomes have become motionless, many have their flagella torn off, and many are rounded up and disintegrated (Pl. XX, fig. 36). In stained specimens there is also evidence of the death of many of the trypanosomes as is shown by the fact that they may be found in all stages of disintegration. In some the nucleus is broken up, in others it is altogether absent. Many stain badly and are filled with vacuoles. However, in fresh specimens very active trypanosomes are still found, the majority of which appear to be of the 'stumpy' type and average about $20\ \mu$ in length (Pl. XIX, fig. 3.) These forms in stained preparations have well defined and well stained nuclei, and a short free flagellum; the protoplasm stains a dark blue and may contain a few fine chromatin granules. Bruce (1911b) figures stout forms during the first few days after the ingestion of *T. gambiense* by *G. palpalis*. The survival of these stout forms was also found by

Roubaud (1909) and Kleine and Taute (1911) in the gut of *G. palpalis* fed on animals infected with *T. gambiense*. Swellengrebel (1911) also states that the stout forms are more resistant to unfavourable influences than the more slender forms in the blood of mice which had died from an infection of *T. gambiense*. This observer suggests that very probably the thick forms found in the gut of *G. palpalis* and the blood of dead mice are the more resistant, and that the predominance of these forms at the beginning of the development in the fly can be thus explained. From this it would appear that the stout forms found in the blood might possibly be forms specially adapted for the further development in the fly, and this may explain the reason why we have found that cultures were more successful if made when the stout forms found in the blood predominate. At the end of forty-eight hours the active trypanosomes are usually fewer in number and difficult to find. The surviving forms do not differ much from those seen in the twenty-four hour old cultures (fig. 35). During these two days, and also during the whole period of cultivation some of the trypanosomes may show numbers of large purple staining granules scattered through the protoplasm, the exact significance of which at present seems to be uncertain (figs. 26, 49, and 52). No appreciable change in the position of the blepharoplast is to be noted. In some cultures on the third, fourth, and fifth days the trypanosomes may be so scanty as to have apparently disappeared entirely from the medium, but usually they may be found in these days although they may not be in great numbers. Irregular clumps of trypanosomes formed around masses of granular débris may be seen which are probably due to agglomeration.

It is usually about this time in cultures, as well as in sub-cultures, that large plasmodial masses with many nuclei and flagella corresponding to 'some of the more exaggerated types of degeneration forms' described by Bruce, etc. (1911b) in the gut of the tsetse-fly appear (fig. 25). That some of these are degeneration forms seems probable, because they are usually more frequently found in cultures which die off quickly, and because they stain badly. But all these multinucleated 'mis-shaped masses of protoplasm' do not appear to be degenerated because some of them stain well, while in fresh preparations some of them have been seen to give off by

a process of multiple fission daughter trypanosomes which are apparently normal (fig. 24).

It is at this period, third, fourth, and fifth days, that any distinct increase in size of the trypanosomes can be made out (figs. 4 and 10). Forms $30\ \mu$ in length by 3 or $4\ \mu$ in breadth are fairly common. The nucleus in these trypanosomes is large, oval in shape, compact, and well stained. The blepharoplast in some forms is rod-shaped, and has moved distinctly nearer to the nucleus giving the trypanosome a 'snouty' appearance. The protoplasm tends to stain a very dark blue and sometimes may contain numbers of purple granules, the so-called 'volutin' granules. The flagellum shows practically no free portion, and is seen to arise in many cases from a small red staining area immediately anterior to the blepharoplast. It is closely applied to the body of the trypanosome so that the undulating membrane is very narrow. These are very similar to the so-called 'female forms' figured by Kleine and Taute (1911).

The sixth, seventh, and eighth days. During this period there is usually marked multiplication of the trypanosomes in the tubes. On the sixth day the trypanosomes may be still scarce in numbers, but in most of the culture tubes this time seems to be the beginning of a period of great activity. The trypanosomes are all very large forms more than $40\ \mu$ in length have been observed (fig. 4), many of which are undergoing unequal longitudinal division (fig. 22). It is during these days that division rosettes are first seen (fig. 50). In the early stages these are composed of only a few trypanosomes (Pl. XXI, fig. 1), but later on colonies, similar to those described by Smedley (1905) in cultures of *T. brucei*, containing as many as a hundred or more, may be seen (Pl. XXI, fig. 2). The trypanosomes which compose these rosettes are attached centrally by their posterior ends, their flagellar ends being directed towards the periphery. This arrangement of the rosettes in *T. gambiense* and *T. rhodesiense*, with the flagella directed towards the periphery, resembles that of *T. brucei* as found by Novy and MacNeal (1904) and Smedley (1905), and differs from that of *T. lewisi*, where the flagella are directed centrally, as has been pointed out by these observers. In fresh specimens the individual trypanosomes show very active movements of the flagellar ends, and their protoplasm contains numerous highly refractile granules

which were not always observed in stained preparations. The movements are so active that it is difficult to watch division take place, but in small rosettes actual increase in numbers can be observed. Frequently in association with the larger rosettes are seen rounded bodies varying in size and filled with refractile granules, but what part these take in the development is at present uncertain. Besides these rosettes numerous free forms occur either singly or in pairs attached by their posterior extremities. The form which predominates at this period is one which corresponds exactly to that which Bruce (1911b) calls the 'healthy normal developing type in the intestine of the fly' (figs. 5 to 10, 43 to 49, and 53 to 61). We cannot do better than give his description of this type. 'This is a long moderately broad form, the protoplasm staining well, without granules or vacuoles, having an oval compact nucleus situated in the centre of the body, a small round micronucleus lying at some distance from the elongated snout-like posterior extremity, the undulating membrane, narrow and simple, and the flagellum proceeding little, if anything beyond the protoplasm of the cell. The flagellum also appears very frequently to arise from a pink-coloured body situated near the micronucleus, an appearance never seen in the normal blood trypanosome. This seems to be the healthy normal developing type in the intestine of the fly.' In cultures these forms were found to measure between $25\ \mu$ and $35\ \mu$ in length, and about $2\ \mu$ to $3\ \mu$ broad. The shape of the posterior extremities of these forms varies. In some the 'snout' is blunt (figs. 5 to 8, 47 and 59), while in others it is sharper (figs. 9, 43 to 46). The position of the blepharoplast in relation to the nucleus in both *T. gambiense* and *T. rhodesiense* varies from forms where it lies in close proximity to the nucleus, to forms in which it is situated midway between the nucleus and the posterior extremity of the trypanosome. We have never seen any true crithidial forms in cultures. Such trypanosomes have a very rapid and characteristic movement like that described by Roubaud (1909) in similar forms found in *Glossina palpalis*. The trypanosomes move forward with the anterior end in advance and this has a peculiar wriggling movement, while the posterior portion behind the blepharoplast remains rigid. About this stage of development very long thin forms ($15\ \mu$ to $30\ \mu$ in length by 0.75 to $1.5\ \mu$ in breadth) may be seen splitting off in fresh preparations

from some of the larger trypanosomes. This mode of division closely resembles the formation of the 'spirillar' forms (figs. 39 to 41) described by Leishman and Statham (1905) in cultures of *Leishmania donovani*. This may be seen occurring at any stage of the culture, but appears to be more prevalent about the eighth or ninth day. The movement of these forms is much more active than that of the stouter forms.

On the ninth, tenth, and eleventh days large numbers of the 'normal developing' forms, many of which are undergoing division, are found, but during this period a number of other types are seen, some of which correspond to the 'small developmental forms' of Bruce and his colleagues (1911b), and others are somewhat similar to the so-called 'male' trypanosomes of Kleine and Taute (1911). Numerous other forms are also present. The 'small developmental forms' (figs. 15 to 17, 42 and 58) are very similar to the 'normal developing forms,' and differ only in the facts that they are more stumpy in character and only measure about $20\ \mu$ by $3\ \mu$, but intermediate types between these two forms may be found (figs. 18 to 20). The slender forms found in cultures resemble to a certain extent only the so-called 'male' trypanosomes of Kleine and Taute in that they are long and very slender, do not stain very deeply (taking up a pinkish colour), and have the blepharoplast fairly close to the nucleus, but differ from them because in no case have we seen the crithidial types depicted by these observers. These cultural forms resemble more closely the slender forms described by Bruce and his colleagues (1911b), and in our cultures were comparatively rare (figs. 11 to 14, 39 to 41 and 46). After the twelfth day the cultures slowly die off, but a few motile trypanosomes may be seen as late as the twentieth day. During this period very large, swollen, multinucleated trypanosomes may be seen which are very similar to those which Bruce calls the 'degenerative forms of the normal reproductive type.' (fig. 24).

The degeneration and disappearance of the trypanosomes in culture tubes is probably due to the exhaustion of the food supply, as well as to the production of waste products, to changes in the haemoglobin, and to evaporation. This is analogous to the statement of Bruce and his colleagues (1911b) that 'when a fresh supply of blood is taken in by the fly, this type (healthy normal developing

type) can be imagined to multiply with extraordinary rapidity. When the blood supply runs low then this type can also be imagined as degenerating and disappearing just as rapidly.' The sequence of changes described above in the cultures has been found in all our successful experiments, but the rate of development seems to depend on various factors, such as the incubation temperature, composition of the medium, etc.

(b) *Further History in sub-cultures.* Sub-cultures made between the seventh and tenth days were found to be more successful than those made at other dates. The reason of this appears to be that about this time the normal developing forms are more numerous. In these sub-cultures during the first days few or no trypanosomes may be seen, but about the seventh and eighth days small symmetrical division rosettes begin to appear attached centrally by their posterior extremities with the flagellar ends directed outwards. During the next few days these masses may increase in size up to many hundreds, and the culture fluid at the same time shows numerous very actively motile trypanosomes.

The duration of life in these sub-cultures varies as in the case of the original cultures from 10 to 17 days. In the case of *T. rhodesiense* the second generation showed very active development, but although the trypanosomes could be found in the third generation for several days, it was impossible to be sure that any actual multiplication had taken place in this sub-culture.

With *T. gambiense* on the other hand, sub-cultures were much more successful, and active multiplication was seen in the fourth generation as late as the thirty-seventh day. This sub-culture is still under observation. In some of these later sub-cultures of *T. gambiense* very large tangled masses, comprising many thousands of struggling trypanosomes, were seen presenting a very wonderful appearance. Such an enormous proliferation occurring at this time would appear to indicate that the trypanosomes had become accustomed to their new environment.

In all stages of the cultures rounded bodies may be present. Some of these, from their marked vacuolated appearance in fresh specimens and from their poorly stained protoplasm and fragmented nucleus, are evidently degenerate forms (fig. 29), but there frequently occur other forms which, from their definite outline, well-stained

protoplasm, compact nucleus and distinct blepharoplast (figs. 30 and 62), we do not think to be degenerate, but rather to be of a similar nature to rounded bodies described by Fantham (1911), who showed that rounded bodies could be inoculated into rats and produce trypanosomiasis. The same observer also showed that rounded or non-flagellated bodies grew and flagellated, and so turned into trypanosomes when placed in fresh, warm uninfected blood. Novy and MacNeal (1904) found similar rounded bodies in *T. brucei* cultures and concluded they were 'death changes,' because attempts to infect animals and obtain sub-cultures with them were negative.

On two separate occasions in sub-cultures of *T. rhodesiense* there was observed a definite cyst-like structure, $41\ \mu$ long by about $35\ \mu$ broad, with distinct capsule apparently composed of several layers, and this cyst was closely packed with hundreds of small oval bodies about $2\ \mu$ in length. Although kept under observation for over twenty-four hours no change in these bodies was observed. In spite of the absence of any evidence of bacterial or fungoid contamination we are unable to say whether this was a stage in life history of *T. rhodesiense* or merely a body of extraneous origin.

(c) *Comparison between the cultural character of T. gambiense and T. rhodesiense.* In our experience *T. gambiense* seems to be more easily cultivated than *T. rhodesiense*. Up to the present time sufficient cultures have not been carefully studied to state definitely whether it is possible to make a diagnosis between cultures of *T. rhodesiense* and *T. gambiense*. The life cycle and general morphology in both seems similar, but in all stages of the cultures of *T. rhodesiense* it is comparatively common to find forms both thin and stout with the nucleus in the posterior third or fourth of the trypanosomes (figs. 37 to 42, 47, 51, 58 and 60), that is posterior nuclear forms as described by Stephens and Fantham (1910). On the other hand in cultures of *T. gambiense*, although the nucleus may not be central, it is very rare to find forms with the nucleus even in the posterior third (fig. 21). This morphological difference has been well marked in the cultures examined during this investigation up to the present. On reference to the accompanying plates these differences are seen. These facts seem to strengthen and support the specific differences between *T. gambiense* and *T. rhodesiense*.

V. INFECTIVITY OF CULTURES

In cultures infectivity was retained as late as the third day, probably owing to the presence of some of the original blood forms inoculated, but after this day we have never succeeded in producing an infection though cultures of varying ages up to twenty-five days have been tried. As Bruce and his colleagues (1911a, 1911b) have found that the infectivity of *G. palpalis* seems to depend on the presence of trypanosomes of the blood type, which appear in the salivary glands about the twenty-fifth day, the non-infectivity of our cultures would seem to be due to the absence of these forms. It is possible that these forms appearing at a later date might render the cultures infective, but up to the present they have never been observed to occur in the cultures.

In the case of *T. lewisi*, Minchin and Thomson (1911) have found that the infectivity of the intestinal contents of an infected flea (*Ceratophyllus fasciatus*) depends upon the presence of stumpy forms which appear about the fifth or sixth day. The life cycle of *T. lewisi* in this insect host appears to differ from that of *T. gambiense* in the fact that the whole cycle is completed in the intestine, whereas in *T. gambiense* the infective forms are only found in the salivary glands. This might possibly be the reason why cultures of *T. lewisi* are infective from a very early date, whereas in *T. gambiense* it is possible that the developing trypanosomes may require some change to an environment similar to that of the salivary glands of the tsetse-fly before they regain their infectivity. Roubaud (1909) found that developmental forms of *T. gambiense*, somewhat similar to those found in the gut of *G. palpalis*, occurred in the intestines of mosquitos and *Stomoxys*, but quickly died out. These flies probably acted like culture tubes, and although they contained the non-infective developmental forms, because these flies were not suitable hosts, the cycle could not be completed and, therefore, infective forms did not develop. It is possible that the initial infectivity of cultures might be prolonged for a longer period than those observed by us, because the nearer an artificial culture medium approaches the normal blood conditions the longer will the trypanosomes retain their original characters and so probably remain infective, but in media resembling the conditions in the fly's stomach a development similar to that

which takes place in the fly will occur earlier and so infectivity will be lost sooner.

VI. COMPARISON BETWEEN THE CULTURAL FORMS AND THE FORMS FOUND IN *GLOSSINA PALPALIS*

From the description given above of the morphology of the cultural forms it will be seen that there is a very close resemblance between the changes which take place in cultures and those which occur in the gut of *Glossina palpalis*. It is interesting to note that Gray and Tulloch (1906) also drew attention to this feature in the cultures of *T. gambiense* attempted by them. A comparison of the cultural characters of *T. lewisi* with its development in the gut of the flea (*Ceratophyllus fasciatus*), Minchin and Thomson (1911), and the resemblance between the cultural forms of *Leishmania donovani* and the forms found in the probable insect host (*Cimex rotundatus*) (Patton, 1907, 1908) seem to strengthen the argument that the development of these Protozoa in cultures is similar to their development in their various insect hosts.

In the case of *T. gambiense*, both in cultures and in the gut of *Glossina palpalis*, the degeneration of a large number of the trypanosomes during the first few days takes place, leaving only a few stumpy forms, which seem to be the starting point of the new types found later in both. The close resemblance between the forms found in both has already been pointed out above in the description of the cultural morphology of the trypanosomes. Bruce and his colleagues (1911a, 1911b) have found that the developing forms found in the gut of *Glossina palpalis* are non-infective, and that it is only when trypanosomes like the blood types appear in the salivary glands that infectivity is found. So far, like these observers, we have been unable to infect animals by the injection of cultures containing only these 'normal developing forms.'

VII. SOME OBSERVATIONS ON THE SO-CALLED MALE AND FEMALE FORMS

Neither in *T. gambiense* nor in *T. rhodesiense* at any period of cultivation have we observed anything which might definitely be termed a sexual phase. No life-cycle such as described by Schaudinn in *T. noctuae* has been seen, and we have not found conjugation

similar to that observed by Prowazek (1905) and Baldrey (1909) in *T. lewisi* in the mid-gut of the rat-louse (*Haematopinus spinulosus*).

The fact that there are two distinct forms of trypanosomes, namely thin forms and stout forms, has suggested to some observers that there is a sexual cycle, and these forms have been called by Kleine and Taute (1911) male and female forms. There seems, however, to be no real reason for calling these male and female forms until it is definitely proved that conjugation takes place between them. Minchin (1908) was unable to find any definite evidence of conjugation between the 'male' and 'female' forms in *T. grayi*. Bruce (1909b) and Roubaud (1909), although finding so-called sexual forms also observed all stages of transition between these two extremes, and think there is not sufficient evidence to identify these forms with any stage in a sexual cycle. Roubaud believes that this differentiation of forms depends rather on the age of the culture and consequently on the composition of the medium. Walker (1910) experimenting with cultures of *Trypanoplasma ranae* comes to a somewhat similar conclusion. The occurrence in our cultures of trypanosomes attached by their posterior extremities was such a common phenomenon at certain periods, that it was at first thought that this might possibly be conjugation, but as the forms thus attached to each other were not always thin and stout forms these are rather to be considered either as the result of simple division or agglomeration. Such forms although watched for many hours have never been seen to fuse or apparently to make any attempt to do so. Baldrey (1911) has found similar forms in the gut of flies (*Tabanus* and *Stomoxys*) fed on animals infected with *T. evansi*, and he believes them to be conjugation forms, and has described the formation of an ookinete.

The very numerous varieties of trypanosomes found in our cultures, showing all stages between thin and stout varieties, seems to make it probable that the thin forms are merely due to the act of division.

In both fresh and stained specimens it would be very easy to mistake division phenomena for that of conjugation. On several occasions a large stout form has been seen with an apparent sphere of attraction thrown out to meet a long thin form which seemed to have its posterior end embedded in the protoplasm in close proximity

to the nucleus. Although this was watched for many hours, however, the thin form did not seem to make any further progress, and thus it is difficult to say whether this was an act of conjugation or not. No stained specimens, however, were obtained of this latter condition.

VIII. SUMMARY AND CONCLUSIONS

(1) *T. gambiense* and *T. rhodesiense* are capable of being cultivated on artificial media. *T. gambiense* has been cultivated for a period of thirty-seven days, and during that time was carried through four generations by means of sub-cultures. *T. rhodesiense* has been more difficult to cultivate and has continued to develop for only twenty-one days. All the flagellates disappeared in the third sub-cultures.

(2) The life history of these trypanosomes in culture tubes is similar to that which occurs in the gut of the insect host. This is shown by a comparison of the morphological characters of *T. gambiense* in cultures with those forms found by Sir David Bruce and his colleagues in *Glossina palpalis*. We find that the developmental forms found in the culture tubes are identical in their chief characteristics with those described by Bruce in the gut of an infected tsetse-fly.

(3) The cultures of *T. gambiense* and *T. rhodesiense* quickly lose their infectivity, and intraperitoneal injections of these into rats after the third day have been unsuccessful in causing an infection. This we have shown to be comparable to the results of Sir David Bruce and his colleagues, who have found that the intestinal contents of *G. palpalis* quickly become non-infective. The infectivity during the first few days appears to be due to the persistence of some of the original blood forms. In successful cultures, therefore, we expect infectivity to cease as soon as these blood forms disappear and the 'healthy developing forms' which have been shown by Bruce to be non-infective appear.

(4) Our cultures of *T. gambiense* and *T. rhodesiense* have remained non-infective after the third day, and we believe that this is explained by the fact that the infective forms such as found by Bruce and his colleagues in the salivary glands of *Glossina palpalis* did not occur in our cultures, and we have suggested that probably

some tranference of the cultures to a new medium or environment similar to that of the salivary glands of the tsetse-fly might be required to permit the full life history of the trypanosomes being completed.

(5) Cultures of *T. rhodesiense* seem to differ from those of *T. gambiense*. In cultures of the former typical posterior nuclear forms such as described by Stephens and Fantham (1910) are relatively common, whereas in the latter they are of very rare occurrence.

(6) Although the so-called 'male' and 'female' forms are present in cultures we were unable to find any definite evidence of a sexual cycle.

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EXPLANATION OF PLATES

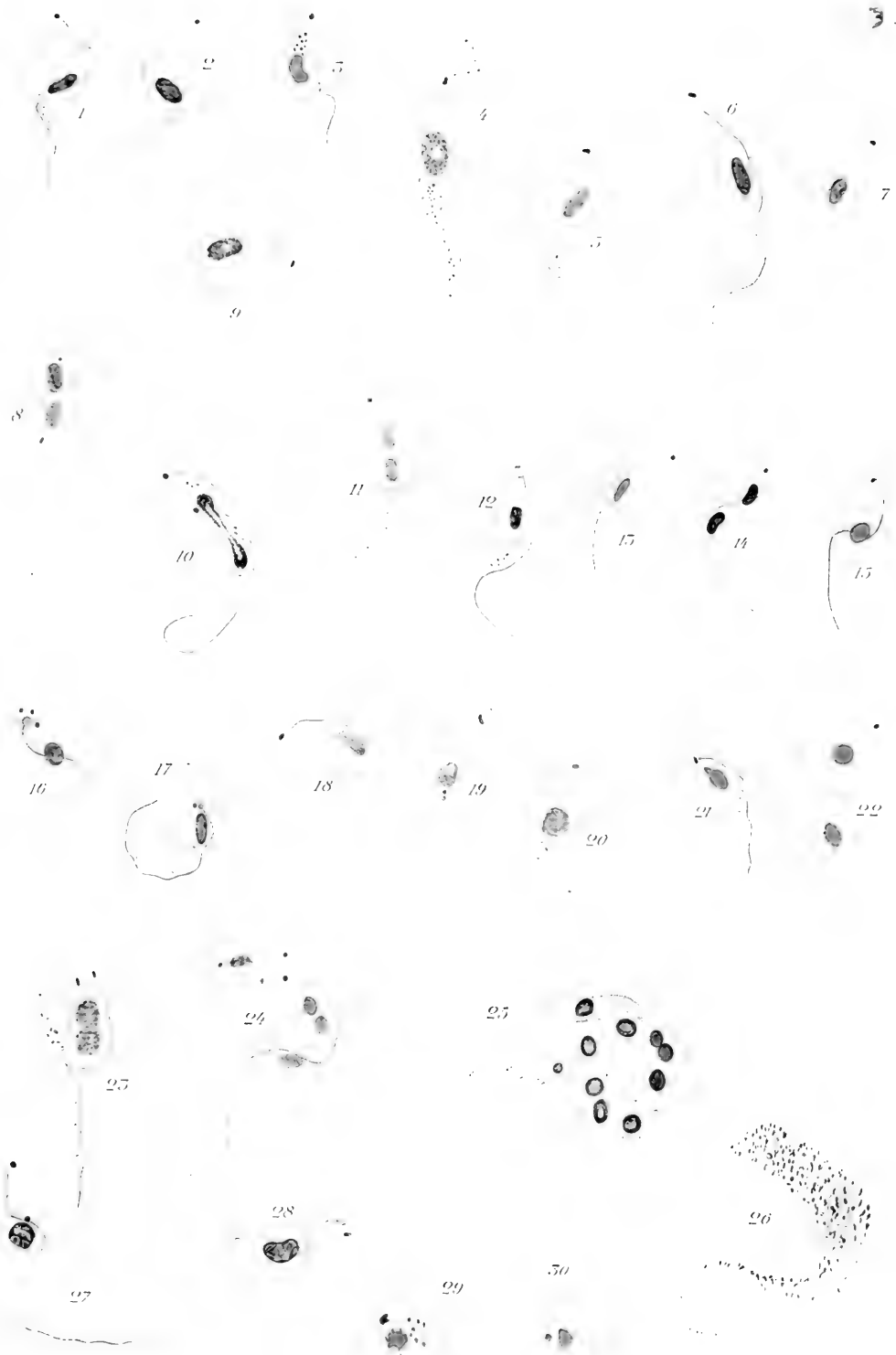
Trypanosoma gambiense

Figures drawn by means of an Abbé camera lucida, using Leitz ocular 4 and objective 1/12 inch. Stained Giemsa's stain. Magnification 1650.

PLATE XIX

- Fig. 1. Slender trypanosome from blood of rat.
 Fig. 2. Stout trypanosome from blood of rat.
 Fig. 3. Stout form surviving after 24 hours in culture.
 Fig. 4. Very large form with nucleus showing a karyosome (4th day of culture).
 Figs. 5-10. Forms corresponding to the 'normal developing forms' described by Bruce in the gut of *Glossina palpalis*, and probably similar to the so-called 'female' forms of some observers. Note the long 'snout,' the position of the blepharoplast, the origin of the flagellum from a pink staining area anterior to the blepharoplast and the short or absent free flagellum. (From 10th, 11th, 18th, 17th, 29th and 5th days of growth). Nos. 7 and 8 from the second and No. 9 from the third generation. Fig. 10, dividing form.
 Fig. 11. Form intermediate between the stout 'normal developing forms' ('female') and the slender forms ('male') (18th day of growth; second generation).
 Figs. 12-14. Slender forms corresponding to the so-called 'male' forms. Note the long 'snouts' and homogeneous nuclei (11th, 10th and 12th days of growth).
 Figs. 15-17. 'Small developing forms.' Forms shorter than the 'normal developing forms.' Note the varying position of the blepharoplast (14th, 14th and 15th days of growth, second generation).
 Figs. 18-20. Forms intermediate between the 'normal developing forms' and the 'small developing forms' (15th, 18th and 31st days of growth, first, second and third generations respectively).
 Fig. 21. Stumpy form with posterior nucleus. One of the very few posterior nuclear forms found in cultures of *T. gambiense* (15th day; second generation).
 Figs. 22-23. Dividing forms (7th and 15th days).
 Fig. 25. Large multinucleated plasmodial mass (4th day).
 Figs. 24 and 26. Forms similar to those which Bruce calls 'degeneration forms of the normal reproductive type' (16th and 6th days).
 Figs. 27-28.—Some forms seen when the cultures begin to die out (11th and 12th days).
 Fig. 29. Rounded body with detached flagellum and fragmented nucleus (5th day).
 Fig. 30. Rounded body with distinct nucleus and blepharoplast (12th day).

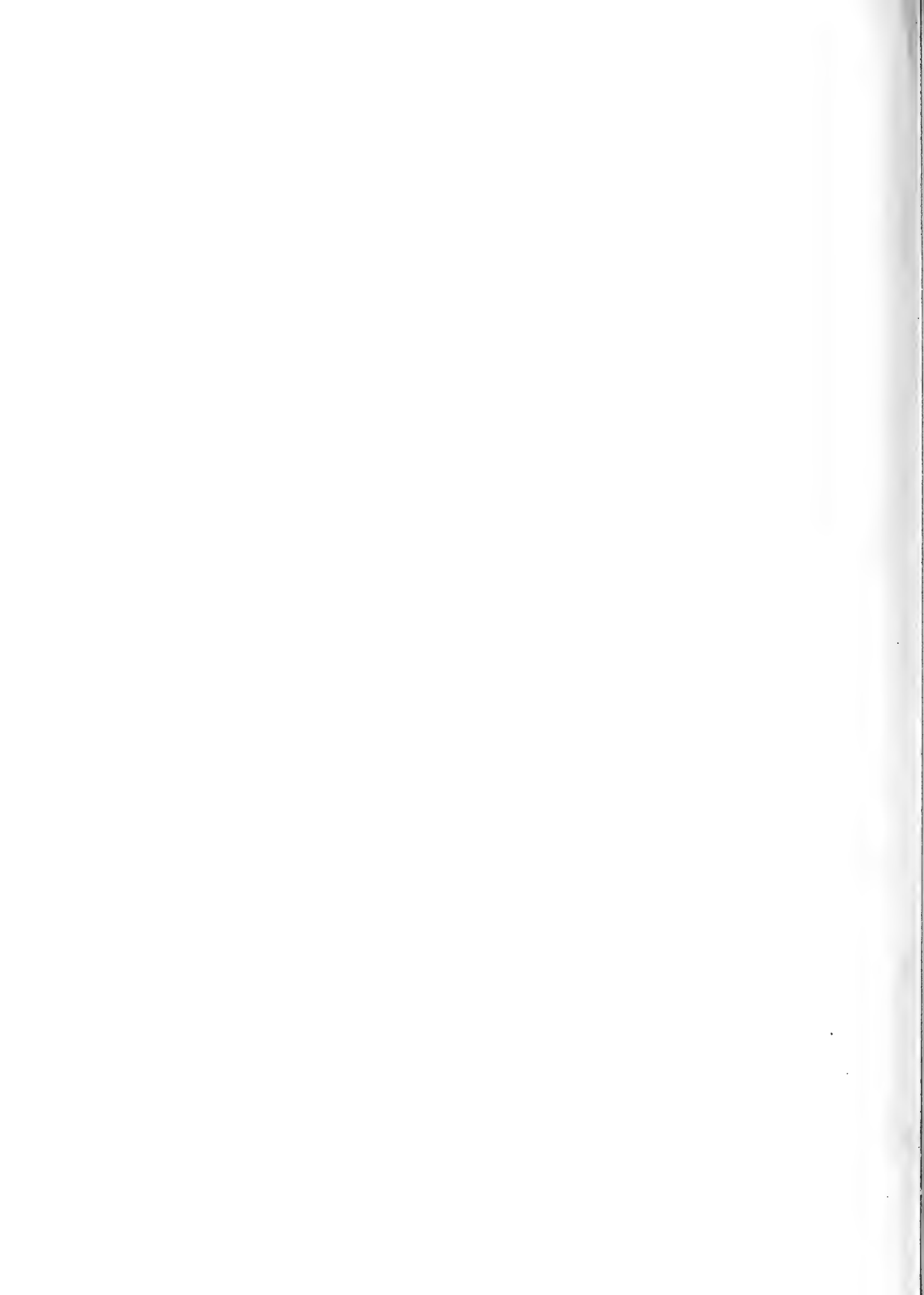
35-2^a



J. A. Sinton, del.

TRYPANOSOMA GAMBIENSE
IN CULTURES

Illustrations by the artist, etc.



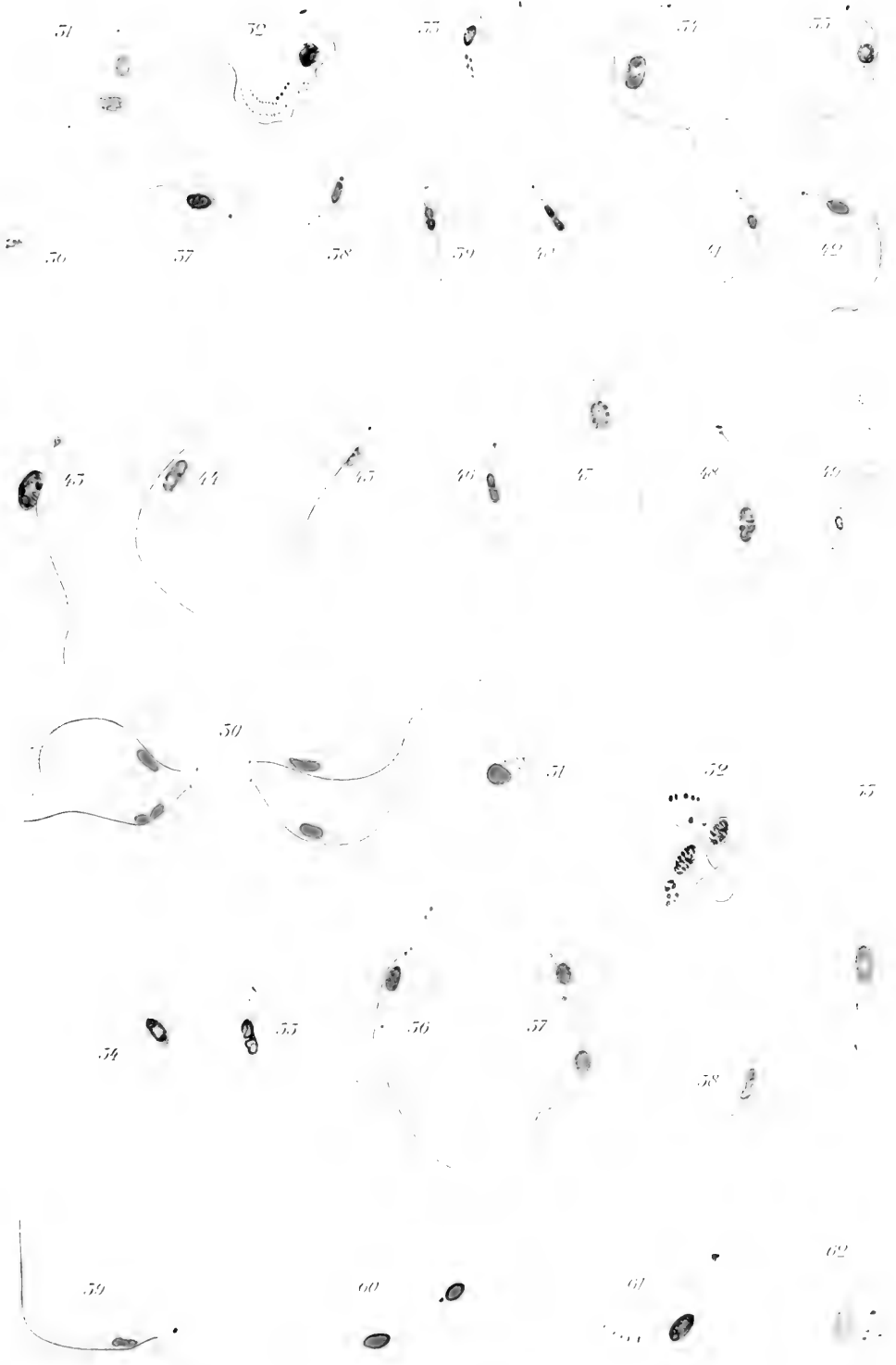
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PLATE XX

Trypanosoma rhodesiense

Slides stained with Giemsa. Figures drawn with Abbé camera lucida. No. 4 ocular and 1/12 inch oil immersion lens (Leitz). Magnification 1650.

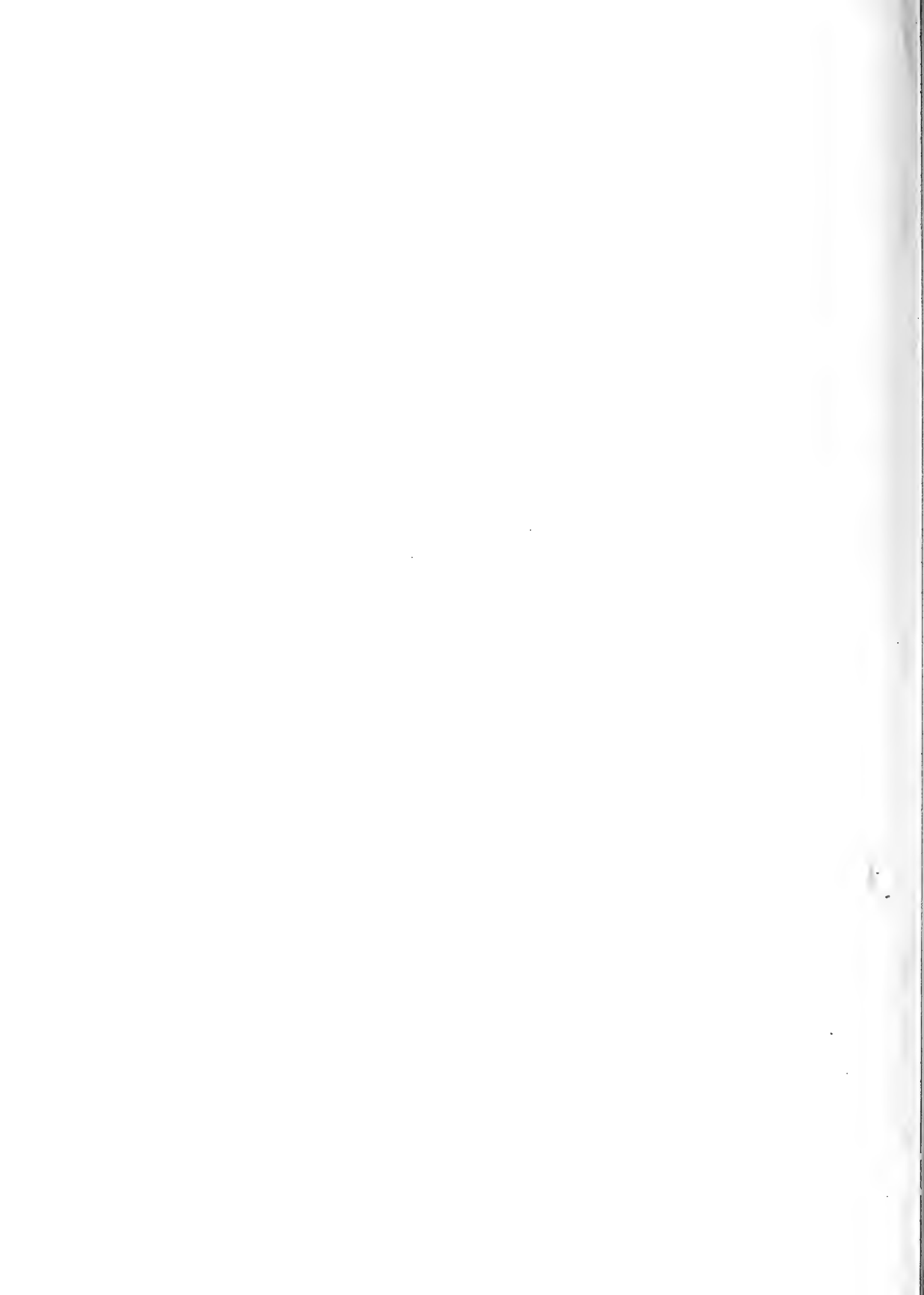
- Fig. 31. Normal blood form from peripheral circulation of a rat. Shows two nuclei and two blepharoplasts and well marked undulating membrane with free flagellum.
- Fig. 32. Stout form from the peripheral blood of a rat showing the nucleus in a posterior position.
- Fig. 33. Blood form after 24 hours in culture tube. Well stained protoplasm and nucleus. Rod-shaped blepharoplast and free flagellum.
- Fig. 34. A stout form with sharp nose from a culture 24 hours old. Protoplasm stained a deep blue. No free flagellum.
- Fig. 35. A short stout form from a culture 48 hours old, showing red stained area immediately anterior to the blepharoplast in which flagellum arises. Very short free flagellum.
- Fig. 36. A degenerate form of long thin blood type after 24 hours in culture tube.
- Fig. 37. Stout form. Posterior nucleus. Blunt aflagellar end. Very short free flagellum (third day).
- Fig. 38. Form with posterior nucleus which is quite close to the blepharoplast. Short free flagellum. Flagellum closely applied to the body of the trypanosome (5th day of culture).
- Figs. 39, 40, 41. Thin 'spirillar' forms, which correspond to the so-called 'male' forms. All show the nucleus in a more or less posterior position. There is no undulating membrane, and a very short free flagellum. (7th, 8th and 9th days of culture).
- Fig. 42. Small developing form (8th day of culture). Posterior nucleus. No free flagellum. Flagellum closely applied to the body of the trypanosome.
- Figs. 43 and 44. Normal developing forms (6th and 7th day of culture) with large oval nucleus, long 'snout,' and the blepharoplast moved away from the posterior end. These correspond to the so-called 'female' forms. They show also the red stained area immediately anterior to the blepharoplast. No free flagellum.
- Fig. 45. Slender developing form, 8th day of culture. Showing nucleus in a posterior position.



J. G. Thomson, del.

TRYPANOSOMA RHODESIENSE
IN CULTURES

Trypanosoma rhodesiense



- Fig. 46. Long thin form with very long 'snout.' No free flagellum.
- Fig. 47. Normal developing form from the 9th day of a sub-culture with well marked posterior nucleus.
- Fig. 48. Long form with oval nucleus and long 'snout' from 8th day of culture.
- Fig. 49. Form showing presence of so-called 'volutin' granules from the 8th day of culture.
- Fig. 50. Young division rosette 7th day of culture.
- Fig. 51. Stout form from 8th day of culture with posterior nucleus.
- Fig. 52. Large form showing division from 9th day of culture. Contains 'volutin' granules.
- Fig. 53. Very large form with two blepharoplasts and a single large oval nucleus from 11th day of culture.
- Fig. 54. Form from 8th day of sub-culture. Blepharoplast moved away from the posterior end, giving the appearance of a very long 'snout.' No free flagellum. The flagellum arises in red stained area immediately anterior to the blepharoplast.
- Figs. 55-56. 'Normal developing forms' (12th and 15th days).
- Fig. 57. Similar form with two nuclei and two blepharoplasts (12th day of culture).
- Fig. 58. Posterior nuclear form. Small developmental form just after breaking away from a division rosette (see fig. 50). From 8th day of culture.
- Fig. 59. Shows very broad 'snout' (from 10th day of culture).
- Fig. 60. Form with two nuclei. One nucleus posterior (8th day of culture).
- Fig. 61. Normal developing form (9th day of sub-culture).
- Fig. 62. Well stained rounded body from a 3rd day's culture. Flagellum still attached.

PLATE XXI

T. rhodesiense in culture

Micro-photographs taken from slides stained with Giemsa.

- Fig. 1. Division rosette from the 7th day of a culture. Shows the trypanosomes attached by their posterior ends. This arrangement in the rosettes is similar to that found in cultures of *T. brucei*.
- Fig. 2. A colony of trypanosomes from a culture of *T. rhodesiense*. Slide made on the 8th day. Flagella are well stained, and also the nuclei. Note that the flagella are directed towards the periphery.



FIG. 1.

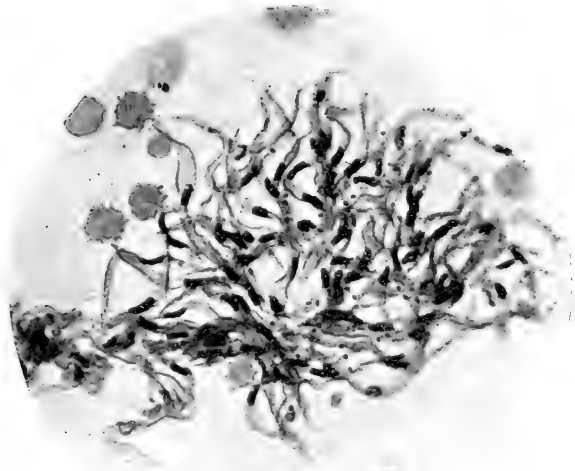


FIG. 2.

Trypanosoma rhodesiense.



SPIROCHAETA CTENOCEPHALI, Sp.
 Nov., PARASITIC IN THE ALIMENTARY
 TRACT OF THE INDIAN DOG FLEA,
CTENOCEPHALUS FELIS

BY

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

ON SPECIAL DUTY

(Received for publication 23 July, 1912)

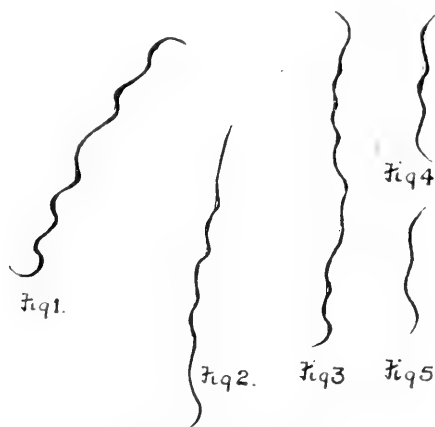
The presence of protozoa in the alimentary tracts of blood-sucking insects deserves special attention, especially when they occur in those insects which may be utilised for feeding experiments on man. In view of the recent work of Basile and others on the supposed development of the parasite of canine Kala Azar in *Ctenocephalus canis*, it is important to study all the natural parasites which may occur in the dog flea, especially in India, where, up to the present, canine Kala Azar has not been found.

In Madras the dog flea is *Ctenocephalus felis*, the species *canis* having only, so far, been found on the jackal, *Canis aureus*. *Ctenocephalus felis*, in addition to being infected with a gregarine and a herpetomonas, also contains a spirochaete. The latter parasite has been found twice in the larva of this flea, and once in the adult insect. The larvae in question were bred from fleas living on two cats which were kept in the laboratory, while the flea was taken from a dog, killed in one of the lethal chambers of Madras. In case this spirochaete may be found in *Ctenocephalus canis* in Italy, I think it will be well to describe it shortly, for there is the remote possibility that it may be mistaken for some supposed unrecognised stage of the parasite of canine Kala Azar.

It will, in the first place, be noted that this spirochaete may be found in the alimentary tract of the larva of *Ctenocephalus felis*, a fact which at once settles its origin. I have already pointed out that the larva of this flea lives almost exclusively on the excreta of the adult insect, and that the youngest as well as the oldest larvae, always contain the black digested blood passed out by the

fleas. This spirochaete has, then, nothing whatever to do with the blood, either of the dog or the cat, that is to say, it is not a parasite of these animals. Further, it should be noted that it is a rare parasite of the flea, for although about 1,500 larvae have been examined it has only been found in two, and only once out of about 500 fleas. The great rarity of this organism would tend to lead the observer to think that it possibly represented the extra-corporeal stages of a rare spirochaete of the blood of the dog or the cat. Again, the very method by which the flea acquires this parasite, namely, in its larval stage, would certainly mislead the unsuspecting observer.

In the fresh condition, this spirochaete is very active, exhibiting all the movements characteristic of this group of parasites, and which it is unnecessary to describe here. In the long forms there were from eight to ten well marked spirals, whereas in the short forms there were about four. Many of the long forms were seen dividing transversely, and I have little doubt, from what was seen in several stained specimens, that they also divide longitudinally; these methods of division have been accurately described by Fantham and Porter in the case of other spirochaetes, and I would refer the reader to their papers on this subject. Many of the long forms were seen coiled up towards their centres, or at one or the other end, an appearance which simulates, and which has been mistaken for the formation of cysts or spores. Figure 1 represents a long, stout form, in which the undulating membrane could be



Spirochaeta ctenocephali, Sp. nov.

seen as a faint pink band at the concavities of the spirals. Figure 2 represents a thinner form, and figure 3 shows such a parasite dividing transversely. Figures 4 and 5 represent short forms, in which distinct chromatic granules could be clearly made out. The long forms measured about $20\ \mu$ in length, and the short forms from $4\ \mu$ to $6\ \mu$. I propose naming this organism *Spirochaeta ctenocephali*.

REFERENCE

- FANTHAM, H. B., and PORTER, ANNIE (1909). 'The modes of division of *Spirochaeta recurrentis* and *S. duttoni* as seen in the living organisms.' Proc. Roy. Soc., B, Vol. 81, pp. 500-505.



REPORT ON AN OUTBREAK OF CANINE
PIROPLASMOSIS DUE TO *PIROPLASMA*
GIBSONI (PATTON) AMONG THE
HOUNDS OF THE MADRAS HUNT,
TOGETHER WITH SOME OBSERVATIONS
ON THE TREATMENT OF THE DISEASE
WITH SALVARSAN

BY

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(Received for publication 23 July, 1912)

In an earlier paper, one of us recorded the discovery of a new piroplasm, *P. gibsoni* (Patton), which was found in the blood of the hounds of the Madras Hunt, and later was proved to be common in the blood of the jackal (*Canis aureus*). It will be remembered that it was then shown that *Piroplasma gibsoni* differs in structure from *Piroplasma canis*, in that it is much smaller, and that the large pyriform stages, so characteristic of the dog piroplasm, were not encountered. It is true that it was thought that this piroplasm in the blood of the hounds was in reality *P. canis* which had become modified by the action of the trypanblau, but this view had soon to be abandoned when the parasite was recovered from the blood of country-bred dogs, which had been inoculated with the blood of an infected hound. Lastly, the parasite was found in the blood of a jackal, and it was later recovered from the blood of clean dogs inoculated from this jackal. There could then be no doubt regarding its origin. We had then to deal with an entirely new piroplasm and a new disease of economic importance.

Although the hounds of the Madras Hunt are known to have suffered from some form of piroplasmosis, it was only in the 1909-1910 season that the disease was accurately diagnosed and the true nature of the parasite recognised. Knowing that this piroplasm is almost certainly transmitted from the jackal to the hounds by the bite of a tick, one of us (W. S. P.) has made repeated attempts to try to transmit it by placing ticks from jackals on clean dogs. So far, *Haemaphysalis bispinosa* has been used, and although a large number have been placed on young dogs, we have so far failed to infect them. We have long known that there is a species of *Rhipicephalus* which is fairly common on the jackal, but unfortunately we have so far failed to obtain suitable specimens for our transmission experiments, a batch of young adults having been lost by an assistant in 1910. This *Rhipicephalus*, Professor Neumann informs one of us, is a new species allied to *Rhipicephalus simus*. We are now making another attempt to obtain this tick, and we hope, in due course, to find out whether it is the natural carrier of the parasite; it is most difficult to get jackals with ticks on them. This is one of the reasons why one of us (W. S. P.) has not given a more complete account of *Piroplasma gibsoni*, but we hope to do so in connection with some other studies on the life histories of the *Piroplasmata* occurring in South India.

Piroplasma gibsoni, and the disease it causes, are not only of interest to the student of Protozoology, but also to sportsmen, for, owing to its virulent nature, it soon disables a pack, and brings hunting to a standstill. We hope, therefore, that this report will appeal to a wide circle, and that if the parasite is found in other parts of the world, those interested in the welfare of their hounds will be able to check the disease by the use of Salvarsan. We propose, then, in this paper, recording the recent outbreak among the hounds of the Madras Hunt, and, at the same time, making a few notes on the action of Salvarsan on the parasite; owing to other more important duties, we have not had the time to carry out any exact experiments, but we hope in due course to do so. In any case, sufficient is now known regarding the great value of Salvarsan in this disease.

The Madras hounds are imported annually from England, and are utilised in hunting jackals in the country round Madras. The

kennels have been constructed regardless of cost, and every possible precaution has been taken to render them tick-proof. They contain scarcely any woodwork, and the main building, together with the several yards, are surrounded with a moat four inches wide and three inches deep, in which a solution of cyllin is always kept. No tick can possibly pass this barrier. Every hound on returning from hunting or from exercise is carefully examined for ticks, and any found are at once destroyed; this operation is carried out daily, under supervision, by the dog boys, in one of the yards, so that the animals are never permitted to enter the kennels proper until they are tick free. These details are mentioned, in order to show that any tick-borne disease must be encountered outside, and not in the kennels.

For the 1911-12 season 19½ couple were landed on October 11th, 1911, and were all in excellent condition; they were taken straight to the kennels in the hunt van. During October they were exercised along the road, and were given an occasional run across country.

All the hounds—only bitches are imported—were very fit when hunting started on November 4th. At the very commencement of hunting, jack was killed, and the hounds appeared to remain in good condition till November 28th, when several couple were noticed to be off their feed, and developed temperatures ranging between 102° F. and 104° F. The day before this change was noticed there had been a very hot run, and it was at first thought, and hoped, that the slack condition of some of the animals was due to a 'touch of the sun.' However, during the following week, on examining some of the sick hounds we noticed that they were anaemic, and that their spleens were readily palpable (the spleen of a dog is an organ which cannot be readily felt, at any rate in health). Further, the sick animals were unmistakably losing condition. From past experience it was at once evident that we had to deal with the old enemy *P. gibsoni*. On December 11th one of us (W. S. P.) took blood films from all the hounds, twenty-one couple in all, 1½ couple having arrived in the meantime from Ootacamund, and found that 15½ couple were infected with the parasite. Two days later one hound died, and at the autopsy all the signs of acute malignant piroplasmiasis due to *P. gibsoni* were

found. We use the term malignant because of the extremely gross changes the parasite produces in these highly susceptible animals, and further, because we know of no cure. Trypanblau, trypanrot, and almost every drug we could think of had been tried in previous years with disappointing results.

On examining the films made from the sick hounds, it was found that some were more seriously infected than others, judging from the large number of parasites. It will be unnecessary here to describe the parasite, but we would like to point out that it is easily overlooked when only a few are present in the peripheral blood. The success of the search depends on making a really good clean film, and we would strongly recommend those who may have to examine the blood of hounds for this parasite to attend to all the little details in making the film. For instance, the slides must be clean and free of grease; the needle, with which the film is made, must be dry and free of rust, and the same with the scissors required for snipping a piece of skin from the ear. It is most important to clean thoroughly the hound's ear after cutting off the hair from the tip. If these details are attended to, and the film fairly deeply stained with Romanowsky's stain, the observer will have no difficulty in finding the parasite; if, on the other hand, the slide is dirty, or the hound's ear not cleaned, the parasite will certainly be missed.

At this stage of the recent outbreak, it occurred to one of us (T. H. S.) to try the effect of Salvarsan. Accordingly, on December 14th, two hounds, one very ill and heavily infected, the other moderately so, were selected, and 0.6 gram of the drug was injected intramuscularly into the hamstrings. A fair amount of local oedema followed the injection, which eventually involved the whole leg. On December 16th, one of us examined blood-films from the two hounds, and noted that the number of parasites was about the same. Clinically the two hounds had unquestionably improved, their temperatures had fallen to normal, and they began to 'feed up,' to use a hunting phrase. On the strength of this experiment we decided to waste no more time, but inject all the hounds which were infected. Hunting was suspended, and with the assistance of Mr. F. Ware, C.V.D., one of us (T. H. S.) injected the sick hounds on December 21st and 22nd. Before

recording the result of this experiment, it will perhaps be useful if we give a short account of the disease as it strikes the lay mind.

The first symptom which is evident, is the intense anaemia which clearly points to the great destruction of the red blood cells. This symptom explains the first sign which the huntsman will notice in the incipient stage of the disease, namely, slackness of the hound in cover, and inability to keep her usual place in the field when the pack is running. We have always been able to pick out the sick hounds when hunting, and later have found the parasite in their blood. Next, the spleen enlarges and the hound loses condition rapidly; it should be noted that in a dog the spleen, when it enlarges, does so by increasing in length more than in its transverse diameter, so that it comes to resemble a big tongue-like organ extending across the abdominal cavity even into the right iliac fossa. In one case the spleen was found doubled on itself. It is very difficult to feel this great enlargement of the organ with one hand; the best way is to grasp it crossways between both hands, no mistake can then be made as to the presence of the enlargement. The liver also hypertrophies, but not to the same extent as the spleen. In spite of the enormous destruction of red blood cells, we have never seen haemoglobinuria. Pyrexia is usually present, but only well marked shortly before death. Several of the hounds developed large sloughing sores along the floor of the mouth and inside the cheek, closely simulating cancrum oris, which is so well known in Kala Azar.

After the injections of Salvarsan mentioned above, the hounds picked up in every way. Their spleens slowly diminished in size, the anaemia was less and less noticeable, till in a few weeks it disappeared altogether. They put on flesh, and after hunting had been suspended for three weeks it was resumed, and continued till February 22nd, 1912. At the last meet of the season sixteen couple were out and killed a jack in the open after a fifty-five minutes' run without a check. We mention this remarkable feat in the case of hounds which only a few weeks previously were extremely ill, in order to show the extraordinary effect of a single dose of Salvarsan.

The blood of the hounds was examined as often as was possible, and it was found that the parasite in the case of those which were

previously heavily infected was present in small numbers; in the moderately infected hounds it was almost impossible to find a single parasite. We regret that this part of the work is far from being complete, and we fully recognise that much has yet to be learnt regarding the action of the drug on the parasite; we hope to fill in the gaps in due course. One of the hounds, which was the first to be injected with Salvarsan, and which was at the time seriously ill, has been kept at the King Institute, and repeated examination of its blood has failed to show a single parasite. We have no doubt whatever that had this animal not been treated with the drug it would have died in a few days.

We must, however, note that some of the hounds died in spite of the treatment, and we will now give a few notes regarding them. Vixen (see chart IV) and Discord died before we started the treatment. Flourish (see chart I), who was very ill at the time, being extremely anaemic, died four days after the injection. Rhapsody, moderately infected, died of double pneumonia, as was seen at the autopsy. Curious (see chart II) undoubtedly died as a result of the disease, and as in the case of Flourish, the drug was evidently given too late. Amy died of diffuse cellulitis of the neck, and at the autopsy no piroplasms could be found in her spleen, the drug having obviously destroyed them. It will be remembered that the spleen usually contains the parasite in large numbers. Ruby died of dysentery, probably following colitis set up by the disease. This makes a total of $3\frac{1}{2}$ couple, of which $1\frac{1}{2}$ may be put down as having died of complications, and of piroplasmosis. It leaves two couple, one hound of which died of pneumonia, and the other of cellulitis of the neck. Out of the $17\frac{1}{2}$ couple left in the kennels, $12\frac{1}{2}$ were found by blood examinations to be absolutely free of the disease, while five couple which originally were infected still showed a few parasites in their blood, but they had obviously recovered.

As far as we know, this is the first time in which salvarsan has been used in the case of an outbreak of piroplasmosis in a large hunting pack, and we think the extraordinary result is of sufficient interest to merit its being placed on record. In the case of the Madras Hunt, it was becoming a question, even if it had not already become so, as to whether it was advisable to go on hunting in view

of the serious losses incurred. Except in the case of one couple, who did not respond rapidly to one dose of the drug, and which were given a half dose thirty days later, we have shown that one injection of 0.6 gram appears to be all that is required. The great drawback to the treatment is the high price of the drug, and we hope that ere long it will be sold at a lower rate, especially when required for animals such as hounds.

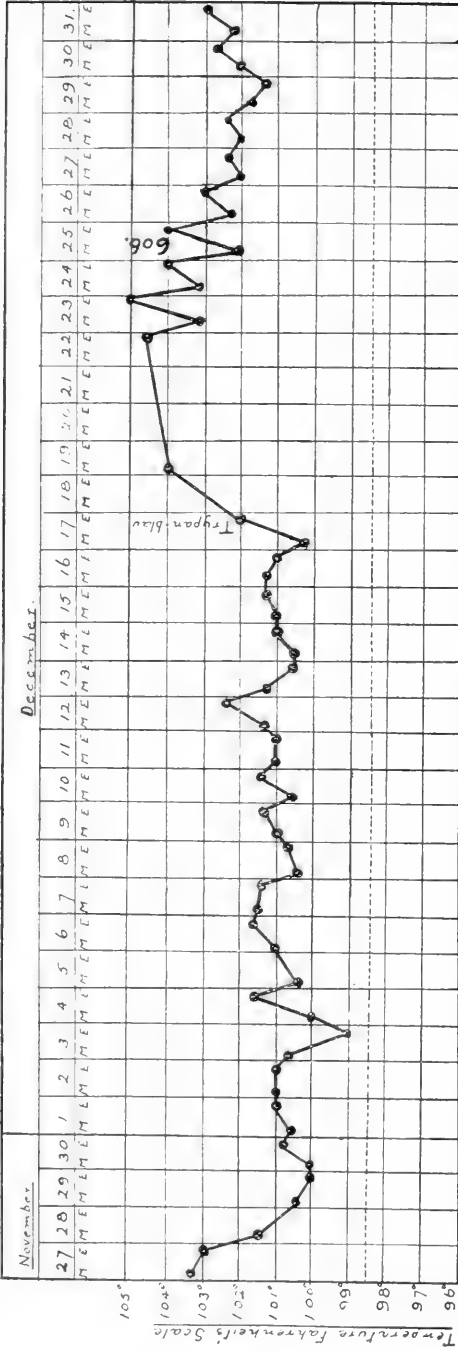
On referring to the temperature charts (pp. 368-370) it should be noted that a dog's normal temperature is about 101°F.

REFERENCE

- PATTON, W. S. (1910). Preliminary Report on a new piroplasm *Piroplasma gibsoni*, sp. nov., found in the blood of the hounds of the Madras Hunt and subsequently discovered in the blood of the jackal *Canus aureus*. *Bull. Soc. Path. exot.* tome 3, No. 4.

NAME—' FLOURISH,'

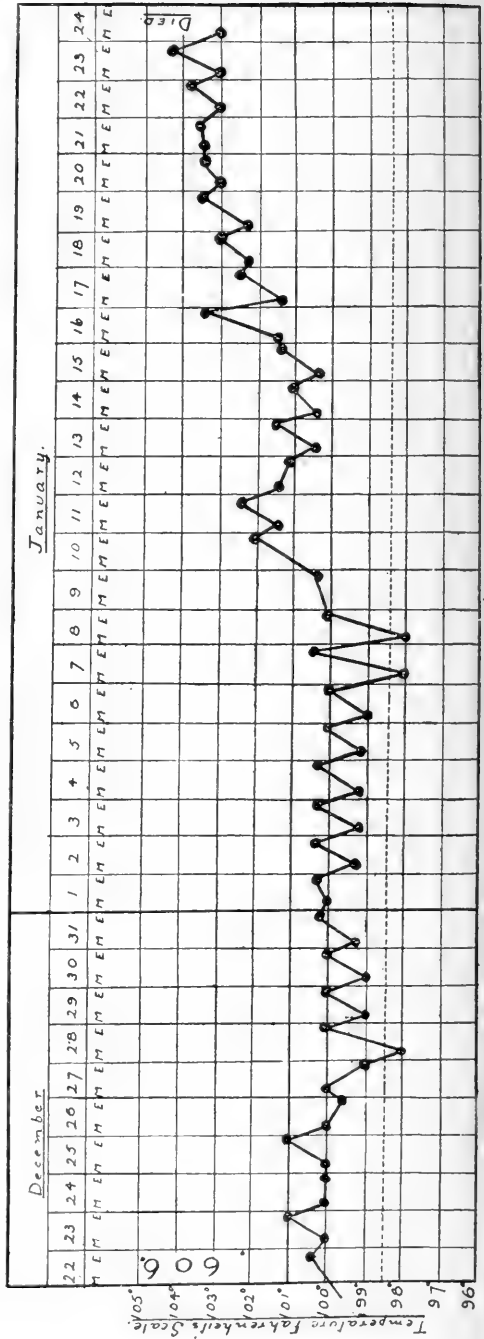
CHART No. I



This hound showed rapid wasting, marked anaemia, great enlargement of the spleen and had stomatitis as a complication; it was given 6 grammes Salvarsan on December 25. It had a dose of Trypanblau on December 17. After the dose of 606 it picked up and came out for its food, which it had not done before. It, however, got worse on December 30 and died the next day.

NAME—' CERIOUS,'

CHART No. II



This hound showed rapid wasting, marked anaemia, great enlargement of the spleen and had stomatitis as a complication; it was given 6 grammes Salvarsan on December 25. It had a dose of Trypanblau on December 17. After the dose of 606 it picked up and came out for its food, which it had not done before. It, however, got worse on December 30 and died the next day.

CHART No. III

NAME—'HANDMAID,'

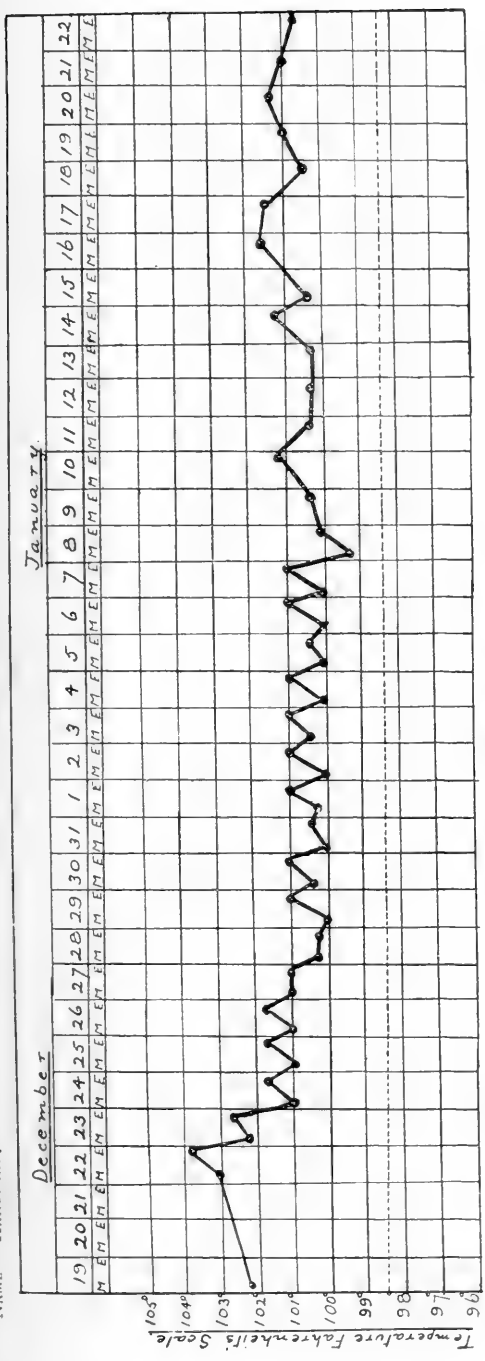
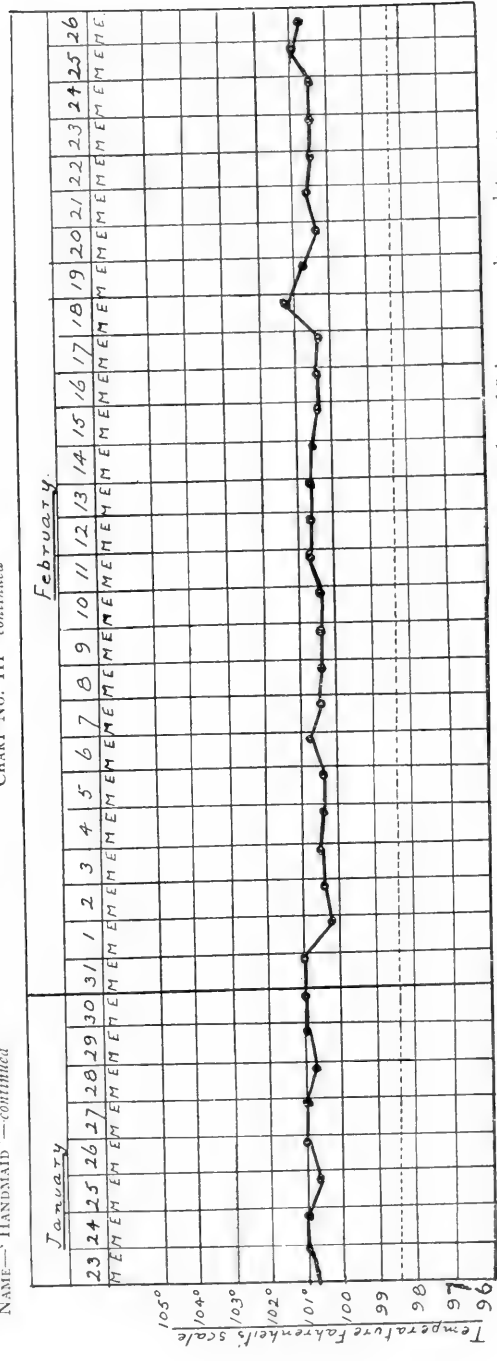


CHART No. III—continued

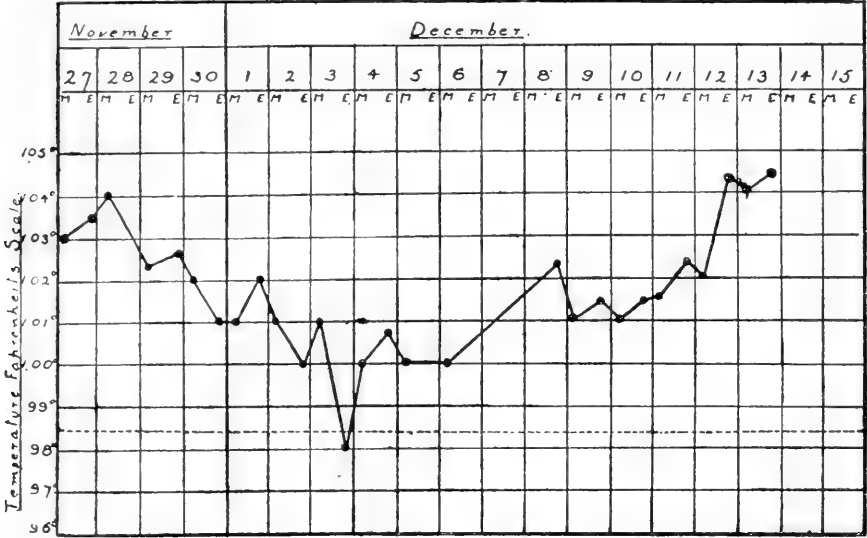
NAME—'HANDMAID'—continued



Hound considerably wasted, anaemia marked, spleen extended to right in nipple line. One dose of Salvarsan, result complete cure.

NAME—'VINEN.'

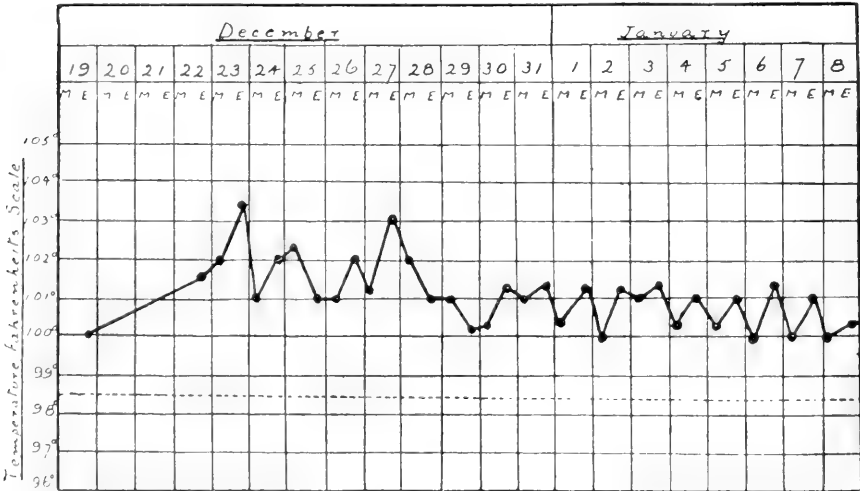
CHART No. IV



Marked and rapid wasting, great anaemia, spleen enormously enlarged, liver considerably so. 606 not given, result death.

NAME—'CHARMER.'

CHART No. V



Marked anaemia, spleen enlarged up to mid-line, condition fair, considerable lassitude. One dose of 606, result cure.

SOME ATTEMPTS AT THE CULTIVATION OF THE MALARIAL PARASITE BY BASS'S METHOD

BY

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(Received for publication 12 August, 1912)

While working at the Liverpool School of Tropical Medicine under a grant from the Post-graduate Research Fund of the Queen's University of Belfast, my attention was drawn by Sir Ronald Ross to an article by Bass (1911) on the cultivation of the malarial parasite, and he suggested that I should make some experiments with the view of verifying the results of that observer. I wish to acknowledge my indebtedness to Sir Ronald Ross for his help in this investigation, and also to Dr. David Thomson under whose care the cases were.

Bass claims to have grown all the three kinds of malarial parasites (*Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium falciparum*) in both citrated and defibrinated blood of malarial patients containing these parasites. His method depends upon the heating of the infected blood to 40° C. for half an hour. This, he states, will, without injuring the parasites, destroy the complement which would otherwise in conjunction with the amoceptor destroy the parasites in the cultures.

This complement-free blood was then incubated under strict anaerobic conditions. Bass states that in these cultures the parasites grew, were successfully transplanted, and, at the time of writing, some were alive in citrated blood after a period of over two weeks.

He believes that the unsuccessful attempts of other observers to cultivate the malarial parasite is due to the presence of complement, and that if complement is destroyed without injuring the parasites, it would be possible to grow almost any blood protozoön.

While trying to confirm these results, I have attempted cultures from five cases of malaria, of which three were infections with *P. falciparum*, one was an infection with *P. vivax*, while the other was a mixed infection with *P. vivax* and *P. falciparum*.

The infected blood was obtained from the median basilic vein of the malarial cases, and half was defibrinated while the other half was citrated. The blood was then heated to 40° C. for thirty minutes, and a little was examined to see if the parasites were still alive. It was found after exposure to this temperature that the parasites still showed active motility inside the red blood cells.

The blood was taken either immediately before or immediately after a rigor, and showed in all the cases numerous parasites.

The blood was now transferred to small sterile test-tubes, about 1/2 c.c. being added to each, so that from each case it was possible to obtain twenty or more tubes of blood, some of which were defibrinated and some citrated. The majority of these were incubated under anaerobic conditions, some at 25° C. and some at 37° C., while the remainder were incubated aerobically at similar temperatures.

All these cultures were examined daily for ten to fourteen days, and afterwards every few days. In some of the cultures movements could be observed in the parasites up to the third and fourth days, after which, although they were still present, it was impossible to say whether they were alive or not, as they were inactive.

After the fifth or sixth days the red blood cells which contained parasites were distinctly paler than the other red cells, and in some cases it was only after careful focussing that it was possible to determine that the parasites were still intracellular; whether this paleness was due to the action of the parasite on the haemoglobin or to the infected cells being more easily haemolysed, I cannot say.

In cultures kept at 37° C. it was usually found that in eight to ten days the parasites were either very scanty or absent, and that the majority of the red cells had become haemolysed. The disappearance of the parasites was probably due to the more rapid haemolysis of the infected cells at the higher temperature.

At 25° C. the parasites appear to persist in the red blood cells as long as the cells which contain them do not undergo haemolysis. In one culture of *P. falciparum* under aerobic conditions the

parasites could still be distinguished both in stained and fresh preparations after 104 days. These parasites in fresh preparations appeared rounder than usual, and in stained specimens, although they took up the stain well, they were bluer than normal, but whether these parasites were still alive or infective it was impossible to say.

In the case of *P. vivax* parasites could still be distinguished both in aerobic and anaerobic cultures for 54 days, but in stained preparations they took up the stain very badly.

Other attempts were made with (1) infected corpuscles which had been washed free of complement and then mixed with sterile human pleuritic fluid which had been heated in one case to 56° C. for half an hour and in another case to 45° C. for one hour to kill the complement; (2) infected blood heated to 40° C. for half an hour was mixed with an equal amount of normal human blood treated in the same way; (3) cultures were attempted on Nicolle's modification of the Novy-McNeal medium made up with human blood.

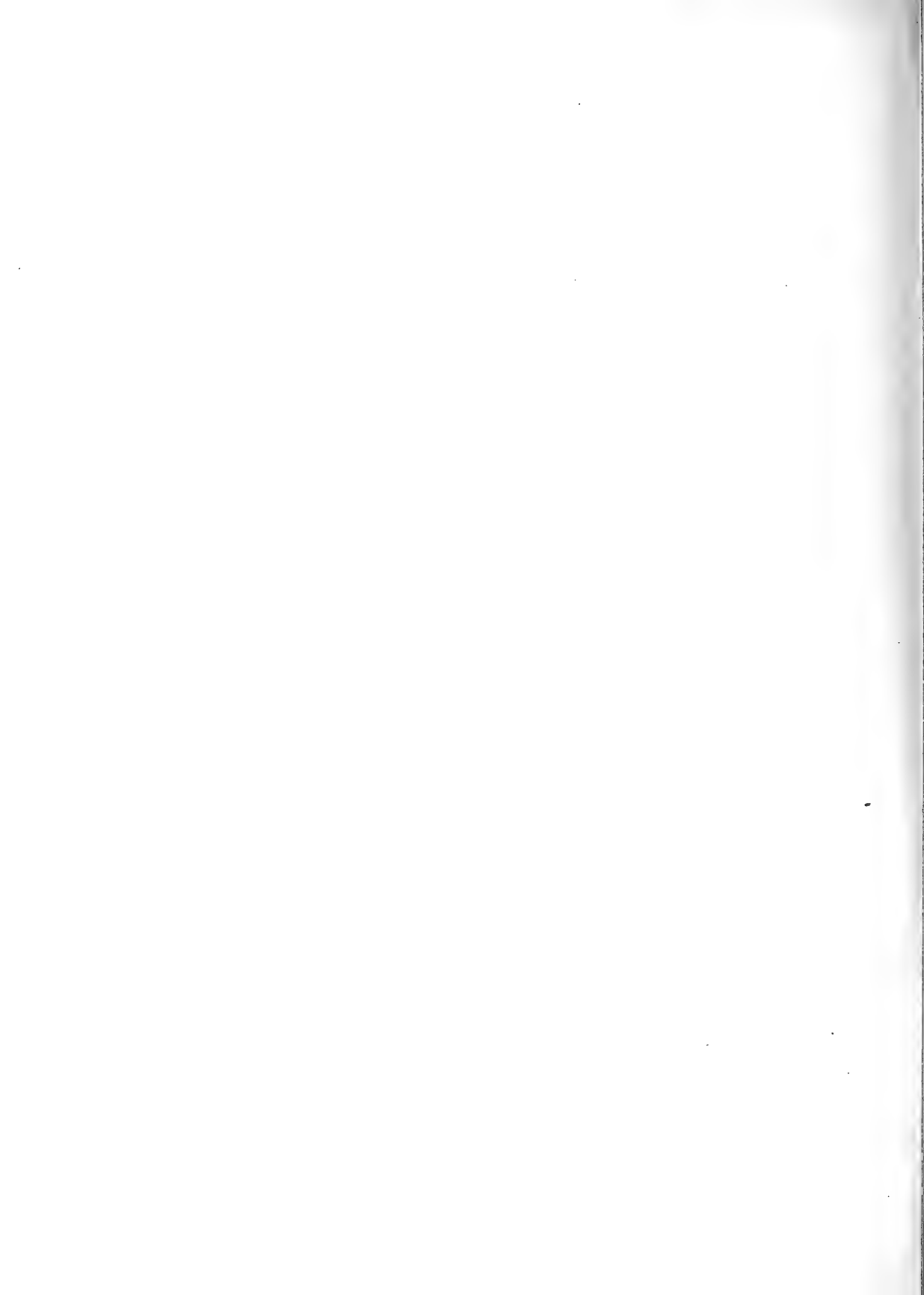
In none of my cultures was I able to satisfy myself that any increase, either in number or size, occurred in the parasites, although they persisted in some of the culture tubes for a very long time, and attempts at subculture gave no satisfactory results, although the parasites could be found for a few days.

In these attempts at cultivation no marked differences were observed between those kept aerobically and those kept anaerobically.

As Bass has not yet published the full details of his technique, and because of the small number of the cases I have tried, it is impossible for me to make any criticism of his work. I can, therefore, only give this short account of my results.

REFERENCE

- Bass, C. C. (1911). Jour. Amer. Med. Assoc., LVII, 19, p. 1534.



URRIOLA'S TEST FOR MALARIAL INFECTION

BY

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Urriola (1911) has described a new urinary test for infection with malaria, regarded by him as pathognomonic. His test is the discovery of malarial pigment in the urine. The urine is centrifuged, and the deposit examined under a high magnification. He states that four types of pigment granules may be found in malarial cases: (1) very fine granules massed together; (2) larger granules arranged in similar groups; (3) large masses varying in form; (4) granules included within leucocytes and hyaline casts. The colour is usually an intense black of the Chinese ink type, blue granules may be seen, and rarely some which are yellow ochre in colour. This malarial pigment cannot be confused with the granules found in other febrile disorders, for it is much more abundant. Notwithstanding this, more than one drop of deposit should be examined, and half an hour may have to be devoted to the search.

To test the value of this sign, the urines of ten cases of malaria have been examined by me at the Liverpool School of Tropical Medicine.

Of the ten cases examined, nine may be classed as having been infected with *Plasmodium falciparum*, of which one had a mixed infection of *P. vivax* and *P. falciparum*, and one had a history of blackwater fever a few days before admission. The tenth case was an infection with *P. vivax*. All these cases showed more or less pigment which might be classified under the first three types of Urriola.

Of the nine malignant cases five were old chronic ones, and showed fairly abundant pigmented leucocytes. Three were cases

which did not date their first infection further back than two months. In one of these cases no pigmented leucocytes were found, while in the other two they were only found after a prolonged search. The ninth case was apparently cured, and had no history of fever for more than two years. In this case the pigment was scanty, and only one pigmented leucocyte was seen. In three of the chronic cases pigmented hyaline casts were found. The case of benign tertian showed free pigment, but none in leucocytes or casts.

Ten control urines were examined from normal persons and from patients with renal disease, anaemia, chronic dyspepsia and bronchitis. In all of these except one, pigment was found in fairly large masses, but no pigmented leucocytes or casts were seen.

The urine which did not contain any pigment was passed directly into the centrifuge tube.

It was found that urine which was collected in the ordinary way contained large amounts of pigment derived from dust in the air, urinals, centrifuge tubes, slides, etc., and that the greater the precautions taken to exclude these sources of contamination the less was the amount of pigment present in normal urines. It was impossible to exclude absolutely these sources of error even when the urine was withdrawn by a catheter directly into the centrifuge tube.

CONCLUSIONS

On account of the small number of cases examined it is impossible to make any definite statement as to the value of Urriola's test, but it would appear to me that:—

(1) It is almost impossible to exclude the possibility of the pigment derived from extraneous sources finding its way into the urine, and therefore the presence of small quantities of fairly large masses of pigment appears to me to be of no diagnostic value.

(2) The trouble necessary to exclude the possibility of extraneous pigment appears to be too great in comparison with the value of the test.

(3) As far as can be concluded from the few cases examined, the presence of pigmented leucocytes or casts appears to be of more

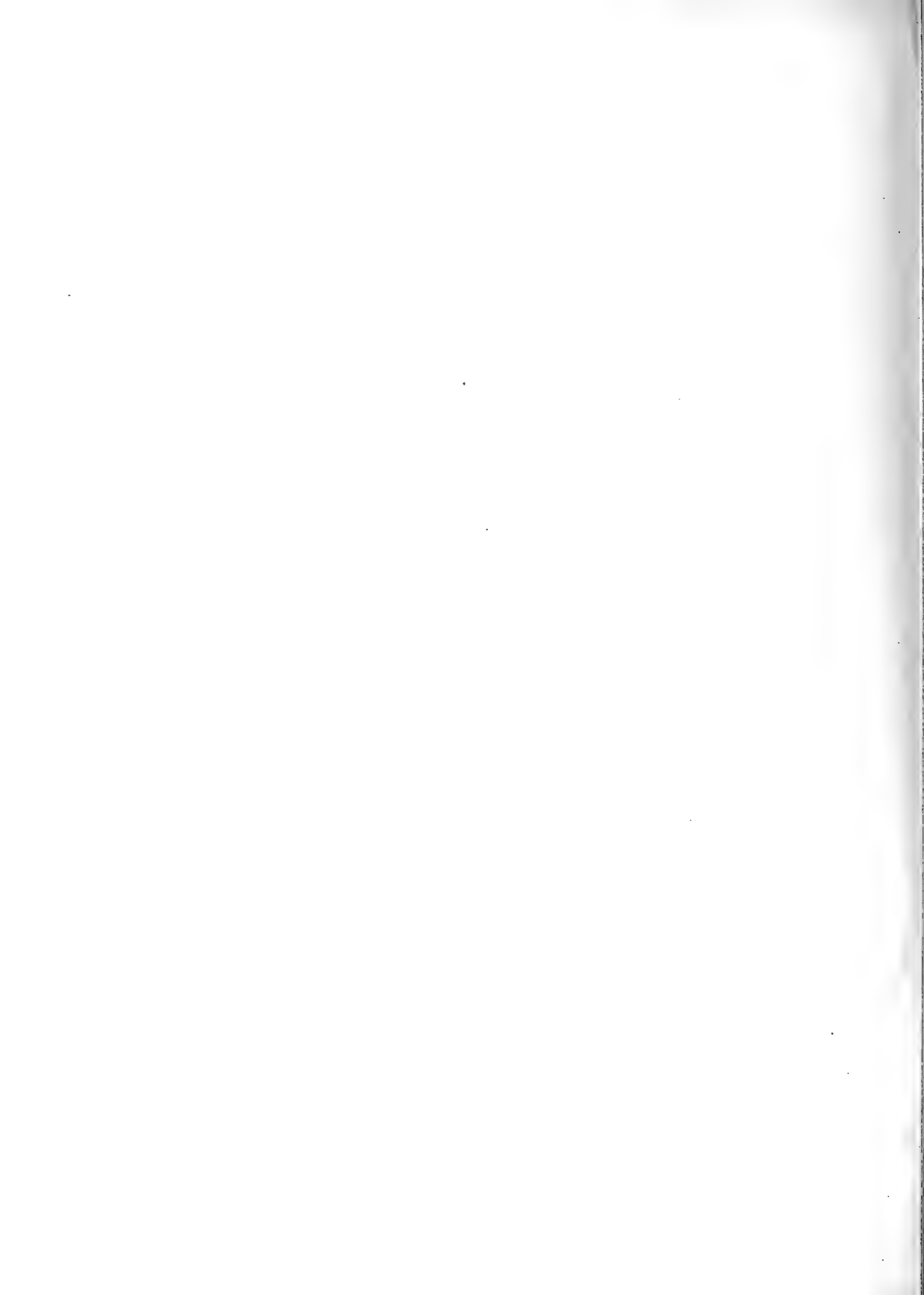
value than free pigment in making a diagnosis of present or past malaria, especially in chronic cases.

(4) The presence of pigmented leucocytes or casts does not seem to be an indication of active malaria, but rather of the fact that the patient has at some time or other had malaria. Two of the above cases were examined just before being discharged as cured, and one of the above cases had had no signs of malaria for two years, yet they all showed pigmented leucocytes. The presence of such pigment in the urine probably represents an attempt by the body, both during an infection and after the infection has passed off, to get rid of the pigment deposited in the tissues.

I wish to thank Dr. David Thomson, under whose care the cases were, for the trouble he has taken.

REFERENCE

- URRIOLA, C. L. (1911) 'Sur un nouveau signe pathognomonique du paludisme.'
Semaine Medicale, Jan. 4.



NOTES ON SOME EARLY REFERENCES TO TROPICAL DISEASES*

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PLATE XXII

IV. ANDRÉ THEVET ON THE IDENTITY OF YAWS AND SYPHILIS—1558

In the case of yaws, as with several other American diseases, we owe our first accounts to the Spanish chroniclers, and notably to Fernandez de Oviedo (Note 1). These first descriptions are, however, scanty, and for clearer statements we must turn to somewhat later authorities. Among these writers a place may be accorded to André Thevet. We have in a previous issue (Note 2) described Thevet's work, 'La France Antarctique,' in which he gives considerable attention to medical matters. It is of interest that in the account of yaws, or 'Pians' as he calls it, this disease is compared to the syphilis then spreading over Europe. Framboesia is still known in the French Antilles as 'Pians,' which is said to be a native term meaning a 'strawberry.' Although Oviedo calls it 'Bubas,' the designation 'Pians' is found in many sixteenth and seventeenth century writers, but the earliest use of the word known to us is in the pages of Thevet.

Thevet's book was published in Paris in 1558, and is a fine piece of printing of the best period of French typography. We have endeavoured in what follows to preserve something of the quaintness of the original by quoting from the anonymous English translation of 1568, making only such corrections as are necessary to preserve the sense.

NOTE 1.—Oviedo first landed in America in 1514, but his books, 'Natural Hystoria de las Indias,' Toledo, and 'Coronica de las Indias,' Madrid, did not appear until 1526 and 1547 respectively.

NOTE 2.—Annals of Tropical Medicine, Vol. VI, p. 96.

* Continued from Annals Trop. Med. and Parasit., VI, (1912), pp. 87-101.

'*The description of a sicknesse named Pians, to the which are subject these people of America as well in the Ilandes as the maine land.* (Note 3.)

Pians a
sicknesse in
America and
his originall.

The wildmen
are very
lecherous and
carnall.

The true
originall of the
French pocks
as the Frenche
men write

' . . . And for that all diseases (as oure Phisitians shew us) come or happen either of the aire, or of the manner of men's living, I am determind to write and set out here a sicknesse or disease very rife and common in these countreys of America and of the West, discovered in our time. Now this sicknesse named Pians by the people of the countrey, cometh not of the corruption of the aire, for it is there verie good and temperat, which [is shewn] by experience [of] the fruites that the earth bringeth forth, with the benefits of the aire, without ye which nothing is made, bee it of Nature or artificiall. Also that the sycknesse [which proceedeth] of the corruption of the ayre, doth hurt as well the young as the olde, the ryche as well as the poore notwithstanding the internall or inwarde disposition (Note 4). Therefore it muste needes bee, that [Pians] proceedeth of some misgovernement, as to much carnall and fleshely frequentation the man with the woman, considering that thys people is very lecherous, carnal and more than brutishe, specially the women: for they do seeke and practise all the meanes to move man to lust. *This sicknesse is no other thyng than the pocks that raigneth and hath power over all Europe, specially among the Frenchemen: For of us it is named the Frenche pockes, the whyche disease as the Frenchmen wright, was first taken at a voyage into Naples, and thether it was broughte by the Spanyardes, from the West Indies. For before it was discovered and made subiecte to the Spanyardes there was no mention thereof. It is not onely here in Europe, but also in Grecia, in Asia and in Affrica.*

'Well, let us returne unto the wylde men's evyll and to the

NOTE 3.—From chapter 48, p. 70, of Thevet's 'France Antarctique.' Words and passages not in the original English translation are placed in square brackets.

NOTE 4.—From the days of Hippocrates to the seventeenth century, and even down to our own times, epidemic and infectious diseases have been regarded as due to some change or corruption of the air. Thus, in Shakespeare's 'Winter's Tale,' Act v, Scene 1, Leontes says:

'The blessed gods
Purge all infection from our air whilst you
Do climate here!'

It is true that Fracastor (*De Contagionibus*, Venice 1546), made a great advance by his conception of the 'seeds' (*semina*) of disease, but in spite of his influence and although his phrases were freely quoted, his idea found no fruitful soil until the middle of the succeeding century. The writer hopes to discuss this point in a forthcoming work.

remedies that they doe use therefore. Nowe this evill taketh the parties, as well wilde men as Christians that are there, by contagion [or] touching, even as the pockes dothe. Also it hath the like Symptomes, and it is daungerous, that if it be waxen old, it is harde (and daungerous) to heale: for sometimes it dothe afflict them even to the death. As for the Christians, whiche doe inhabite in the lande of America, if they couple themselves with the women, they shall never [escape infection], but shall fall into the daunger thereof muche more sooner than they of the countrey. For the curing of this disease, likewise for a certain alteration that oftentimes cometh wyth this evill, they make a certaine decoction of the barke of a tree, named in their language *Hiuourahe*, of the which they drinke, more easier to cure than with our medicine, and they are more easier to be healed than others to my judgement, for their temperatenesse and complection, which are not broken out with infections, as [ours] are.'

The curing of
this disease.

Hiuourahe
a tree.

The passage here given in italics is an amusing mis-translation. It forms an incident of that game of battledore and shuttlecock played by writers throughout the sixteenth century, in which the charge of originating the venereal plague was made in turn against each western nation and in due course rebutted on to a neighbouring nation. The final responsibility has been left with the poor Indian inhabitant of the Western Isles, whose exclusion from the republic of letters has prevented his views from reaching us.

A just translation of the indignant Thevet's actual words in the italicized paragraph would run as follows: 'Which also makes me think and say that this disease is probably no other than that beautiful pox now so rife in Europe which has falsely been fastened on the French nation. By the way that foreigners have labelled it as the *French disease* one might think that none but Frenchmen suffered from it. We all know how it flourishes in France, but so it does elsewhere. It took rise first in an expedition against Naples whither some Spaniards from the Western Isles [i.e., the West Indies] had carried it. Before these islands were discovered and conquered by the Spanish there was no mention made of it either in this country or in Greece or in any part of Asia or Africa. I have myself often talked this matter over with

the late Monsieur Sylvius (Note 5), one of the most learned doctors of our age. To my judgment it would be more in keeping with the facts and more rational to call it the Spanish disease, having regard to the country of its origin.'

There are a number of points in this account to which we would draw the reader's attention.

(1) Thevet straightway identifies pians with syphilis. This was commonly the case with early writers, and the discovery of a form of spirochaete in both diseases goes some way toward justifying our unscientific forebears. Framboesia as it presents itself to the modern observer is not, we suppose, very frequently confused with syphilis, though to judge from the earlier accounts, the disease, like many others, has perhaps changed its type.

(2) 'The pockes . . . taken at a voyage into Naples . . . and thether brought by the Spanyardes from the West Indies,' refers to the terrible outbreak which burst upon Italy in 1493-4. The syphilis, not then exclusively a venereal disease, or at least not regarded as such, spread with terrible rapidity throughout Europe, and left perhaps a deeper impression on medical literature than any event in the world's history.

The work of Fracastor (Note 6), the inventor of the term syphilis, and one of the earliest scientific writers on the subject, leaves open the question whether or no the disease was brought from the New World. Fracastor's composition is in the form of an allegorical poem, which tells how a shepherd, Siphilus by name, blasphemed against the Sun god, who in anger smote him down with the disease. Perhaps into this reference to the Sun god we may read a belief that the disease was of tropical origin. The balance of opinion among syphilographers on this difficult point is still that the disease was indeed brought from tropical America in 1493 by Columbus. In the absence, however, of human remains in America of unequivocal pre-Columbian date, and bearing traces of the ravages of the disease, the question can never be finally settled in this sense (Note 7).

NOTE 5.—The Sylvius referred to is Jacques Dubois (Jacobus Sylvius), 1478-1555, Professor of Anatomy at Paris, and teacher of Vesalius.

NOTE 6.—Girolamo Fracastoro, 'Syphilidis sive Morbi Gallici, libri tres,' Verona, 1530.

NOTE 7.—See Dr. Norman Moore's summing up of this question at a recent discussion at the Royal Society of Medicine. *Lancet*, June 15th, 1912, p. 1600. While this article was in the press, a masterly summary of certain aspects of the subject appeared from the pen of Sir Henry Morris, *Lancet*, August 24th, 1912, p. 497. Sir Henry Morris produces evidence, previously unknown to the present writer, of the discovery in America of syphilitic bones of early date.

(3) 'The bark of the tree Hiuourahé' is Guaiaca which remained in use for the treatment of syphilis long after the introduction of mercury as an internal remedy about the middle of the sixteenth century. Guaiaca was probably brought to Europe from the West Indies by the Spaniards in 1508. It was described with some fulness by Oviedo in 1526 (Note 8), and recommended by Ulrich von Hutten in his classical work 'De morbi Gallici curatione per administrationem ligni guiaci,' published in 1519 (Note 9). The remedy was lauded by Fracastor and by many contemporary writers. At these early dates it was the *wood* or *bark* of the Guaiacum officinale that was used, as described by Thevet. The *resin* did not enter pharmacy until the following century, but has now replaced the wood, which is utilised in only one official preparation, the Decoctum sarsaparillae compositum.

The administration of guaiacum for syphilis is perpetuated in the German terms for the plant, Pockenholzbaum and Franzosenbaum; although gradually superseded by mercury, it was long reckoned an almost infallible remedy, so that a sixteenth century predecessor of Mr. Bernard Shaw assures us that 'the physitions wolde not allowe it, perceyvyng that theyr profite wolde decay thereby.' (Note 10.) The therapeutic value now set on guaiacum forms at least a partial excuse for these recalcitrant physicians.

The use of guaiacum as a routine treatment of yaws survived its vogue for the sister disease. In the eighteenth century it was still commonly administered (as 'Lignum vitae' or 'Lignum sanctum') to infected negroes by planters in the West Indies and other parts of tropical America. A recipe in our possession used by an eighteenth century slave-owner of South Carolina runs as follows:

'Two pounds of Lignum Vitae; four ounces of bark of Sassafras root; four ounces Aniseeds; half a pound of brown sugar; boiled in four gallons of water till reduced to three. The patient to take a pint a day mixed with three pints of water for twenty days.'

NOTE 8.—Oviedo, 'Natural Hystoria de las Indias,' Toledo, 1526, fo. xxxvii.

NOTE 9.—There are at least two works earlier than von Hutten's on the use of Guaiacum for Syphilis. One is the 'De Cura morbi Gallici' of Nicolaus Poll, printed in 1535, but dated Dec. 19th, 1517; the other is the 'De morbo Gallico tractatus' of Leonard Schmaus, printed Nov., 1518, and reprinted in the 'Aphrodisiacus' of Luisinus, 1728.

NOTE 10.—Thomas Paynel, Canon of St. Marten's Abbey, 'Of the wood called Guaiacum that healeth the Frenche Pockes and also helpeth the goute in the fecte, the stoone, the palsey, the lepre, dropsey, falling evyll, and other dysceases.' London, 1533, p. 8. There are several later editions. The book purports to be a translation of von Hutten.

The recipe is accompanied with minute directions for washing the sores with tobacco juice.

We cannot but think that the evidence that the New World was the source from which the venereal plague reached Europe is strengthened by the early identification of syphilis with yaws,—a disease of admittedly exotic origin,—and by the universal treatment of both diseases with an American drug in the years when they were first recognised. That the curative value of this drug is negligible appears to us to rather strengthen the case for the American origin of both diseases.

The argument that the widespread use of a tropical American remedy suggests a tropical American origin of the disease was used by Oviedo himself, who wrote: 'I was often amused in Italy by hearing the Italians speak of the "French disease" whilst the French call it the "disease of Naples." But of a truth they would both hit its name off if they called it the disease of the Indies. And that this is the truth may be gathered from this chapter and from *many experiments already made with holy wood and guayacan*, wherewith especially, better than with any other medicine, this terrible pustulous disease is cured and healed.' (Note 11.)

(4) It has been urged against the American origin of syphilis that the natives of that continent show no immunity to the disease, but are indeed liable to an even severer type than attacks Europeans. Whatever may be the case nowadays, we have the evidence of Thevet that in the past 'the Christians whiche doe inhabite in the lande of America, if they couple themselves with the women, they shall never escape infection [i.e., by the diseases syphilis, yaws, or both], but shall fall into the daunger thereof much more sooner than they of the country.' This statement certainly seems to apply better to syphilis than to yaws, to which Europeans seldom fall victim.

(5) Lastly, it is interesting to have evidence that Sylvius believed syphilis to be of American origin. Sylvius (see Note 5) was born in 1478 and was therefore quite old enough to remember details of the great outbreak of the disease in 1494, and to this extent he has the advantage of the two great early writers on the French disease, for Fracastor was only ten or eleven years old at the time while von Hutten was but a child of six.

NOTE 11.—Oviedo 'Coronica,' folio xx.

Before finally leaving Thevet's book we give his account of native medical practice. The line of treatment adopted on the advice of the medicine men—'prophètes' as he calls them,—would in itself, suffice to explain the wide spread of a contagious disorder.

'The foolish opinion of the wilde men in their prophets and of their diseases.'

These prophets make them to beleve that they do speake unto spirites and soules of their parentes, and that nothyng

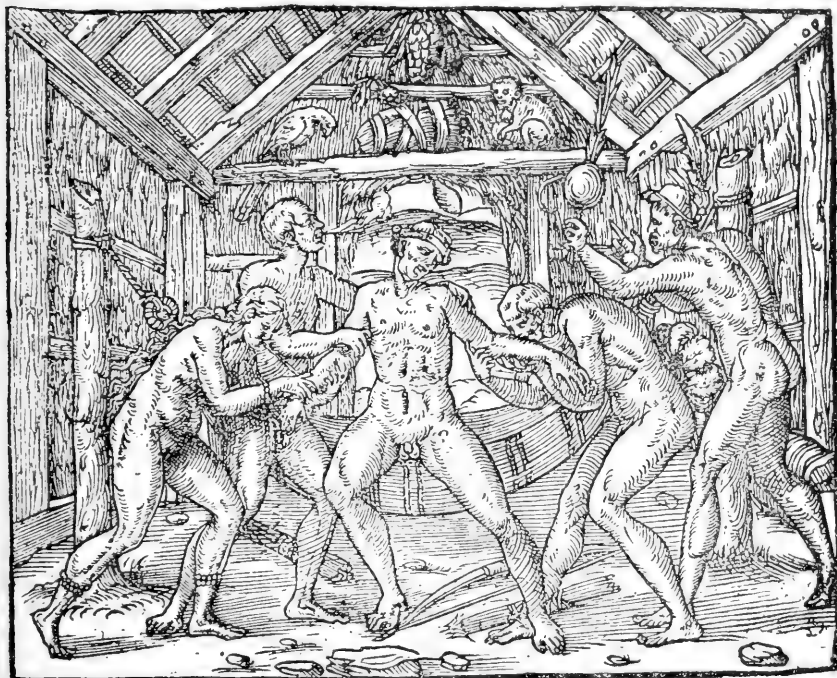


Photo by Donald Macbeth, London.

FIG. I

to them is impossible, also that they can cause the soule to speake within the bodie.' (Note 12.)

'Moreover when soever any syche man feeleth his stomache to swelle by the occasions of some humours in the stomacke and liver, the whyche by debilitie or otherwyse he cannot cast or vomite up, he thinketh that it is his soule that complaineth. Now these goodly

NOTE 12.—An interesting account of the beliefs of the American Indians in their medicine men, closely parallel to the narrative of Thevet, may be found in Francis Parkman's 'Jesuits in North America,' Toronto and London, 1901.

Prophetes for to heale this disease wil suck with their mouth the place where the sore or disease lieth, thinking that by this meanes they draw it oute. Lykewyse they sucke one an other. The women use other meanes; they will put into the pacientes mouth a threede of cotton a two feet long, the whiche afterwarde they suck thinking also by thys threede for to get away thys disease or sicknesse. If one of them doe hurte an other in earnest or otherwyse, he is bounde to sucke his wounde untill the tyme that he bee healed.'

Text-fig. 1 is a reproduction of Thevet's woodcut, illustrating an Indian undergoing this pytotherapeutic treatment. On one side of the patient a woman is seen with a thread issuing from her mouth, while a man on the other side sucks the lesions. Beyond is another Indian offering a medicinal plant to the sufferer.

V. ON CERTAIN EARLY REFERENCES TO DRACONTIASIS, THE GUINEA-WORM DISEASE

It has long been known that the Guinea-worm disease was recognised in very early times, and some have even interpreted the fiery serpents which tormented the Children of Israel in the desert as of the nature of filariae. (Note 13.) Certainly Plutarch (circ. 50-117 A.D.) makes mention of the disease, and Galen (131-210 A.D.) refers to it, though his practice in Rome placed him away from its endemic centres, and he expressly says that he has himself seen no cases. The condition finds a place in the writings of Aëtius (6th century), while Paulus of Aegina (7th century) devotes a whole chapter to the *Dracunculus*. (Note 14.)

Among mediaeval writers, the Arabian physicians, coming in direct contact with the disease, give the fullest and best accounts. We need not discuss the general question of the scientific value of their works, but it is certain that on this particular point they show accurate and first-hand clinical knowledge. Among them, the most important for our purpose is Avicenna (980-1037), who was born and spent much of his life in Bokhara, then included in the Persian empire. Avicenna, or to give him his true name, Abu

NOTE 13.—Numbers, chapter 21, v. 7. This passage is regarded by critics as part of E, the Ephraimite or Northern Israelite document, and therefore to be dated about the 8th century B.C.

NOTE 14.—The knowledge of the Guinea-worm by ancient writers is discussed fully by that sound old scholar Dr. Francis Adams of Banchory, in his edition of 'Paulus Aegineta,' Sydenham Society, London, 1846, Vol. II, p. 151.

Ali el-Hosein Ibn-Abdallah Ibn-Sina, wrote in Arabic, but his 'Canon' was frequently translated into Latin, and exercised an immense influence on European medicine as late as the 17th century. The passage which follows is from a Latin rendering, published in 1674. (Note 15.)

Canon of Ibn-Sina, Sectio III, Tractatus ii, Capitum xxi.

'Concerning the Vena Medinensis.

'The signs of this condition are as follows. A pustule first appears and swells up, but afterwards contracts down again to a mere bleb. Soon, however, the bleb perforates and dark red matter is continuously exuded. In the meanwhile a vermicular movement can be distinguished beneath the skin as though some live thing were there, and indeed as we shall see, a worm is present, for so at least some regard the thing that has arisen. There are others, however, who think that the nerves of the part have become corrupted and thus the part itself affected. For the most part it is the legs that are involved, but I have seen cases in which the hands and even the sides were affected, while in children it is not uncommon to find both flanks invaded. Should the worm be ruptured, much pain and trouble ensue, and even if rupture does not take place the condition is tiresome enough . . . The disease is commonest at Medina, whence it takes its name. It occurs also in Chavorstana and in the country beyond, and is found in Egypt and in other regions.'

In the following chapter, Avicenna gives advice as to treatment, with directions as to blood letting from the saphenous vein (Note 16), diet, purgation, etc. He lays emphasis on cold applications:— 'As soon as the symptoms have been recognised, the part should be treated with wet, cold compresses, as of Sandal or Caphura, and the body purged and leeches applied.' Then 'if there is no contra-indication, an opening may be made and the worm extracted whole.'

NOTE 15.—By G. H. Velschius, 'Exercitatio de Vena Medinensi,' Vienna, 1674.

NOTE 16.—The Arabians paid much fanciful attention not only to blood-letting, but also to the particular vein selected for the purpose.

The next writer to whom we refer is João Rodriguez de Castell-Branco (1511-1568), better known as Amato the Portuguese (Amatus Lusitanus). This interesting character exercised considerable influence on the medical thought of his day, and was in many respects a link between the mediæval physicians and the great seventeenth century fathers of modern clinical medicine. Amatus still claims recognition by reason of his discovery of the valves in the veins, an honour which he shares with Fabricius of Acquapendente. This discovery led to the demonstration of the circulation of the blood by Harvey, who was the pupil of Fabricius. (Note 17.) Amatus was a Jew by religion, and his hunted existence during a period of great intolerance had, at least, the advantage of giving him a knowledge of the diseases of many lands. He acted for a time as medical adviser to Pope Julius III, on whose death he was forced to leave Italy. He settled in Salonica, under the more tolerant rule of the Turk, and here he observed the disease that is the subject of these pages.

Amatus wrote as follows, in one of his 'Centuries of observations':—'A certain Ethiopian slave, 18 years of age, when first brought to Salonica from Cairo was seized with pain in the leg. An ulcer developed, in which vein-like structures became prominent. This condition is known as the *Turkish disease*, a dangerous malady which develops not only in the country from which it takes its name, but also in Egypt, India and other countries, as the Arabian physicians especially Avicenna and Avenzoar, do teach, describing it as the Medina vein. For its cure we may follow Dr. Parahyas (?Pareira) who is learned in Arabic and advises thus:—

'First, the patient ties the end of the vein or nerve round a small piece of wood, and this he winds little by little till the last part of the worm is drawn out. As the structure is often three cubits long, the treatment may last many days before the sufferer is altogether free from pain and inconvenience. Many adopt a

NOTE 17.—Amatus has been the subject of a recent monograph in Portuguese 'Amato Lusitano a sua vida e sua obra,' by Maximiano Lemos, Oporto, 1907. The 'Centuries of observations' of Amatus were published at various times between 1551 and his death, and the dates of their first appearances seem uncertain. They are mainly a series of clinical notes on cases. The translation here given is from the seventh century, case 64, and is from the Leyden edition of 1570, the earliest that we have been able to find of this seventh output. All his works are in Latin.

cataplasm or cold suffusion, as Soranus, Leonides, and Paulus of Aegina recommend.' (Note 18.) To this passage Amatus adds Scholia, or commentary, which runs as follows: 'Authors are in doubt whether this is a nerve, a vein or a worm. But I have seen the condition with my own eyes, and can bear witness that a thin, white worm in many coils was drawn forth . . . and if it should be broken in the process much pain and general disturbance ensues.'

Another sixteenth century writer who refers to the Guinea-worm disease is the Dutch explorer Linschoten. Linschoten made his voyages to the East in the latter half of the century, and on his return to Holland he issued a series of folio volumes with accounts of his experiences. These books were among the most popular of the period. They passed through many editions, and were translated into nearly every European language. The first edition appeared in Dutch in 1595-6 (Note 19), and in 1599 there were two Latin editions. All the early editions of Linschoten are illustrated, but the figure we reproduce (Plate XXII, fig. 1) is only to be found in the Latin editions of 1599. This we believe to be the earliest representation in a printed book of the Guinea-worm disease.

Attached to the engraving is a legend that runs as follows: 'Armsium [Hormuz (Note 20)] is the name of an island and of its principal city . . . Such is the heat of the sun there, that no tree can put forth leaves nor can grass grow, so that the earth is barren and brings forth nothing but scorpions and sandstone. The houses are built with a single large aperture in the ceiling for the admission of light and air. The inhabitants, in order to avoid the great heat, sleep at night, men and women together, immersed but for their heads in troughs of water. Thus it comes about that they are infected by worms, which grow in their legs, and are two or three feet long.' In the reproduction of the plate (Plate XXII, fig. 1) the figure outside may be seen winding the worm from his leg, and a

NOTE 18.—Soranus of Ephesus was a contemporary of Galen. Only fragments of his work have survived, but reference is made both to him and to Leonides by Paul of Aegina. The sentence is practically quoted from Paul.

NOTE 19.—Jan Huygen van Linschoten, 'Reys-Gheschrift Vande Navigatien der Portugal-oysers in Orienten,' Amsterdam, 1595, and 'Itinerario voyage ofte schipvaert,' Amsterdam, 1596. The Latin translation from which our figure is taken is entitled 'Vera Descriptio regni Pars Indiae Orientalis in qua Johan. Hugonis Linscotani Navigatio in Orientem . . . accurate propununtur,' by Teucrides Annaeus Lonicer, Frankfurt, 1599. Folio. There is a separate Atlas of plates to this Latin work, by the brothers de Bry.

NOTE 20.—Hormuz, an island at the mouth of the Persian Gulf.

hiatus in the wall introduces us to the ménage of other natives of 'Armusium.'

During the course of the seventeenth century the French were very active in missionary enterprise. About the middle of the century, Mgr. de la Motte Lambert, Bishop of Beirut, undertook a pastoral tour to the far East, and an interesting record of his experiences was published. (Note 21.) Crossing Persia from Ispahan to the coast, he passed through the town of Lar, the capital of Laristan, and not very far from Bandar Abbas. He says . . . 'It is well provided with fruits, but the water of the town is very bad and the cause of severe and mortal diseases. To this bad water supply throughout the country between Lar and Gomeron (Note 22) may be attributed certain worms of a prodigious length, which engender in the thighs and legs. These worms lie hid, tortuously entwined in the flesh. When detected, a little incision is made to get at the head, this is attached to a piece of stick, and the stick turned a little each day. The worm, which is often six feet long, is thus wound out into a skein. Should it break, the part which remains will cause a dangerous corruption. *The way to avoid this worm is to drink only wine, or if water is used, only such as has been carefully filtered through linen.*'

We have yet to refer to one other early writer on the Guinea-worm, who transcends and includes all the others. The whole field of early references to the *Dracunculus* has been traversed by G. H. Velschius, who in a portly volume under the title 'De Vena Medinensi' (see Note 15), brings together every possible and a great many impossible references to that parasite. Velschius, though a dreary and not very accurate writer, is valuable for his extensive first-hand acquaintance with Arabic literature. A man of great learning and antiquarian interest, he was yet fully acquainted with the rapidly accumulating scientific knowledge of his day, and in this work he has thrown down a gauntlet of unattractive learning, which none are likely to take up.

He seems to have suffered from a sort of dracuncular obsession,—

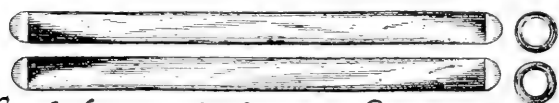
NOTE 21.—Relation du Voyage de Mgr. l'Eveque de Beryte, Vicaire Apostolique du royaume de la Cochinchine, par la Turquie, la Perse, les Indes, etc., jusqu'au Royaume de Siam et autres lieux. Par M. de Bourges, Prêtre, Missionnaire Apostolique. Paris, 1666.

NOTE 22.—Gomeron, a town on the Gulf of Oman which we are unable to identify.

Guinea-worm on the brain,—and saw the object of his study everywhere. In some respects his book reminds one of the Garden of Cyrus of his contemporary, our own Sir Thomas Browne, but he writes like Browne with all the magic left out. As the great stylist pursued his 'quincunx' through heaven and earth and into the waters under the earth, so does Velschius his *Dracunculus*. He sees it everywhere, on ancient Roman emblems, in the signs of the zodiac, among marine nemertines and polychaets, in the serpent on many antique coins, in Arabic lettering, in many Greek

Instrumenta Chirurgica ad extractionem dracunculorum. Pag. 310.

I. *Hemicylindria aperta, cum annulis suis.*



II. *Hemicylindria annulis suis conjuncta et clausa cum dracunculo circumvoluto.*



III. *Cylindrus alius medio fissus, qui annulo digiti protruso aridatur.*



IV. *Collabus tractorius simplex*



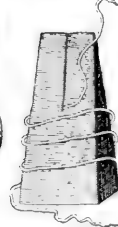
VII
*Synecismus
Aristophanis
Scholiasta*



V. *Vertibulum trochleare*



IX
*Synecismus
Pamblichi
Ioniaca*



VI. *Cylindrus alius, forcipis linteary figura.*



Melchior Haffner sc

sculptures. The famous Laocoon group with its three figures struggling with gigantic serpents are for him a very personification and apotheosis of his Medina worm. He discerns a suggestion of the creature in the thecae of the serpulidae that in some places litter the rocks of the seashore, in the intestinal worms of men and animals, in the convoluted viscera of decapod crustacea, in architectural devices, and in designs of Albrecht Dürer and other artists. The very emblem of our profession gives him one of his best opportunities, and in the serpents coiling round the staff of Aesculapius he distinguishes a Guinea-worm which that distinguished physician has just extracted by entwining it around a piece of wood!

We give in Plate XXII, fig. 2, a reproduction of Velschius' idea of a patient suffering from dracontiasis. The figure, evolved from his inner consciousness, represents in one devoted and saddened individual a number of accounts of the condition.

We part with our subject in Text-fig. 2, which displays the instruments for the extraction of the worm recommended, if we may believe Velschius, by a formidable array of authorities.

VI. MEASURES TAKEN IN PARIS IN 1533 TO PREVENT SPREAD OF THE PLAGUE

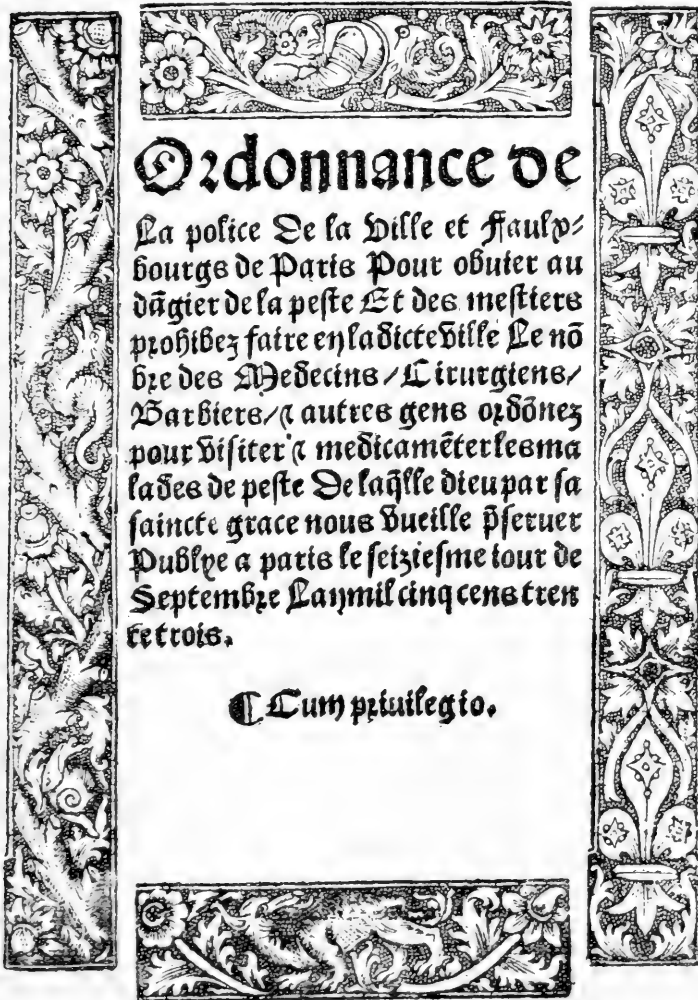
During the centuries when the Plague was a regular visitor to Europe, it was the custom to distribute plague leaflets to the inhabitants of stricken cities, giving the symptoms, directions for treatment, and instructions for avoiding infection. The 'Pestblätter' date back to the very early days of printing, and a number are known which were issued in the fifteenth century. (Note 23.) These leaflets are frequently illustrated. A favourite theme was a figure exhibiting buboes and other signs of the plague, and pierced by arrows according to the words of the Psalm (Ps. XCI) 'Thou shalt not be afraid . . . for the arrow that flieth by day nor for the pestilence that walketh in darkness.' The pamphlet here described was however a more ambitious and official attempt to regulate the public health, and was issued as a

NOTE 23.—Many of these leaflets are most beautifully reproduced and described in a volume entitled 'Pestblätter des XV Jahrhunderts,' by P. Heitz and W. L. Schreiber, Strassburg, 1901.

black letter booklet, and circulated in Paris in 1533. We give below a translation of nearly the whole pamphlet, but we have added headings for the reader's guidance, and we have mitigated the length by omitting the constant repetitions which appear necessary to all legal documents.

Our cordial thanks are due to Mr. Osman Edwards for assistance in translating some of the obscurer passages.

The general regulations are similar to those adopted in the Great Plague of London of 1665, which may be pleasantly followed in



Defoe's Journal of the Plague Year. In reading the rules for cleansing the streets, it should be remembered that the gutters were in those days in the middle of the roadway, and conducted a flowing stream which acted as the common sewer.

In the course of the 'Ordonnance' the doctrine of fomites will be found implicitly involved; although it was several years before Fracastor gave it precise formulation.

POLICE ORDINANCES FOR THE CITY AND SUBURBS OF PARIS to prevent spread of the Plague, to regulate trades, to provide for Physicians, Surgeons, Barbers and others to visit and treat those sick of the disease, from which may God preserve us by His holy grace. Published at Paris, September 16th, 1533.

EXTRACT FROM THE REGISTERS OF PARLIAMENT

The Chamber, convoked by his Majesty during the recess, (after recitation of the deliberations of the council for provision against the plague now raging in this city of Paris and its suburbs, and having summoned the Procureur-General of the King, the Deputy-Provost of Police, the Inspector of traders and the Aldermen) commands and enjoins as follows:

GENERAL ENACTMENTS

. . . All proprietors or tenants of houses in which there have been cases of plague during the last two months, and all who after the publication of this ordinance may have such cases in their houses, shall place in the window or other conspicuous place a wooden cross, and shall fix or raise a second cross in the middle of the front door or entrance, so that everyone may know of it and may abstain from entry. It is forbidden to remove these crosses or cause them to be removed within two months of the time when they were affixed,—on pain of summary fine. Furthermore all who have suffered from this disease and who may be hereafter afflicted thereby, and all members of households and families where there have been or may hereafter be patients sick of the plague

are commanded and enjoined that they carry in their hands when going to and fro a white rod or staff,—on the same penalty.

The Chamber forbids anyone of any rank, quality, or condition whatsoever to carry or cause to be carried to this town or its suburbs from other places, or to transport or cause to be transported from one house, tenement, dwelling or lodgement where there has been a death from or risk of plague, to other houses or lodgements within the town any bed-coverings, coverlets, quilts, or woollen, serge, linen or similar goods, capable of harbouring the plague, whether such goods belong to them by succession or otherwise. All are enjoined to leave the said goods in the places where the death or danger from plague has occurred, until they receive permission from the Chamber or from the aforesaid officers to transport them,—on pain of confiscation of liberty and goods. Further the Chamber forbids all old clothes dealers, tailors, clothes-repairers, hawkers, etc., male or female, and all subjects of the king or of other states to sell or expose for sale any bed furniture or articles of costume or other goods, in which plague or noxious air may be harboured,—on the same penalty.

Similarly the Chamber forbids any person of whatsoever rank to visit any public bathing establishment until after next Christmas day,—on pain of corporal punishment.

ENACTMENTS CONCERNING MEDICAL MEN AND VETERINARIES

The Chamber forbids all surgeons and barbers to throw blood of patients suffering from any malady whatsoever into the river Seine in its course through Paris, (the horse knackers' establishment being regarded as the boundary of the town), but they are enjoined to send or carry it outside the town,—on penalty of imprisonment and summary fine.

All surgeons and barbers who have bled patients suffering from the plague are forbidden to practise their art, profession or trade on the bodies of healthy persons until after the elapse of the period enacted by law,—*on pain of the halter.*

Furthermore, when horses are bled, the use merely of lime or earth is forbidden; but the blood is to be caught in a vessel, and carried to the sewers outside the town.

RULES FOR CLEANING THE STREETS, ETC.

The Chamber commands all persons of every rank and condition that they shall henceforth pave and repair broken and cracked roadway in front of their houses. They shall keep the roadway in good repair and ensure its cleanliness by throwing down water, morning and evening, each in front of his own door. And they shall see that the streams of the gutters have an unimpeded course past their houses, so that no filth may accumulate. Nevertheless when any rain or other thing may fall from heaven, all cleaning, sweeping and scouring shall be withheld until the rain has passed and has drained away, so that the water have as free a course as may be . . . It is forbidden to throw out of window infectious filth or garbage or dirty water, or to keep it within the house, but such refuse must be carried to the stream, and a bucket of pure water thrown in after. Whoever carries urine to the doctor to be examined, is forbidden to cast it into the streets, but must carry it back to his house and there throw it into the stream in front of his door, casting after it at least three buckets of water. These things shall be done on pain of corporal punishment and summary fine according to the exigencies of the case.

The Chamber expressly forbids any person of whatsoever rank to empty or throw filth or garbage unsuitable for the gutter into the open street or to burn it there, but enjoins them strictly to carry it or to have it carried into the fields to the appointed places, permitting them if they have not dung carts, vehicles or drivers immediately available, to enclose it in baskets or hampers and place these alongside their houses that they may be carried off as soon as possible.

The Chamber forbids stone-cutters, masons, tilers, and others working for them to empty or cause to be emptied from houses gravel or other things which may harbour infection, unless they have vehicles and drivers ready to carry away the said refuse as fast as it is taken from the houses to the appointed places in the fields,—on pain of imprisonment and rigorous punishment at the pleasure of the law. Nevertheless the Chamber ordains that masters and mistresses shall be responsible for their varlets, servitors and maids on the subject of this and the preceding article, beyond the punishment which may ensue to the said varlets, servitors, and maids.

Similarly the Chamber commands the sanitary inspectors and other such persons that with no further excuses they shall apply themselves to the scouring and cleansing of the roads of this town, that all filth and garbage may be carried away,—on pain of prison and summary fine. Further, the dung carts shall be ready with their horses, and shall be well closed up so that nothing can issue from them. And the tailpiece of the cart shall be as high or higher than the front,—on pain of imprisonment and confiscation of the horses and dung carts and of summary fine. All police and citizens of Paris who shall see or find such dung carts not properly closed shall take them or cause them to be taken to the prison to be suitably dealt with.

Further, the Chamber forbids all butchers, cooks, bakers, hucksters, dealers in game and poultry, taverners, labourers, traders, and other persons of whatsoever station or condition, to keep or rear in this town or its suburbs any hogs, sows, fowls, geese or pigeons, either for their own nourishment, for sale or for any other reason, excuse or plausibility whatsoever. It enjoins that if any persons possess such live-stock at the time of publication of this present ordinance, they shall convey the said live-stock or cause it to be conveyed into the fields beyond the town,—under penalty of imprisonment and severe punishment at the discretion of the magistrate and of confiscation of the live-stock. Similarly the Chamber commands all who shall have knowledge of any contravention of this ordinance that they shall make it known to the magistrate with all diligence, for the execution of suitable punishment.

The Chamber enjoins all proprietors of houses with middens or cesspools that they shall with all speed and diligence wall them up,—on pain of the rent of such houses being seized by summary order of the Chamber and applied to the walling up of the said cesspools or middens. And it is moreover forbidden in future to any cleaner of cesspools to empty or clean them out without express permission of the magistrate.

This Chamber expressly forbids all sojourners and inhabitants of this town of Paris to place or cause to be placed any cloth or other hanging from the window sills of their houses.

THE DUTIES OF MAGISTRATES AND OTHERS

Moreover, the said Chamber commands the officers examining magistrates of the prison of Paris, that they cause this present ordinance to be strictly observed and fulfilled, and empowers them to imprison all those who contravene the same, that they may be punished according to the exigencies of the case. The Chamber enjoins the divisional police to render every assistance in their power to the said officers, and to inform them of any trespass or crime which comes to their knowledge. And that the commissioners be the more zealous to maintain this ordinance and to execute arrests and imprisonments, and that the divisional police may make the necessary accusations, the Chamber ordains that they shall have letters-patent of compensation.

ENACTMENTS FOR THE MEDICAL FACULTY

This Chamber orders the faculty of Medicine to depute four doctors of medicine, expert in theory and in practice, to visit and treat the plague-stricken patients in the town and its suburbs. For this service they shall each receive three hundred pounds for the current year, of which one fourth shall be paid to them in advance. Further, the College of Surgeons of this said town shall elect two sworn master surgeons, fit to visit, treat and consider those sick of the plague, and each shall be guaranteed one hundred and twenty pounds during this present year, of which they shall similarly receive one quarter in advance. Similarly, the congregation and assembly of sworn barbers of this town shall elect six sworn master barbers to visit, comfort, treat and support the said sufferers from plague, and each of them shall be paid during this year eighty pounds. The Chamber hereby pledges itself to the payment of all the above charges.

Also the Chamber commands the said four doctors, two surgeons and six barbers who shall be elected and commissioned, that during the time aforesaid and for forty days thereafter or until it shall be otherwise ordained by the Chamber, they shall abstain from seeing, visiting or treating other persons not plague-stricken; and the said surgeons and barbers are enjoined that they keep their offices shut during the time aforementioned,—on pain of corporal punishment, deprivation of status and summary fine.

POLICE ORGANISATION

The Chamber ordains that the commissary of each quarter, with the assistance of two churchwardens of the parish, shall depute persons in every parish to remove plague-stricken corpses from the houses, to bury them, to remove their furniture to the appointed place, to cleanse the houses, to set open the windows and apertures of these houses, to shut the doors and to attach crosses to them. The pay of such persons shall be six pounds of Paris per month.

Also the commissaries shall locate in each quarter four beadles armed with staves, who shall enforce the execution of these duties on the persons deputed by the commissaries and churchwardens.

And the Chamber forbids all physicians, surgeons, barbers, apothecaries, nurses and others who shall have visited, aided, helped, served or assisted any of the plague-stricken, to communicate with any others until forty days have elapsed from the day when they visited, nursed, aided or assisted the plague stricken . . .

REGULATIONS FOR CERTAIN TRADES

All curers and tanners of hides are forbidden to carry on their trades in the city and suburbs, but they are permitted to work beyond the suburbs at the river Seine below the town on the St. Germain's side of the meadows. They are to keep at a distance of over two bowshots from the town,—on pain of banishment from the realm and confiscation of property. Curriers and tanners are moreover forbidden to sell their merchandize within the town, even though the hides are free from infection and bad odour.

Until otherwise ordained, all furriers, leather dressers, cloth-dyers and those of similar estate are forbidden to do their muling, dressing or dyeing within the town and its suburbs. They may not carry woollen stuffs or fabrics to be washed in the Seine above the Tuileries, nor may they pour the washings of such materials into the river within the said limits, nor may they air them nor expose them for sale, though these favours are permitted them below the town on the St. Germain's side, as with the hide-dressers . . .

All butchers, pork butchers and those of similar estate are forbidden to make abattoirs or tripe-dresseries within the town and its suburbs, except in those ancient spots set aside from time immemorial for the royal butchery.

All sellers and hawkers of sea-fish and other similar traders are forbidden to perform any gutting operations within the town and its suburbs.

All undertakers are forbidden to spread in the churches, houses, gates or doors, cloths or other articles used in mortuaries—on pain of loss of license and rights, and confiscation of goods and of the said cloths. . .

CONCLUSION

Further, the Chamber enjoins on all sojourners and inhabitants of this town and its suburbs that if there should be hereafter found any defilement or suspicion of plague, they shall reveal it immediately to the local heads of police without respect of persons or exception for husband, wife, child, servant, master or mistress; so that the commissary may be informed and that he may at once take steps as enjoined by the Chamber.

And finally the Chamber enjoins on the Provost of Paris or the Deputy Provost of Police to cause the present ordinance to be read and published at the cross-roads of this town, that none may pretend ignorance but that it may be kept and observed strictly.

Enacted by the Chamber by command of the King during Vacation the thirteenth day of September in the year One thousand five hundred and thirty-three.

EXPLANATION OF PLATE XXII

Figure 1.—Natives of the island Armusium (Hormuz). On the left they are represented as sleeping in troughs of water; on the right the figure in the foreground is engaged in removing a Guinea-worm from his left leg. Another worm coiled round a piece of stick is seen in the calf of the right leg.

From Lonicer's 'Vera Descriptio regni Pars Indiae Orientalis,' Frankfort, 1595.

Figure 2.—From G. H. Velschius 'Exercitatio de Vena Medinensi,' Vienna, 1674. The figure shows a man extensively infected with Guinea-worms, and to it is attached a legend to the effect that it is 'adapted from the accounts of Guenocius, and shews the Medina veins lying coiled in the thighs, legs, arms and trunk.' It is supposed to represent a West African scene.

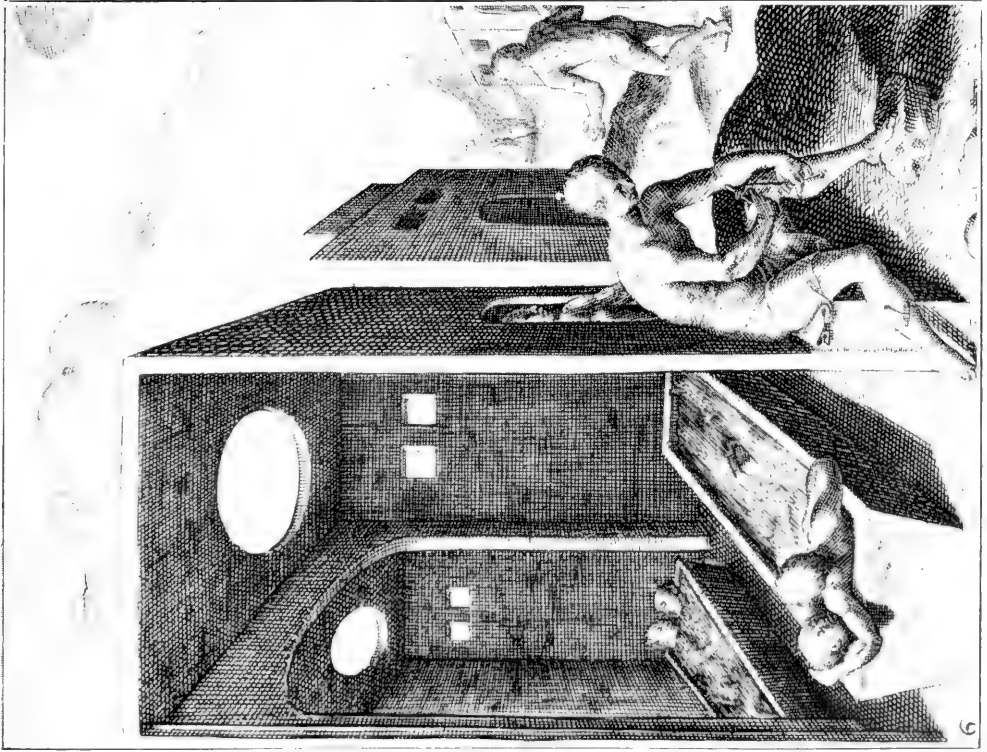


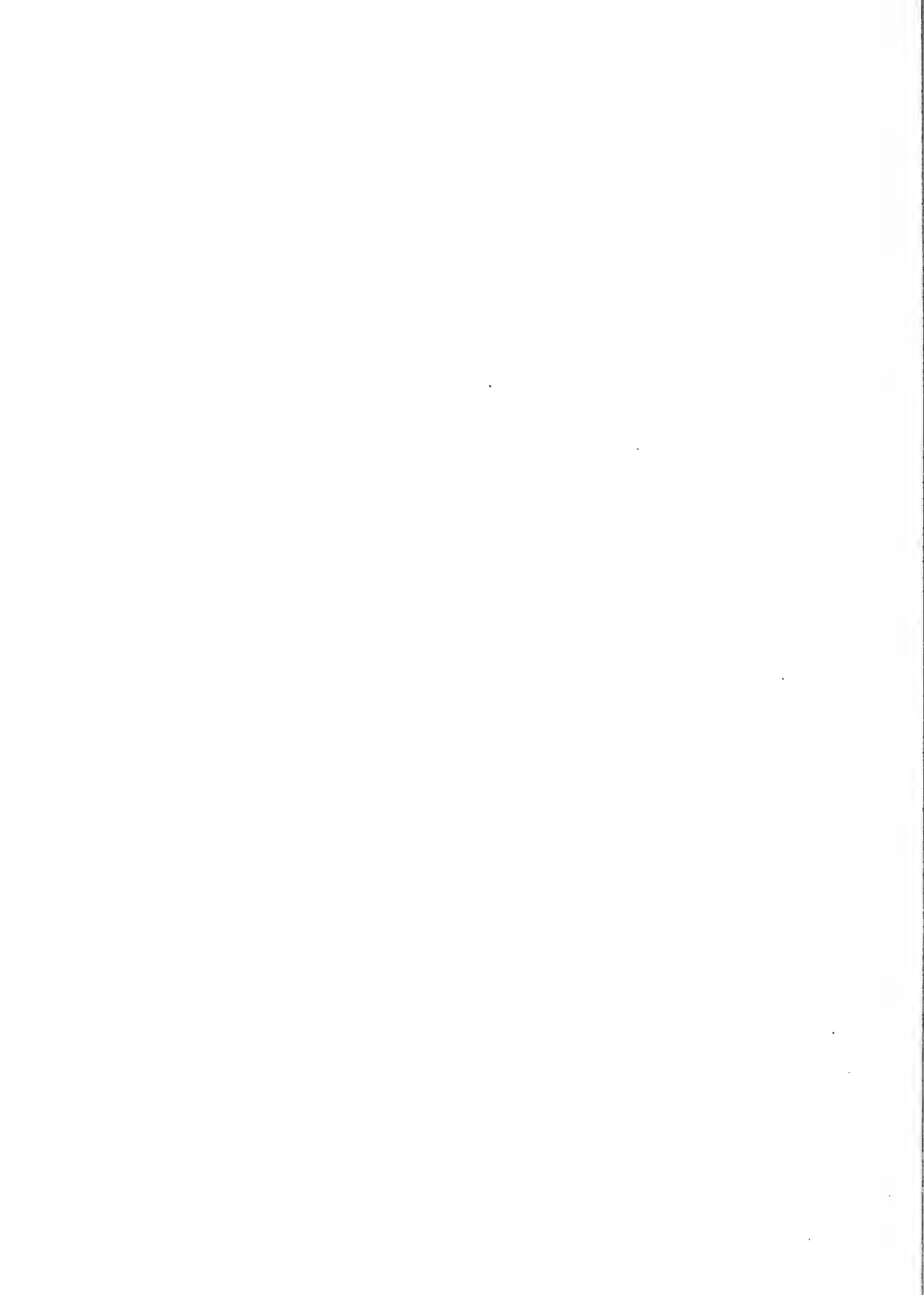
Photo by Donald Macbeth, London.

FIG. 1



Photo by Donald Macbeth, London.

FIG. 2



NOTE ON THE OCCURRENCE AND DISTRIBUTION OF *HERPETOMONAS* *PEDICULI*

BY

H. B. FANTHAM, D.Sc., B.A.

(Received for publication 30 August, 1912)

As much attention is now being paid to the problems of the transmission of leishmaniasis, spirochaetosis and trypanosomiasis, it may be of interest to add a few notes regarding *Herpetomonas pediculi*, a parasite recently described by me from the digestive tract of the human body louse, *Pediculus vestimenti*, in England.

H. pediculi is a flagellate Protozoön passing through three stages in its life cycle, namely, preflagellate, flagellate and post-flagellate stages. In its resting condition (preflagellate or post-flagellate) it greatly resembles both in size and structure the Leishman-Donovan body, and the non-flagellate stages of trypanosomes (Fantham, 1911). As I have been careful to point out, after breeding and feeding experiments with infected lice on my own body, it has no connection with *Leishmania* or *Trypanosoma*, but is a natural flagellate of the louse.

Since publishing my account of the parasite, I have found it in head lice (*P. capitis*) in England. The *Herpetomonas* occurs in the digestive tract of the head lice, and is apparently identical with *H. pediculi* from the body louse.

From personal communications, I gather that *H. pediculi* has been seen by Captain Mackie, I.M.S., in body lice in Bombay, and by Dr. Blaizot, in lice in Tunis. In each case, the main object of the examination of the Pediculi was in connection with the transmission of human spirochaetosis. Apparently the herpetomonad infections in lice in the tropics are heavier than those in England.

Herpetomonas pediculi, then, is now known to occur in human body lice and head lice in England, and in body lice in India and in Tunisia. The geographical distribution, consequently, is much wider than was at first thought when I published my paper, and, hence, the occurrence of natural flagellates in lice is of greater importance than was at first supposed in dealing with the problems of the transmission of kala-azar and allied diseases.

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ON THE INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE DEVELOPMENT OF *TRYPANOSOMA RHODESIENSE* IN *GLOSSINA MORSITANS**

BY

ALLAN KINGHORN

AND

WARRINGTON YORKE

(Fifth interim report of the Luangwa Sleeping Sickness Commission of the British South Africa Company)

(Received for publication 23 September, 1912)

In a former report†, attention was drawn to certain points which seemed to indicate that meteorological conditions, in the Luangwa Valley, had a considerable influence on the development of *Trypanosoma rhodesiense* in *Glossina morsitans*. As, however, the experiments were few in number, and were carried out during only a single dry and wet season, no definite conclusions could be drawn. Since the Commission established its headquarters on the Congo-Zambesi watershed, evidence has accumulated which indicates decisively that such conditions, more particularly the temperature, exert a very pronounced influence on the development of the trypanosome in *Glossina morsitans*.

In Tables 1 and 2 the meteorological conditions at Nwalia (Luangwa Valley) and at Ngoa (Congo-Zambesi Watershed) are summarised.

At Nawalia, eight transmission experiments were made, four with laboratory-bred, and four with 'wild' *Glossina morsitans*. At Ngoa, five experiments have been carried out to date, four with 'wild,' and one with bred flies. It is unfortunate that the bred

* Reprinted with additions from the Brit. Med. Journ., October 5th, 1912.

† Kinghorn and Yorke, Annals Trop. Med. and Parasit., Vol. VI, p. 275, July, 1912.

TABLE 1.—Meteorological observations at Nawalia, N. Rhodesia, 12° 25' S., 32° 2' E., altitude 2,100 feet (approximately).

1911-1912	External shade tem. mean	Absolute maximum	Absolute minimum	Laboratory tem. mean	Relative humidity per cent.	Rainfall inches	Days on which rain fell
June	67.2	89.0	44.3	—	48.6	0	—
July	68.7	91.0	44.6	67.4	45.7	0	—
August	73.3	96.8	51.2	71.2	35.8	0	—
September ...	77.5	103.3	54.0	71.5	31.5	0	—
October.....	86.1	107.6	62.3	84.5	31.8	0.26	2
November.....	87.1	107.8	67.9	84.6	41.1	1.61	8
December ...	82.3	101.7	67.8	79.6	69.1	8.54	20
January	80.6	101.1	67.0	78.4	77.7	14.97	16
February	79.2	96.0	66.1	77.1	73.8	5.55	16
March	79.0	97.6	61.0	72.0	62.5	5.10	6
April (to 9th)	79.5	97.9	59.8	77.2	58.0	0.01	1
						36.04	69

TABLE 2.—Meteorological observations at Ngoa, N. Rhodesia, 11° 40' S., 31° 30' E., altitude 4,400 feet (approximately).

1912	External shade tem. mean	Absolute maximum	Absolute minimum	Laboratory tem. mean	Relative humidity per cent.	Rain fall inches	Days on which rain fell
April (20th—30th)	72.0	89.7	54.1	68.0	53.7	0	—
May	69.0	90.5	45.1	66.2	51.3	0	—
June	61.6	85.5	34.3	60.5	53.0	0.01	1
July	62.4	83.0	42.3	60.9	53.0	0	—
						0.01	1

flies are not more numerous, but owing to the low temperature the majority of the flies did not emerge from the puparia and many of those which did were malformed, and quickly died. In all the experiments in which 'wild' flies were used, however, the possibility that they were already infected with the trypanosome was excluded by first feeding them on healthy monkeys.

The details of the Nawalia experiments have already been published*, while those of the Ngoa series will appear in a later report. For the purposes of the present paper synopses of the two series will suffice, and will be found in Tables 3 and 4.

TABLE 3.—Synopsis of transmission experiments with *Trypanosoma rhodesiense* and *Glossina morsitans* carried out at Nawalia, N. Rhodesia.

No.	Date on which started	Season	Number flies used	Variety flies used	Result	Duration of developmental cycle in days	Mean temperature during developmental cycle†	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity† per cent.
1	20/8/11	Dry	26	bred	Infection	13	75·1	90·0	60·5	35·0
2	14/11/11	Commencement of rains	16	"	"	15	83·5	93·5	74·5	44·8
3	14/11/11		57	wild	"	11	83·8	93·5	74·5	44·8
4	29/12/11	Rainy	20	bred	Negative	—	78·2	87·1	72·8	74·5
5	12/1/12	"	42	wild	"	—	78·0	85·7	72·8	74·2
6	12/1/12	"	42	"	"	—	78·0	85·7	72·8	74·2
7	12/1/12	"	23	bred	Infection	19	77·9	85·7	72·8	75·4
8	13/2/12	"	104	wild	"	25	77·3	86·0	71·0	66·5

It will be seen from these tables that, whereas in the Luangwa Valley, *Trypanosoma rhodesiense* was successfully transmitted by *Glossina morsitans*, all efforts in this direction on the Congo-Zambesi watershed have so far been in vain. Of 330 flies used in the Valley experiments, six, and probably ten, became infective. The larger figure is based on the number of salivary gland infections found in the flies. Our experience indicates that the

* Kinghorn and Yorke, *Annals Trop. Med. and Parasit.*, Vol. VI, No. 1 A, 1912.

† In the case of the unsuccessful experiments, the mean temperature, and the relative humidity have been calculated for the first 30 days only.

implication of these structures is intimately connected with the ability of *Glossina morsitans* to transmit *Trypanosoma rhodesiense*, and that until they are invaded by the organisms the flies are non-infective. Salivary gland infections have been found in all the flies which were capable of transmitting the parasite. In Experiment 3 (Table 3) six flies were found to harbour trypanosomes in the glands, but of these only two were actually proved to transmit *Trypanosoma rhodesiense*. As in all other instances it was shown conclusively that those flies in which trypanosomes were found in the salivary glands were infective, it may be concluded that the remaining four flies in this experiment were also capable of transmitting the parasite. Invasion of the salivary glands has not been observed, except in those flies which were known to transmit *Trypanosoma rhodesiense*.

TABLE 4.—Synopsis of transmission experiments with *Trypanosoma rhodesiense* and *Glossina morsitans*, carried out at Ngao, N. Rhodesia.

No.	Date on which started	Season	Number flies used	Variety flies used	Result	Duration of developmental cycle in days	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity per cent.
1	18/5/12	Dry	116	Wild	Negative	—	62·7	74·5	50·5	52·0
2	13/6/12	"	90	"	"	—	59·1	72·4	42·0	52·0
3	14/6/12	"	119	"	"	—	59·1	72·4	42·0	52·0
4	26/6/12	"	19	Bred	"	—	60·2	71·8	42·0	52·0
5	11/7/12	"	176	Wild	"	—	62·0	72·0	49·1	50·0

In the five plateau experiments, 520 *Glossina morsitans* were employed without a single fly becoming infective.

The explanation of these apparently contradictory results is at first sight not very obvious, more particularly in view of the fact that even on the plateau, 'wild' *Glossina morsitans* capable of infecting healthy monkeys with *Trypanosoma rhodesiense* were occasionally encountered. If the climatic conditions under which the valley experiments were carried out be compared with those

obtaining during the plateau experiments, it will be seen at once that the most striking difference is one of temperature. As a rule, the temperature during the former series of experiments was roughly from 15-20° F. higher than during the latter series.

With a view to ascertaining the influence, if any, exerted by temperature on the developmental cycle of *Trypanosoma rhodesiense* in the tsetse fly, a further series of experiments were performed on the plateau, in which, by means of an incubator, the flies were kept at a temperature approximating to that of the valley at the most favourable season.

TABLE 5.—Synopsis of experiments to transmit *Trypanosoma rhodesiense* by means of *Glossina morsitans* kept in incubator.

No.	Date on which started	Season	Number flies used	Variety flies used	Result	Duration of developmental cycle in days	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity per cent.
1	30/6/12	Dry	61	Wild	Infection	14	80.6	87.8	74.5	36.0*
2	1/7/12	"	72	"	"	13	80.6	87.8	74.5	36.0*

* Approximate.

No water was placed in the incubator, and the warm, dry air was found to have a very deleterious effect on the insects. Within the first seven days, 25 of the 61 flies with which Experiment No. 1 was commenced, and 53 of the 72 in Experiment No. 2 had died. Notwithstanding the small number alive at the end of the second week, two infective flies were obtained in Experiment 1 and one in Experiment 2.

It seems obvious, therefore, that a high temperature favours the development of the trypanosome in *Glossina morsitans*, and that the comparatively low temperatures obtaining during May, June and July on the Congo-Zambesi watershed are very unfavourable to the completion of the developmental cycle of *Trypanosoma rhodesiense* in this fly, and would account for the unsuccessful experiments at laboratory temperatures.

Other observations afford corroborative evidence in support of this contention. An analysis of the valley transmission experiments shows that the largest number of infective flies was obtained at the hottest season of the year.

TABLE 6—Showing number of infective flies obtained, and meteorological observations at Nawalia (Luangwa Valley)

No.	Date on which started	Season	Duration of developmental cycle in days	Number infective flies found	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity per cent.
1	20/8/11	Dry	13	1	75·1	90·0	60·5	35·0
2	14/11/11	Commencement of rains	15	1	83·5	93·5	74·5	44·8
3	14/11/11		11	6	83·8	93·5	74·5	44·8
4	29/12/11	Rainy	—	0	78·2	87·1	72·8	74·5
5	12/1/12	„	—	0	78·0	85·7	72·8	74·2
6	12/1/12	„	—	0	78·0	85·7	72·8	74·2
7	12/1/12	„	19	1	77·9	85·7	72·8	75·4
8	13/2/12	„	25	1	77·3	86·0	71·0	66·5

The fact that an occasional infective 'wild' fly was encountered on the plateau during a period (May, June and July) when attempts to transmit in the laboratory were invariably unsuccessful requires some explanation. A possible solution may be that the flies in question were infected during the warmer season of the year and had survived into the cold season.

If the results obtained by feeding freshly-caught flies on healthy monkeys in the valley are compared with those from flies caught on the plateau, a marked difference in the number of infections resulting is apparent. In the Luangwa Valley, 3,202 flies were fed in 29 batches and *Trypanosoma rhodesiense* was isolated in six of the experiments, giving a ratio of 1 infective fly to 534, whereas on the Congo-Zambesi watershed, 5,041 freshly-caught *Glossina morsitans* were fed in groups on 39 monkeys, with four positive results—1 infective fly to 1,260. As tsetse flies and game

are about equally numerous at Nawalia and Ngoa, and as the disease was presumably introduced into the two localities, which are less than 70 miles apart, about the same time, it appears to us that the only essential difference which can account for the fact that the percentage of infective 'wild' flies at Nawalia is two and a half times as great as at Ngoa is the difference in the climatic conditions. It will be seen from Tables 1 and 2 that the temperatures experienced on the Congo-Zambesi watershed during May, June and July are very much lower than those at Nawalia from September to March. It was during the months named that our experiments were carried out at the two places.

Finally, it might be mentioned that the percentage of infective 'wild' flies caught in the valley was greater in the hot than in the cold season. This point is illustrated in Table 7.

TABLE 7—Percentage of *Glossina morsitans* found infected with *Trypanosoma rhodesiense* at Nawalia at different seasons of the year.

1911-1912	Mean external shade temperature	Number flies fed	Number infections with <i>T. rhodesiense</i>	Ratio of infective to non-infective flies
June... ..	67.2	18	0	} 0 : 790
July	68.7	385	0	
August	73.3	193	0	
September	77.5	194	0	
October	86.1	—	—	} 1 : 338
November	87.1	270	1	
December	82.3	205	0	
January	80.6	538	2	
February	79.2	104	0	} 1 : 466
March	79.0	823	2	
April (to 9th) ...	79.5	472	1	

In addition to temperature, there is one other factor in the climatic conditions which may possibly influence the developmental cycle of the trypanosome in *Glossina morsitans*. We refer to the relative humidity of the atmosphere. At the most favourable

season of the year in the Luangwa Valley for transmission experiments, and also in the case of those carried out in the incubator, the relative humidity was extremely low. Whether this is a mere coincidence we are, at present, unable to form an opinion, but hope to be able to write more definitely on the subject in a later communication.

SUMMARY

The developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans* is, to a marked degree, influenced by the temperature to which the flies are subjected. High temperatures (75-85° F.) favour the development of the parasite, whilst low temperatures (60-70° F.) are unfavourable.

NGOA, N. RHODESIA,

August 3, 1912.

ADDENDUM

Since writing the above, a further experiment has been completed which, besides affording support to the conclusions arrived at, indicates in a rather more definite manner the influence of temperature on the developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans*.

Two batches of 'wild' *Glossina morsitans* (Batch A consisting of 90 flies and Batch B of 119) in which the possibility of the presence of an infective fly had been previously excluded by feeding the insects on clean monkeys, were fed for three consecutive days on a guinea-pig infected with *Trypanosoma rhodesiense*. After being starved for a day, each batch was fed on a healthy monkey until the 40th day after the first feed on the infected animal. Neither of the monkeys became infected. Batch A, in which there were then 42 flies still alive, was placed in the incubator, whilst Batch B, in which there were now 58 flies, was kept as before at laboratory temperature. The sudden change from the laboratory to the warm, dry air of the incubator proved very fatal to the flies in Batch A, and on the 43rd day only six were alive. From the 41st to the 47th day the flies in this batch were fed on a monkey,

and from the 48th day on a rat. The rat became infected on the 53rd day, so that allowing five days for the incubation of the disease in the animal, Batch A contained an infective fly on the 48th day after the first feed on the infected guinea-pig, and eight days after being placed in the incubator. As the monkey died on the 47th day, we are unable to state whether the fly became infective before the 48th day. The four flies still living on the 53rd day were fed on four clean rats, and three of these became infected.

The monkey on which Batch B was fed was still negative at the end of 60 days, when there were 38 flies alive.

From this experiment it appears that the first part of the developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans* can occur at comparatively low temperatures (60° F.), whereas a considerably higher temperature (75-85° F.) is necessary for the completion of the cycle.

It is interesting to note that the flies in Batch A became infective eight days (possibly less) after being placed in the incubator. This is three days less than the shortest incubation period observed in any of our successful transmission experiments,—a fact which supports the view that the developmental cycle of the parasite in the fly had proceeded to a certain point at the laboratory temperature (60° F.) before the insect was subjected to the higher temperature (80° F.) of the incubator.

NGOA, N. RHODESIA,
August 10, 1912.



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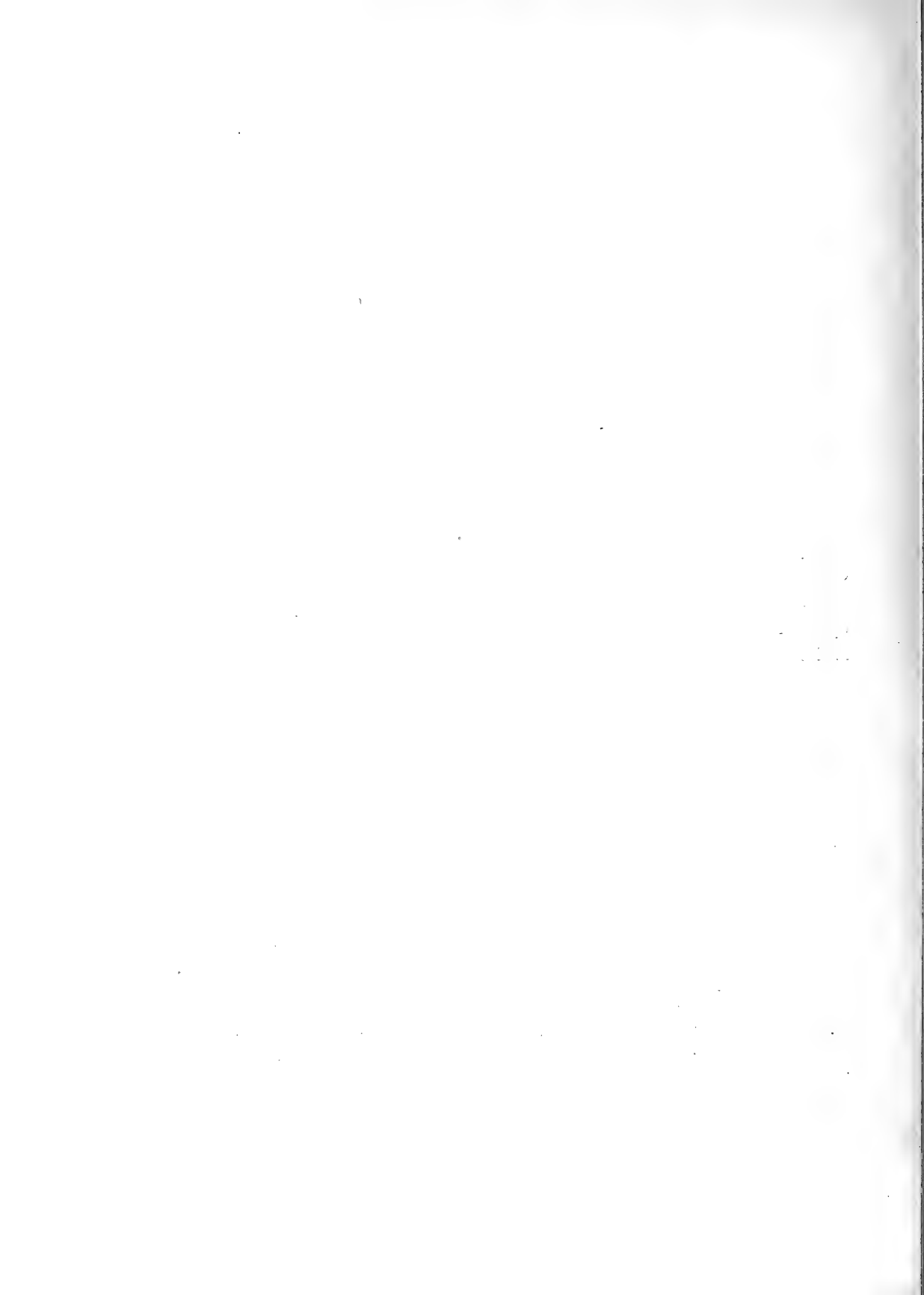
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By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the fourth number of the sixth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.



ON THE RESISTANCE OF *CIMEX LECTULARIUS* TO VARIOUS RE-AGENTS, POWDERS, LIQUIDS AND GASES

BY

B. BLACKLOCK, M.D., D.P.H.

*From the Runcorn Research Laboratories**(Received for publication 25 September, 1912)*

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I. INTRODUCTION

The experiments on the bed-bug described in this paper were undertaken at the suggestion of Sir Edwin Durning-Lawrence, who wished for an investigation on the best method of killing vermin and who also generously supplied the necessary funds, the research being placed under Sir Ronald Ross. In the first place the habits of the bed-bug were studied, and its food supplies and life-history. Next, experiments on bed-bugs were done with powders of many kinds, beginning with well-known proprietary and patent insecticides, and continuing with other powders of vegetable and mineral origin. Liquid preparations were then tested, and finally the action of certain gases.

II. GENERAL OBSERVATIONS ON THE HABITATS, FOOD SUPPLIES, AND LIFE HISTORY OF *CIMEX LECTULARIUS*

Habitats. It is unnecessary to dwell here on the very great prevalence of *Cimex lectularius*. In certain quarters of most villages and cities the bed-bug is accepted by a considerable portion of the inhabitants as a necessary and unavoidable evil. Many people even do not consider it in the light of an actual evil, but merely as a somewhat unpleasant cohabitant in their dwellings. This attitude of toleration towards the bed-bug is encouraged no doubt by the belief which many people entertain that this parasite of man is a comparatively harmless creature. Otherwise one can hardly explain why it is that householders, who in regard to general ideas of sanitation in other respects attain a fairly high standard, frequently appear to regard this tenacious insect with little abhorrence. Whether the belief in the harmlessness of *Cimex lectularius* is justified appears problematical, and it would be of considerable interest to have fuller information as to the bed-bug's capabilities in regard to the transmission of human disease. There is evidence that it can transmit kala-azar in India, and many observers consider that it plays an important part in the spread of relapsing fever in Europe. These facts are sufficient to render us somewhat sceptical of the innocuousness of this animal.

In houses where it dwells, the bed-bug chooses as its headquarters those positions which secure to it, in the first place, safety from attack, and in the second place ready access to steady food supplies. The latter consist preferably of the blood of human beings. It is, therefore, the rule of the bed-bug to take cover in crevices or cracks behind wallpaper, adjacent to a bed, or if possible, as in the case of wooden bedsteads, in the framework of the bed itself. Where suitable cover is not obtainable in close proximity to beds, the bed-bug is content to take up a position of safety at some more remote corner of the room. Distance affords very little obstacle to its nocturnal activities in withdrawing its nourishment from the circulation of man. The bed-bug is a very rapid walker, and can cover the ground at a remarkable pace. Hence the adoption of iron bedsteads (a very great advantage where bugs are prevalent) is not in itself sufficient means of dealing with them. They certainly

have to travel a little further for their meals but they will generally arrive in time. Unlike ticks, the bed-bug does not take a long time to feed, nor does it remain attached to one place during its whole meal. It prefers to run rapidly from place to place, pausing here and there for a few seconds to puncture the skin with its stylets and suck up some blood. In a few minutes it will be swollen and shiny. If bed-bugs are placed on the shaved skin of an animal, e.g., a rat, they will frequently feed immediately until they are quite gorged with blood, and will then run round inside the feeding-glass trying to escape. If the glass be kept on for a considerable time they will, as a rule, wait some time before they begin to defaecate on the skin. On the other hand, if as soon as they are seen to be gorged they are placed in a petri dish, they will generally at once defaecate all over it. They appear to prefer not to defaecate on the living animal, and this probably accounts for the fact that traces of them on sheets and bed-clothing are comparatively seldom to be seen, even where, on careful examination of crevices and wall bindings, the bugs are found alive in large numbers. One must not, therefore, conclude that a house is free from bed-bugs simply because no traces of them can be found on the bed-clothes.

As regards food supplies, it is well known that bugs can subsist without blood of human beings for long periods. During these experiments bugs have been kept in the laboratory in test tubes under varying conditions. Some were placed in tubes with cotton wool and dust, some in tubes without dust, the cotton wool plug being quite inaccessible to them. After three months they were still alive and active. Some, placed in a short test tube in which a cork was used as stopper, cling always to the cork and never leave it; these appear to derive some nourishment from the cork, and several of them have shed their skins. Thirty newly-hatched larvae were placed in a tube. Occasionally one or two died, and the dead bodies disappeared, fed on by the others apparently. There are now after three months' time only fourteen left: these are alive and active, there are no dead ones present. It is mentioned* that Geer kept a bug in a glass tube for a year without any food at all; it lived quite comfortably for that period.

* Butler, *Our household Insects*, p. 293.

Bed-bugs' eggs are very easily obtained in a clean condition by first feeding the bugs well on an animal and then placing them in a petri dish with a piece of clean blotting paper. The eggs will generally be found at intervals after the first twelve hours from the time of feeding. A female, fully fed, was opened thirty-six hours after its feed on a rat. It contained twelve eggs in various stages of development. The eggs when deposited adhere rather tightly to the surface of the blotting paper. If blotting paper or some absorbent material is not placed in the dish, not only are the eggs often adherent to faeces in the bottom of the dish, but also many of the bugs will become entangled in the faeces and die. In the experiments made, of which a summary is given in Table I, the

TABLE I.—Showing incubation period of eggs of *Cimex lectularius* at different temperatures.

Temperature at which kept		Period of incubation in days		
		Min.	Max.	Average
I.	Laboratory temperature	14	25	17
II.	22° C.	11	17	14
III.	25° C.	6	14	10

eggs hatched on an average in seventeen days at laboratory temperature (June, July, August), in fourteen days if kept at 22° C., and in ten days at 25° C. Eggs which had been immersed for twenty-four hours in cold water did not hatch out, the controls hatching normally. The larvae on being hatched are most active at the higher temperatures, and can be fed on an animal within an hour or two of emerging from the egg. In order to feed them safely, however, it is necessary that the shaved skin should be as dry as possible, otherwise many of them become covered with moisture, the antennae and legs all becoming glued together, and they die. It is advisable, therefore, to shave the skin some time before the larvae are to be fed. The rate at which the larvae grow

depends on the amount of food they obtain and the temperature at which they are kept. These two factors influence also the intervals at which successive sloughs are cast, a plentiful supply of food and a moderate temperature, 23° C., being favourable. The larvae are incapable of resisting much heat. For example, in one experiment one hundred and fifty unfed larvae all died within five minutes on being exposed to a temperature of 45° C. Adult bugs can resist this temperature somewhat longer.

III. THE EFFECT OF VARIOUS PROPRIETARY 'INSECTICIDE' POWDERS AND OTHER POWDERS

The experiments with powders were carried out in the following manner, each powder to be tested being subjected to the same conditions.

Adult and newly-hatched bed-bugs.

Method I. The first application was made by placing the bugs in a clean petri dish and sprinkling the powder over them.

Method II. The second experiment consisted in sprinkling a fine layer of the powder over the bottom of petri dishes, and then placing adult or newly-hatched bugs in each dish. They were left there for varying lengths of time and examined at intervals to observe the effect produced. Thereafter they were further examined at intervals of a day.

Method III. The third experiment was carried out by making a ring of powder in the bottom of the petri dishes, the clear centre of the ring being the size of a five-shilling piece and the width of the ring a quarter of an inch, the powder lying about one-sixteenth of an inch deep. In the clear centre of the ring adults or newly-hatched bugs were placed, and each one which passed through the ring was collected and deposited in a clean dish, with blotting paper, for observation.

Method IV. The fourth experiment consisted of making an island of powder, the size of a penny, in the centre of the petri dish, and depositing the bugs in the clear peripheral portion of the dish. Each bug which approached the island of powder and, after touching or walking over it, succeeded in regaining the clear area, was collected and placed in a clean dish with blotting paper.

Method V. This was the same as IV, but pieces of blotting paper were placed in the peripheral portion of the dish in which the experiment was done to give shelter to the insects. Observations were made from time to time of all insects used for each experiment.

Eggs. Experiments were made with a view to studying the effect of the powders employed upon the eggs of bed-bugs. First, a number of eggs was placed in each powder for a period of an hour, then they were removed and carefully brushed with a fine camel-hair brush and placed in small capsules. Secondly, other eggs were laid upon a layer of each powder on the bottom of petri dishes and the effect upon the hatching observed and the results noted.

The list of proprietary powders used was as follows: Keating's powder, Hawley's I.K. Insect-killer, Danzo powder, Cimo bane, Jacko dust, Vals Beetle Cure, Steiner's genuine Dalmatian Insect powder.

The results obtained. Considerable variation resulted according to the method of application of the individual powders. Methods I and II were most effective, owing to the prolongation and certainty of contact, and the insects rarely escaped death by these methods. Methods III and IV, however, were not quite so successful, and although many individuals which were transferred to the clean dishes died ultimately, several did not die, but recovered and were able to feed on an animal afterwards and deposit ova. In Method V many insects never touched the powders.

Mode of action. The certainty with which a given powder will kill bed-bugs depends upon several factors, the chief of which appear to be:—

- i. Fine sub-division of the powder.
- ii. Dryness.
- iii. Lightness.

Given a powder which has these properties, an application by Methods I and II will kill bed-bugs with great certainty, the action being apparently in most cases purely mechanical. If a bug be sprinkled with the powder, it becomes coated over, the fine particles adhering to the body, antennae and legs. After struggling for a

time, the insect turns over on its back, and in this position it struggles until it dies, the period of death varying from a few minutes to several days. An experiment with Keating's powder may be taken, for example, using Method II on ten adult bugs. After twenty-four hours five were dead, after forty-eight hours all dead. Frequently in its struggles in the supine position the insect passes faeces, and this, drying on the glass and the posterior extremity of the animal, tightly anchors it down so as to render escape quite impossible. Other of the proprietary powders have a similar action. It is claimed for most of the powders that they are a speedy and certain death to insects. Some are said to have other actions. Vals Beetle Cure, for example, is stated to be a food which the insects eat eagerly, after which they die. This powder also, when applied by Methods I and II, produces somewhat similar effects to Keating's, and kills a considerable number of the bugs within twenty-four hours. As to whether it is an attractive food for the insects we shall see more fully in dealing with the other methods of application, especially Method V. The third method is an intermediate one, in which the bug is not placed actually in contact with the powder. In the vast majority of cases, however, the first thing bugs do when placed in the centre of a ring of powder is to make their way through it, and escape to the side of the petri dish. They do this apparently in their desire to find shelter from the light. The mortality of bugs after passing through the powders, and becoming covered more or less with them, is still high. Thus, in an experiment of ten placed in a ring of Jacko dust, nine escaped from the ring, one stuck and died in it after half a day. Of the nine which escaped, eight were alive after twenty-four hours, but all were dead in three days, the controls living for long periods.

The fourth method. In these experiments the majority of bugs came in contact with the island of powder sooner or later within the first twenty-four hours, and many died ultimately. Some, however, not only did not die, but were fed on animals some days after they were removed to the clean dishes.

Method V. From many points of view the fifth method seems to be the most practical. In this the bugs were provided with shelter in the shape of blotting paper, which they could leave or not as they chose. Many of them preferred to remain under

cover, and consequently did not come in contact with the powders at all, the experiments being watched for forty-eight hours. In this connection attention must be called to the experiments with Vals Beetle Cure, which is considered to be a food which bugs eat eagerly. In one experiment of ten adults placed with blotting paper round an island of this powder, five came in contact with it and four of these subsequently died. One remained alive for several days after touching the powder, then died. The five others remained sheltered under the blotting paper and were not attracted by the nutritious properties of this insect food. Other similar experiments with this powder had similar results. As regards these insecticide powders in general, it may be said that they are very fatal to bed-bugs, and will destroy them with considerable certainty if applied by Methods I and II. By Method III they are partially successful, and also by Method IV, their success being dependent chiefly upon chance contact in these last cases. Method V, however, which most nearly approaches the state of affairs which exists in the natural habitats of bed-bugs, gave results which show that these powders must fail to radically exterminate bed-bugs under natural conditions. Even if they were applied very liberally to the floors, bedding, etc., of an infected house they would have the effect of cutting short the existence of only the more reckless and adventurous individual bugs. Large numbers would escape death by the simple means of remaining where they were, safely ensconced in nooks and crevices. Later we shall see that bugs can remain without animal food for long periods without obvious ill effects. They are not compelled to come out of their hiding places frequently in search of food, and consequently may escape the powders which are strewn in their supposed path.

Eggs. The eggs of bed-bugs placed in contact with these powders for a period of an hour, and brushed clean, are capable of hatching out successfully afterwards. Not only that, but eggs placed on a layer of these powders actually hatch out upon the surface. In this case, however, the newly-hatched bug often becomes entangled and may die.

Besides the insecticide powders mentioned above, various other powders were used to experiment with, namely, French chalk, Pulv. rhei, Pulv. jalapae, Dalmatian flower powder, Pulv. cort.

ulmi, Pulv. cinchonae, Pulv. sod. brom., Pulv. embel., Pulv. acid tannic, Pulv. amyli, Barium sulphate, powdered.

Certain of these powders also have marked effects on adults and newly hatched bugs when applied by Methods I and II. Dalmatian flowers and fine French chalk are good insecticides. Some of the others which were coarsely divided, such as were the samples used of cubeb and jalap, were not greatly effective. Pulv. cinchonae quickly renders bed-bugs incapable of progression.

IV. THE EFFECT OF VARIOUS LIQUID PREPARATIONS

The parasites were tested by complete immersion for varying lengths of time in each liquid.

1. *Water.*

A. Cold. Bed-bugs placed in cold tap water soon become motionless. If the water is drained off, the parasites, after being dried and warmed, speedily recover. They show very definite resistance to cold water. For example, a bed-bug was placed under cold tap water in a test tube for one minute. Dried with blotting paper it became at once active. It was immediately immersed again for two minutes, and again dried and so on, the insect being replaced under water each time as soon as it showed signs of activity. In this way it successfully resisted successive immersion for one, two, three, ten, twenty-five minutes, one hour, two hours. Finally, it was submerged for four hours and it did not recover from this. Twenty-four hours complete immersion was sufficient to kill all the bed-bugs in ten different experiments, ten adults being used in each experiment. Shorter exposure failed to kill. Seventeen hours immersion killed only twenty-two out of thirty adults in three experiments, the remainder being alive and active twenty-four hours after the completion of the experiments. It is of interest to note in this connection that it is commonly reported that where bed-bugs infect the woodwork of old barges, very prolonged complete immersion of the woodwork fails to destroy the insects. In these cases it is probable that the water does not actually come in contact with the bugs.

B. Hot. Boiling-water has the effect of killing instantaneously

bugs upon which it is poured, or which are dropped into it. Water at 80° C. kills the parasites on an application of sixty seconds duration. Even at 70° C. none recovered after immersion for one minute. At lower temperature, however, recovery was frequent. At 40° C. for one minute, seventeen out of twenty recovered, while at 60° C. for a similar period, nine recovered out of ten.

2. *Carbolic acid.*

A. A 5 % solution of carbolic acid killed the insects in ten minutes, shorter periods not always sufficing. Five minutes immersion, for example, killed nine out of ten, two minutes only nine out of twenty.

B. A 10 % solution killed after an immersion of two minutes, but after one minute five out of thirty remained alive.

3. *Perchloride of Mercury.*

A. A solution of 1 in 1,000 had very little effect upon the parasite. Two hours complete immersion failed to kill any of five adults, five and a half hours killed only one out of five. Experiments made for shorter periods than two hours produced no mortality.

B. A strength of 1 in 200 for one minute failed to kill at all: after five minutes immersion nine of ten recovered, after ten minutes seven of ten, after thirty minutes thirteen of twenty recovered.

4. *Paraffin Oil.*

A. Immersion in paraffin oil for ten minutes, five minutes, one minute, thirty seconds and five seconds killed all parasites in the experiments done (ninety bugs). After five seconds, one out of ten in one batch, two out of ten in another were still moving, but at the end of twenty-four hours all were dead.

B. Bed-bugs are capable of walking over wood smeared with paraffin oil and are apparently none the worse. Recovery in all experiments was estimated by the bugs being active at the end of twenty-four hours from the completion of the application.

Paraffin oil is the most efficient of all the liquids tested and it has the advantage of cheapness compared with most of the other preparations.

V. THE EFFECT OF VARIOUS GASES

1. *Chloroform vapour* rapidly immobilises bed-bugs, but unless the action is continued for some time they recover. Thus, thirty seconds exposure failed to kill any of ten bugs in one experiment, sixty seconds also did not kill. After three minutes exposure to chloroform vapour nine of ten parasites recovered, after five minutes eight of ten, after ten minutes treatment seven of ten. In fact, after twenty minutes two of ten recovered. One of three hours killed all parasites.

2. *Vapour of paraffin oil* for eighteen hours had little effect.

3. *Carbonic acid gas*. This gas quickly renders the parasites motionless, but does not kill them readily. Batches of twenty bugs were exposed to the gas for periods of two, four, five and ten minutes. They all recovered.

4. *Coal gas*. Application of coal gas failed to kill in times varying from thirty seconds to five minutes. Fifteen minutes' application was effective in killing the parasites.

5. *Hydrocyanic acid gas*. This proved a rapid and effective insecticide, but experiments with it were discontinued, owing to the difficulties attending the safe use of it in practice.

6. *Sulphur dioxide gas*. Large numbers of experiments were made with this gas on adults, newly hatched bugs and on the eggs.

A. *Adults and young forms*. (a) Batches of parasites were placed in test tubes and the gas played upon them under slight pressure for varying periods of time. After the exposure to the gas, each batch was transferred to a fresh tube and observed up to twenty-four hours. On exposure to the gas, the insects became violently active for a few seconds and then became motionless. The longest period during which the bugs were observed to move under exposure to the gas when thus applied was ten seconds. Eighteen experiments were carried out in this manner, comprising nearly two hundred adults and young forms, the time exposure varying from sixty seconds down to five seconds. No parasites recovered.

(b) The insects were placed in petri dishes and the gas simply sprayed over them, holding the funnel close to the bottom of the

dish containing them. Thirty seconds application by this method proved fatal, large numbers of the parasites being used. Two hundred bugs sprayed with the gas gently from a funnel held six inches above the petri dish containing them were all dead in two minutes.

(c) The gas was poured into a deep jar, and bed-bugs were dropped into it, the top being left open. Thirty seconds, twenty and fifteen proved fatal to the bugs by this method.

(d) Parasites were wrapped in cotton wool and enclosed in cardboard pill boxes. These were placed in a bottle and gas admitted for thirty seconds. In one experiment when five bugs were thus treated, one was alive at the end of the experiment, but died shortly after. Sixty seconds application killed all the parasites in six experiments, forty-five bugs being used.

(e) A cork, one and a half inches long and one and a quarter inches in diameter at the narrow end, had a hole bored in it half an inch in diameter down to the centre. In the cavity ten bugs were placed and the aperture plugged with a cork a quarter of an inch thick. The end was sealed by thickly coating with paraffin. This cork was placed in a jar and the gas was admitted under slight pressure for three minutes. All the bugs were dead at the end of this time. This experiment was repeated several times with the same result.

(f) A large wooden box was filled with sawdust. Among the sawdust the following packages were placed in various positions, near the centre. (1) Twenty bugs in a petri dish. (2) Twenty bugs in a petri dish wrapped round with smooth brown paper. (3) Thirty in a test tube between plugs of cotton wool. (4) Five sets of twenty in test tubes plugged with wool, and wrapped in brown paper. (5) Twenty bugs in wool in a pill box wrapped round with brown paper. The box, tightly packed with sawdust, was then sealed up and round it brown paper was wrapped. Through a small aperture the gas was admitted for three minutes under moderate pressure from a glass pressure bottle, the aperture sealed and the box left for ten minutes. At the end of this time, all the parasites were dead with the exception of two individuals which were moving very slightly. They were contained in the test tubes plugged and wrapped in brown paper. They died

within an hour, so that on the usual examination at the end of twenty-four hours all the parasites were dead.

B. *The effect of the gas on the eggs.* Any remedy intended to be of service in eradicating bed-bugs (from a dwelling house or other place) must be capable of destroying the eggs with certainty and preventing hatching out. Various experiments carried out with the eggs are shown in the table below, which gives details as to the duration of exposure to the gas and the results obtained by the application of the gas by two different methods. The two methods used were the open method, which consisted in spraying the gas over the eggs by means of a funnel attached to the gas-bottle by a tube, no pressure being applied. For the second method of application slight pressure was used, the gas being admitted to a test tube containing the eggs by a glass tube passing through a loose plug of cotton wool in the mouth of the tube. The gas was then able to escape through the loose wool plug fairly rapidly. It will be observed from the table that after using the open method for one minute, of fifty-eight eggs used for experiment only three hatched, while after application for two minutes one of seventeen hatched at laboratory temperature. Kept at 22° C. after treatment none of fourteen eggs treated hatched out.

TABLE II.—Showing the effects of treating the eggs with SO₂ gas.

A. Open method, gas poured gently over eggs: no pressure.

Duration of exposure to gas	No. of eggs used	Temperature at which bugs kept	Time observed	Result.	
				Exps. hatched	Controls hatched
One minute	58	Laboratory temperature	6 weeks	3	13 out of 19 hatched
2 minutes	17	Laboratory temperature	6 weeks	1	6 out of 7 hatched
2 minutes	14	At 22° C.	6 weeks	None	9 out of 14 hatched

B. Gas applied with slight pressure.

1 minute	62	Laboratory temperature	6 weeks	None	85 out of 93 hatched
1 minute	16	At 25° C.	6 weeks	None	5 out of 6 hatched
2 minutes	15	..	6 weeks	None	13 out of 15 hatched

Using slight pressure gave even better results, both at laboratory temperature and at 25° C. In no case did an egg hatch out after exposure for one minute to the action of the gas under slight pressure as described above. The controls to all these experiments are given in the table.

VI. CONCLUSIONS

1. *Cimex lectularius* whether in the larval or adult stage is not readily killed by depriving it of human blood.
2. It may thus remain alive and active for months in houses which have ceased to be inhabited.
3. Houses on being re-occupied after being empty for months may still be found infested with bugs.
4. Bed-bugs may transmit certain diseases from one human being to another. Therefore, it is inadvisable that bugs and human beings should occupy the same house.
5. Human beings must be protected from the attacks of bed-bugs.
6. There is no evidence that bed-bugs can be cleared out of a house by insecticide powders. Experiment suggests that powders are of very limited utility.
7. The same applies to liquid remedies.
8. Gaseous substances present the best prospect of success.
9. Of such substances, sulphur dioxide is cheap and effective.
10. Sulphur dioxide gas under pressure for two minutes, kills with certainty all stages in the cycle of development of the bug, including the egg

THE RESISTANCE OF *ORNITHODORUS MOUBATA* TO VARIOUS SHEEP-DIPS

BY

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*From the Runcorn Research Laboratories**(Received for publication 2 October, 1912)*

The following experiments were carried out (under the Sir Edwin Durning-Lawrence fund as stated in the previous paper) with each of the dips as described below, the preparation of the dip solution being carried out as stated in the directions on each sample.

A. Animal experiments.

1. *Prophylactic.* The skin of the animal (goats were most frequently used for the purpose of feeding the ticks) having been shaved, was soaked with the solution to be tested. While the skin was still moist the parasites were placed on it and observations made. Again, the skin was allowed to dry before the ticks were placed on it, and the effect noted.

Many experiments were done to ascertain whether the dip solution used, Cooper's, Little's, Savar's, Hayward's Yellow Paste, and MacDougall's dip, were capable of completely preventing ticks feeding on animals, either when the dip was still moist on the skin of the animal, or when it had dried. Frequently, the ticks fed well, but sometimes they refused to feed under these conditions. But as those ticks which would not feed, refused usually to do so when placed on normal skin used as a control, their failure to feed can hardly be attributed to the prophylactic action of the applications, but should more probably be attributed to the condition of the ticks themselves.

2. *Curative.* The ticks were allowed to feed on the shaved skin of an animal, and while still feeding, the various dip solutions were applied to them at varying intervals after the commencement of the meal, the dips being poured into the feeding glass, completely covering over the parasites.

When ticks of this species were permitted to attach themselves

to an animal, and the solution of dip poured over them, the results varied, not only as regards the immediate effect upon the tick, but also as regards the after effects. Generally speaking, the sooner after the commencement of the feed the application was made, the more likelihood there was of the ticks becoming loosened, and apparently also the more chance of the parasites subsequently dying. But these are points which scarcity of material prevented working out as completely as was desirable.

B. *In vitro*.

Ticks were placed in test tubes and the dip solutions poured over them, care being taken that the parasites were completely covered by the solution; this was effected by means of a piece of blotting paper which prevented the ticks rising to the surface of the fluid. The experiments of Group B were usually carried out until a definite effect was obtained on the ticks, either by increasing the time of application, or by increasing the strength of the solution used. Several consecutive treatment experiments were carried out with individual ticks, of which the following is an example. Using Little's dip in a strength of 1 : 50, a tick was covered over with the fluid in a tube for a period of two minutes. It was taken out, dried and warmed and was found to be alive and active. It was then subjected to the following periods of complete immersion in the solution, four minutes, eight minutes, sixteen minutes, and thirty-two minutes, being dried and warmed after each application and at once re-immersed. At the end of this series it was active. It was then rested for ten hours for observation, when a further immersion, this time for sixty-four minutes, was made; it was still alive and active, but at the end of the next period of application—a hundred and twenty minutes—it was found to be dead. Using Cooper's dip in a strength of 1 : 50 a tick survived immersion for thirty-two minutes, followed by immersion for two hundred and sixty minutes. Fuller details of experiments are given under the head of each dip. Experiments carried out as described above brought out certain very interesting facts in connection with the powers of resistance of *Ornithodoros moubata*, to various liquid preparations. It is well to draw attention here to the fact that ticks belonging to the family *Argasidae* are generally supposed to possess greater powers of

resistance than other ticks. Whether this is so or not, certainly the resistance of *Ornithodoros moubata* to the lethal action of these common sheep dips is noteworthy. If this property is the exceptional possession of ticks of this family it will naturally not diminish the value of the applications tested in their behaviour towards other ticks. At any rate, it would be of interest to have a very complete set of experiments done with other species in order to establish this point. Meanwhile, making every allowance for possible variations in other species, and admitting that fuller experiments are necessary, it appears evident that these ordinary applications fail to affect *Ornithodoros moubata* so as to kill it either with rapidity or certainty.

Cooper's dip. 'The best known means of destroying all the ticks. To kill ticks and prevent the fly striking. The best time to dip is about a month after shearing, but sheep thrive better if dipped again in the autumn, to keep them clean through the winter.' It will be observed that here both prophylactic and curative properties are attributed to this dipping preparation. The strength recommended is about 1 to 100 parts of water, the time of application one minute.

A. Animal experiments. This dip applied to shaved skin did not completely succeed in preventing ticks feeding, either when they were placed on the animal while the skin was damp, or after the skin had dried. When applied to ticks already feeding it frequently failed to stop them or make them relax their hold. Even when applied for longer times and in much greater strength than recommended it sometimes failed to kill. For example, ticks feeding on a dog were treated fifteen minutes after the commencement of the feed with a strength of 1 : 50 for two and a half minutes. They went on feeding, and were alive and active twenty-four hours after removal.

B. In vitro. One hour's complete immersion failed to kill ticks; two hours also failed. Five hours was effective in one experiment.

Little's dip. 'A greatly improved cattle wash and sheep dip for scab, lice, ticks and all parasites. Ticks and ordinary dipping. One gallon dip to 100 to 120 gallons of water.' The time of application recommended is one minute.

A. Animal experiments. This dip also did not act as a certain prophylactic when used as described above. Nor did it cause ticks already feeding to stop. Many experiments were done, the ticks being fed on rabbits, goats, dogs. In one experiment a strength of 1 : 50 was applied to two ticks feeding on a goat and the application was maintained for twenty minutes. At the end of this time both ticks were fast. One moved away after ten minutes further feeding and both were alive and active after twenty-four hours. In some of the experiments the application seemed to disturb the ticks, and they would then walk away.

B. In vitro. Ticks survived very prolonged treatment, two hours with no difficulty and much longer periods. In one experiment, five hours application failed to kill, using a strength of 1 : 50, i.e., twice the strength recommended.

McDougall's sheep dip and cattle dressing. Scab and insect exterminator. One part dip to fifty parts water.

A. Animal experiments. The results were rather variable. Some parasites fed on the treated skin. But in some cases parasites feeding, especially when they had only just commenced to feed, were dislodged, and some of these died within twenty-four hours.

B. In vitro. Ticks survived thirty-two and sixty-four minutes. In one experiment a tick died after two hours immersion, but others survived four and a half and five hours.

Savar's dip. One gallon of dip to seventy gallons of water.

A. Animal experiments. The prophylaxis conferred by this dip did not prove effectual. For example, the skin of a goat was moistened thoroughly with a solution of 1 : 70. Two minutes after, while the skin was still moist, four ticks were placed on it. Of these, two refused to feed, the other two fed well and were apparently none the worse. Again, when the skin had dried, four ticks were placed upon it: these all fed well. In curative experiments ticks were killed in thirty minutes.

B. In vitro. Ticks survived two hours immersion in the solution, but not five hours.

Hayward's Yellow Paste.

A. Animal experiments. The prophylactic action was here again by no means reliable. In the case of feeding ticks this

application was also frequently ineffective. Experiments were done, using the strength 1 : 100 and applying it to feeding ticks on a goat for times varying from sixty seconds to thirty minutes, the parasites surviving such applications. The ticks suffered so little inconvenience that they frequently could be fed again after a short interval. Thus, a tick which had been so treated for thirty minutes while feeding on a rabbit, was removed and after an interval of fifteen hours was able to feed again on a dog.

CONCLUSIONS

1. The dips tested failed very frequently to prevent *Ornithodoros moubata* feeding on an animal.
2. Feeding ticks were not easily caused to loosen their hold by them.
3. In test tube experiments the resistance of this species of tick to these substances in solution is marked.
4. Used in the strength recommended and for the time suggested these dips appear to have very slight effects on this tick.
5. Possibly other ticks behave in a different manner under these applications.



DESCRIPTIONS OF THREE NEW
AFRICAN SPECIES OF THE GENUS
TABANUS

BY

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(Received for publication 14 November, 1912)

PLATE XXIII

Tabanus nagamiensis, n. sp.

Medium-sized dark greyish species. Dorsum of thorax greyish-black with two admedian longitudinal stripes of a paler shade, expanding posteriorly; also a short, pale, median stripe anteriorly. Dorsal surface of abdomen smoky-brown to rich dark brown with greyish apical bands expanding into median and somewhat ill-defined triangles and broad lateral areas. Wings hyaline. Legs dark, tibiae ochraceous-buff with black tips. Eyes hairy.

Head (Pl. XXIII, fig. 4): Face, jowls and subcallus, pale greyish clothed with long, greyish-white hairs; posterior region slightly darker, bearing short, greyish-white hairs. *Front* dark brown with greyish pruinose pubescence, about three and one-half times as long as the breadth at the lower extremity; the vertex usually denuded and shining, dark brown. *Frontal callus* (fig. 9) large and shining, dark brown to reddish-brown, almost square, with the lower corners rounded and narrowly separated from the eye on each side, extending to the central region of the front more or less in the form of a spindle; when partly denuded this part of the callus frequently appears as a long, narrow rectangle, the sides being almost parallel. *Palpi* obscure pinkish-white, terminal segment broad at the base and tapering rapidly from the mid-region to the apex; first segment and base of the second clothed with fairly long white hairs, remaining portion of second with minute black and white hairs intermixed, the latter usually preponderating. *Antennae* pale reddish-buff, and slightly pruinose; basal joint clothed with minute black hairs distally, white hairs basally and laterally, the latter being moderately long, upper extremity of joint projecting con-

siderably and markedly prominent; expanded portion of third segment broad, almost as broad as long.

Thorax (fig. 5): Dorsum greyish-black with two well-marked, grey, admedian, longitudinal stripes on the anterior region; clothed with sparse, short, white hairs. Scutellum similar in colour to the thorax, with the posterior margin paler. Humeral calli greyish-buff, becoming more decidedly grey behind, clothed with fairly long white and black hairs, the former preponderating. Post-alar calli grey, with long dense white hairs on the outer margins. *Pleurae* greyish-buff in the region of the humeral calli, merging into dusky grey and clothed with long white hairs.

Abdomen (fig. 5): General coloration of the dorsal integument smoky-brown to rich dark brown, with greyish apical bands which form a line of pale, median, triangles with bases resting on the apical margins of the segments, and expanding laterally into large indefinite blotches; distally the pale markings become somewhat irregular in outline and less distinct. Hairs on grey areas dusky-white, those on the darker portions black (except at the middle of the apical margin of the first segment, where they are whitish), becoming longer distally at the outer angles of the segments and on the border of the last segment, where both black and white hairs are present, the former preponderating. In several examples the integument is distinctly dull orange-brown at the sides and along the apical margins.

Wings hyaline, stigma pale brownish and elongated. Squamae opaque, whitish. *Halteres* dark brown, the knobs slightly paler.

Legs: Integument of the coxae dark brown, pruinose, with whitish hairs. Fore femora dark brown or black, mid and hind femora slightly paler, clothed with white hairs. Tibiae ochraceous-buff, dark brown to black at the tips, clothed with white hairs on the pale portion and black on the extremities; the dark area in the fore tibiae is more noticeable than in the others, and occupies almost one-third of the length. Tarsi very dark brown to black, clothed with short black hairs.

Length, 11 to 13.25 mm.; length of wing, 9 to 10.75 mm.; width of head, 3.7 to 4.75 mm.; width of front, 0.5 to 0.65 mm.

Habitat: Ngamiland, South Africa; Dr. W. R. W. James, 10.12.1910; fourteen females.

In the form of the frontal callus, *Tabanus nagamiensis* somewhat resembles *Tabanus diversus*, Ric., but it is clearly distinct from this and, as far as I can judge, also from any other allied species.

Tabanus fulvicapillus, n. sp.

Large dark species, distinctly palearctic in form, and closely related to *Tabanus trigonus*, Coq.* Dorsal surface of thorax dark (partly denuded); pleurae dark, clothed with dull golden-yellow hairs. Dorsum of abdomen, rich, dark, reddish-brown shading to very dark brown, with broad, dull, golden-yellow apical bands and median triangular spots. Legs dark, tibiae reddish-brown with dark apices, and clothed with short golden-yellow hairs.

Head (fig. 2): Dark brown to black, jowls clothed with relatively long golden-yellow hairs, posterior surface with short similarly-coloured ones. *Front* about four times as long as broad, the margins almost parallel. *Frontal callus* (fig. 7) pitchy black, strongly dilated distally with a long narrow shaft, the latter reaching approximately to the centre of the frontal stripe. *Antennae* with the two basal joints very dark brown, and with numerous short black hairs. Third segment, as far as the base of the dorsal projection, reddish-brown, the remaining portion blackish. *Palpi* somewhat slender and of a dull ochraceous-buff colour, terminal joint clothed with small black hairs in the central and distal regions, yellowish hairs towards the margin and base, first segment clothed with numerous long yellow hairs.

Thorax: Integument very dark brown, almost black, clothed with yellow hairs anteriorly and over the insertions of the wings (unfortunately the median and posterior areas in both specimens are denuded). *Pleurae* dark brown, clothed with long yellow hairs.

Abdomen: Dorsum of first segment with dense yellow hairs slightly darker on the apical margin than on the basal; succeeding segments rich dark reddish-brown, somewhat darker medially—the second and third slightly paler than the rest—apical margins with broad bands of dark golden-yellow hairs, and expanding laterally where the hairs become longer and denser; on the middle

* We are greatly indebted to Mr. E. E. Austen for the loan of specimens of this species, for comparison with *Tabanus fulvicapillus*.

segments yellowish, median, triangles are formed, the bases of which rest on the distal margins. *Venter* similar to the dorsum, but with a dark blackish-brown median area on each of the segments, clothed with numerous yellow hairs.

Wings faintly and uniformly infuscated, stigma long, narrow, and deep brown in colour. *Halteres*: Knobs dark brown, paler and yellowish below, stems dark.

Legs: Coxae very dark brown, clothed with golden-yellow hairs, those on the front coxae longer and denser. Femora dark brown to black, thickly clothed with long golden-yellow hairs. Tibiae reddish-brown, with dark, almost black, apices, clothed with dull golden-yellow and black hairs, the latter on the distal extremity. Tarsi dark brown, second, third and fourth joints of front pair with small median areas of short golden-yellow hairs.

Length, 21 and 23 mm.; length of wing, 19 mm. (second specimen not perfect); width of head, 7·15 and 7·6 mm.; width of front at vertex, 0·7 and 0·8 mm.

Habitat: Banana, Congo Free State; Dr. Etienne; two females.

This very striking species cannot possibly be mistaken for any other hitherto recorded from the African Continent. As already stated, this species is very closely related to *Tabanus trigonus*, Coq., which it resembles in general colour and pattern. It is slightly smaller, but the median triangles on the dorsum of the abdomen are yellow and not greyish; the hairs on the thorax and abdomen also are decidedly golden-yellow in colour, whereas in *T. trigonus* the corresponding thoracic hairs are black, merging into others of pale greyish or buff colour. Furthermore, there are appreciable differences in the form of the frontal callus, in the width of the front (in *T. trigonus* the front is from five to six times as long as broad), and in the shape of the antennae, as may be gathered by comparing the figures (figs. 3 and 8). The hairs on the femora also are shorter in *T. trigonus*. It is important to add, however, that the two specimens we possess were both preserved in alcohol, so that the colours may have undergone some slight change, and as the median area of the thorax in both examples is denuded, it is impossible to say whether any distinct markings were present.

Tabanus donaldsoni, n. sp.

A somewhat robust bright umber-brown species, rather over medium size. Dorsal surface of thorax slightly paler in colour, with two obscure greyish-brown, lateral, longitudinal stripes. Dorsum of abdomen rich umber-brown, clothed with short similarly-coloured hairs; venter greyish-white, darkening towards the apex. Wings large and heavily tinged with brown; anterior branch of the third longitudinal vein bearing a well-marked appendix.

Head (fig. 1): Face and jowls yellowish, clothed with yellowish hairs which become longer and paler towards base of palpi, those at the base and behind being whitish, posterior region greyish-white, with a series of short, stout, dark brown hairs along the dorsal eye margin. *Front* (fig. 6) yellowish, rather more than four times as long as its breadth at the lower extremity, clothed with sparse dark brown hairs and paler yellowish hairs intermixed. *Frontal callus* conspicuous, deep reddish-brown, and in the shape of a long narrow triangle, the apex of which reaches to a point slightly beyond the middle of the front. *Palpi* ochraceous-buff, the first segment clothed with long whitish hairs, the terminal broad at the proximal end and rapidly tapering towards the apex, with numerous minute black hairs on the outer surface (there are also a few whitish hairs intermixed towards the base). *Antennae* long and slender, first two segments pale reddish-buff, clothed with short black hairs, which increase in length ventrally; third joint dull orange-buff, the annulate portion about half the length of the segment with circles of short black hairs at the rings; dorsal angle only moderately pronounced, and bearing a few minute black hairs.

Thorax: Dorsal surface yellowish-umber-brown, with two inconspicuous greyish-brown lateral stripes, becoming paler and fainter posteriorly, clothed with short yellowish-brown and black hairs intermixed, the latter especially noticeable anteriorly. Humeral and post-alar calli somewhat paler than the rest of the thorax, the former with long brown hairs, the latter with long yellowish-brown hairs on the outer margin. *Pleurae* greyish-white, clothed partly with yellowish and partly with whitish hairs.

Abdomen: Dorsum rich umber-brown, becoming slightly darker laterally and distally, clothed with short yellowish-brown hairs, which are longer and intermixed with dark brown to black ones on the apical segments. *Venter* slightly darker than the pleurae,

especially the terminal segments, and clothed with short white hairs which are denser on the apical margins, almost forming narrow pale bands when viewed in certain lights.

Wings relatively long and of a dull brown colour, the infuscation being more pronounced along the veins and towards the tip of the wing, especially at the apices of the second longitudinal vein and the upper branch of the third long vein; the latter with a relatively long, backwardly directed, appendix. Costal cells raw-umber-coloured; stigma long and narrow, slightly darker than the costal cells. *Squamae* of a similar colour to the thorax, but rather darker. *Halteres* yellowish-brown, the stems gradually darkening towards the base.

Legs: Coxae greyish-white, clothed with long whitish hairs. Femora greyish-white at the base, shading into greyish-brown distally, the hairs on the paler portion whitish, on the darker portion of a brownish or yellowish-brown colour; hind femora with longer and glistening white hairs. Tibiae of much the same shade as the body, becoming slightly darker at the tips, and clothed with yellowish-brown hairs. Tarsi dark brown to blackish, the base of the first joint being paler and approaching the colour of the tibiae.

Length,* 17.5 mm.; length of wing, 16 mm.; width of head, 6.15 mm.; width of front, 0.75 mm.

Habitat: Ashanti, Bromassie (two hours from Coomassie), West Africa; Dr. Anson S. Donaldson; one female.

Tabanus donaldsoni apparently belongs to Surcouf's sixth group (*T. combustus*, *T. thoracinus*, etc.), and in regard to the shape of the antennae and frontal callus, approaches *T. obscurehirtus*, Ric. It may easily be distinguished from this species, however, by its somewhat larger size, greyish-white ventral surface, pale femora and unicolorous tibiae—the front tibiae in *T. obscurehirtus* being partly black and partly white. From the latter also, and all other species in this group, it may be separated by the relatively long, heavily-infuscated wings.

The types of all three species are in the collection of the Liverpool School of Tropical Medicine.

* We have recently received three additional examples of this well-marked species from Dr. T. B. Adam, Calabar, Southern Nigeria, to whom we are greatly indebted. They show a somewhat considerable variation in size, measuring 20.5 mm., 19 mm., and 17 mm. in length respectively.

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EXPLANATION OF PLATE XXIII

- Fig. 1. Head of *Tabanus donaldsoni*, n. sp. × about 12.
Fig. 2. Head of *Tabanus fulvicapillus*, n. sp. × about 12.
Fig. 3. Antenna of *Tabanus trigonus*, Coq. × about 12.
Fig. 4. Head of *Tabanus nagamiensis*, n. sp. × about 12.
Fig. 5. *Tabanus nagamiensis*, n. sp. × 3.
Fig. 6. Front of *Tabanus donaldsoni*, n. sp. × about 11.
Fig. 7. Front of *Tabanus fulvicapillus*, n. sp. × about 11.
Fig. 8. Front of *Tabanus trigonus*, Coq. × about 11.
Fig. 9. Front of *Tabanus nagamiensis*, n. sp. × about 11.

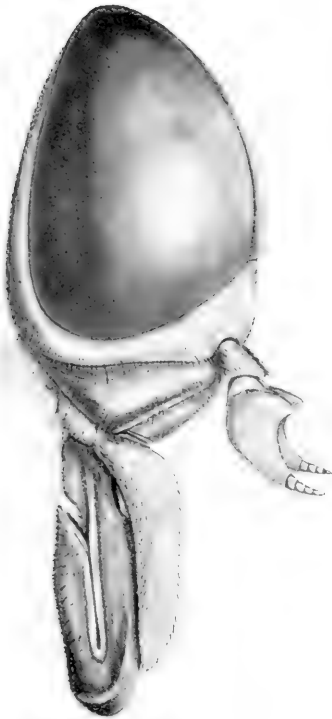


FIG. 2

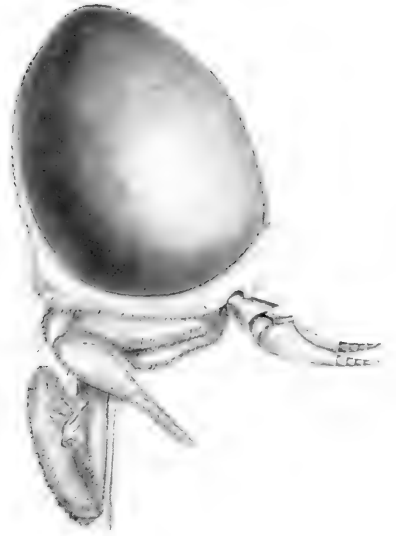


FIG. 1



FIG. 3



FIG. 5



FIG. 4

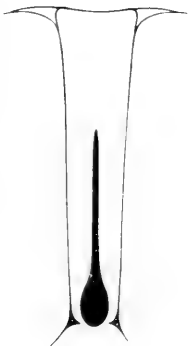


FIG. 8



FIG. 7

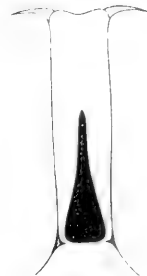


FIG. 6



FIG. 9



ON HAEMOGLOBIN METABOLISM IN MALARIAL FEVER

PART II. THE INFLUENCE OF QUININE

BY

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AND

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(*Received for publication 30 November, 1912*)

In a previous paper¹ it was shown that the amount of haemolysis proceeding in the body can be measured to some extent by the total excretion of urobilin, the end product of the pigment of the blood: the intestinal elimination is more important than the urinary.

In malaria a high degree of haemolysis is followed by a high excretion of urobilin in the faeces, and sometimes also in the urine; in blackwater fever the amount of liberated haemoglobin excreted as this derivative is far larger than the amount passing unchanged through the kidneys.

In another communication² it was shown that a haemolytic principle can be demonstrated occasionally in malarial serum; this action is most likely to be shown by the serum of a benign tertian case drawn at the onset of a paroxysm.

Clinical evidence would seem to favour the view that quinine is a determining factor in many cases of blackwater fever, but Barrett and Yorke³ have shown that this drug, in strengths comparable with those likely to be present in serum after its administration, has no haemolytic action *in vitro* on normal or malarial human corpuscles. Deeks and James,⁴ in the Report on haemoglobinuric fever in the Canal Zone, give cases which demonstrate clearly that quinine does influence the production or action of haemolysins and cytolysins in malaria.

It is possible that, though quinine has no haemolytic action on red corpuscles *in vitro*, it may have such a power in the human body: this may be due to some reaction with the living bioplasm,

or to metabolites of quinine. Only about one-third to one-half of the amount of quinine ingested is excreted unchanged,⁵ and the rest must be broken down in the body—many of the quinoline antipyretics proved so likely to cause haemolysis that their therapeutic use was abandoned.

Deeks and James have suggested that quinine may act as complement to haemolytic amboceptors produced by the body or by the malarial parasite.

We have undertaken a series of experiments to ascertain whether the administration of quinine to animals or man causes any increase in the excretion of urobilin, which would show that quinine has a haemolytic effect, direct or indirect.

EXPERIMENTS ON ANIMALS

The normal excretion of urobilin by rabbits was first determined; then the effects produced by injection of haemoglobin itself and various haemolytic drugs.

As was to be expected, an increased urobilin excretion was observed after injection of red corpuscles or haemoglobin and of haemolytics, including haemolytic antipyretics. Increase was also noted after the administration of quinine.

The accuracy of the figures, obtained by dilution methods, for the urobilin content of the faeces of these animals is somewhat unsatisfactory, owing to the interference of other pigments from the food and to the small increases obtained.

Normal average excretion of urobilin, 0.006 milligrammes per diem	Milligrammes
Total increase of excreted urobilin after injection of 1.836 grams of haemoglobin	0.12
Total increase of excreted urobilin after administration of 0.64 " antipyrin	0.032
" " " 3.0 " "	0.123
" " " 0.013 " mowrin	0.040
" " " 0.05 " "	0.018
" " " 0.70 " quinoline	0.054
" " " 0.04 " quinine	0.054
" " " 1.65 " "	0.086

Similar increases were obtained in dogs after administration of haemolytics, but only doubtful results were obtained after quinine and quinoline derivatives.

Normal average daily excretion of urobilin, 0.018 milligrammes		Milligrammes
Total increase from administration (intra-peritoneal) of	10 grams of haemoglobin	0.160
Total increase after administration of	0.125 " toluene-diamine	0.381
"	0.250 "	0.278
"	0.400 "	0.214
"	0.250 " quinine	0.006
"	0.400 " "	0.004
"	1.0 " thallin sulphate	0
"	1.0 " kairalin	0

It is interesting to note that the injection of toluene-diamine in dogs gives rise to increased urobilin excretion (without haemoglobinuria unless large doses are given); in a puppy we obtained jaundice with excretion of large amounts of bile in urine and faeces, and in cats the injection gave a slight increase of urobilin followed on the third day by severe haemoglobinuria.

In the puppy the dose was 0.4 gram, and the result may have been due to diarrhoea or to lack of the urobilin organisms in its intestinal flora. The difference between dogs and cats shows some difference in the mechanisms of blood destruction.⁶

The experiments on animals gave some support to the view that quinine might have a haemolytic action in the organism, and experiments were now made on man.

MAN

1. In a hospital patient (non-malarial) the average excretion of urobilin was 50 mgms. The patient received on each of two successive days a dose of 2 grams (30 grains) of quinine. The average daily excretion in the subsequent period was 49 mgms. (It is probable that some stools were not saved.)

2. Healthy adult. Average daily excretion of urobilin 75 mgms.

Total increase of urobilin after 1 gram antipyrin per diem for two days, 72 mgms. The main increase was on the fifth day.

3. Healthy adult. Average daily excretion of urobilin 72 mgms.

(a) Quinine, 1.3 grams per diem for two days. Average daily excretion of urobilin 94 mgms. Total increase of urobilin 110 mgms. The main increase was on the fifth day. 110 mgms. urobilin equals 2.7 grams haemoglobin.

(b) Quinine, 2 grams on one day. Subsequent average excretion 82 mgms. Total increase of urobilin 50 mgms., = 1.2 grams haemoglobin.

In this individual quinine caused marked headache and indisposition on each occasion.

4. Healthy adult. Average urobilin excretion 40 mgms. per diem.

(a) Quinine, 3.3 grams on one day (marked indisposition). Average subsequent urobilin 53 mgms. per diem. Total increase of urobilin 59 mgms. = 1.47 grams haemoglobin.

(b) Quinine, 1 gram per diem for three days (no indisposition). Average subsequent urobilin = 42 mgms. per diem. Decrease of urobilin excretion.

5. Healthy adult. Average normal output of urobilin 113 mgms. per diem. 0.6 gram quinine per diem for three days (no indisposition). Subsequent average urobilin excretion 92 mgms. per diem. Decrease of urobilin.

6. Healthy adult. Average normal output of urobilin 112 mgms. per diem. One gram quinine daily for three days (slight symptoms of quininism). Average subsequent urobilin 107 mgms. per diem. No result from quinine.

7. Healthy adult. Average normal output of urobilin 86 mgms. per diem. One gram of quinine daily for three days (headache and malaise). Average subsequent urobilin 105 mgms. per diem (maxima on fourth and seventh days). Total urobilin increase 152 mgms., equivalent to 3.6 grams of haemoglobin.

8. Healthy adult. Average normal output of urobilin 175 mgms. Two grams of quinine on one day (marked headache and malaise). Average subsequent urobilin 205 mgms. Total urobilin increase 270 mgms. = 6.4 grams haemoglobin.

These experiments on the whole support the results obtained

from experiments on animals, and it appears probable that administration of quinine may give rise to destruction of red corpuscles. The increase is more likely to occur when symptoms of quinism are produced.

The experiments require to be repeated on a larger scale, preferably where both malarial and non-malarial individuals are regularly taking prophylactic doses of quinine. The claims of other work have prevented us making many observations.

To be conclusive, the subjects of experiment should be on a constant diet with little meat (as foreign blood pigment from the diet exercises some influence); the experiments require to continue for seven to ten days after the administration of quinine has ceased.

Graham⁷ has shown by photographic methods a regular increase of urobilin in the urine after prophylactic doses of quinine. His experiments did not cover the important faecal channel of elimination, but the regularity of the increase is very marked, and faecal determinations would probably have confirmed the urinary ones.

CONCLUSIONS

1. Increased excretion of urobilin may occur after the administration of quinine in doses of ten to thirty grains a day. A similar result follows injection of blood pigment or haemolytic drugs.

2. Quinine probably possesses the power of determining haemolysis in the body, though the exact mechanism of its action must be further investigated.

3. Individual or pathological idiosyncrasy may exaggerate this action and so account for the influence of quinine in blackwater fever.

These researches were carried on in the Liverpool School of Tropical Medicine. The funds for the research were allotted by the Advisory Committee for the Tropical Diseases Research Fund (Colonial Office).

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THE CULTIVATION OF ONE GENERATION OF MALARIAL PARASITES (*PLASMODIUM FALCIPARUM*) IN VITRO, BY BASS'S METHOD

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(Received for publication 25 November, 1912)

PLATES XXIV, XXV

INTRODUCTION

From 1880, when Laveran discovered the malarial parasites, numerous workers have attempted their cultivation in vitro. It was not, however, until 1911 that Bass was able to state that he had succeeded in cultivating *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium falciparum*. In this preliminary paper the technique was not given in any detail, though certain factors were noted, such as incubation temperature of 40° C. or higher, strict anaerobic conditions and culture media of defibrinated or citrated human blood. Working on these lines, J. A. Sinton (1912), of the Liverpool School of Tropical Medicine, carried out a series of experiments, but in no instance was he successful in obtaining any development of the parasite, and it was only in October, 1912, that Bass's full technique became available.

After studying this paper, which appeared in the American Journal of Experimental Medicine for October, 1912, and after the examination of a series of eight slides kindly sent to the Liverpool School of Tropical Medicine by Dr. Bass, the results appeared so noteworthy that on the suggestion of Sir Ronald Ross we decided to make an immediate attempt to prove or disprove

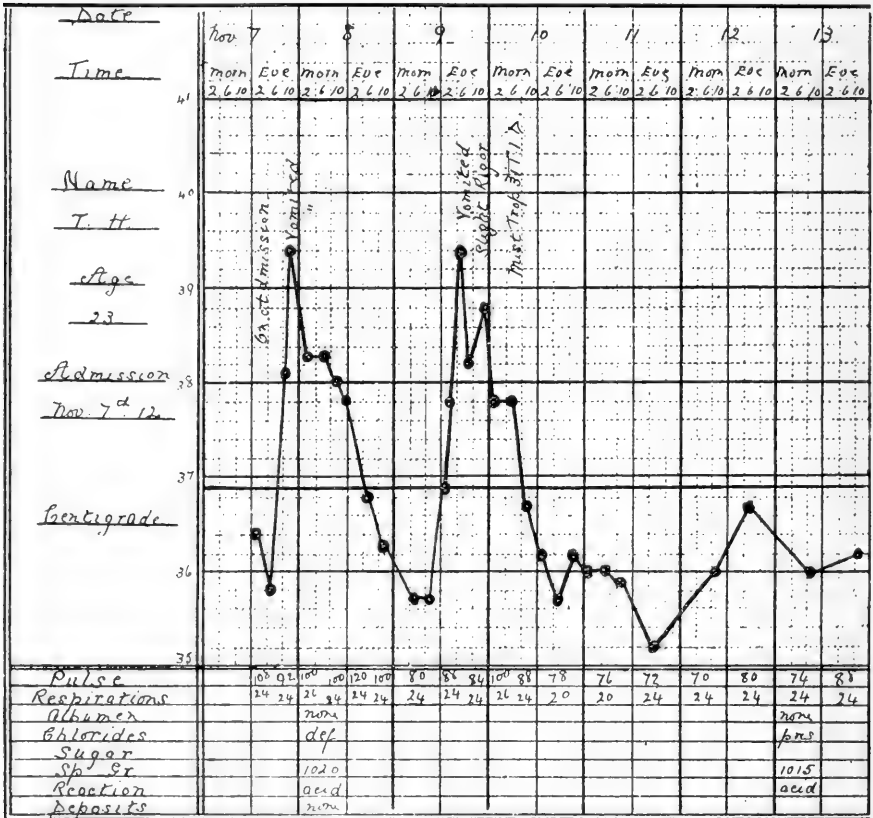
Bass's statements. We have much pleasure in thanking Sir E. Durning-Lawrence for generously supplying funds for this investigation.

With this end in view, therefore, we commenced our first set of experiments, and the results appear sufficiently remarkable to warrant this preliminary note.

CASES

The first case selected as suitable for attempting the cultivation of the parasite was a male, T. H., aet. 23, admitted to the Royal Southern Hospital in Sir Ronald Ross's clinic, November, 1912.

CHART I*



Mist. Trop. 3i = 10 grains of quinine hydrobromide.

* Charts kindly prepared by Sister Marshall of the Tropical Ward, Royal Southern Hospital.

The following history was elicited. The patient had left the West Coast of Africa six weeks previously, having had a severe attack of malaria whilst at Sierra Leone. On the way home he had another attack and also suffered from gastric disorder and headache. Three weeks after his arrival in England, as he did not markedly improve in health and had several febrile attacks, he came to the Royal Southern Hospital, where his blood was examined, numerous small ring parasites being found. The temperature, which had been subnormal on admission, rose the same evening to 39.4°C ., but next day again fell gradually to subnormal and remained so until 12 noon on the following day, when it again began to rise. On the blood being again examined, parasites were found to be numerous and 8 c.c. of blood were drawn off for cultivation from the median basilic vein. As the temperature rose the same night to 39.4°C ., it will be seen that the blood was taken just at the onset of a second attack of fever. Ten grains of quinine hydrobromide were administered thrice daily, and the patient made a rapid and excellent recovery. See Chart I.

TECHNIQUE

Eight c.c. of blood were drawn from the median basilic vein, at the bend of the elbow, into a sterile glass syringe under aseptic precautions. In order to obtain and preserve this asepsis it is advisable that the arm be not washed with any watery solution, as two dangers have always to be guarded against, namely, that no micro-organisms (usually the *Staphylococcus albus*) be either carried into the vein or deposited in the culture tube, and, secondly, that no antiseptic shall be carried into the culture tube. We have always, therefore, found it advisable first to scrub thoroughly the arm with an alcoholic solution of biniodide of mercury, and secondly to wash off all the antiseptic and to fix the epidermal scales by a 70 per cent. solution of alcohol, afterwards allowing this to evaporate before puncturing. When the vein was punctured the blood was allowed to flow into the syringe, slowly forcing up the piston by means of its own pressure, after which it was transferred as quickly as possible (in order to prevent clotting) into a sterile culture tube. This tube was fitted with a thin glass rod running through the woollen plug and contained 1/10 c.c. of

a sterile 50 per cent. solution of Merck's dextrose. This dextrose is, according to Bass, a most important factor in the cultivation of the *Plasmodium*, and as it was not mentioned in his original paper, it is probable that Sinton's failure was due to its omission.

The blood is next gently defibrinated and at the same time mixed with the dextrose by gently stirring with the glass rod, care being taken to avoid air bubbles in any of the manipulations.

We next transferred it to two culture tubes, the column of blood being one to two inches thick, and incubated at 38° C. The red blood cells gradually settled and about half an inch of serum accumulated on the surface.

The above technique is practically identical with that described by Bass, the only points of difference to be noted being:—

(a) The slight increase in the amount of dextrose employed relative to the quantity of blood—(1/10 c.c. dextrose to 10 c.c. blood is recommended by Bass).

(b) The lower temperature at which we incubated (40° C. advised by Bass).

The tubes so prepared contain roughly three layers, namely:—

(1) A top layer of varying depth, preferably about half to one inch of clear serum.

(2) A thin intermediate layer of loose red corpuscles and white cells—the culture layer.

(3) A bottom layer consisting mainly of red cells, in which the parasites die in periods varying from two to twenty hours.

EXAMINATION OF CULTURES

By means of a drawn-out capillary pipette, a few of the surface cells from the intermediate layer in our cultures were drawn off at twelve hours, twenty-five hours, twenty-seven hours and thirty-two hours. Small drops were placed on clean glass slides and smears made, fixed in methyl alcohol for twenty minutes, and stained in the usual way with Giemsa or modified Romanowsky solution. Controls were made in each case.

It was found that the smears stained well, but it is advisable to make somewhat thick smears, owing to the amount of serum mixed with the cells.

MORPHOLOGY

(A) *Control*. The blood examined before incubation showed numerous typical small ring parasites with nucleus and vacuole. The character of the nucleus varied in shape, being either circular or rod-shaped, and was situated in the vacuole, as a rule near one margin. The parasites varied in size with a maximum diameter of about 3μ . They also varied in shape, though the ring form predominated. Amoeboid movement was seen in some of the irregular forms, but pigment, segmenting forms and crescents were all absent. There was no enlargement of the red blood corpuscles and no stippling of the red cells.

The amoebae were typical of the malignant tertian parasite, *Plasmodium falciparum* (Pl. XXIV, figs. 1-5).

It is to be noted, in regard to the description of the forms cultivated by us in vitro, that the malignant tertian parasite is seldom or never seen undergoing schizogony in the peripheral circulation; as a rule, only small rings are present in this situation, segmentation occurring almost entirely in the internal organs. The active small rings grow larger in size and become the less amoeboid trophozoite, finally reaching the stage of the mature schizont with a single nucleus. The nucleus then divides up by a primitive form of mitosis, and, as the nuclei increase in number, division becomes simpler (Schaudinn). Immediately previous to the division of the nucleus the presence of pigment makes itself manifest. It is round this central mass of pigment that the merozoites formed by the nuclear division are arranged, until the final stage of the fully segmented parasite, when the enclosing corpuscle shews signs of breaking down.

By the cultivation of the parasite in vitro we have been enabled to observe the process of segmentation actually occurring in the culture tubes, and thus the old, difficult, and mostly inaccurate method of studying it in the internal organs has been avoided.

(B) *Culture*, twelve hours duration; 38° C.; fixed specimens examined. A definite increase in the size of the parasite was found to have occurred, the measurements varying from 5μ to 6.5μ . A round mass of pigment about 1μ in diameter was present in almost every case—usually at one pole of the parasite. The chromatin

had also increased, in some cases retaining its original compact form, in others spread out irregularly (see figs. 6-13). No distinct amoebae could now be seen. At this stage of the cultures an interesting and well-marked feature was the tendency which the corpuscles containing parasites shewed to clump together into irregular masses (see Pl. XXIV, fig. 10, and Pl. XXV, figs. 2 and 3). On one slide a mass of as many as sixty parasites was made out. There was no marked increase in the size of the containing corpuscles.

The shape of the organisms varies, being circular, irregular and (most frequently) oval. One young crescent was found (fig. 14). This, however, may have been originally in the peripheral blood, as we have noted in other cultures that crescents persist for many hours without shewing any apparent change.

(C) *Culture*, twenty-five hours incubation, 38° C.

A most remarkable appearance was now noted in fixed specimens prepared from two culture tubes, definite segmenting forms being seen in large numbers, shewing the nucleus broken up into fragments varying from two to thirty in number. A microphotograph is herewith reproduced shewing a portion of a slide on which hundreds of these schizonts were seen clumped together (Pl. XXV, figs. 4 and 5). The diameter of these forms varied according to the amount of segmentation that had taken place, the maximum being about 7 to 8 μ . In many the corpuscle could not be seen, but in others the margin of the enveloping red cell could be distinctly seen (Pl. XXIV, figs. 15-30, and Pl. XXV, figs. 4, 5). The pigment mass is quite distinct, and where segmentation is nearly complete the merozoites are arranged concentrically round it, thus forming the so-called 'rosette.' Fig. 23 shews the formation of thirty merozoites.

No evidence of these merozoites having entered new corpuscles, and so beginning another generation of parasites, was obtained.

(D) *Culture*, twenty-seven hours incubation.

Only a few segmenting forms were now found, the probability being that the corpuscles had burst and the merozoites dispersed, thus allowing the destructive action of the serum and leucocytes to

take place. Of the few found, fig. 32 shews a parasite with early segmentation, and fig. 31 the nucleus broken into sixteen daughter portions.

(E) *Culture*, sixty-six hours incubation.

It now became increasingly difficult to find parasites, but one is shewn with the nucleus divided into four (fig. 30). Slides examined after this time shewed only disintegrated and degenerate forms, all the parasites having apparently perished.

A second series of experiments was carried out on the blood of a patient, W.B., from the West Coast of Africa, who gave a history of six weeks' illness. He habitually took five grains of quinine per diem, and after landing at Liverpool came direct to the Royal Southern Hospital.

On admission his temperature was 38.7° C., and he seemed very ill with definite anaemia. The blood examination shewed a few small ring parasites (*Plasmodium falciparum*). No quinine was given, and the temperature fell gradually to subnormal on the following day and then began to rise. 10 c.c. of blood was drawn off, as in the previous case, just as the temperature was rising, and cultures prepared as already described. (See Chart II.)

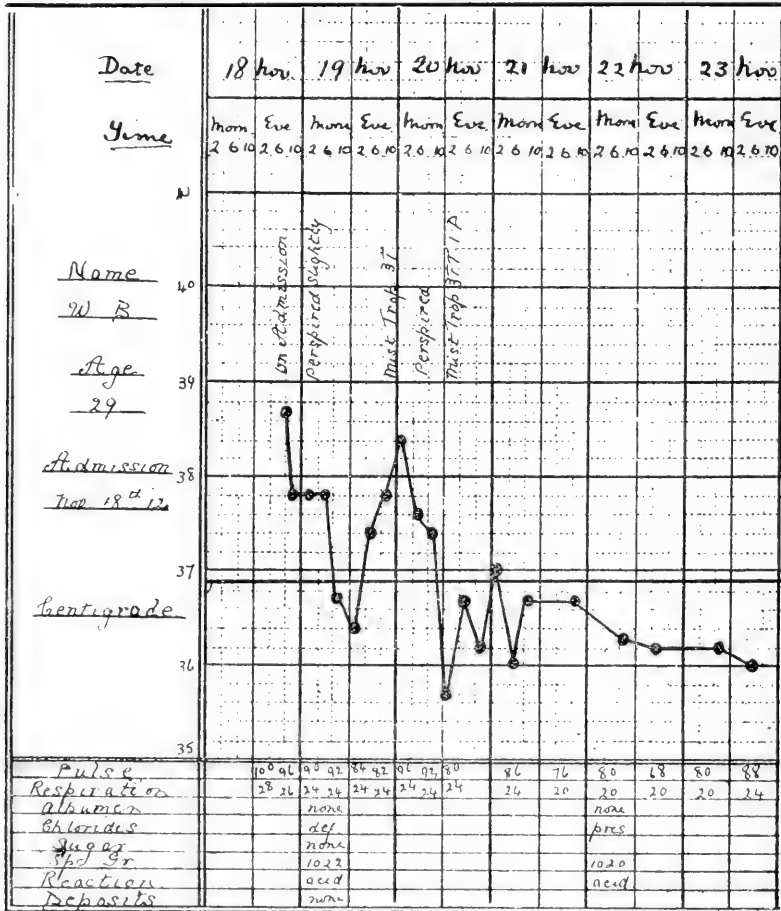
(A) *Control*. Before incubation.

A few small ringed parasites were found, but much less numerous than in the previous case.

(B) *Cultures*. After seven hours incubation, temp. 39° C., the parasites were distinctly enlarged. In eighteen hours many early segmentation forms were found with the nuclei broken up into two or four. Segmentation proceeded steadily, slides being examined after twenty-two, twenty-five, thirty and thirty-two hours, after which development apparently ceased. In some of these later slides small parasites were seen without a vacuole, but with distinct nucleus and protoplasm, suggesting the possibility that several of the merozoites had escaped destruction and had entered a fresh corpuscle. It is impossible, however, to be dogmatic about this, as no definite increase in the number of small rings was noted in any of our slides after maximum segmentation had occurred.

In this second series of culture tubes the segmentation did not appear to proceed so rapidly as in those of the first case, nor did the nucleus break up into as many segments. The schizonts also appeared smaller in diameter and did not fill the corpuscles so completely. In this connection it is interesting to note that the patient, up to the time of admission, had been taking quinine with great regularity. This fact leads us to suggest that possibly the vitality of the parasites had been impaired by the drug, sufficiently at any rate to prevent full segmentation and to account for the difference in growth noted between the two cases.

CHART II



Mist. Trop. 5i = 10 grains of quinine hydrobromide.

The patient made a good recovery on quinine being pushed, and is almost well at the time of writing.

REMARKS

Such is the morphology of our two cases of *Plasmodium falciparum* infection, cultivated in vitro. If our observations are correct, it appears to afford a simple method of studying some of the hitherto difficult problems of malaria. For example, the segmentation and sporulation of *P. falciparum* may be thus studied. It will be remembered that Sir Ronald Ross (1910) in his book 'The Prevention of Malaria,' states that 'the number of spores produced by each species of parasite is variable,' and that 'different authors give different figures, and in all probability the number has never been accurately estimated.' Sir Ronald adopts the figures of Welch (1897), who says that the 'malignant parasite produces six to twenty or more spores every two days.' In the work of Marchiafava, Bignami and Mannaberg (1894) the following description of the segmentation of the aestivo-autumnal parasite is given. 'The forms which precede fission (*i.e.*, segmentation) are represented by round or ovoid parasites which are in size between a quarter and half that of the red blood corpuscle, having pigment collected at the centre or slightly excentric in a small mass or in a cluster of granules in motion. The forms of fission vary in size; they may be as large as two-thirds of the red blood corpuscle, and are composed of one or two circles of spores (usually ten or twelve, seldom fifteen to sixteen) arranged round the central mass of pigment.'

Stephens and Christophers, in 'The Practical Study of Malaria' (1908), say that the malignant tertian parasite is rarely seen segmenting in the peripheral circulation, and state that there are eight to ten chromatin masses.

It is thus apparent that different observers have, in their studies of segmentation, encountered parasites with varying numbers of chromatin masses. This diversity of number is capable of various explanations:—

(1) That there are two or three varieties of the malignant parasites.

(2) At the time the observations were made segmentation may have been more or less complete.

(3) The administration of quinine may have in some way lowered or modified the vitality of the parasite, and thus prevented complete segmentation.

It is obviously impossible to dogmatize on such a limited number of cases and in such a short paper. We are still proceeding with our experiments with a view to elucidating these and several other important points which are not explained in Bass's paper. Thus:

(1) Why should the parasite only grow on what we have called the thin intermediate layer in the culture tubes, that is, the layer immediately beneath the serum and above the main mass of red blood cells; in other words, the layer in most immediate contact with the potentially destructive leucocytes and serum?

(2) What is the function of the apparently inert mass of red cells immediately beneath the growing layer, and of the serum immediately above? In both the cases we mention, one would expect the serum to be filled with antibodies, produced by repeated sporulations of parasites, and yet it is apparently powerless to stop in any way parasitic growth as long as such growth remains intracorporeal.

(3) Do the parasites grow better or worse in normal human serum? In other words, is there any specific affinity between the vitality of the individual's red cells and his own serum?

Such, and many other questions, can only be settled by repeated experiment. We have ventured to bring forward these two consecutive cases of the growth of a single generation of one particular variety of malarial parasite mainly because they show many interesting morphological points, which so far have not been touched on by Bass in his published papers. Also, they appear to prove to us, and to various competent observers to whom the slides have been shown, that the growth of the malarial parasite *in vitro* has at last been successfully accomplished.

We wish to thank Dr. H. B. Fantham for his valuable advice and help during this investigation.

NOTE

I have examined both the specimens sent to me by Dr. Bass from Tulane University, U.S.A., and those made by Drs. Thomson and McLellan in Liverpool, and am quite convinced that they give indisputable evidence of the successful cultivation of *Plasmodium falciparum* up to the sporulating forms. These forms are much too numerous in the specimens to admit of the supposition that the parasites have not developed since the blood was taken from the patient. The advance thus made is one of great importance, as all efforts to cultivate the parasites of malaria have hitherto failed since their discovery by Laveran in 1880.

RONALD ROSS.

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EXPLANATION OF PLATE XXIV

Figures drawn by means of an Abbé camera lucida, using Leitz ocular 4 and objective 1/12 inch. Stained with Giemsa or Romanowsky. Magnification 1,600 diameters.

Case T. H.

Figs. 1-5.—Parasites from the peripheral blood before incubation. Stained with Romanowsky. Fig. 5 shows elongated form.

Figs. 6-13.—Parasites after twelve hours cultivation. These show definite increase in size. The chromatin is more spread out, and a distinct circular mass of pigment is to be seen. Fig. 10 shows four corpuscles clumped together, each containing a parasite. (Romanowsky stain.)

Fig. 14.—A young crescent stained with Giemsa. This was found after twelve hours incubation, and was probably present in the original blood.

Fig. 15.—A schizont with the chromatin divided into two. After twenty-five hours incubation (Giemsa stain).

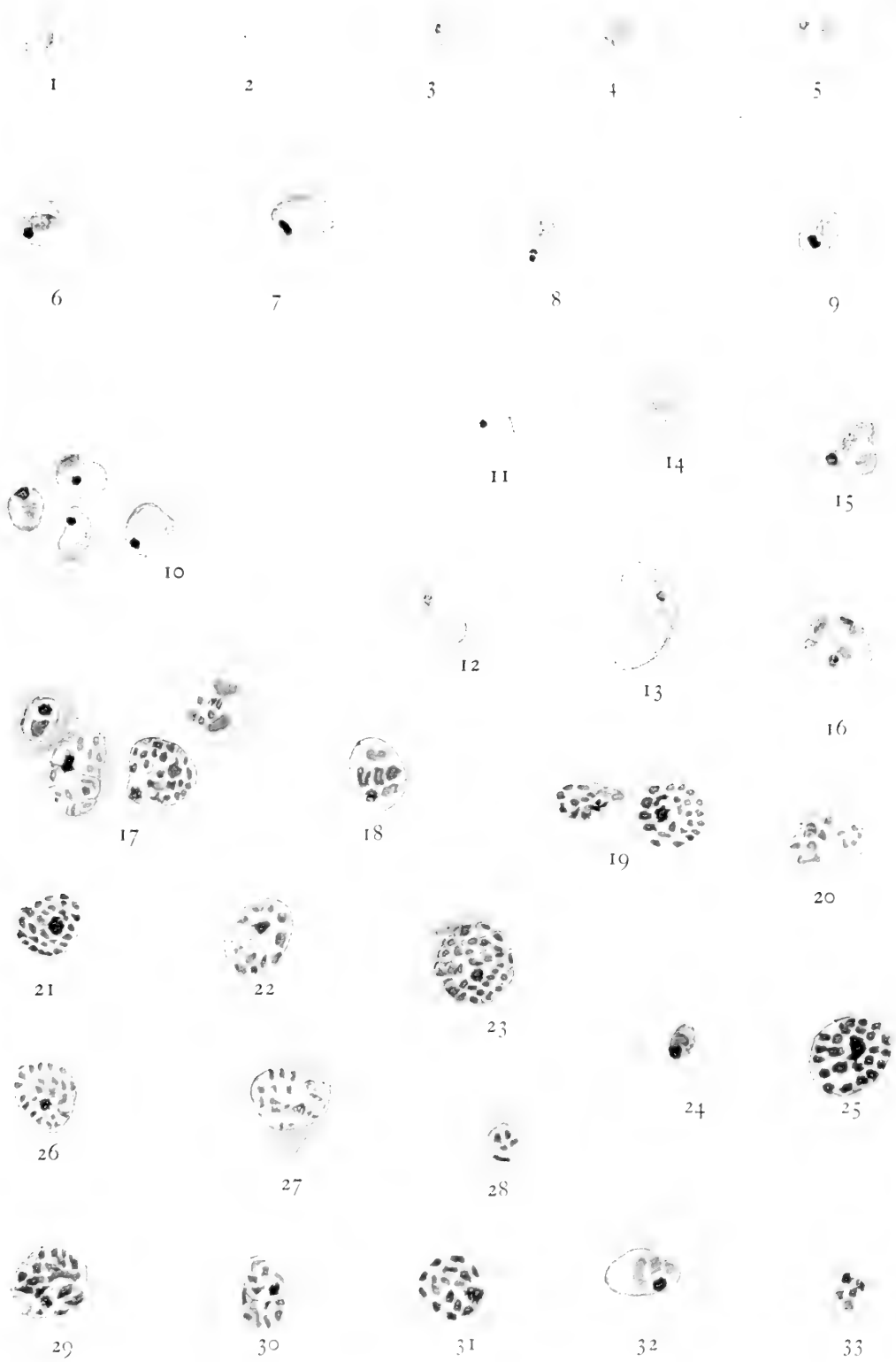
Fig. 16.—A schizont with the chromatin divided into four. After twenty-five hours incubation (Giemsa stain).

Figs. 17-30.—Various stages of segmentation of nucleus—all found in a twenty-five hours culture. Fig. 23 shows thirty chromatin particles.

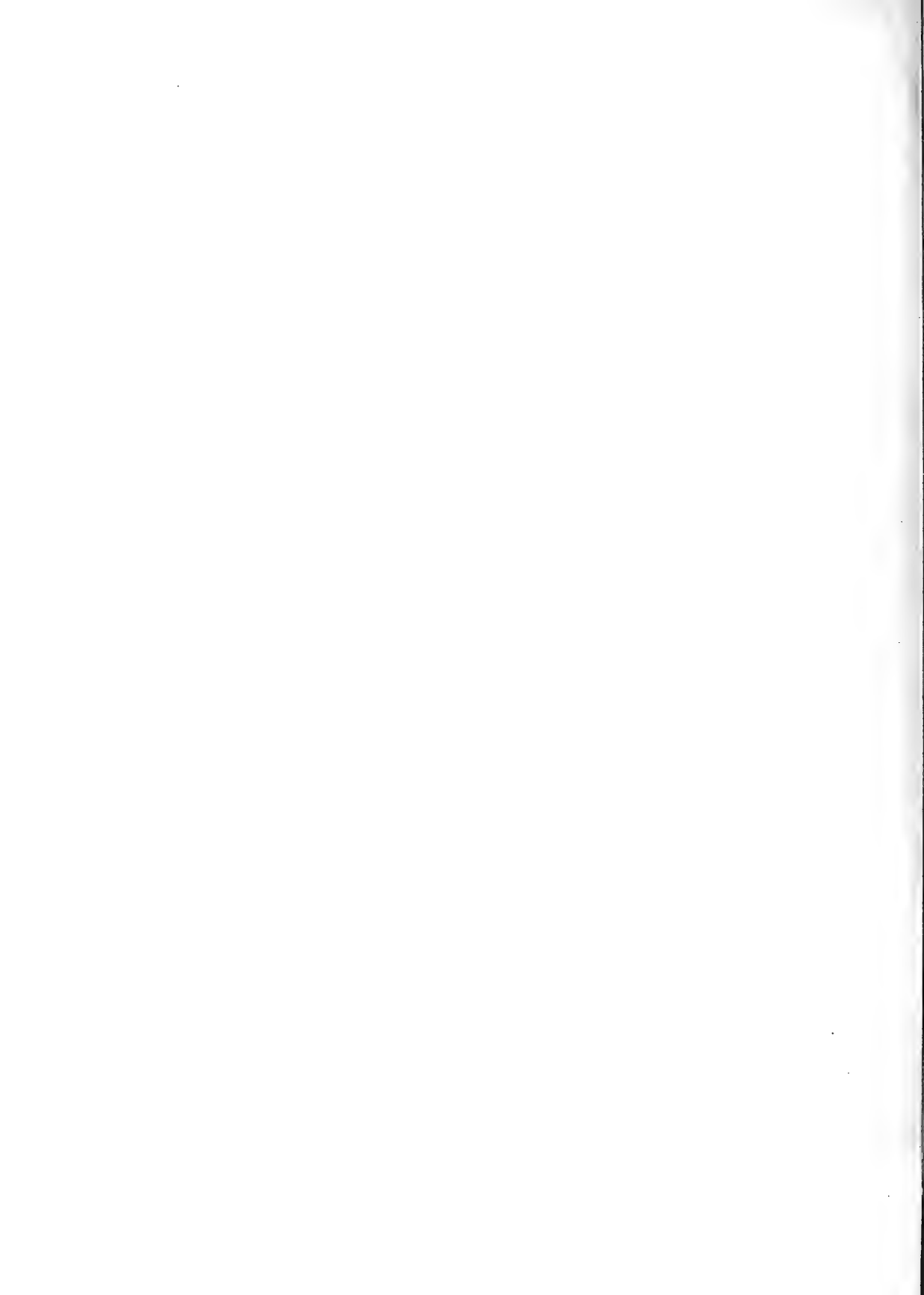
Fig. 31.—A segmenting form found after twenty-seven hours culture.

Fig. 32.—Form after twenty-seven hours culture.

Fig. 33.—Form after sixty-six hours in culture.



CULTIVATION OF *PLASMODIUM FALCIPARUM*



EXPLANATION OF PLATE XXV

Microphotographs showing development of one generation of *Plasmodium falciparum*.

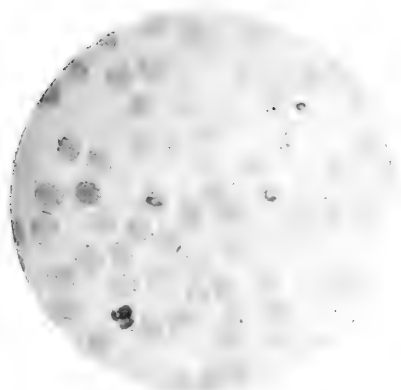
Figs. 1, 2, 3 and 4 are all of the same magnification. Fig. 5 is taken at a higher magnification.

Fig. 1.—This photograph shows two parasites before incubation.

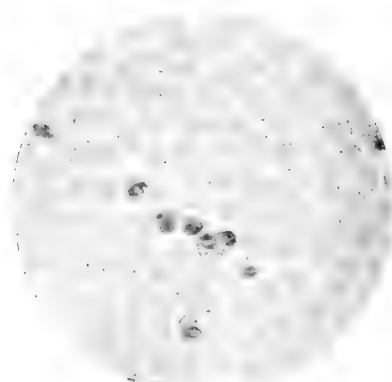
Figs. 2 and 3.—The parasites after twelve hours incubation at a temperature of 38° C. These show increase of size; the pigment is collected together into a circular mass, and the chromatin is spreading out into the protoplasm. The photographs also illustrate the tendency of the parasites to group together.

Fig. 4.—Shows schizogony. The chromatin is split up into varying numbers forming merozoites. The pigment is collected into a mass, usually circular. Specimens prepared after twenty-five hours incubation.

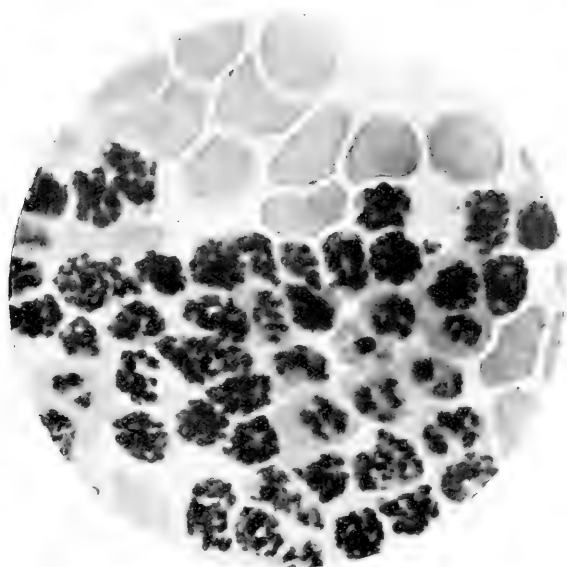
Fig. 5.—Schizogony after twenty-five hours incubation. The higher magnification shows the daughter nuclei more distinctly. Figs. 4 and 5 also show the tendency of the fully developed schizonts to clump together into masses when a smear is made.



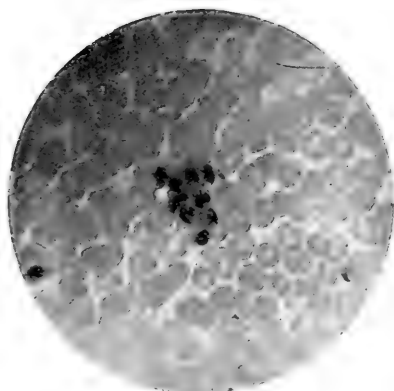
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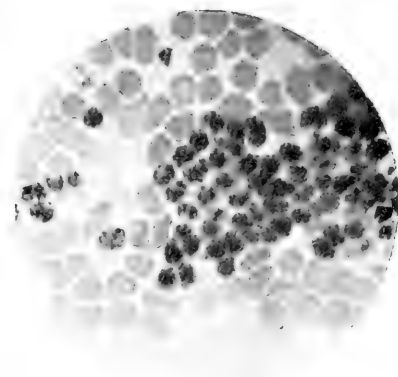
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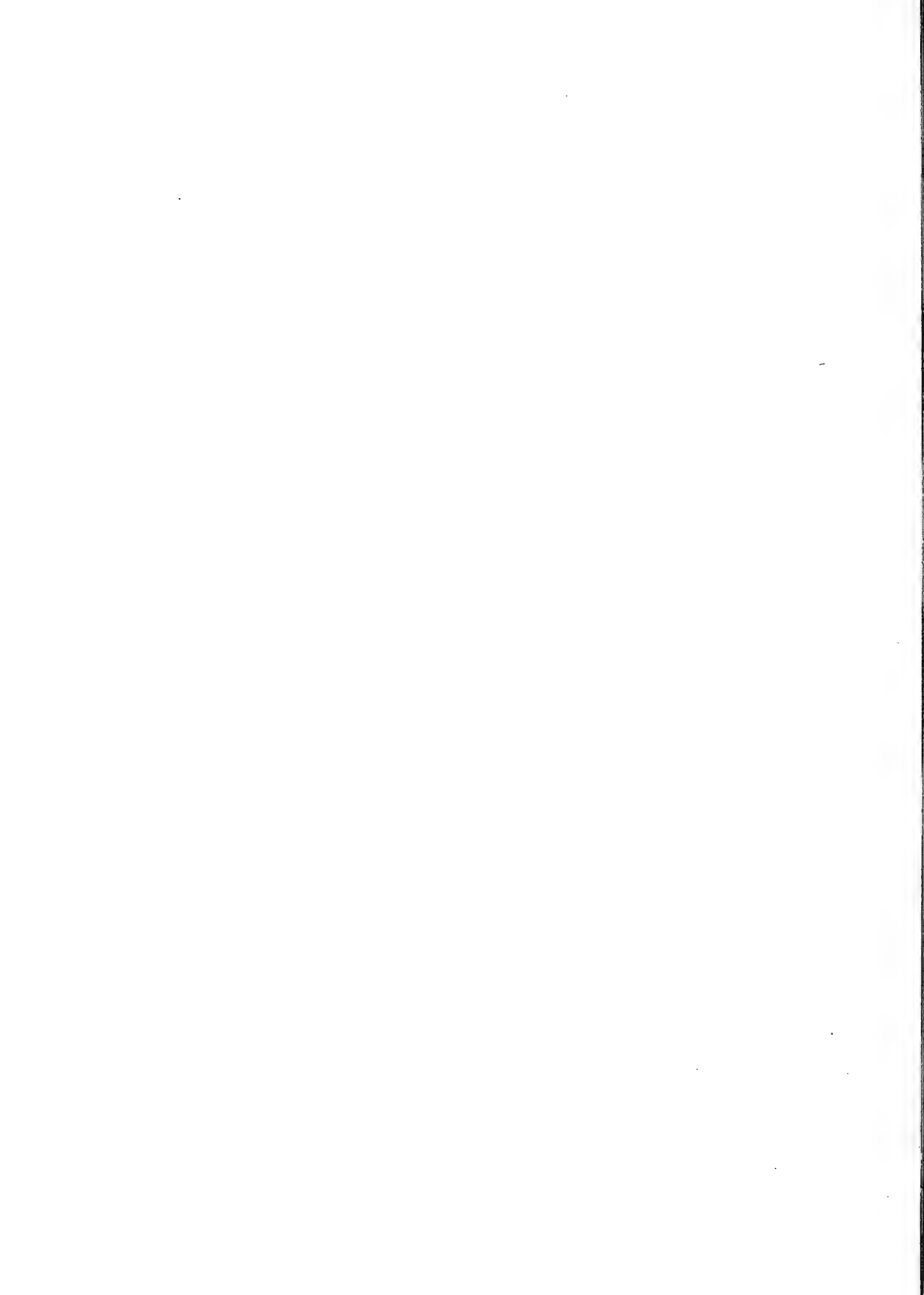
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NOTE ON THE SEX OF MOSQUITO LARVAE

BY
HELEN A. ADIE

(*From the Malaria Laboratory, Lahore*)

(*Received for publication 9 November, 1912*)

PLATE XXVI

In the *Lancet* of March 30, 1912, I described very shortly how it was possible to distinguish sex in anopheline larvae. The following contribution, the result of subsequent dissections, goes a little further into the matter.

With regard to anophelines, generally speaking the dark or brown testis can be distinguished in living larvae by a lens; or, if the larva is very young, say about three days, by a two-thirds lens. Often the testes are more easily seen from the ventral surface. If the larva is lying dorsal surface up, the simplest way to obtain a ventral view is to invert the slide. Fig. 1 (Pl. XXVI) shows the testes in the sixth abdominal segment, and the vasa deferentia extending downwards. The testis is enclosed in a hard brown sac (fig. 2*b*). On pressure it is seen to contain both immature and fully developed spermatozoa. The latter are found in various interesting stages of development. The testis is frequently marked out into areas, the posterior of which is seen to enclose coils of spermatozoa. Fig. 11 shows the areolation. In films stained with Romanowsky, the younger tailed spermatozoa resemble adult ones.

A simple way to dissect out the testis is to drag out the stomach by the ordinary method, and then to stroke gently the abdominal segments downwards with the needle held flat. In the pupa the dissection is of course easier, and the entire male apparatus can be got out, as shown in fig. 3. The spermatheca and ovaries are also well formed in the pupa.

Fig. 4 represents the adult male organs, and is introduced for the sake of comparison with the culicine arrangement.

In the female larva the ovary lies in about the same situation as the testis in the male, but stretches through perhaps two or three segments owing to its length.

With regard to culicines, in the larva the testis is not always visible. Working in Kashmir lately, I have, however, noticed a species (probably *C. fatigans*) in which the testis, often of a greenish tint, is easily recognised by the naked eye.

There is no brown enclosing sac, and the shape is more like a spindle. It contains spermatozoa. Many dissections were made to see when the earliest appearance of testes occurred. Fig 7a is from a specimen twenty-four hours old. Fig. 7b from another fourteen hours old.

Fig. 8 represents the male organs of a culicine pupa; the arrangement, it will be seen, is as in the adult.

The ovaries are recognisable in early larval life, and can occasionally be seen with the microscope in the living specimen.

The commonest anophelines examined were *Myzomyia culicifacies* and *M. listoni*, *Nyssorhynchus fuliginosus* and *Neocellia stephensi*.

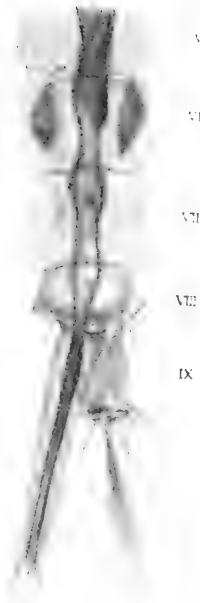
In this connection it might be mentioned that, in the case of *Dixa* larvae taken in Lahore, and in another species found in Kashmir, and described by Miall in 'Aquatic Insects,' the testes were even more conspicuous than those of anophelines.

465

EXPLANATION OF PLATE XXVI

All these figures, except No. 11, have been drawn with a Leitz Zeichenokular.

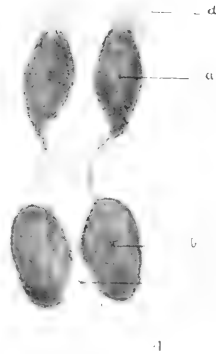
- Fig. 1. Hinder portion of advanced stage of last instar of the larva of an anopheline (*Neocellia willmori*) mounted in canada balsam. The two pear-shaped testes visible in sixth segment. The three dark masses in the middle line are the contents of the intestinal tube.
- Fig. 2. Testis and vas of anopheline larva dissected out.
(a) Body of testis. (b) Brown sheath. (c) Vas deferens. (d) Spermatozoa pressed out.
Specimen dried and stained.
- Fig. 3. Male organs of anopheline pupa.
(a) Testes. (b) Vas deferens. (c) Gland. (d) Vesicula passing into ductus.
Specimen dried and stained.
- Fig. 4. Male organs of adult anopheline.
(a) Testes. (b) Glands. (c) Vesicula passing into ductus.
Specimen mounted in formalin.
- Fig. 5. Ovary of fairly young anopheline larva.
- Fig. 6. Testis and vas deferens of culicine larva dissected out.
Fresh.
- Fig. 7a. Dissection of culicine larva twenty-four hours old, showing last segment and part of syphon.
(a) The two testes. (b) Débris, the result of dissection. (c) Syphon.
Specimen mounted in formalin.
- Fig. 7b. Dissection of culicine larva fourteen hours old, showing the two testes.
- Fig. 8. Culicine pupa, male organs.
(a) Testis with vas deferens (broken off). (b) Vas deferens. (c) Vesicula. (d) Gland.
Note different arrangement from anopheline.
Specimen mounted in formalin.
- Fig. 9. Male organs of adult culicine.
(a) Spermatozoa pressed out. (b) Testes. (c) Vas deferens. (d) Vesicula. (e) Gland.
Note attachment of upper ligament of gland to vas deferens.
Specimen stained and mounted in canada balsam.
- Fig. 10. Ovaries of culicine larva seen attached to a fragment of an abdominal segment.
- Fig. 11. Figure of larva testis showing areolation and spermatozoa in different stages of development, as seen in a fresh specimen. Area (a) contains ripe spermatozoa greatly magnified. Areas (b), (c), (d), spermatozoa in various stages of development.
- Fig. 12. Hind portion of abdomen of a culicine larva, showing testes.



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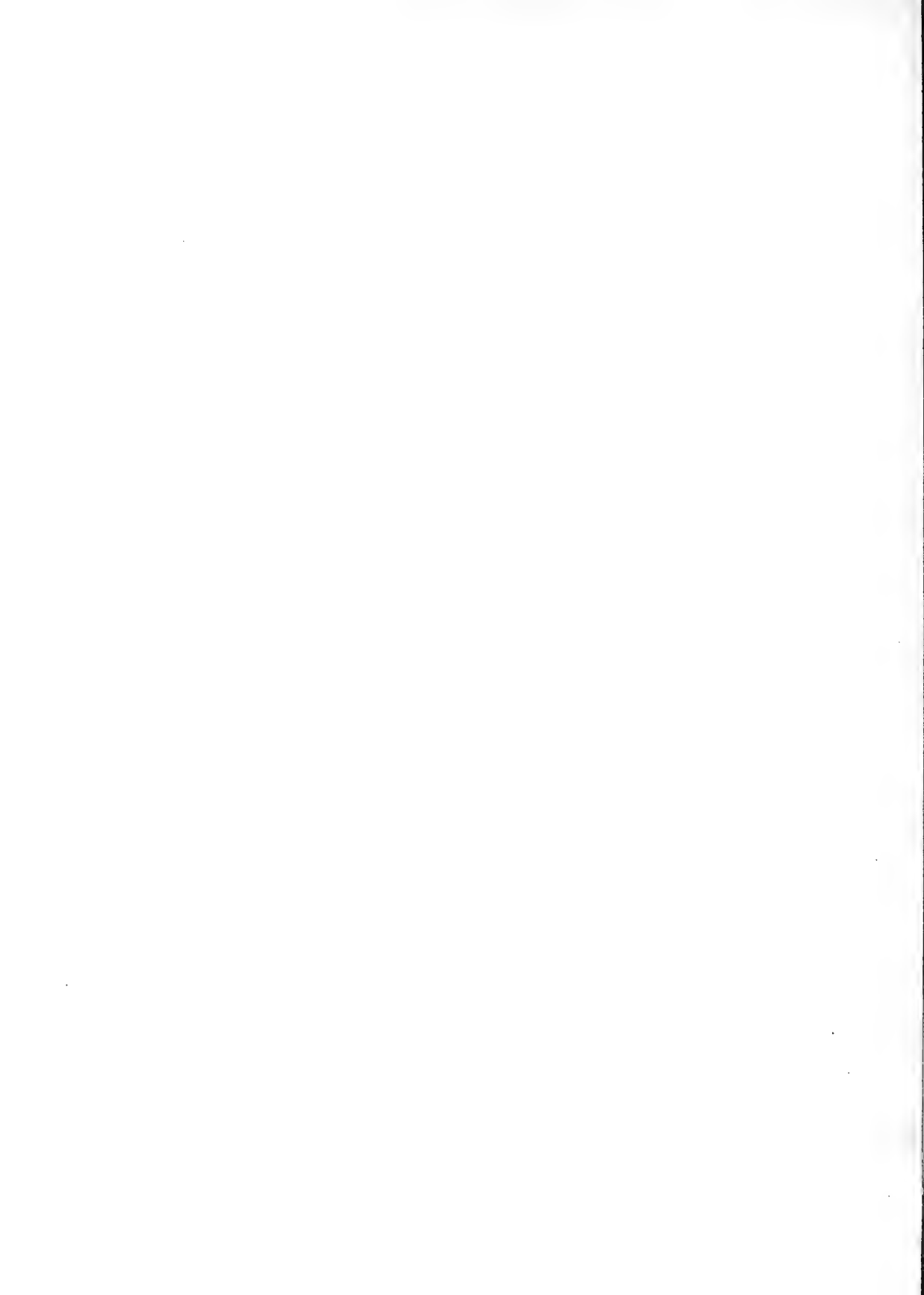
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SOME EFFECTS OF THE OCCURRENCE OF *MYXOSPORIDIA* IN THE GALL BLADDER OF FISHES

(PRELIMINARY COMMUNICATION)

BY

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AND

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(Received for publication 26 October, 1912)

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I. INTRODUCTION

Recently, while working on various protozoal parasites of marine fishes, occasion was made to investigate the more direct effects of these parasites upon the hosts harbouring them. The present paper is a short account of some results obtained when studying the effects of certain *Myxosporidia* inhabiting the gall bladders of various fishes.

II. MATERIAL

The fish in question harboured species of *Myxidium*, *Ceratomyxa*, *Chloromyxum* and *Leptotheca*. Mixed infections of

Myxosporidia occasionally occurred, but the effect of such co-existences was the same as that of either factor considered separately, and so is included here.

The hosts were examined as soon as possible after the fish were taken. Frequently they were killed for the purpose of examination. Our results have been arranged so as to exclude any post-mortem effects. The fish were opened rapidly and the contents of the gall bladders siphoned off, as a rule. The quantity of bile could be thus estimated, as well as its fluidity or viscosity. Sizes of fish—judged usually by length as a better criterion of age than weight, though the latter was also considered—were kept for purposes of comparison. It is to be regretted that more published data as to the normal variation in size and weight of fishes of the same age are not available.

The examinations here recorded extended over five weeks, during which time we were the guests of the Laboratoire Maritime at Luc-sur-mer, Normandy, through the kindness of the Director, Professor Joyeux-Laffuie, to whom we return cordial thanks. We would especially thank heartily Dr. C. Lebailly, who was in charge of the Laboratory, and who spared neither time nor trouble in procuring us materials for research. We also are indebted to Mr. Tate Regan, of the British Museum, for kindly naming some of the fishes (young *Raja*).

III. EFFECTS ON BILE AND GALL BLADDER

Our work embraced both *Elasmobranchii* and *Teleostei*, and we propose to tabulate the results obtained in both groups. The fish investigated is given a serial number and the degree of infection with *Myxosporidia* is indicated. A brief statement as to the colour and density of the bile, and one on the condition of the gall bladder, follows. Thin-walled gall bladders were usually transparent, and only where the transparency was more noticeable than usual is this recorded in the tables.

As the majority of the fish examined (over 100) belong to the *Teleostei*, they are considered first, members of the same tribe of hosts being arranged together for convenience.

(A) TELEOSTEI

TABLE 1.—*Gadus pollachius* (= *Merlangus pollachius*). Young fish.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Heavy	Yellow, thickish	Slightly thick walled	Fish weighed 350 grams.
2	Slight	Yellowish	"	
3	"	"	"	
4	"	Green	"	Weight 350 grams.
5	Uninfected	"	Transparent	" 350 "
6	"	Bright green	Large, thin	(These fish, except No. 23, were 27 to 34 cm. long).
7	"	"	Small, thin	
8	Slight	Yellowish green	"	
9	Uninfected	Greenish orange	"	Examined 5 hours after death.
10	Fair number of parasites	Yellowish orange, thick, turbid	Thick walled	Weight 340 grams.
11	Uninfected	Very pale yellow	Thin	" 370 "
12	Very slight infection	"	"	" 370 "
13	Many parasites	Yellow, thickish	Thick walled	" 360 "
14	Uninfected	Green	Very thin	" 370 "
15	Heavy infection	Yellow	Thick walled	" 350 "
16	"	Orange, thick	"	" 350 "
17	Uninfected	Greenish, very fluid	Thin, transparent	" 375 "
18	Very heavy infection	Brownish yellow	Large, thick walled	
19	Uninfected	Bright green	Thin walled	
20	"	"	"	
21	Slight infection	Yellow, thin	"	
22	Heavy "	Exceedingly thick, straw-coloured purée	Very thick walled indeed, small cavity	Weight 340 grams.
23	Uninfected	Very fluid, green	Thin walled	Large fish, weight 811 grams, length 46 cms.
24	"	Yellowish green, very fluid	Thin	Many worms present
25	Fairly heavy infection	Yellowish brown, lumps of spores in it	Thick, opaque	Weight 350 grams.
26	Very heavy infection	Little bile; very thick, straw-coloured purée	Thick, white, extremely tough, small cavity	Shortest fish of the series, 27 cm. long. Wt. 340 grams. Observed for 12 days.

From the above table it is seen that fifteen of the twenty-six *Gadus pollachius* examined were infected in their gall bladders by a species of *Myxosporidia*, the percentage of infection being 57.7. Five cases only could be described as heavy infections, a percentage of 19.2. Examination of the bile of the uninfected *Gadus pollachius* showed that in every case the colour predominating was green. Nine of the eleven uninfected fish had bile that was vivid green—almost moss-green—in colour. One case had greenish-orange bile, but here the results were possibly invalid, as the fish had been dead for five hours before it was examined. The bile of uninfected *Gadus pollachius* was also very limpid.

When *Myxosporidia* were present, the bile coloration underwent profound changes, and became yellow to orange, and even brown. When the parasites were present in very large numbers, the condition of the bile was one of high concentration, and the contents of the gall bladder resembled a thick, straw-coloured purée. As a general statement, the larger the number of parasites the more opaque, viscid and light-coloured was the bile.

The effect on the gall bladder appears to be no less marked. Ordinarily in uninfected fish it is thin-walled and transparent. With progressive infection it becomes thicker and its cavity smaller, while its opacity increases enormously. The condition is discussed in Section III (c), page 477.

TABLE 2.—*Gadus merlangus* (= *Merlangus vulgaris*).

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Thin, green	Thin	Fish 26.5 cm. long
2	Moderate infection	Brownish yellow, thick	Thick	" " "
3	Uninfected	Fluid, limpid, green	Thin	" " "
4	Slight infection	Brownish yellow	Somewhat thicker	" " "

In this case, the percentage of infected fish was high, 50 per cent., but judging from other experiences, it is likely that a

different result would have been obtained had it been possible to examine more fish. The point of interest, however, that the *Myxosporidia* in the gall bladder induced thickening of its walls and alteration of the bile, remains the same as in the case of *Gadus pollachius*.

TABLE 3.—*Gadus luscus*.

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Green	Thin walled	All these fish were young, and belonged to the same 'school.' They were about 16 cm. long.
2	"	Yellowish Green	"	
3	"	"	"	
4	"	Green	"	
5	"	"	"	
6	"	"	"	
7	"	"	"	
8	"	"	"	
9	"	"	"	
10	"	"	"	
11	"	"	"	
12	"	"	"	
13	"	"	"	
14	"	"	"	

No *Gadus luscus* examined harboured *Myxosporidia* in its gall bladder. In twelve cases out of fourteen the bile was of a bright green hue, and in the remaining two cases a faint yellowish tinge only was perceptible.

The *Gadidae*, as exemplified by *Gadus pollachius*, *Gadus merlangus* and *Gadus luscus*, appear to have normally green bile

contained in a thin-walled gall bladder, and under the influence of Myxosporidian infection the bile alters in colour and composition and the wall of the gall bladder becomes thickened.

TABLE 4.—*Trachinus trachinus*. (= ? *T. vipera*).

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Very green, thin	Large, thin	Large mature ♀
2	"	"	"	" " "
3	"	Slight yellowish tint to the green	Small, thin	" " "
4	Early infection	Yellowish	Thick and very tough walled	Mature ♀, 26 cm. long
5	Uninfected	Bright green	Thin	Mature ♀, 28 cm. long

Thus, in 80 per cent. of the fishes examined, the bile was bright green and the walls of the gall bladder thin, and no *Myxosporidia* were present in the gall bladder or bile. Thickening of the wall and alteration in the colour of the bile coincided with the presence of parasites.

TABLE 5.—*Trachinus draco*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Thin, green	Thin walled	
2	"	Yellowish green	"	Cestode larvae present in gall bladder

Uninfected bile appeared to be green. The presence of Cestode larvae in the gall bladder had caused a bile reaction somewhat resembling that produced in other fishes by the presence of *Myxosporidia*.

TABLE 6.—*Lepadogaster bimaculatus*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Moderate	Yellow, slightly turbid	Thin	Very few of these fish were available for examination.
2	Slight	Yellow, thin	"	
3	Early infection, slight	Yellow, slightly turbid	"	
4	Uninfected	Green	Very thin	

While the gall bladders of the *Lepadogaster* were thin when infected, they were thicker and less transparent than those of uninfected fish. The bile also was yellow and thicker than that of the normal fish.

TABLE 7.—*Blennius ocellaris*

One specimen of this fish only was examined. It contained a *Myxidium* in its gall bladder, which was thin and had greenish yellow bile. The degree of infection was slight.

The tribe *Jugulares* of the *Acanthopterygii* as judged by *Trachinus trachinus*, *Trachinus draco*, *Lepadogaster bimaculatus*, and *Blennius ocellaris*, apparently undergo digestive derangements as a result of the presence of *Myxosporidia* in the gall bladder, the parasites producing alterations in the contents of the gall bladder.

TABLE 8.—*Cottus bubalis*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Thin, green	Thin	Few trypanosomes in blood.
2	"	Vivid green	"	
3	Moderate infection	Faint greenish yellow	Thicker walled	

Cottus bubalis, family *Cottidae*, of the tribe *Scleroperai*, was the only member of the tribe available for examination. The presence of *Myxosporidia* in the gall bladder was shown by a change of colour of the green bile to yellow, and an increase in thickness in the wall of the gall bladder.

TABLE 9.—*Callionymus lyra*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Very slight infection	Pale, yellowish green	Thin	Fish 13 cm. long
2	Heavy	Thick, orange	Very thick, opaque	16 cm. long
3	Uninfected	Yellowish green	Thin	12.8 " "
4	"	"	"	22.5 " "
5	Fairly heavy	Viscid, orange	Thick walled	12 " "
6	"	"	"	14.5 " "
7	"	"	"	13.5 " "
8	Uninfected	Thin, orange bile, but apparent fatty degeneration of liver	Thin	13.5 " "
9	Heavy	Blue-black	Thick	13.5 " "

Two-thirds of the *Callionymus* examined were parasitised and underwent profound changes in bile and gall bladder as a result. The *Gobiidae*, a family of the *Gobiiformes*, when parasitised thus, behave much as do other families among the *Acanthopterygii*.

TABLE 10.—*Solea vulgaris*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Very green, thin	Thin walled	Very few trypanosomes in blood.
2	"	Green	"	
3	"	"	"	

The sole, as a representative of the *Pleuronectidae*, family of the tribe *Zeorhombi*, when normal showed green bile contained in a thin-walled gall bladder. The condition if *Myxosporidia* were present could not be determined, as material was lacking. Some normal plaice resembled the normal soles in the condition of their bile and gall bladder.

TABLE 11.—*Carassius auratus*.

No. of fish	Degree of infection with Myxosporidia	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Bright green	Thin walled	All these fish were 9.5 cm. long and came from the same hatch. They were examined for purposes of comparison and experiment.
2	"	"	"	
3	"	Green	"	
4	"	Green, very fluid	"	
5	"	Green	"	
6	"	"	"	
7	"	"	"	
8	"	"	"	

The *Ostariophysi* as represented by the goldfish (*Carassius auratus*) agree with most other Teleosts in possessing green bile.

It may be mentioned that goldfish, from the same stock,

artificially infected with *Myxosporidia*, had yellowish bile and thickened gall bladders.

TABLE 12.—*Scomber scomber*. (= *S. scombrus*.)

No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder	Other remarks
1	Uninfected	Yellowish red, thin	Thin walled	Fish 20 cm. long
2	"	"	"	24 cm. long
3	"	"	"	24 " "
4	"	"	"	15 " "
5	"	"	"	20 " "
6	Infected	Red to blood-red	Slightly thicker walled	24 " "
7	Uninfected	Blood colour, thin	Thin	30 " "
8	"	"	"	24 " "
9	"	"	"	24 " "
10	"	"	"	22 " "
11	"	"	"	25 " "
12	"	"	"	27 " "
13	"	"	"	29 " "
14	"	"	"	25 " "
15	Slight infection	Blood colour, thin, small quantity	Slightly thicker walls	28 " "
16	Uninfected	Thicker, yellowish red	Thin	28 " "
17	Infection moderate	Abundant bile, fairly fluid	"	30 " "
18	"	Small quantity, red, fluid	"	27.5 " "
19	Uninfected	Blood colour, thin	"	29.5 " "
20	"	"	"	24 " "
21	"	"	"	24 " "
22	"	"	"	24 " "
23	"	"	"	24 " "
24	"	"	"	24 " "
25	"	"	"	24 " "

Scomber scomber—the mackerel—the representative of the *Scombriformes* examined, differs from the other families so far recorded, in that its bile coloration showed little or no difference

when *Myxosporidia* were present. Its colour is, then, not markedly associated with Myxosporidian infection, and neither the colour nor the extremely slight thickening of the walls of the bladder were of use as rough criteria of the infected or normal conditions.

Among the *Apoda*, both *Anguilla* and *Conger* were examined. The bile was green in all the cases, and no *Myxosporidia* were found in the clear, transparent gall bladders.

Ammodytes tobianns afforded an example of the *Percesoces*, but proved uninfected, though a fair number were examined.

(B) ELASMOBRANCHII

Among the *Selachii*, members of the sub-orders *Squali* and *Raji* were examined, to see whether the same conditions obtained as in the *Teleostei*.

TABLE 13.—*Selachii*.

Name of fish	No. of fish	Degree of infection with <i>Myxosporidia</i>	Condition of bile	Condition of gall bladder
<i>Scyllium catulus</i>	1	Uninfected	Greenish	Thin
<i>Galeorhinus galeus</i> (= <i>Galeus canis</i>)	1	Slight infection	Greenish, limpid	Slightly thickened
„	2	Heavy infection	Orange, much mucus—like table-jelly	„
„	3	Uninfected	Green	Thin
<i>Acanthias vulgaris</i>	1	Moderate infection	Yellowish	Thick
<i>Raja batis</i>	1	Uninfected	Thin, green	Thin
„	2	„	„	„
„	3	„	„	„
„	4	„	„	„
<i>Raja maculata</i>	1	Moderate infection	Pale yellow	Slightly thickened
„	2	Uninfected	Greenish	Thin
„	3	„	„	„
„	4	„	„	„

As with the *Teleostei*, so with the *Selachii*, it seems, so far as our experience goes at present, that the presence of *Myxosporidia* in the gall bladder produces some thickening and also changes in the colour and composition of the bile.

(C) PATHOLOGICAL EFFECTS

As most previous workers on the *Myxosporidia* occurring in piscine gall bladders have considered that the free floating parasites usually have little or no direct effect upon the host, we wish to record very briefly the results of our preliminary examination of preparations of the *Myxidium*-infected gall bladders of *Gadus pollachius*.

(a) There is a leucocytic infiltration around the small blood vessels in the fibro-muscular coat of the infected gall bladder.

(b) The leucocytic infiltration is especially pronounced in some places in the sub-serous layer, and in the immediately adjacent portions of a peculiar tissue which covers part of the outside of the gall bladder. This tissue has, according to Dr. Seidelin, a glandular structure and represents, in his opinion, pancreas.

(c) The leucocytic infiltration is also considerably increased at the bases of some of the papillae of the mucosa. In connection with this leucocytic infiltration there appears to be a considerable number of young fibroblasts, but it is difficult to state whether the presence of such is a normal occurrence or not.

These leucocytic infiltrations indicate a condition of inflammation of the gall bladder.

(d) Desquamation of the epithelium of the mucosa occurs in some places, and degenerating epithelium is found in the bile.

(e) Numerous mucous cells are found at various points in the epithelium. The distribution of such areas is irregular.

(f) There is no doubt that the *Myxosporidian* parasites are adherent to the mucosa. In some cases, processes from *Myxosporidian* trophozoites can be seen clearly penetrating the epithelium of the gall bladder. These parasitic intrusions have been seen by other observers to whom we have shown our sections.

(g) Regarding the possibility of small intra-epithelial stages of the parasites, we have seen certain structures—and so have some of

our colleagues—which could be interpreted as such. These intra-epithelial bodies seem identical with those figured by Auerbach (1910)* in the case of *Myxidium bergense*, parasitic in *Gadus virens*. We prefer, however, to defer a definite pronouncement on this difficult matter.

(h) In fine, it would appear that the presence of Myxosporidia is apt to irritate the gall bladder, leading to a catarrhal secretion of mucus and desquamation of epithelium, and to inflammatory conditions throughout the wall. The increase of the mucus accounts for the viscosity of the contents of many infected gall bladders. To some extent the discoloration of the bile is probably due to the presence of parasites.

The existence of a catarrhal cholecystitis seems to explain satisfactorily the described alterations in consistence and colour of the contents of infected gall bladders.

We have much pleasure in thanking Dr. Seidelin and Dr. G. C. Simpson for examining our sections and for suggestions used in writing this sub-section.

It should be mentioned that the results previously tabulated, both gross and pathological, appeared to be in no wise different whether the fish in question had fed recently or was fasting. In every case there was sufficient bile present for determination of its colour and consistency, even though the wall of the gall bladder were thickened. Empty gall bladders were not found in any of the fish examined. It may be of interest to add that most of our results were obtained in the month of August, 1912.

Some effects of Myxosporidia on other organs of fish with biliary infection are briefly recorded in Section V, page 481.

Parenthetically, we may mention that, although anxious to give as correctly as possible the names of the piscine hosts for purposes of reference, we have had some difficulty in certain cases. English and French authorities differ on the classification and nomenclature of fishes. Hence the use of synonymous names in various parts of this paper.

* 'Die Cnidosporidien,' p. 105. Leipzig: Werner Kleinhardt.

IV. TABULAR SUMMARY

To complete the survey of the distribution of the parasites in the gall bladders of various fishes, together with the effects observed, we may tabulate the results obtained thus:—

TABLE 14.—*Teleostei*.*

Sub-order	Tribe	Family	Genus	General result
Apodes		Anguillidae	Anguilla	Examination negative. Effect of Myxosporidia undetermined. Normal bile green.
			Conger	
Percesoces		Ammodytidae	Ammodytes	Fish uninfected, bile green.
Anacanthini		Gadidae	Gadus (3 spp.)	Normal bile green, gall bladder thin; infected bile yellow, gall bladder thick.
Acanthopterygii	Scombriformes	Scombridae	Scomber	Normal and infected bile much alike, gall bladder slightly thicker if infected.
	Zeorhombi	Pleuronectidae	Solea	Normal bile green.
	Gobiiformes	Gobiidae	Callionymus	Normal bile green, and gall bladder thin. Infected bile thick, orange to blue-black, gall bladder thickened.
	Scleroparei	Cottidae	Cottus	Normal bile green, gall bladder thin. Infected bile yellowish, gall bladder thicker.
	Jugulares	Trachinidae	Trachinus (2 spp.)	Normal bile green, thin; gall bladder thin. Infected bile yellowish, gall bladder thicker.
		Gobiesocidae	Lepadogaster	Normal bile green, infected yellow. Normal gall bladder thin, infected thicker.
		Blenniidae	Blennius	Normal bile green, thin; infected bile yellowish, thicker; gall bladder thicker when infected.
Ostariophysi		Cyprinidae	Carassius	Normal bile green, thin; infected bile yellowish.

* Classification based on that given in Sedgwick's 'Zoology,' Vol. II (1905).

TABLE 15.—*Elasmobranchii*.

Order	Sub-order	Family	Genus	General result
Selachii	Squali	Scylliidae	Scyllium	Normal bile greenish.
		Spinacidae	Acanthias	Infected bile yellow, gall bladder thick.
		Carchariidae	Galeus	Normal bile green, thin; infected bile very thick, gall bladder thickened if infected.
	Raji	Rajidae	Raja (2 spp.)	Normal bile green, thin; infected bile thicker, yellow; gall bladder slightly thickened if infection present

From the 'distribution' summary it is seen that in a fairly wide range of fishes the presence of *Myxosporidia* in the gall bladder is associated with thickening of the organ and with changes in the contents.

It may be of interest to note that green bile removed from a freshly-killed fish remained green for as long as a couple of days, while the discoloured bile in an infected fish was obviously discoloured at the moment of the death of the fish. Decapitated fish, whose muscles still executed vigorous movements, if infected, had discoloured bile. In other words, the discoloration of the bile was certainly not a post-mortem effect.

Direct experiments showed that the change in colour was induced by the Myxosporidian parasite. *Gadus luscus* was never found naturally infected with *Myxosporidia*. But two *Gadus luscus* artificially infected by way of food showed yellowish colour of the bile when killed a few days later, and in the bile there were found young stages of the parasite administered to them. The control fish had clear, green, parasite-free bile.

V. OTHER EFFECTS

Other effects of Myxosporidian infection also briefly noted were as follows:—

Infected *Gadus pollachius* had larger livers and gall bladders than normal *G. pollachius* of the same length. Those containing *Myxosporidia* were thinner as a rule than uninfected specimens of the same length. An infected male was thinner than a normal male, and the same was true for the females.

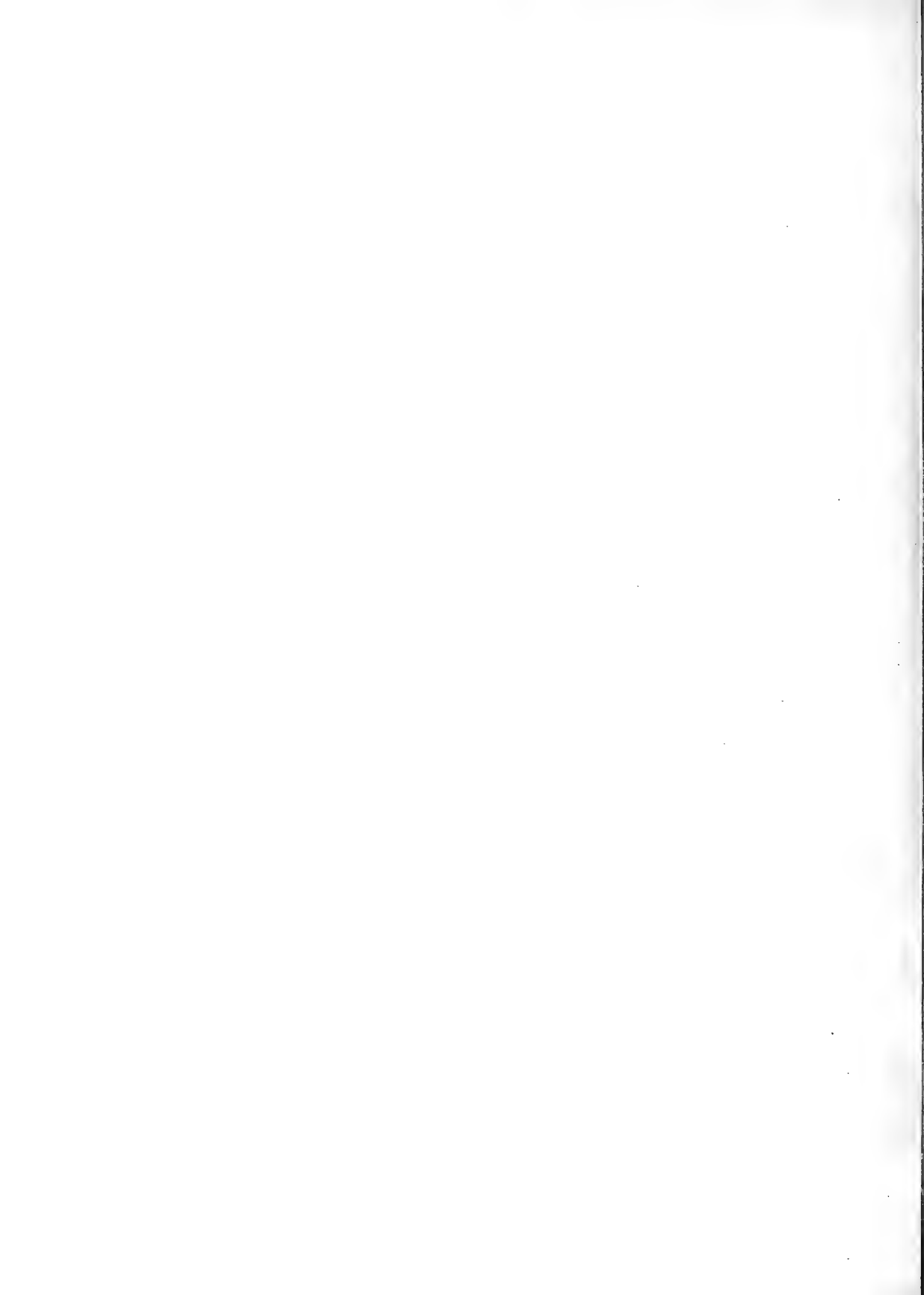
In the case of *Scomber scomber*, the bile, when normal, contained a fair amount of fat. Infected bile showed much less fat.

Acute inflammation of the intestine was sometimes noticed in infected fish.

In some cases the condition of the bile may have hindered pancreatic digestion also, for in a few fish the bile gave an acid reaction instead of the usual alkaline one. The interference with pancreatic digestion would probably, in part, explain the thinner musculature of the body of an infected fish.

VI. CONCLUDING REMARKS

It is obvious, then, that biliary Myxosporidiasis has an economic as well as a biological interest, and hence may be added to the catalogue of little-known diseases well worthy of more detailed investigation. Fish which appeared healthy externally often showed, on closer examination, slight emaciation when Myxosporidia were present. We hope to deal with some of the Myxosporidian parasites in greater detail shortly, and, if possible, to discuss more fully the various other changes occurring in infected fish.



FURTHER OBSERVATIONS ON THE
TRYPANOSOMES OF GAME AND
DOMESTIC STOCK IN NORTH EASTERN
RHODESIA

BY

ALLAN KINGHORN

AND

WARRINGTON YORKE

*(Seventh Interim Report of the Luangwa Sleeping Sickness
Commission, British South Africa Company)*

(Received for publication 7 December, 1912)

In a former report* we described the various trypanosomes found in game and domestic animals in the vicinity of Nawalia in the Luangwa Valley. At the end of April the headquarters of the Commission were removed from Nawalia to Ngoa on the Congo-Zambesi watershed; and during our stay at the latter place the game and domestic animals were examined for trypanosomes in a similar manner.

In this communication we give the complete results obtained by examination of the big game, domestic stock and small vermin during our sojourn in Rhodesia.

The routine method of examination adopted by us was that described in our previous paper.

EXAMINATION OF GAME AT NAWALIA AND NGOA

A total of 127 head of game, comprising 19 genera, was examined at Nawalia, and trypanosomes were found by direct examination, by inoculation, or by both methods, in 33.

* Kinghorn and Yorke, *Annals of Tropical Medicine and Parasitology*, 1912, Vol. VI, No. 3A, p. 301.

At Ngoa, 124 wild animals, belonging to 16 genera, were examined, and trypanosomes were found in 21—a percentage of 16.9. Details are given in Tables 1 and 2.

TABLE 1.—Results of examination of game for trypanosomes at Nawalia.

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculation
1. Elephant	1	0	1	0	0	0
2. Rhinoceros	1	0	1	0	0	0
3. Hippopotamus	1	0	0	0	0	0
4. Zebra	5	0	3	0	0	0
5. Roan	8	1	2	0	0	1
6. Wildebeest	2	0	1	0	0	0
7. Kudu	7	3	3	1	1	4
8. Hartebeest	6	0	1	0	1	1
9. Waterbuck	28	16	14	5	1	17
10. Puku	10	1	6	0	0	1
11. Mpala	20	1	13	1	1	2
12. Bushbuck	9	4	6	1	2	6
13. Bushpig	4	0	1	0	0	0
14. Warthog	9	0	3	0	1	1
15. Lion	2	0	0	0	0	0
16. Hunting dog	1	0	1	0	0	0
17. Giant rat	1	0	0	0	0	0
18. Genet	2	0	0	0	0	0
19. Squirrel	1	0	0	0	0	0
	127	26	56	8	7	33

TABLE 2.—Results of examination of game for trypanosomes at Ngoa.

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculation
1. Rhinoceros	6	0	3	0	0	0
2. Zebra	17	0	5	0	0	0
3. Buffalo	6	0	3	0	0	0
4. Eland	15	0	12	0	4	4
5. Roan	5	0	3	0	1	1
6. Hartebeest	8	0	4	0	0	0
7. Waterbuck	27	12	15	3	0	12
8. Puku	8	1	6	0	0	1
9. Sitatunga	2	1	0	0	0	1
10. Duiker	9	2	4	0	0	2
11. Klipspringer	2	0	1	0	0	0
12. Warthog	12	0	3	0	0	0
13. Hyaena	2	0	1	0	0	0
14. Caracal	2	0	0	0	0	0
15. Galago	1	0	0	0	0	0
16. Reedbuck	2	0	0	0	0	0
	124	16	60	3	5	21

It will be seen that parasites were found at Nawalia by direct examination in 26 cases, a percentage of 20·4, while at Ngoa trypanosomes were found in the peripheral blood of only 16 buck—13·0 per cent. These are high figures for single observations, and it is probable that had several preparations from each buck been searched, the percentage of successes would have been much greater.

In several instances, only a single trypanosome was found in a film covering the greater part of a slide, and this after a very careful examination extending over two hours.

A more accurate estimate of the percentage of animals harbouring trypanosomes is afforded by considering only those from which inoculations were made. An analysis of these gives the following figures:—

	Nawalia	Ngoa
Number of inoculations made	56	60
Number of positive inoculations in which parasites were found in buck's blood...	8	3
Number of positive inoculations in which no parasites were found in buck's blood	7	5
Number of negative inoculations in which parasites were found in buck's blood...	6	6
Total number infected	21	14

These figures show that at least 37·5 per cent. (Nawalia) and 23·3 per cent. (Ngoa) of the local fauna were infected with the trypanosomes of man or domestic stock. The percentage of big game found to be infected with the human parasite (*T. rhodesiense*) was, at Nawalia 16, and Ngoa 3·3. Both *T. vivax* and *T. nanum* have been found in game, and to both these species monkeys and rats are refractory, so that no conclusions can be drawn regarding the presence or absence of these trypanosomes in animals in which parasites were not found in the blood smears. Had sheep and goats been available for inoculation, it is probable that many more buck would have been shown to harbour the two organisms in question. As a conservative estimate, the percentage of game actually infected with trypanosomes (pathogenic to man or domestic stock) in the vicinity of Nawalia might be placed at 50, and at Ngoa 35.

A further point which is brought out in the tables is that different species of buck appear to vary widely in their susceptibility. Amongst the commoner varieties, trypanosomes were never found, either by direct examination or by inoculation, in zebra, buffalo, wildebeest and bushpig, and only rarely in roan, hartebeest, puku, mpala, and warthog. Waterbuck, eland, bushbuck, and kudu were the species found to be most heavily infected.

TABLE 3.—Percentages of various species of game found infected with trypanosomes at Nawalia.

Animal	Number examined	Percentage harbouring trypanosomes
Bushbuck	9	66.6
Waterbuck	28	60.7
Kudu	7	57.1
Hartebeest	6	16.6
Roan	8	18.5
Warthog	9	11.1
Puku	10	10.0
Mpala	29	6.9

TABLE 4.—Percentages of various species of game found infected with trypanosomes at Ngoa.

Animal	Number examined	Percentage harbouring trypanosomes
Sitatunga	2	50
Waterbuck	27	44.4
Eland	15	26.6
Duiker	9	22.2
Roan	5	20
Puku	8	12.5

To a certain extent, perhaps, these differences may be accounted for by the habitats affected by the various species of game. Kudu and bushbuck, and waterbuck to a lesser extent, are usually found in thick cover, from which they seldom emerge, and where they are more constantly exposed to the bites of tsetse flies. Mpala, puku and wildebeest are usually found in open country, frequently remaining for the greater part of the day on wide, bare plains, and here the flies are less noticeable than in the bush. Specific differences in the amount of immunity enjoyed by buck are probably, however, of much greater importance.

In Tables 5 and 6 are given the species of trypanosomes occurring in each animal in which parasites were found. In

TABLE 5.—Trypanosomes found in game at Nawalia

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Bushbuck ... 1 ...	Negative	<i>T. pecorum</i>	<i>T. pecorum</i>
" ... 2 ...	<i>T. pecorum</i> or <i>T. nanum</i>	Negative	<i>T. nanum</i>
" ... 3 ...	<i>T. multiforme</i> , sp. nov.	<i>T. multiforme</i> , sp. nov.	<i>T. multiforme</i> , sp. nov.
" ... 4 ...	<i>T. pecorum</i> or <i>T. nanum</i>	no inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
" ... 5 ...	<i>T. pecorum</i> or <i>T. nenum</i>	"	<i>T. pecorum</i> or <i>T. nanum</i>
" ... 6 ...	Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
Waterbuck ... 1 ...	<i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i>	<i>T. pecorum</i>
" ... 2 ...	<i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>
" ... 3 ...	<i>T. pecorum</i> or <i>T. nanum</i> and <i>T. vivax</i>	Negative	<i>T. nanum</i> and <i>T. vivax</i>
" ... 4 ...	<i>T. pecorum</i> or <i>T. nanum</i>	"	<i>T. nanum</i>
" ... 5 ...	Negative	<i>T. pecorum</i>	<i>T. pecorum</i>
" ... 6 ...	<i>T. vivax</i>	Negative	<i>T. vivax</i>
" ... 7 ...	<i>T. vivax</i>	"	<i>T. vivax</i>
" ... 8 ...	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
" ... 9 ...	<i>T. pecorum</i> or <i>T. nanum</i> and <i>T. vivax</i>	Negative	<i>T. nanum</i> and <i>T. vivax</i>
" ... 10 ...	<i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
" ... 11 ...	(?) <i>T. rhodesiense</i>	Animal died day after inoculation	(?) <i>T. rhodesiense</i>
" ... 12 ...	<i>T. rhodesiense</i>	<i>T. rhodesiense</i> and <i>T. pecorum</i>	<i>T. rhodesiense</i> and <i>T. pecorum</i>
" ... 13 ...	<i>T. vivax</i>	No inoculation	<i>T. vivax</i>
" ... 14 ...	<i>T. rhodesiense</i> and <i>T. vivax</i>	<i>T. rhodesiense</i>	<i>T. rhodesiense</i> and <i>T. vivax</i>

TABLE 5 continued.—Trypanosomes found in game at Nawalia

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Waterbuck ... 15 ...	<i>T. vivax</i>	No inoculation	<i>T. vivax</i>
„ ... 16 ...	(?) <i>T. rhodesiense</i> and <i>T. vivax</i>	„	(?) <i>T. rhodesiense</i> and <i>T. vivax</i>
„ ... 17 ...	(?) <i>T. rhodesiense</i>	„	(?) <i>T. rhodesiense</i>
Kudu ... 1 ...	Negative	<i>T. pecorum</i>	<i>T. pecorum</i>
„ ... 2 ...	<i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
„ ... 3 ...	<i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i>	<i>T. pecorum</i>
„ ... 4 ...	<i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
Roan ... 1 ...	<i>T. pecorum</i> or <i>T. nanum</i>	„	<i>T. pecorum</i> or <i>T. nanum</i>
Warthog ... 1 ...	Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
Puku ... 1 ...	<i>T. vivax</i>	No inoculation	<i>T. vivax</i>
Mpala ... 1 ...	Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
„ ... 2 ...	<i>T. pecorum</i> or <i>T. nanum</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>	<i>T. pecorum</i> and <i>T. rhodesiense</i>
Hartebeest ... 1 ...	Negative	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>

compiling the tables, information obtained from the result of inoculations, where these were made, has been utilised. This enables a differentiation to be made between such parasites as *T. pecorum* and *T. nanum*, which are morphologically indistinguishable. *T. vivax* has a characteristic morphology, and can thus be identified in blood smears without difficulty.

As would be expected, double infections are not uncommon amongst game, and several instances of this are recorded in the Tables.

No data exist as to the ultimate effect of infection on game. All the animals which were shot appeared to be in perfect condition, and presented no objective signs of disease. Whether or not buck succumb to trypanosomiasis it is impossible to say, but as they have

increased steadily since rinderpest swept through the country, it may be assumed that their tolerance to trypanosomes is very great.

TABLE 6.—Trypanosomes found in game at Ngoa.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculations into monkeys and rats	Diagnosis
Waterbuck ... 1 ...	<i>T. vivax</i>	No inoculation	<i>T. vivax</i>
" ... 2 ...	"	"	"
" ... 3 ...	"	"	"
" ... 4 ...	"	Negative	"
" ... 5 ...	"	"	"
" ... 6 ...	"	<i>T. rhodesiense</i>	<i>T. vivax</i> and <i>T. rhodesiense</i>
" ... 7 ...	"	"	<i>T. vivax</i> and <i>T. rhodesiense</i>
" ... 8 ...	"	Negative	<i>T. vivax</i>
" ... 9 ...	"	<i>T. pecorum</i>	<i>T. vivax</i> and <i>T. pecorum</i>
" ... 10 ...	"	Negative	<i>T. vivax</i>
" ... 11 ...	"	No inoculation	"
" ... 12 ...	"	"	"
Eland ... 1 ...	Negative	<i>T. pecorum</i>	<i>T. pecorum</i>
" ... 2 ...	"	"	"
" ... 3 ...	"	"	"
" ... 4 ...	"	"	"
Roan ... 1 ...	"	"	"
Puku ... 1 ...	<i>T. vivax</i>	Negative	<i>T. vivax</i>
Sitatunga ... 1 ...	<i>T. ingens</i> (?)	No inoculation	<i>T. ingens</i> (?)
Duiker ... 1 ...	<i>T. vivax</i>	Negative	<i>T. vivax</i>
" ... 2 ...	<i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>

EXAMINATION OF DOMESTIC STOCK

The domestic animals examined, and the species of trypanosomes found in them, are given in Tables 7 and 8.

TABLE 7.—Examination of domestic stock for trypanosomes at Nawalia.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Cow	<i>T. pecorum</i> or <i>T. nanum</i>	No inoculation	<i>T. pecorum</i> or <i>T. nanum</i>
"	<i>T. pecorum</i> or <i>T. nanum</i>	"	<i>T. pecorum</i> or <i>T. nanum</i>
Goat	<i>T. vivax</i>	Negative	<i>T. vivax</i>
"	<i>T. vivax</i> and <i>T. nanum</i> or <i>T. pecorum</i>	"	<i>T. vivax</i> and <i>T. nanum</i>
"	<i>T. pecorum</i> or <i>T. nanum</i>	"	<i>T. nanum</i>
"	<i>T. vivax</i>	"	<i>T. vivax</i>
Dog	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>	<i>T. rhodesiense</i>
"	<i>T. pecorum</i>	<i>T. pecorum</i>	<i>T. pecorum</i>
"	"	No inoculation	"
"	"	"	"
"	<i>T. sp. (montgomeryi ?)</i>	Negative	<i>T. sp. (montgomeryi ?)</i>

TABLE 8.—Examination of domestic stock for trypanosomes at Ngoa.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Goat	<i>T. nanum</i> or <i>T. pecorum</i>	No inoculation	<i>T. nanum</i> or <i>T. pecorum</i>
"	<i>T. vivax</i> and <i>T. nanum</i> or <i>T. pecorum</i>	"	<i>T. vivax</i> and <i>T. nanum</i> or <i>T. pecorum</i>
"	<i>T. vivax</i> and <i>T. nanum</i> or <i>T. pecorum</i>	<i>T. pecorum</i>	<i>T. vivax</i> and <i>T. pecorum</i>

The only native village in which cattle were found was Kambwiri's, some 40 miles south-west of Nawalia. At present there are only two head, all that are left of a big herd which existed there some four or five years ago. One of the two appeared to be in good condition when seen, but the other beast was obviously ill. The cow in which trypanosomes were found at Fort Jameson was bred on the Government Farm, and had never been beyond the limits of the township. Tsetse flies have never been seen within some miles of the place, but *Stomoxys* is abundant in the kraals, and at certain seasons of the year various species of *Tabanidae* are common.

In several of the villages on the main road from Nawalia to Fort Jameson a number of goats were found at the end of August, 1911, and again at the beginning of April, 1912, but at the end of that month not a single animal was alive. *Glossina morsitans* was found around all these villages. The four goats mentioned in Table 7 were under observation at Nawalia for a considerable length of time. During this period, parasites were found in the peripheral blood only at rare intervals. Two were rather thin, but not markedly so, and, apart from this, there were no signs of disease. Goat No. 258 was examined at frequent intervals for two months before parasites were first found, while in the others, trypanosomes were seen on the first occasion. Nos. 39 and 258, after having been under observation for nine and four months respectively, died on the road when the Commission left Nawalia, most probably from being over-driven. The other two are still alive, seven and four months after the diagnosis was made.

The dog in which *T. rhodesiense* was found came from a village just on the Nyasaland border. The natives said that it had been out of the village for over a year previously. As the disease runs an extremely acute course in these animals, there can be no doubt that the dog was infected locally.

EXAMINATION OF SMALL VERMIN

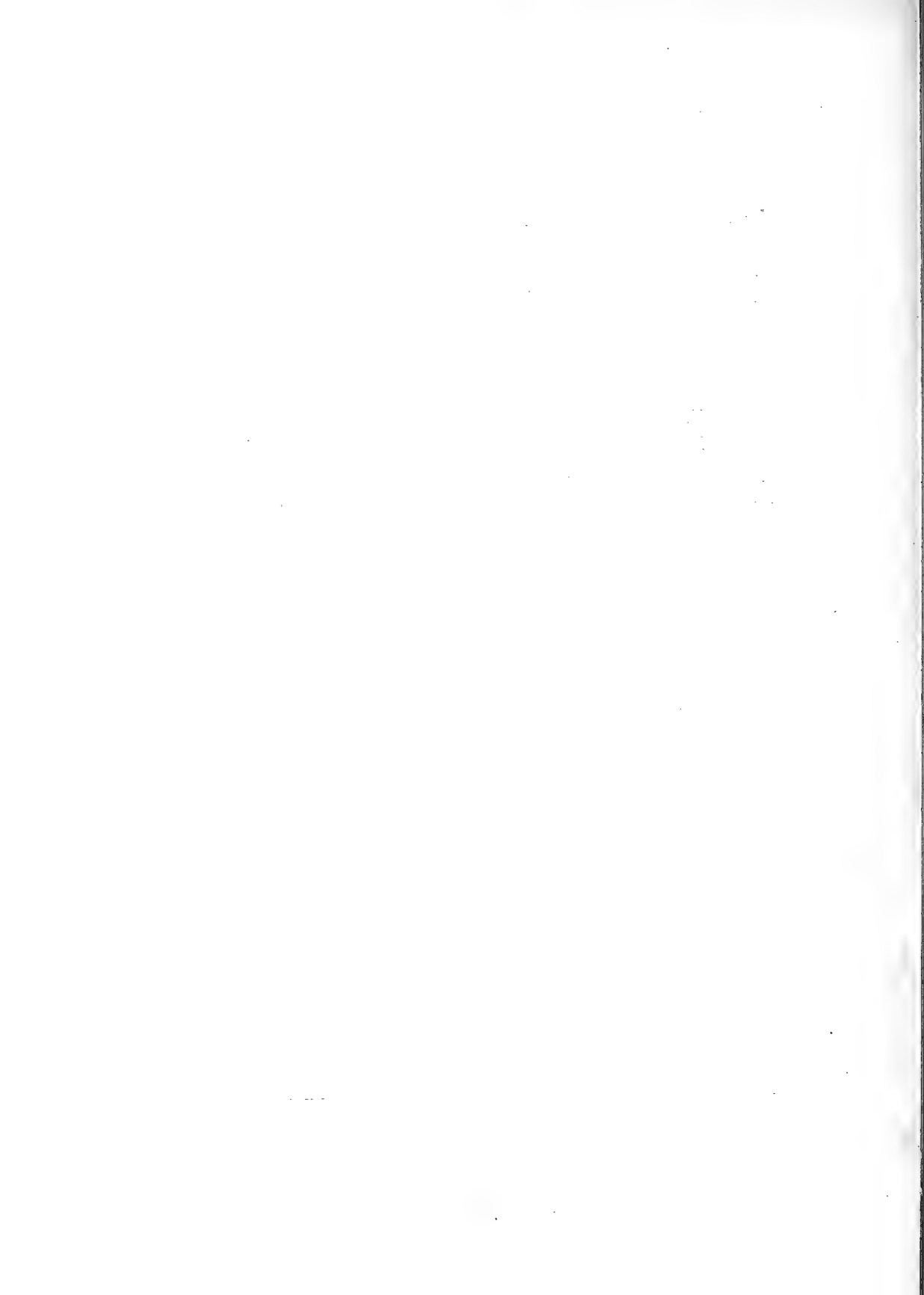
It has been suggested* that the small vermin might also act as reservoirs of trypanosomiasis. It must be remembered, however, that many of the small vermin of Tropical Africa are nocturnal, and are, therefore, not subjected to the bites of *Gl. morsitans*. At Nawalia and at Ngoa we examined in all 142 wild rats, 15 wild mice, 1 wild rabbit, 1 giant rat, 1 squirrel, 1 galago and 2 genet; the results were uniformly negative.

Not a single case of infection with trypanosomes was found in the 256 monkeys (*Cercopithecus pygerythus*) examined by us, although infection with filaria and plasmodium kochi was common.

The probable explanation of this is that the monkeys during the daytime catch the tsetse fly before the insects have time to feed on them, whereas, on the other hand, they are frequently bitten by mosquitos whilst they are asleep at night.

NGOA, NORTH RHODESIA,
August 31st, 1912.

* British Medical Journal, 1912, July 6. Report of Mr. Harcourt's speech in the House of Commons.



ON THE DEVELOPMENT OF
TRYPANOSOMA RHODESIENSE
 IN *GLOSSINA MORSITANS*

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 Commission of the British South Africa Company*)

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In the course of our investigations, we endeavoured to accumulate information regarding the development of the human trypanosome in *Glossina morsitans*. Reference has already been made to this subject in a previous report,* and it is here intended to correlate the facts at our disposal. It may be remarked at once that owing to the comparatively small number of laboratory-bred *Gl. morsitans* available, the information we have collected is by no means so definite as could have been desired.

Up to the present, comparatively little work has been done on this subject, and the records are more or less contradictory. Kleine,† who was the first investigator to write on the development of *T. gambiense* in *Gl. palpalis*, is of the opinion that the complete cycle takes place in, and is limited to, the intestine, whereas the Royal Society Commissioners‡ in Uganda consider that involvement of the salivary glands is essential. They state that without invasion of the salivary glands there is no infectivity of the fly.

* Kinghorn and Yorke. *Annals of Tropical Medicine and Parasitology*, 1912, Vol. VI.

† Kleine. *Trypanosomenstudien*. Arb. aus d. Kaiserl. Gesundheitsamte, Bd. XXXI, Heft 2.

‡ Bruce, etc. *Reports of the Roy. Soc. Commission in Uganda*, 1911.

TECHNIQUE

The method of dissection of the flies used by us was that described by one of us in a previous paper.* Briefly, it consists in splitting the dorsum of the thorax longitudinally, and, after separating the muscles and loosening the tissues with needles, drawing out the salivary glands attached to the pharynx through the waist. This method has obvious advantages over that described by the Royal Society Commission, in which after snipping off the terminal segment of the abdomen, the whole contents were expressed on to a glass slide and the salivary glands subsequently separated from the mass of intestines and other structures. We claim for the technique adopted by us that the process is quicker, more certain, and that the danger of contamination from the intestines is reduced to a minimum. In fact the only lesion in the alimentary canal accompanying the operation occurs in the anterior portion of the oesophagus.

To a certain point the information obtained from our dissections is exceedingly definite. We found that in every fly capable of infecting animals with the human trypanosome (*T. rhodesiense*) the salivary glands were invaded. Of the 160 laboratory-bred *Glossina morsitans* utilised in various experiments to transmit *T. rhodesiense*, 132 were dissected as they died. The remaining 28 were too dry when discovered to allow of dissection. Twenty-seven of those dissected were found to be infected with trypanosomes. The day of the experiment on which the flies died and the results of dissection are given in Table 1.

A glance at the table shows that, of these 132 flies, 5 became capable of infecting animals with the human trypanosome. In each of these there was an enormous invasion of the salivary glands by trypanosomes. In the 127 flies which remained incapable of transmitting the parasite, the salivary glands were not involved, although trypanosomes were found in the intestine of 22.

A precisely comparable state of affairs was observed in dissection of 'wild' *Gl. morsitans* which had become infective after feeding on infected animals. The salivary glands were found to

* Lloyd, Ll. Bull. Entoml. Research, 1912.

TABLE I.—Results of Dissection of Laboratory-bred *Glossina morsitans* which were found to contain Parasites after being fed on Infected Animals.

No.	Date of infecting feed	Day of dissection after infecting feed	RESULTS OF DISSECTION			Remarks
			Proboscis	Intestine	Salivary Gland	
1	9.9.11	4th	o	+++	o	
2	10.8.12	4th	o	+	o	
3	22.1.12	5th	o	+	o	
4	9.8.12	5th	o	++	o	
5	10.8.12	5th	o	+++	o	
6	23.6.12	5th	o	+	o	
7	8.8.12	6th	o	+++	o	
8	9.8.12	6th	o	+++	o	
9	8.8.12	7th	o	+++	o	
10	23.6.12	7th	o	+	o	
11	10.8.12	8th	o	+-	o	
12	8.8.12	9th	o	+	o	
13	10.8.12	10th	o	+++	o	
14	8.8.12	11th	o	+++	o	
15	25.8.11	12th	o	+++	o	
16	31.8.11	12th	o	+++	o	
17	8.8.12	13th	o	+++	o	
18	5.3.12	15th	+	+++	o	
19	11.8.12	19th	++	+++	+++	Infective on 12th day
20	10.8.12	20th	+	+++	o	
21	9.8.12	21st	2 tryps. seen	+++	o	
22	9.8.12	21st	o	+++	o	
23	8.8.12	22nd	1 tryp. seen	+++	o	
24	8.8.12	22nd	+	+++	+++	Infective on 17th to 21st day
25	14.11.11	28th	o	+++	+++	Infective on 15th day
26	22.1.12	29th	o	+++	+++	Infective on 19th day
27	9.9.11	40th	o	+++	+++	Infective on 13th day

N.B. o = Negative, + = Scanty, ++ = Considerable numbers, +++ = Swarming.

TABLE 2.—Results of dissection of Wild *Gl. morsitans* found to be capable of infecting animals with *T. rhodesiense*.

No.	Date of infecting feed	Day of dissection after infecting feed	RESULT OF DISSECTION			Remarks
			Proboscis	Intestine	Salivary Glands	
1	21.11.11	25th	o	+++	+++	Infective on 11th day
2	21.11.11	25th	o	+++	+++	Infective on 11th day
3	1.7.12	28th	o	+++	+++	Infective on 13th day
4	21.11.11	30th	+	+++	+++	Not proved to be infective, as the monkeys died prematurely
5	21.11.11	30th	+	+++	+++	
6	21.11.11	30th	o	+++	+++	
7	21.11.11	30th	+	+++	+++	
8	14.2.12	39th	o	+++	+++	Infective on 25th day
9	4.10.11	40th	o	+++	+++	Infective in nature
10	30.6.12	42nd	o	+++	+++	Infective on 14th day
11	30.6.12	47th	o	+++	+++	Infective on 14th day
12	13.6.12	58th	o	+++	+++	Infective on 48th day after infecting feed, or 8 days after having been placed in the incubator
13	13.6.12	58th	o	+++	+++	
14	13.6.12	59th	o	+++	+++	Not proved to be infective, but inoculation of trypanosomes from gut and salivary glands was followed by positive results
15	14.6.12	71st	o	+++	+++	

be infected only in those insects which were capable of transmitting the human trypanosome. In all, 906 'wild' *Gl. morsitans* were used in these experiments, and of this number 620 were dissected. The remainder were for various reasons too dry to admit of dissection. Of these 620 flies the salivary glands of 14 were invaded by trypanosomes. All except 4 of these were definitely proved to transmit the human trypanosome. In the case of the other 4, the animals upon which the flies had been allowed to feed died before a diagnosis could be made. None of the 607 flies in which the salivary glands were not involved were able to infect animals with the human trypanosome. Again, the infectivity of

Gl. morsitans in nature was examined, as mentioned in a previous paper, by feeding batches of freshly-caught flies on healthy monkeys. Certain of the groups infected monkeys, and from one of these infective groups the actual infective fly was isolated. This, on dissection, was found to have the salivary glands swarming with trypanosomes. The remaining 242 flies in this group, which had been shown to be non-infective when fed on monkeys, were dissected, and in no instance was an infection of the salivary glands observed.

DISSECTION OF INFECTIVE 'WILD' FLIES

In all, 20 *Gl. morsitans* were found to have invasion of the salivary glands by trypanosomes, and of these 16 were definitely found to be capable of infecting animals with *T. rhodesiense*. Owing to unavoidable circumstances, we were unable to prove the point in the case of the remaining 4, but there is no reason to doubt that, had the animals on which these flies were fed survived beyond the necessary five or six days, they would have proved to be infected.

In order to anticipate the criticism that the trypanosomes were not really inside the salivary glands, but simply lying outside these structures, and due to contamination from the gut, our examinations were conducted with extreme care. In the first place, the glands were removed uninjured and attached to the pharynx, placed on a microscopic slide, and gently covered with a coverslip. By careful focussing it could easily be decided that the parasites were actually in the lumen of the tubes, and not outside. Moreover, they were usually present in such enormous numbers as absolutely to exclude the possibility that they were the result of contamination from the intestine. Again, the glands of other flies were removed with care and immediately fixed, and subsequently imbedded and cut. In the sections the parasites could be seen to be inside the glands. Finally, in order to remove any possibility of doubt, sections of the whole abdomen of these infective flies were made, and the glands found to be loaded with trypanosomes.

It will be seen from Table 1 that it was by no means a rare occurrence for trypanosomes to be present in the intestines in the earlier stages, especially in the case of those flies examined within

a few days of the infected meal. As a general rule, however, most of the insects dissected after the first five or six days were negative. In a certain proportion multiplication of the parasites took place in the intestine.

As to the reason for this multiplication in the gut of occasional flies only, and as to the manner in which it occurs, we have obtained but little information. On one occasion a fly, which died on the twelfth day after having been fed on a guinea-pig infected with *T. rhodesiense*, was found to have an enormous gut infection. Possibly there were also a very few trypanosomes in the salivary glands, but on this point we could not be absolutely certain, as the insect had been dead for some time before the dissection was made. In the mid-gut were found a number of cysts containing swarms of trypanosomes. Some of the cysts had thin walls and were filled with a seething mass of flagellates, while others had thicker walls and the contents were quiescent. The cysts ranged in diameter from 27 to 32 μ . Unfortunately, we are unable to state whether the fly was infective at the time of death. It had refused to feed for two or three days previously, and the animal on which it had last fed (ninth day of the experiment) did not become infected. The gut contents were inoculated into a monkey, but the animal died from some unknown cause a couple of days later.

Although multiplication of the parasites occurred in the guts of a proportion of the flies, we met no instance in which a fly was infective and in which inoculation of the gut parasites into experimental animals gave rise to infection unless there was an accompanying invasion of the salivary glands. On the other hand, it appears that on every occasion on which the salivary glands are involved, the trypanosomes, both in these structures and also in the intestines, are virulent, i.e., the fly infects when on a healthy animal, and inoculation of the parasites from either the salivary glands or the intestine gives rise to infection.

The results of inoculation of trypanosomes from laboratory-bred flies in different stages of infection, and also from the wild flies which were proved to be infective, are given in Table 3.

The manner in which the salivary glands become infected is uncertain, but there is a certain amount of evidence which would cause one to believe that it is secondary to the intestinal infection,

TABLE 3.—Result of inoculation from laboratory-bred *Gl. moritans* which had been fed on infected animals.

No.	Result of feeding on normal animal the day before the fly was dissected	Day on which dissected after the infecting feed	RESULT OF DISSECTION			Result of inoculation of trypanosomes from different portions of the fly into clean monkeys or rats
			Proboscis	Intestine	Salivary gland	
1	Negative	4th	o	+++	o	Gut contents; monkey not infected.
2	"	5th	o	+	o	" "
3	"	8th	o	+++	o	" "
4	"	9th	o	+	o	" "
5	"	10th	o	+++	o	" "
6	"	11th	o	+++	o	" "
7	"	12th	o	+++	o	" "
8	"	13th	o	+++	o	" "
9	Positive from 12th day onwards	19th	++	+++	+++	{ Proboscis contents; monkey infected { Gut contents; rat infected { Salivary glands contents; monkey infected { Gut contents; monkey not infected
10	Negative	21st	2 tryps. seen	+++	o	" "
11	"	21st	o	+++	o	" "
12	"	22nd	1 tryp. seen	+++	o	" "
13	Positive from 17th—21st day onwards	22nd	+	+++	+++	{ Gut contents; monkey infected { Salivary glands contents = monkey infected { Gut contents; monkey infected { Salivary glands used for embedding
14	Positive from 15th day onwards	28th	o	+++	+++	{ Gut contents; monkey infected { Salivary glands contents; monkey infected { Gut contents; monkey infected
15	Positive from 19th day onwards	29th	o	+++	+++	{ Salivary gland contents; monkey infected { Hind gut contents; rat infected { Fore gut contents; monkey infected { Salivary gland contents; rat infected
16	Positive from 13th day onwards	29th	o	+++	+++	{ Gut contents; rat infected { Salivary gland (right) contents; monkey infected { Salivary gland (left) contents; monkey infected { Gut contents; rat infected
17	Positive from 8th day after putting in incubator and 48th day after first infective feed onwards	58th	o	+++	+++	{ Gut contents; rat infected { Salivary gland (right) contents; monkey infected { Salivary gland (left) contents; monkey infected { Gut contents; rat infected
18	(?) *	71st	o	+++	+++	{ Gut contents; rat infected { Salivary gland contents; rat infected

* Owing to the unfortunate death of the monkey on which this fly was fed, we were unable to ascertain whether the insect was infective or not. The fly was one of the series which was kept for 60 days after the infective feed at laboratory temperature, and on the 61st day placed in the incubator. In view of the fact that the parasites both in the salivary glands and in the intestine were infective to sub-inoculated rats, it is highly probable that had the animal, on which the fly was fed, lived long enough, it would have been found to be infected.

and that it only occurs when the trypanosomes in the gut have reached a certain stage of development, and only then when the conditions of temperature are suitable for the further development of the parasites. In the first place, of 752 flies dissected at various intervals after having fed on infected animals, we never found trypanosomes in the salivary glands in the earlier stages before the flies were infective. Again, whenever trypanosomes were found in the salivary glands there were also enormous numbers present in the intestine. Moreover, it is significant that whenever trypanosomes were found in the salivary glands they were always infective, as were also those present in the gut.

Attention has already been drawn in a previous report to experiments which suggest that although the parasites can multiply and develop up to a certain stage in the intestine at comparatively low temperatures (55°—65° F.), yet the flies do not become infective until the temperature to which they are subjected is raised to at least 75°—80° F. In none of our experiments were trypanosomes found in the salivary glands of flies which had not been subjected to the higher temperatures. Probably the salivary glands become invaded by parasites which have reached a certain stage in their developmental cycle in the intestine. The remarkably short period (eight days or less) in which three flies, which had been kept forty days after the infecting feed at laboratory temperature, became infective after being placed in the incubator at 85° F. can be best explained on the assumption that some portion of the cycle must have occurred in the gut during the first forty days at laboratory temperature.

In conclusion, we might remark that invasion of the salivary glands was only observed in the case of flies infected with the human trypanosome (*T. rhodesiense*), and not in the case of any of the other trypanosomes with which we had to deal in either the Luangwa Valley or on the Congo-Zambesi watershed. This was the case both with the strain of *T. rhodesiense* derived from man, and also with that found in 'wild' *Gl. morsitans* which had been infected in nature.

It will be observed that trypanosomes were found occasionally in the proboscis of both infective and non-infective flies. We do not believe, however, that the presence of the parasites in this

structure has any special significance, but rather that it is fortuitous, depending on the passage of the infected salivary secretion, or due to regurgitation from the gut resulting from manipulation during dissection.

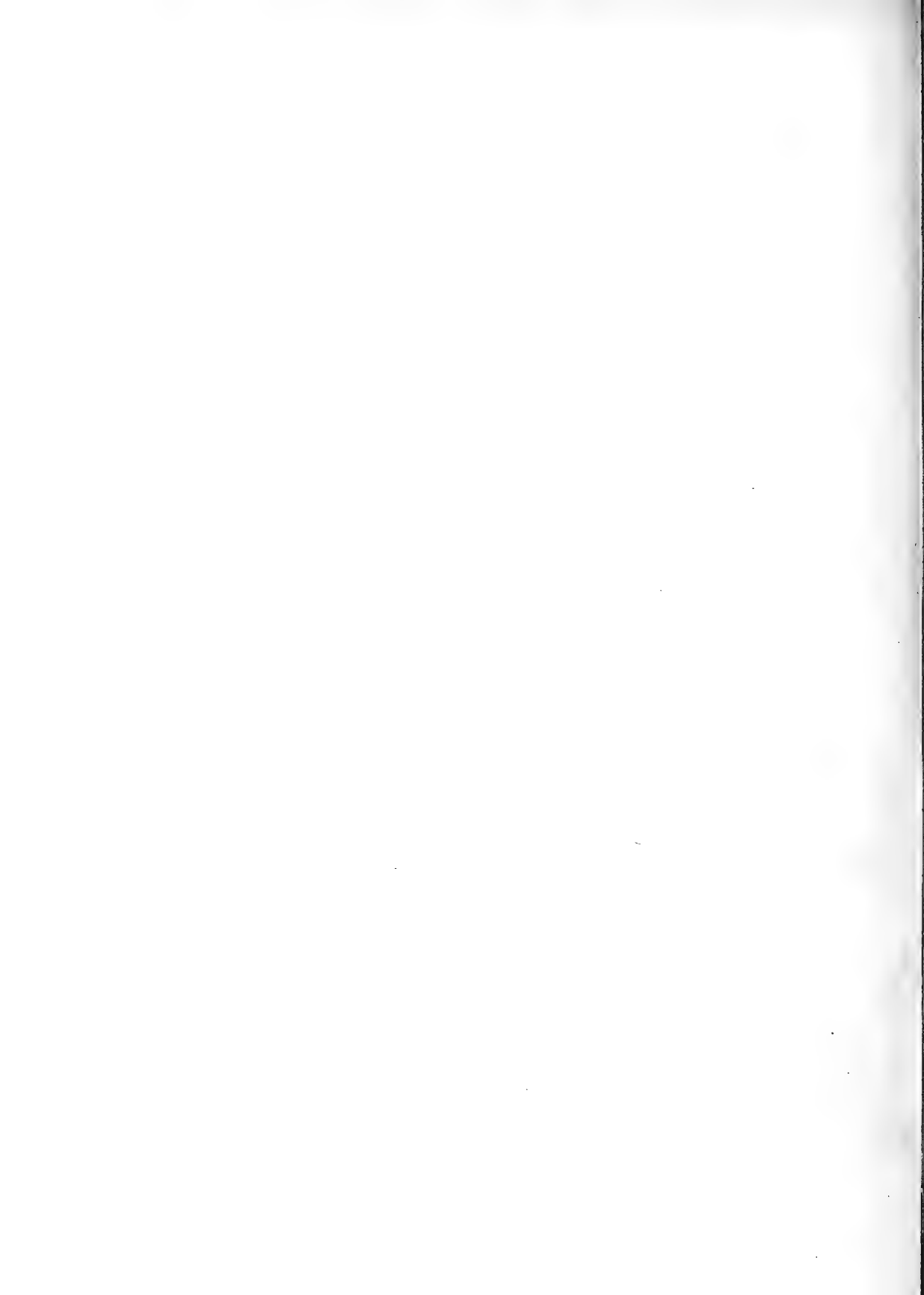
It is of interest to note that of 310 'wild' *Gl. morsitans* which were dissected as they were brought into the laboratory, recognizable mammalian red corpuscles were found in the intestine of 70, whilst nucleated red corpuscles were only found on 4 occasions.

MORPHOLOGY OF THE TRYPANOSOME IN *GLOSSINA MORSITANS*

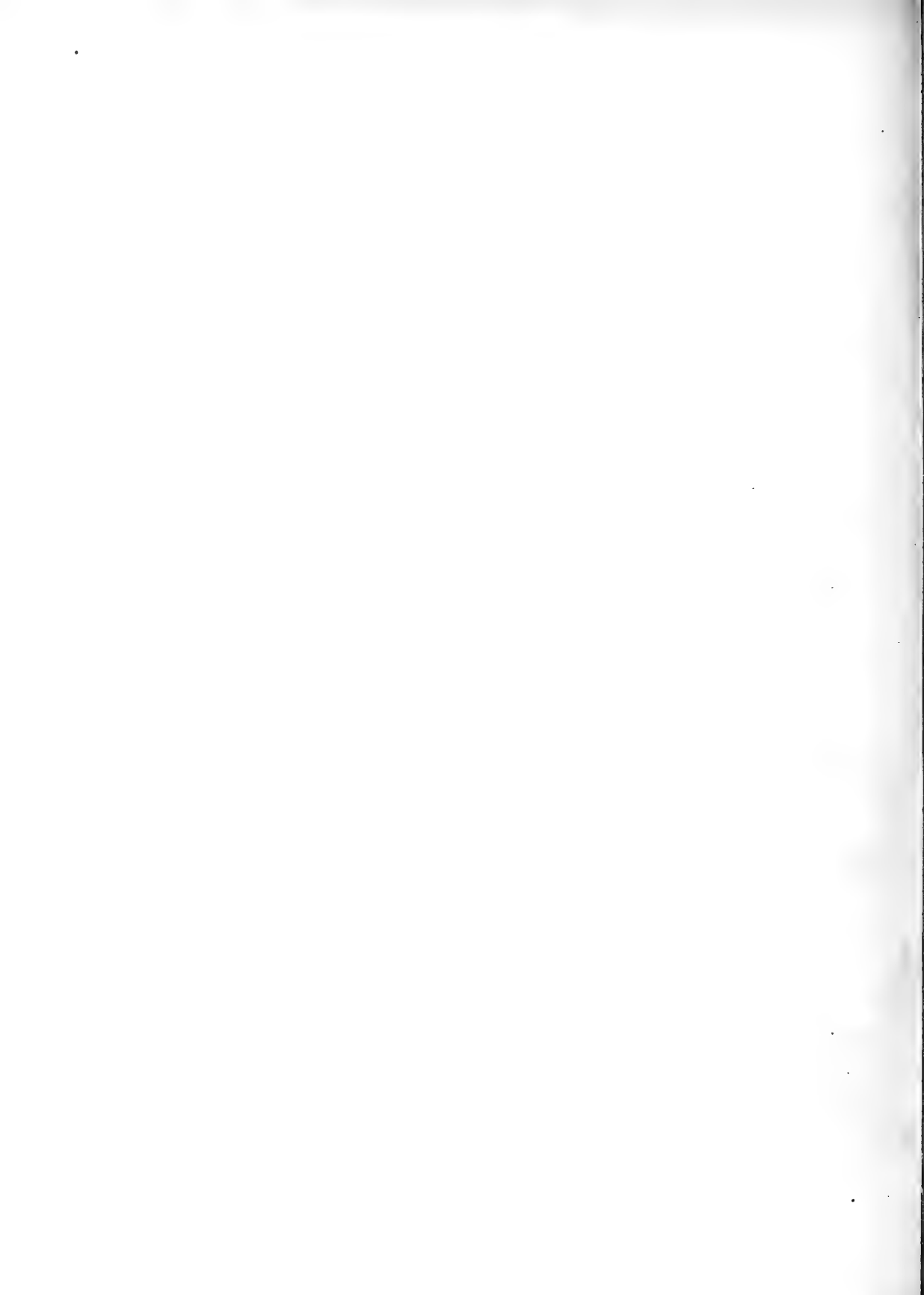
A description of the parasite as it appears in different portions of the tsetse fly must be left for a further communication. It may be stated, however, that the predominant type of the parasite in the salivary glands is quite different from that found in the various portions of the intestine. The form occurring in the salivary glands approximates rather closely to the short variety of the trypanosome in the mammalian blood; nevertheless it is not identical with this. The predominant type in the intestine is a large broad flagellate; the undulating membrane is feebly developed, and there is little, if any, free flagellum. The nucleus is usually fairly central in position, but not infrequently it lies in a more posterior position.

NGOA, NORTH RHODESIA,

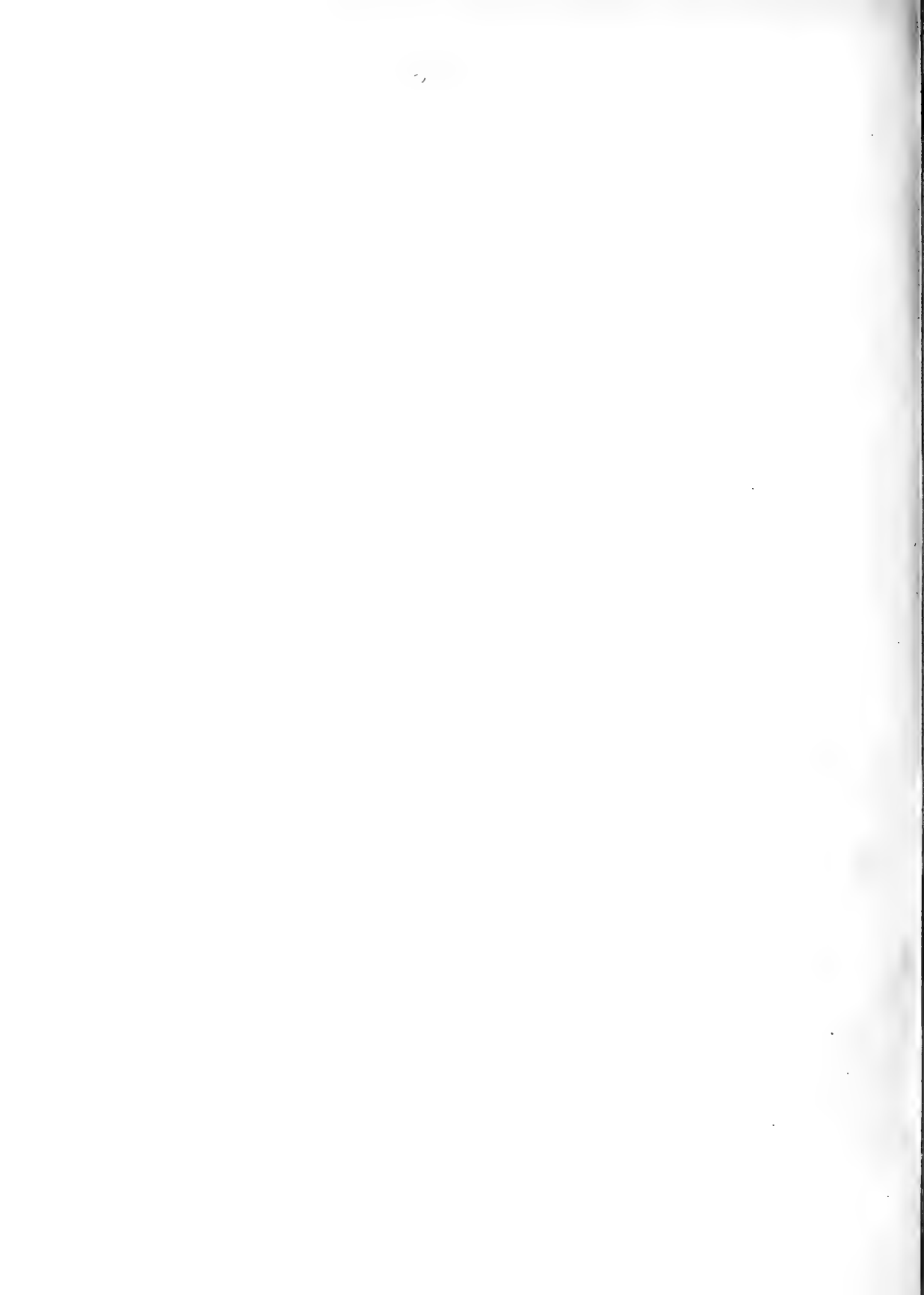
August 31st, 1912.







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