Collected papers on trypanosomiasis / by Sir David Bruce and others.

COLLECTED PAPERS

ON

TRYPANOSOMIASIS.

BY

SIR DAVID BRUCE,

AND OTHERS.

1909-1911.

CONTENTS.

CONTENTS (continued).

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The Development of Trypanosoma gambiense *in* Glossina palpalis.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908.)

(Received July 5, 1909.)

[PLATES 10 AND 11.]

The following experiment is so complete in itself that no apology is offered for publishing it by itself. In 1903 the Sleeping Sickness Commission of the Royal Society came to the conclusion that the carrying of infection from a sleeping sickness patient to a healthy person by the *Glossina palpalis* was a mechanical act, and required no previous development of the parasite within the fly. The Commission also held that the power of transferring the disease was lost to the fly 48 hours after it had fed on an infected person.

Koch and Stuhlmann, in German East Africa, described developing forms in *Glossina*, but did not succeed in infecting healthy animals by the injection of these forms.

Kleine, in German East Africa, at the end of 1908, succeeded first in showing that *Glossina palpalis* could convey *Trypanosoma brucei* some 50 days after the fly had fed on an infected animal.

It seems, at first, strange that this fact should have escaped notice for 15 years, and can only be accounted for by assuming that it is an event of the rarest for a fly to be found which fulfils the unknown conditions necessary for the development of the trypanosomes in its interior. If we assume that it is only one fly in a hundred or in a thousand in which this

development takes place, then the difficulty of observing the phenomenon can be understood.

Take the following experiments, for example:—

Table I.—Flies caught in an Infected Area, kept for some days, and then fed on Healthy Animals.

Trypanosoma brucei—Glossina morsitans.

Expt.	Place.	Observer.	No. of flies fed.	No. of times flies fed.	No. of days before infection or under observation.	Result.
210	Zululand	Bruce	5	32	64	Negative.
242	,,	,,	30	11	56	,,
232A	,,	,,	50	15	34	,,

These experiments seemed to show that if flies caught in a highly infected district, into which a horse could not be taken even for a few hours without contracting nagana, are kept without food for a few days—say three to five—they are then incapable of conveying infection. This appeared to be a strong proof that the duration of infectivity in the fly was a short one, since, if this were not the case, 1 of the 85 flies ought to have been in a condition capable of infecting, having, of course, been infected at some previous date in the "fly country." It may be repeated, that these flies were caught in a most highly infected district, so that if *Glossina morsitans* can remain infective for 50 or 60 days, 1 at least of the 85 ought to have been in the condition which made it capable of conveying the disease.

This development of the trypanosomes in the fly is strikingly like what occurs in the test-tube with Novy's medium. A thousand tubes are inoculated with *Trypanosoma brucei*: the trypanosomes all appear to die off, but 20 days afterwards a peculiarly resistant individual is found in one tube of the thousand, who has adapted himself to the new enviroment, and soon multiplies into myriads. What it is which enables this particular individual to adapt itself to such altered conditions is unknown. It is the merest speculation to call it a sexual act and pick stout forms as females and slender forms as males.

Again, because this late development of the trypanosomes enables a particular fly to remain infective for 100 days, or even possibly for the remainder of its life, it by no means follows that this is the usual method of infection. The mechanical transference of the disease is proved up to the hilt, and for every case which falls a victim to the rare late-infected fly, a thousand must be infected by direct mechanical transference.

Summary of the Experiment which forms the Subject of this Paper.

Before describing at length the experiment which forms the subject of this paper, we may summarise it as follows:—

1. On March 5, 1909, 60 *Glossinæ palpalis* caught on the lake shore were placed in two cages, 30 in each. The flies were fed on two infected monkeys for 2 days. They were then starved for 72 hours to get rid of mechanical transference. The following 5 days they were placed on a healthy monkey, and every successive period of 5 days, or thereabouts, on a fresh monkey, up to 86 days, when the experiment came to an end. The result was, that the first two monkeys remained healthy, but that all the following monkeys, up to 75 days, became infected with *Trypanosoma gambiense*.

2. If 7 days be deducted for the incubation period, then the flies first became infected 18 days after their first feed on an infected animal.

3. There is some evidence that among the 60 flies only 1 was infective. Fifty-four days after the beginning of the experiment each cage was placed on a separate monkey. Up to that time both the cages of flies had been fed on the same animal. Cage A contained, after 54 days, 11 flies. Cage B, 4 flies. Cage A continued to infect monkeys for 21 days more, making a total of 75 days. Cage B did not infect. Again, as was natural, the flies gradually died off during the experiment, and as each fly died it was carefully dissected and examined for trypanosomes. Not a single trypanosome of any kind whatever was seen in any dissected fly up to 75 days, when a fly died in Cage A which was found to be swarming with trypanosomes similar to *Trypanosoma gambiense*. After the death of this fly, Cage A ceased to be infective, and when the experiment was stopped the remaining flies were killed off and dissected, but among them not a sign of a trypanosome could be seen. In the same way the flies remaining in the non-infective Cage B were examined, with a similar negative result.

4. Here follows an interesting and unique observation. A tiny drop of fluid taken from the gut of the 75-day fly injected under the skin of a monkey gave rise to Sleeping Sickness after an incubation period of eight days. This, so far as we are aware, is the first time this has been recorded.

5. It will be seen from the detailed experiment that the flies were starved for three days between several of the experiments. This, of course, was to get rid of the fallacy of mechanical transference.

6. It may be said that perhaps these monkeys became infected by some other means than the flies in the cage—for example, by other biting flies, or by contact. To this it may be answered that there are more than 200

monkeys under observation here, sick and healthy. They are all examined twice a week, but during the last eight months not a single case of accidental infection has taken place.

Details of the Experiment.

Experiment 663.

To ascertain if development of *Trypanosoma gambiense* takes place in the interior of *Glossina palpalis*, and if so, how long does the fly remain infective.

March 5, 1909.—Two batches of *Glossina palpalis* caught on the Lake shore, consisting of 30 flies in each batch, were fed on monkeys, Experiments 568 and 214, whose blood contained numbers of *Trypanosoma gambiense*.

March 6.—The flies again fed as on the 5th, to ensure that as many as possible should get a feed of the infected blood. Nearly all the flies fed on one or other occasion. The flies are kept in a moist atmosphere at 22° C.

The following table gives the principal details of the experiment:—

Table II.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Mar. 5	—	Flies fed on infected monkey			
6	1	„ „ „			
7	2	Flies starved 72 hours			
8	3	„ „			
9	4				
10	5				
11	6	Fed on Monkey 579		−	
12	7				
13	8				
14	9				
15	10				
16	11	„ „ 651		−	
17	12				
18	13				
19	14				
20	15				
21	16	„ „ 652	+		
22	17				
23	18				
24	19				
25	20				
26	21	„ „ 653	+		
27	22				
28	23				
29	24				
30	25				
31	26	„ „ 654	+		
Apr. 1	27				
2	28				

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Apr. 3	29				
4	30				
5	31	Fed on Monkey 655	+		
6	32				
7	33				
8	34				
9	35				
10	36	„ „ 672	+		
11	37				
12	38				
13	39				
14	40	„ „ 722	+		
15	41				
16	42				
17	43	Starved for 72 hours			
18	44				
19	45				
20	46				
21	47				
22	48	Fed on Monkey 727	+		
23	49				
24	50				
25	51				
26	52				
27	53	Starved for 76 hours			
28	54	Cage A fed on Monkey 735 ...	+		
29	55				
30	56				
May 1	57				
2	58	„ B „ „ 736 ...		−	
3	59				
4	60	Starved for 74 hours			
5	61	Cage A fed on Monkey 749 ...	+		
6	62				
7	63				
8	64				
9	65	„ B „ „ 748 ...		−	
10	66				
11	67	Starved for 72 hours			
12	68	Cage A fed on Monkey 765 ...	+		
13	69				May 13.—Flies remaining in Cage B killed and dissected.
14	70				
15	71				
16	72	„ B „ „ 764 ...		−	
17	73				
18	74	Starved for 72 hours			
19	75				May 19.—Fly 866 found dead in Cage A and dissected. Did not feed on Monkey 848.
20	76				
21	77	Cage A fed on Monkey 848...		−	
22	78				
23	79				
24	80				
25	81	Starved for 72 hours			
26	82				
27	83				
28	84	Cage A fed on Monkey 911 ...		−	Expts. 848 and 911 healthy on 7th June, 1909.
29	85				
30	86				
31	87	Experiment stopped.			Remaining flies killed and dissected.

Remarks on the Experiment.

Everyone will agree that this is a most interesting experiment. It is evident that a single infected fly did all the mischief, and by good luck this fly was detected. Captain A. E. Hamerton, D.S.O., had charge of the experiment at first, and on his leaving Mpumu about the beginning of May, it fell to Sergeant A. Gibbons, Royal Army Medical Corps. Both are to be congratulated on the results, which are the outcome of care and thoroughness. Captain F. P. Mackie had the good fortune to dissect the fly which did the injury, and which will be fully described later.

Incubation Period.

From the experiment may be drawn the incubation period in monkeys bitten by a late-infected fly.

It is remarkable how regular this is in those monkeys which gave a positive result. This shows how very infective Fly 866 was. Apparently each time it bit it infected.

The following table gives the period of incubation in each case :—

Table III.

Date.	Experiment.	Flies first fed.	Trypanosomes appeared in blood.	Number of days before trypanosomes appeared in blood.
1909.		1909.	1909.	
March 19	652	March 19	March 30	11
„ 24	653	„ 24	April 2	9
„ 29	654	„ 29	„ 6	8
April 3	655	April 3	„ 13	10
„ 8	672	„ 8	„ 15	7
„ 13	722	„ 13	„ 20	7
„ 18	727	„ 18	„ 24	6
„ 28	735	„ 28	May 5	7
May 5	749	May 5	„ 11	6
„ 12	765	„ 12	„ 17	5

Leaving out the first experiment, 652, as it is doubtful as to the exact day Fly 866 became infective, this gives an average incubation period of seven days. It would therefore appear that Fly 866 probably infected each animal on the first day it bit it, showing how dangerous such an infected fly is.

DESCRIPTION OF THE *Glossina palpalis*, FLY 866, WHICH WAS DISSECTED 75 DAYS AFTER HAVING FED ON A MONKEY WHOSE BLOOD CONTAINED *Trypanosoma gambiense*.

Experiment 866.

May 19, 1909.—Dissected a *Glossina palpalis*, which was found dead to-day in Cage A of Experiment 663. On removing the viscera by the usual method, the mid-gut was seen to be of a pale salmon-pink. A small quantity of its contents, examined in the fresh condition, was found to contain enormous numbers of trypanosomes. The tube of this part of the intestine was absolutely crammed with active, seething masses of these flagellates. In regard to the other parts of the fly, nothing was seen in the proboscis. In the proventriculus one trypanosome only was found. The salivary glands contained large numbers of altered-looking trypanosomes, the fore-gut many large stout forms, with bright granules. The crop was empty and showed nothing. The Malpighian tubules, hind-gut, and proctodæum also were drawn blank.

In addition to examining these organs in the fresh condition, smears were made and stained. The examination of these stained specimens gave the following results:—

The salivary glands.—These had been carefully removed before the intestine was opened, and therefore had no chance of being fouled. As will be seen from the coloured drawing (Plate 10, fig. 1), the trypanosomes found in these glands differed from those seen in the intestine. The bodies are very irregular in shape, and contain, besides a reddish-stained nucleus, dark deeply-stained coarse chromatin granules. The other cell contents remain unstained. Free chromatin granules and flagella are to be seen scattered over the field. Sometimes the bodies are definitely pear-shaped, with a flagellum coming from the narrow end, and rarely a more definite trypanosome shape can be seen; but never a true trypanosome.

[It is a matter of deep regret that an inoculation experiment was not made with an emulsion of part of the salivary glands.]

The fore-gut.—The fore-gut contained many trypanosomes. The cytoplasm stains a pale blue, and the nucleus a reddish-purple. The micronucleus is not distinctly seen in some of the trypanosomes, but when it is, it is always distinctly posterior to the nucleus. The protoplasm contains many coarse darkly-stained chromatin granules. The undulating membrane is less marked than in the normal blood trypanosome, and the flagellum, which usually springs from a micronucleus-like body, is less deeply stained (Plate 11, figs. 6—13).

The mid-gut.—The mid-gut contained innumerable trypanosomes of the *gambiense* type. Some are dividing, and all have a well-marked nucleus and micronucleus, the latter at or near the posterior extremity. The protoplasm contains many chromatin granules, and an undulating membrane and flagellum are present (Plate 10, figs. 6—16). Many groups, or rosettes, composed of 15 to 20 individuals, occur, the flagella pointing outwards (Plate 11, fig. 1).

The *proboscis, proventriculus, thoracic gut, crop, hind-gut,* and *Malpighian tubes* contained no trypanosomes.

The most interesting thing in this description of the examination of Fly 866 is the condition of the salivary glands. How these trypanosome-like bodies, or derivatives of trypanosomes, got into them is a mystery, and we will content ourselves at present with merely placing the bare fact on record until the salivary glands of similarly infected flies are examined.

There is one fallacy which might be pointed out. It is assumed that Fly 866 became infected on the first or second day of the experiment. It is possible that it became infected when feeding on the fifth day on an animal which showed trypanosomes in its blood a day or two later. This, however, is unlikely, as no other fly showed trypanosomes on dissection.

In order to make the story more complete, on Plate 10, figs. 1—5, is represented the *Trypanosoma gambiense* from the blood of one of the monkeys on which the flies were fed at the beginning of the experiment, and on Plate 11, figs. 2—5, are shown *Trypanosoma gambiense* from the monkey which became infected from the contents of the mid-gut of Fly 866.

Proportion of Infected Flies to Non-Infected in Nature.

In the experiment under consideration it is seen that, in artificially-infected flies, only 1 in 60 showed the phenomenon of late infectivity. In nature the proportion must be less, as many of the flies, in many places at least, can never have fed on an animal whose blood contained *Trypanosoma gambiense.*

That there can be but few under natural conditions Table IV shows. The table is made by subtracting the flies fed on the animal during the last seven days, before trypanosomes were found in the blood, this being the incubation period, from the total number. The experiments consist in catching tsetse flies in the infected area, bringing them to the laboratory and placing them straightway on healthy animals.

The first two experiments were made with *Trypanosoma brucei* and *Glossina morsitans,* and it would appear from them that 104 and 108 flies

Table IV.—Table to show Probable Number of Naturally infected Flies per thousand.

Expt.	Place.	Observer.	No. of flies fed before infection took place.	Result.		Probable No. of naturally infected flies per thousand.
				Positive.	Negative.	
		Trypanosoma brucei—Glossina morsitans.				
225	Zululand	Bruce	104	+		9·6
236	„	„	108	+		9·2
		Trypanosoma gambiense—Glossina palpalis.				
94	Uganda	Bruce and Nabarro	89	+		11·2
130	„	Bruce, Nabarro, and Greig	850	+		1·2
131	„	„ „	506	+		1·9
136	„	Nabarro and Greig	723		−	
228	„	Greig and Gray	866	+		1·2
301	„	„ „	2299		−	
45	Leopoldville	Dutton, Todd, and Hannington	457		−	
46	„	„ „	552		−	
128A	River	„ „	25		−	
139	„	„ „	262		−	
141	„	„ „	52		−	
182	Kasongo	„ „	211		−	
198	„	„ „	2659	+		0·4
203	„	„ „	1789		−	
213	„	„ „	717		−	
52	Uganda	Bruce, Hamerton, Bateman, and Mackie	41		−	
214	„	„ „	3284	+		0·3
568	„	„ „	178	+		5·6
571	„	„ „	850	+		1·2
53*	„	„ „	21		−	
612	„	„ „	615	+		1·6
674	„	„ „	2315	+		0·4

* Animal died.

were used respectively before an infective one was found. This perhaps explains why Bruce's 85 flies failed to infect.

In the experiments with *Trypanosoma gambiense* and *Glossina palpalis* the average is 2·5 per thousand. It is, of course, impossible to tell how many of these positive experiments were infected by mechanical transference or by a late-infective fly; but, in any case, the proportion is small. If this were not so, all the native population of the Lake shore, and most of the Europeans in Uganda, would long ago have been blotted out.

DESCRIPTION OF PLATES.

Plate 10.

Smear preparation of salivary glands of *Glossina palpalis*, Experiment 866, stained Giemsa, showing irregularly shaped trypanosomes, with unstained protoplasm, reddish-coloured nuclei, and deeply stained chromatin granules. Note the chromatin granules scattered singly about the field, each surrounded by a pale area, fig. 1. × 2000.

Normal *Trypanosoma gambiense* from monkey, Experiment 568, on which the flies were fed at the beginning of the experiment, figs. 2, 3, 4, and 5. × 2000.

Trypanosomes from the mid-gut of infected fly, Experiment 866, figs. 6—16. × 2000.

Plate 11.

Rosette form from the mid-gut, fig. 1. × 2000.

Trypanosoma gambiense from the blood of monkey, Experiment 868, into which a tiny drop of the contents of the mid-gut of Fly 866 had been injected, figs. 2—5. × 2000.

Trypanosomes from the fore-gut of Fly 866, stained Giemsa, figs. 6—13. × 2000.

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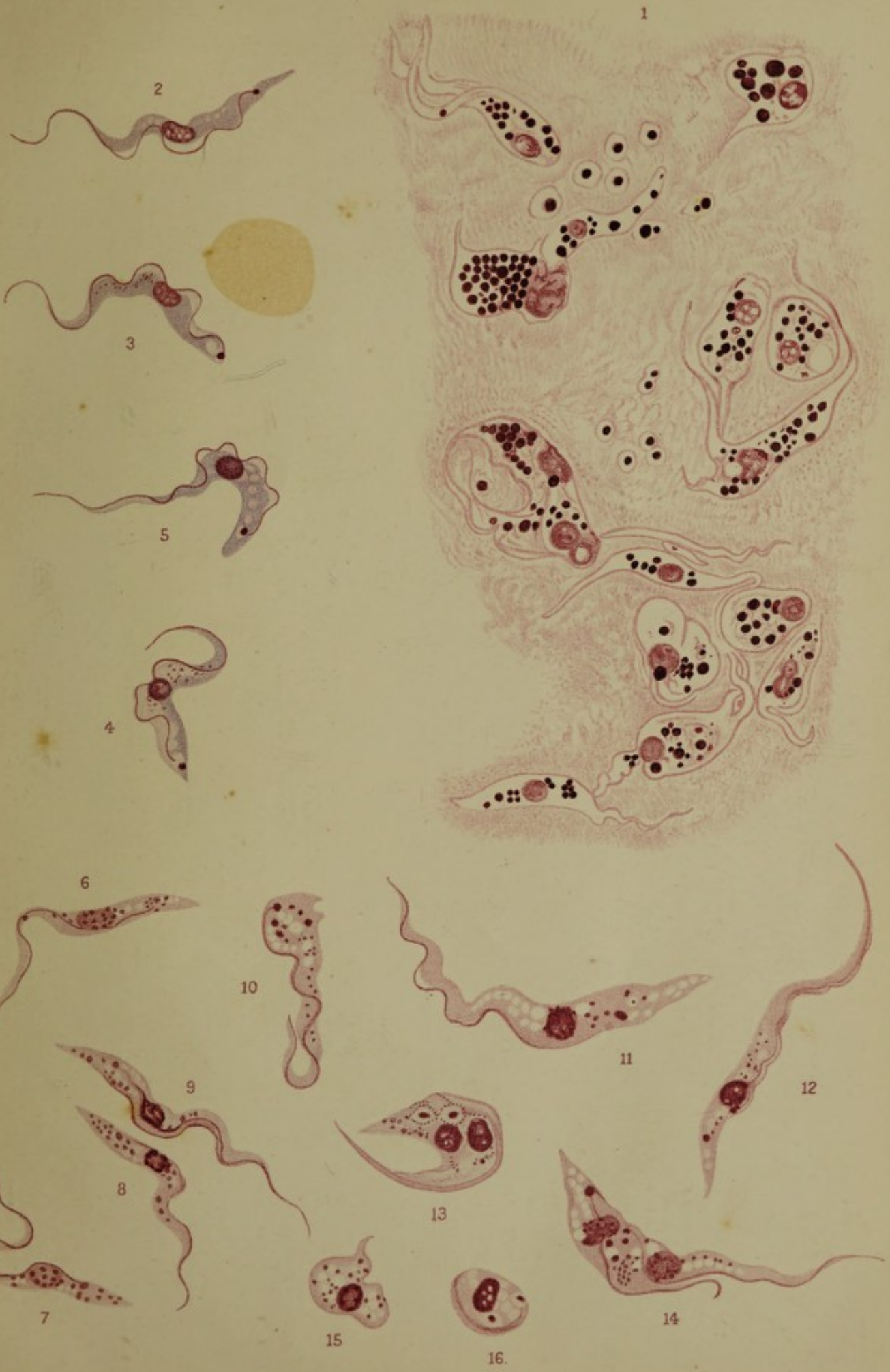

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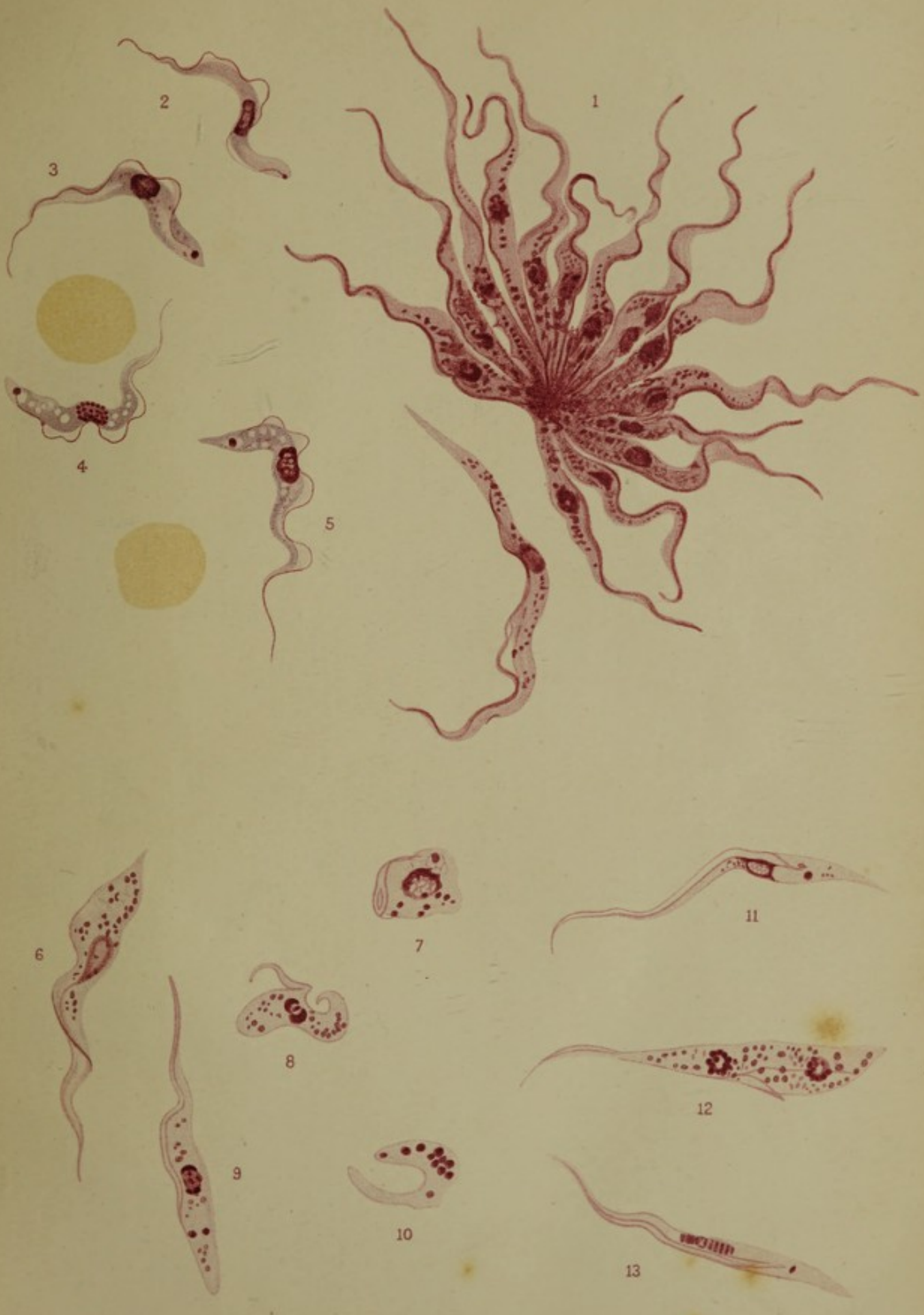

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The Development of Trypanosomes in Tsetse Flies.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service.
(Sleeping Sickness Commission of the Royal Society, 1908–10.)

(Received April 18,—Read May 5, 1910.)

In the 'Proceedings' of the Royal Society (B, vol. 81, 1909) a paper was published describing a single experiment illustrating the development of *Trypanosoma gambiense* in *Glossina palpalis*. This experiment was carried out at Mpumu, Uganda, near Lake Victoria, in the spring of 1909. Since that date many experiments, on the same lines, have been made, not only with *Trypanosoma gambiense* but also with *Trypanosoma dimorphon*, *Trypanosoma nanum*, and *Trypanosoma vivax*.* It is proposed to describe these further experiments in this paper.

It will be remembered that Kleine, in German East Africa, at the end of 1908, made the discovery that *Glossina palpalis* could convey *Trypanosoma brucei* for some 50 days after the fly had fed on an infected animal. Following Kleine's lead, our experiments were carried out, at first with Lake-shore flies, afterwards with flies bred in the laboratory.

A. *The Development of* Trypanosoma gambiense *in* Glossina palpalis *caught on the Lake-shore.*

These experiments were carried out with ordinary wild tsetse flies caught on the Lake-shore, and therefore open to the doubt that some of them may have been naturally infected when they were captured. As there is some evidence that one fly in 400 or 500 of the wild Lake-shore flies is found to be naturally infected, it is evident that these previously infected flies may lead into error. It will be seen later that this risk is not run when flies bred in the laboratory are used.

The flies when brought up from the Lake-shore were kept in small boxes, with mosquito-netting sides, and placed over dishes containing water, to imitate, as far as possible, their natural conditions. It may be remarked here that these tsetse flies are so numerous on the shores of Victoria Nyanza, and the supply so unending, that the fly-boys brought up some 500 every day, and these usually caught at only one or two spots.

* These names may require to be changed, when the trypanosomes affecting domestic animals in Uganda come to be described.

The method of carrying out these experiments was always the same. The flies were fed for some days on a highly infected monkey, whose blood on microscopical examination was seen to contain numerous trypanosomes of Sleeping Sickness, and afterwards on a series of healthy monkeys.

The following table gives the number of flies used in each experiment, the number of days they were fed on a monkey whose blood contained *Trypanosoma gambiense*, the number of days which elapsed before the flies became infective, and the number of days the flies remained infective. The minus signs signify that the flies failed to become infected, or at least failed to infect; or, in other words, that the experiment was negative.

Table I.—Development of *Trypanosoma gambiense* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
624	60	3	–	–
656	280	3	–	–
663	60	2	18	75
676	500	3	29	47
721	50	3	–	–
980	350	3	–	–
986	100	2	19	35
987	50	2	40	51
989	50	2	45	45
1020	100	3	–	–
1023	50	3	37	51
1026	70	3	34	48
1198	20	40	–	–
1372	100	3	–	–

Of these 14 experiments, seven are positive and seven negative. In the positive experiments 880 flies were used, an average of 126; in the negative 960, an average of 137. The shortest time which elapsed before a fly became infective was 18 days, the longest 45 days, and the average 32 days.

It may be well to give some of these experiments more in detail, in order to show the methods used, and draw attention to various interesting points.

Experiment 624.

To ascertain if development of *Trypanosoma gambiense* takes place in the alimentary canal of Wild or Lake-shore *Glossina palpalis*.

March 31, 1909.—Two batches of *Glossina palpalis*, caught on the Lake-shore, consisting of 30 flies in each batch, were fed to-day on a monkey whose blood contained numbers of *Trypanosoma gambiense*.

The following table gives the principal details of the experiment :—

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Mar. 31	—	Flies fed on infected monkey.			
Apr. 1	1	,, ,,			
,, 2	2	,, ,,			
,, 3—4	3—4	Flies starved 72 hours.			
,, 5—8	5—8	Flies fed on healthy monkey, 657		—	
,, 9—12	9—12	Flies fed on healthy monkey, 677		—	
,, 13	13	Flies fed on healthy monkey, 702		—	
,, 14	14	Flies fed on healthy monkey, 703		—	
,, 15	15	Flies fed on healthy monkey, 704		—	
,, 16	16	Flies fed on healthy monkey, 705		—	
,, 17—21	17—21	Flies fed on healthy monkey, 706		—	
,, 22—May 22	22—52	Flies fed on healthy monkey, 728		—	

Remarks.—These 60 Lake-shore or wild flies, although fed on an infected monkey for three days, failed to convey the infection to healthy monkeys. As the flies died they were dissected. Only in one were flagellates found, and these appeared to be of the *Trypanosoma grayi* type. An emulsion was made of the contents of the alimentary canal of this fly and injected into monkey 914. Monkey 914 never showed trypanosomes in its blood, although kept under observation for a month.

Experiment 656.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Apr. 3—5	1—2	280 flies fed on infected monkey.			
,, 6—7	3—4	Flies starved 72 hours.			
,, 8—17	5—14	Flies fed on healthy monkey, 675		—	
,, 18	15	Flies fed on healthy monkey, 712		—	
,, 19	16	Flies fed on healthy monkey, 713		—	
,, 20	17	Flies fed on healthy monkey, 714		—	
,, 21	18	Flies fed on healthy monkey, 715		—	
,, 22—25	19—22	Flies fed on healthy monkey, 716		—	
,, 26—28	23—25	Flies starved.			
,, 29—June 17	26—75	Flies fed on healthy monkey, 744		—	June 8, 8 flies alive. June 18, 2 flies alive.

Remarks.—Two hundred and eighty wild tsetse flies, fed for three days on an infected monkey and then on healthy monkeys, failed to transfer the disease. The experiment lasted from April 3 to June 17, and seven healthy monkeys were used. After 66 days eight flies remained alive ; after 75 days only two. None of the flies which died, or were killed and dissected, showed flagellates in the alimentary canal.

Experiment 663.

This is the experiment described at length in the 'Proceedings' (B, vol. 81, 1909). Sixty wild flies were used. One fly became infective after 18 days, and remained infective 75 days, when it died. A small quantity of fluid from the gut of this fly injected into a healthy monkey gave rise to Sleeping Sickness.

Experiment 676.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Apr. 6—8	1—2	500 Lake-shore flies fed on infected monkey.			
„ 9—10	3—4	Flies starved.			
„ 11—16	5—10	Flies fed on healthy monkey, 696		−	
„ 17—18	11—12	Flies fed on healthy monkey, 707		−	
„ 19—20	13—14	Flies fed on healthy monkey, 708		−	
„ 21	15	Flies fed on healthy monkey, 709		−	
„ 22	16	Flies fed on healthy monkey, 710		−	
„ 23—May 12	17—36	Flies fed on healthy monkey, 711	+		April 23, 67 flies alive
May 13—14	37—38	Flies starved.			
„ 15—16	39—40	Flies fed on healthy monkey, 766	+		
„ 17—21	41—45	Flies fed on healthy monkey, 770	+		
„ 22—23	46—47	Flies starved			May 23, 22 flies alive. Infected fly found.
„ 24—28	48—52	Flies fed on healthy monkey, 901		−	
„ 29—30	53—54	Flies starved.			
„ 31—June 3	55—58	Flies fed on healthy monkey, 941		−	June 3, 1 fly alive.

Remarks.—Five hundred Lake-shore tsetse flies were fed for three days on an infected monkey. As none of the five healthy monkeys on which these flies were fed during the first 16 days showed any sign of Sleeping Sickness it may be assumed that there was no naturally-infected fly among the 500. On or about the 29th day the cage of flies became infective, and remained infective up to the 47th day. On this day a dead fly was found on dissection to contain flagellates, and after the death of this fly no further infection took place. The injection of the infected fly failed, however, to give rise to Sleeping Sickness when injected under the skin of a healthy monkey.

Experiment 721.

Remarks.—This experiment lasted from April 12 to July 6, a period of 85 days, and remained negative throughout. On the 30th day, 12 flies out of the 50 remained alive; on the 73rd day only six flies were left. As the flies died they were dissected, but no infected fly was found.

Experiment 980.

Remarks.—This experiment lasted 66 days, and remained negative. At the end 15 flies remained alive. These were killed and dissected. All proved negative.

These experiments on the development of *Trypanosoma gambiense* in Lake-shore or wild *Glossina palpalis*, given somewhat in detail, will suffice to show the method employed, and make it unnecessary to explain the remaining experiments further than is done in Table I.

It would appear from the fact that none of the healthy monkeys became infected before the 18th day, that not a single fly of the 1840 used was infective when captured. That is to say, that among nearly 2000 Lake-shore flies, not one was naturally infected. On referring to Table IV, in the previous paper in the 'Proceedings'—a table showing the probable number of naturally-infected flies—this is seen to be by no means exceptional.

Other points of interest arising out of these experiments are the number of flies which became infective, and the result of injecting their body-contents into healthy animals.

The following table shows this:—

Table II.—Number of Flies found Infected with Trypanosomes in the Experiments with Lake-shore Flies and *Trypanosoma gambiense*.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
624	60	–	1	Negative	*T. grayi* type.
656	280	–	0		
663	60	+	1	Positive.	
676	500	+	1	Negative.	
721	50	–	0		
980	350	–	0		
986	100	+	1	Not injected.	
987	50	+	2	Negative	1 *T. gambiense* and 1 *T. grayi* type.
989	50	+	1	Negative.	
1020	100	–	0		
1023	50	+	5	2 positive. 3 negative.	
1026	70	+	4	3 positive. 1 negative.	
1198	20	–	0		
1372	100	–	0		

Thus it is seen that the infected flies found in three of the positive experiments when injected into healthy monkeys gave negative results, while those found in three others gave positive results. The infected fly found in the seventh successful experiment was not injected.

It is difficult to understand why the results of injecting infected flies into healthy animals are so irregular. The only theory brought forward is that the trypanosomes introduced under the skin along with the tissues of the fly may give rise to a reaction at that point, which will so damage the parasites that they fail to infect.

In these experiments, 1840 flies were used, and of these 16 became infected, or, at least, were found to have flagellates in their gut. This works out at less than 1 per cent. The smallness of the percentage is due to the fact that less care was taken to dissect the flies which died during the course of the experiments.

B. *The Development of* Trypanosoma gambiense *in Laboratory-Bred* Glossina palpalis.

The pupæ of the fly were found on the Lake-shore, and hatched out in the laboratory. For a long time the Commission failed to find any pupæ, although days were spent in turning over soil and decaying vegetable matter in those places where the fly most abounded. At last, Lieutenant A. D. Fraser, Royal Army Medical Corps, found them in numbers in patches of sand on the edge of the Lake in the Sesse Islands. After the Sesse Islands were emptied of their inhabitants, Fraser's native collectors came into the service of the Commission, and from that time there was no lack of pupæ. These natives found them in large numbers. One day they brought up as many as 7000. These pupæ proved to be much healthier than those obtained from flies in captivity. The flies bred from larvæ born in the laboratory rarely showed any marked vitality. Many of the larvæ were immature, and those which hatched out were rarely a success as experimental flies. On the other hand, the flies hatched out from pupæ found on the Lake-shore were fairly strong and vigorous, and lived in captivity for a couple of months or more. It was, however, difficult to get them to feed at first, and very few became infective, as the following table shows. The flies were fed chiefly on infected monkeys. In one negative experiment (1431) they were fed on a case of Sleeping Sickness in man, and in five—two positive (1566 and 1602) and three negative (1269, 1452, and 1672)—on oxen. Numerous observations went to show that there is no hereditary transmission of trypanosomes in *Glossina palpalis*; and no evidence was gained that the flies became infected with any flagellate by contact with other flies or fouled cages. Any trypano-

somes found in laboratory-bred flies may therefore be considered to be derived from the infected animal they had fed upon.

Table III.—Development of *Trypanosoma gambiense* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
725	8	3	—	—
733	8	3	—	—
750	15	6	—	—
768	9	5	—	—
858	9	8	—	—
915	6	10	—	—
947	9	5	—	—
975	16	5	34	49
991	27	6	—	—
1266	11	12	—	—
1269	22	7	—	—
1368	14	12	—	—
1397	7	19	—	—
1428	50	6	—	—
1431	30	20	—	—
1452	90	10	—	—
1549	50	4	37	46
1558	60	4	—	—
1559	50	4	—	—
1566	35	4	53	53
1602	50	4	27	27
1604	35	12	—	—
1640	40	10	—	—
1651	60	4	41	41
1664	50	12	—	—
1665	50	12	—	—
1671	45	12	—	—
1672	28	12	—	—
1680	45	4	—	—
1686	60	3	—	—
1688	60	3	—	—
1693	50	4	—	—
1706	60	7	—	—
1712	50	12	32	32
1758	50	3	—	—
1760	60	4	29	29
1761	50	4	38	38
1769	60	3	—	—
1801	70	12	—	—
1802	75	12	—	—
1860	60	12	—	—
1868	60	12	—	—

Among these 42 experiments there were only eight positive, while there are as many as 34 negative. In the eight positive experiments, 371 flies were used, an average of 46; in the 34 negative experiments, 1323 flies, an average of 40. The shortest time which elapsed before a laboratory-bred fly

became infected with *Trypanosoma gambiense* was 27 days, the longest 53 days, and the average 36 days.

Here follow the experiments, given somewhat more in detail, which gave positive results :—

Experiment 975.

To ascertain if any development of *Trypanosoma gambiense* takes place in the alimentary canal of laboratory-bred *Glossina palpalis*.

The following table gives the principal details of the experiment :—

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
June 8—12	1—4	Flies fed on infected monkey			16 flies used.
„ 13—15	5—7	Flies starved.			
„ 16—July 19	8—41	Flies fed on healthy monkey, 1008	+		
July 20—21	42—43	Flies starved.			Aug. 4, 12 remaining flies killed and dissected.
„ 22—Aug. 3	44—55	Flies fed on healthy monkey, 1374	+		

Remarks.—Sixteen laboratory-bred flies were fed on infected monkeys for five days. Thirty-four days after their first feed they became infective, and remained so for at least 49 days. On the 56th day the remaining 12 flies were dissected and examined for flagellates. None were found, but the contents of the alimentary canals of the 12 flies, pooled and injected into a healthy monkey, gave rise to Sleeping Sickness.

Experiment 1549.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Aug. 23—27	1—4	Flies fed on heavily-infected monkey			50 flies used.
„ 28—29	5—6	Flies starved.			
„ 30—Oct. 5	7—43	Flies fed on healthy monkey, 1617	+		Oct. 8, 20 remaining flies dissected, 4 found infected.
Oct. 6—7	44—45	Flies starved.			

Remarks.—Fifty laboratory-fed flies were fed on an infected monkey daily for five days. Thirty-seven days after their first feed they became infective. Twenty flies remained alive on the 46th day, and on dissection four were found infected with flagellates. A drop of fluid from the alimentary canals of two of these infected flies injected into a monkey gave rise to Sleeping Sickness.

Experiment 1566.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Aug. 29—Sept. 1	1—3	Flies fed daily on infected ox			35 flies used.
Sept. 2—3	4—5	Flies starved.			
„ 4—Oct. 19	6—51	Flies fed on healthy monkey, 1566	+		Oct. 21, 20 remaining flies killed and dissected; 9 found infected.
Oct. 20	52	Flies starved.			

Remarks.—Thirty-five laboratory-bred flies were fed daily for four days on an ox infected with *Trypanosoma gambiense*. Fifty-three days after their first feed the flies became infective. Nine of the 20 remaining flies, when dissected, showed infection with flagellates: three of these had infection of the proboscis. The contents of the alimentary canal of one infected fly injected into a monkey and goat gave negative results, as also did the probosces of two infected flies when injected into a goat.

Experiment 1602.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 1—4	1—3	Flies fed on infected ox daily			50 flies used.
„ 5—6	4—5	Flies starved.			
„ 7—14	6—13	Flies fed on healthy monkey, 1620		—	
„ 15—20	14—19	Flies fed alternately, daily, on healthy monkey, 1620, and goat, 1690		—	
„ 21—29	20—28	Flies fed alternately, daily, on healthy monkey, 1703, and goat, 1690	1703 +	1690 —	Sept. 29, 32 remaining flies killed by accident and dissected; 5 found infected.

Remarks.—Fifty laboratory-bred flies were fed daily for four days on an infected ox, as in the previous experiment. From the 15th to the 28th day the flies were fed alternately on a monkey and goat. Twenty-seven days after their first feed the flies infected monkey 1703 with Sleeping Sickness. Five of the 32 remaining flies showed infection with flagellates, and one of these injected into a monkey gave rise to Sleeping Sickness.

Experiment 1651.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 11—14	1—3	Flies fed on infected monkey			60 flies used.
,, 15—16	4—5	Flies starved.			
,, 17—24	6—13	Flies fed on healthy monkey, 1651		−	Monkey 1651 died on 13th day.
,, 25—Oct. 29	14—48	Flies fed on healthy monkey, 1720	+		Nov. 1, 32 remaining flies killed and dissected; 4 found infected.
Oct. 30—31	49—50	Flies starved.			

Remarks.—Sixty laboratory-bred flies were fed daily for four days on an infected monkey. Forty-one days after their first infected feed they became infective. Four flies infected with flagellates were found among the 32 which remained alive on the 51st day. They were not injected into animals.

Experiment 1712.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 23—Oct. 5	1—12	Flies fed on infected monkey			50 flies used.
Oct. 6—7	13—14	Flies starved.			
,, 8—Nov. 2	15—40	Flies fed on healthy monkey, 1790	+		Nov. 4, 31 remaining flies killed and dissected; 1 found infected.
Nov. 3	41	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed daily for 13 days on an infected monkey. The flies became infective 32 days after their first feed. On dissection of the remaining 31 flies on the 41st day, one was found to be infected with flagellates, but it did not give rise to the disease when injected into a healthy monkey.

Experiment 1760.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Oct. 1—5	1—4	Flies fed on infected monkey			60 flies used.
,, 6—7	5—6	Flies starved.			
,, 8—Nov. 6	7—36	Flies fed on healthy monkey, 1760	+		Nov. 4, 1 infected fly injected into healthy monkey.
Nov. 7	37	Flies starved.			Nov. 8, 28 remaining flies killed and dissected; all negative.

Remarks.—Sixty laboratory-bred flies were fed daily for five days on an infected monkey. They became infective on the 29th day. On the 34th day one fly which had died was found to be infected with flagellates, but on injection into a monkey it failed to give rise to Sleeping Sickness. No infected flies were found among the 28 remaining alive on the 38th day, when they were dissected.

Experiment 1761.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Oct. 1—5	1—4	Flies fed on infected monkey			50 flies used.
,, 6—7	5—6	Flies starved.			
,, 8—Nov. 8	7—38	Flies fed on healthy monkey, 1761	+		Nov. 11, 31 remaining flies killed and dissected; all negative.
Nov. 9—10	39—40	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed daily for five days on an infected monkey. They became infective on the 38th day. No infected flies were found among the 31 remaining alive on the 41st day, when they were dissected.

Table IV.—Number of Flies found Infected with *Trypanosoma gambiense* in the Experiments with Laboratory-Bred Flies.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
975	16	+	0	Positive.....	12 remaining flies pooled.
1269	22	—	2	Negative.	
1452	90	—	1	,,	
1549	50	+	4	Positive.	
1566	35	+	9	Negative	1 fly injected.
1602	50	+	5	Positive.	
1651	60	+	4		Flies not injected.
1672	28	—	2	Negative.	
1680	45	—	1	,,	
1693	50	—	2	,,	
1706	60	—	4	,,	
1712	50	+	1	,,	
1760	60	+	1	,,	
1769	60	—	2	Positive.	
1801	70	—	1		Fly not injected.

There are some curious results to be noted here. In Experiment 975 the twelve remaining flies were dissected and examined. None was found

infected. They were then pooled and injected into a healthy monkey, which became infected with Sleeping Sickness. This shows that the infected fly may escape detection by the microscope.

In Experiments 1269, 1452, 1672, 1680, 1693, and 1706, flies were found containing flagellates. These flies had not given rise to disease in the monkey they had been fed on, nor did their injection prove successful. The flagellates must be considered to be *Trypanosoma gambiense*, and therefore a cage of flies may become infected without causing disease either by biting or injection.

In Experiments 1549 and 1602, flies were found containing flagellates, and these flies succeeded in infecting monkeys both by biting while alive and injection after death.

Lastly, in Experiments 1566, 1712, and 1760, flies were found with flagellates which had infected the monkey fed on, but which failed to give rise to disease when their body-contents were injected into healthy animals.

In these experiments 746 laboratory-bred flies were used. Thirty-nine became infected—that is to say, more than 5 per cent.

C. *The Development of* Trypanosoma dimorphon *in Lake-shore* Glossina palpalis.

This is the commonest cattle trypanosome in Uganda. During 1909 it caused epidemics among the Government transport oxen at Entebbe, Mr. Walsh's cattle at Kabula Muliro, and the Uganda Company's cattle at Namukekera, all of which were investigated by the Commission.

The name *Trypanosoma dimorphon* is used for this species, although two forms have not been found. It belongs to the short, stumpy type of trypanosomes, without free flagella, and is probably identical with that found in Zanzibar by Edington ; in Portuguese East Africa, described by Theiler; in Northern Rhodesia by Montgomery and Kinghorn; and in Southern Rhodesia by Bevan.

Table V.—Development of *Trypanosoma dimorphon* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
574	500	3	21	21
996	100	3	—	—
1010	120	3	—	—
1022	100	3	—	—

Only one experiment out of four was successful. The flies became infective 21 days after their first feed on the infected dog.

Experiment 574.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Mar. 4—6	1—2	Flies fed on infected dog			500 flies used.
„ 7—25	3—21	Flies fed on a fowl.			
„ 26—30	22—26	Flies fed on healthy monkey, 649	+		Mar. 25, 120 flies alive,
„ 31—Apr. 3	27—30	Flies fed on healthy monkey, 650		—	Apr. 14, 60 flies alive.
Apr. 4—8	31—35	Flies fed on healthy monkey, 660		—	May 3, 30 flies alive.
„ 9—13	36—40	Flies fed on healthy monkey, 678		—	May 14, 22 remaining flies dissected; all negative.
„ 14—May 13	41—70	Flies fed on healthy monkey, 723		—	

Remarks.—Five hundred wild flies were fed for three days on a dog whose blood contained numerous *Trypanosoma dimorphon*. Twenty-one days after their first feed they became infective. By the 27th day they lost the infection and did not regain it, although kept under observation for 71 days. None of the flies which died or were killed and dissected showed any flagellates. It appears as if the infected fly had died early in the experiment and had escaped notice.

D. *The Development of* Trypanosoma dimorphon *in Laboratory-Bred* Glossina palpalis.

Table VI.—Development of *Trypanosoma dimorphon* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1642	50	4	14	14
1675	50	3	—	—
1676	50	3	—	—
1843	140	4	—	—

Four experiments were carried out, as in the Lake-shore group, and one also was successful. The flies became infective on or about the 14th day.

Experiment 1642.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 8—11	1—3	Flies fed on infected oxen			50 flies used.
„ 12—13	4—5	Flies starved.			
„ 14—28	6—20	Flies fed on healthy ox, 870	+		
„ 29	21	Flies starved.			
„ 30—Oct. 11	22—33	Flies fed on healthy monkey, 1741		—	Oct. 13, 35 remaining flies dissected; all negative.
Oct. 12	34	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed on two infected oxen for four days, and then on a healthy ox. Fourteen days after their first infected feed this ox took the disease.

These experiments with *Trypanosoma dimorphon* are not very satisfactory. Experiment 574 appears to be fairly free from fallacy, and from it, it would seem probable that *Trypanosoma dimorphon* can develop in *Glossina palpalis* and infect a healthy animal after a period of 21 days. Ox 870, in Experiment 1642, became infected at a time when several of the cattle at Mpumu became naturally infected with this trypanosome disease, so that this experiment is not free from doubt. It is evident that more work must be done before anything definite can be said regarding this species.

E. *The Development of* Trypanosoma vivax *in Lake-shore* Glossina palpalis.

Table VII.—Development of *Trypanosoma vivax* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
997	60	4	11	48
998	45	4	11	44
1014	200	4	21	60?

As *Trypanosoma vivax* does not affect monkeys, naturally cattle or goats were used in these experiments. The three experiments with Lake-shore flies were all successful; two became infected in 11 days, and one in 21 days.

Here follow the experiments in detail :—

Experiment 997.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
June 15—18	1—3	Flies fed on infected goat			60 flies used.
„ 19—20	4—5	Flies starved.			
„ 21—July 3	6—18	Flies fed on healthy calf, 1030	+		July 6, injected infected fly into goat; negative.
July 4—5	19—20	Flies starved.			
„ 6—21	21—36	Flies fed on healthy bull, 1268	+		Aug. 10, remaining flies dissected. Infected fly injected into goat; negative.
„ 22—23	37—38	Flies starved.			
„ 24—Aug. 10	39—56	Flies fed on healthy calf, 737	+		

Remarks.—Sixty wild flies were fed for four days on a goat infected with *Trypanosoma vivax*. Eleven days after their first feed they became infective, and remained so during the experiment. Two flies were found infected with flagellates, one on the 21st day, and one on the 56th day, both of which when injected into goats failed to give rise to the disease.

Experiment 998.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
June 15—18	1—3	Flies fed on infected goat			45 flies used.
„ 19—20	4—5	Flies starved.			
„ 21—July 3	6—18	Flies fed on healthy calf, 1030	+		Aug. 6, pooled contents of 14 flies remaining; injected into goat, which became infected with *T. vivax*.
July 4—5	19—20	Flies starved			
„ 6—Aug. 5	21—51	Flies fed on healthy calf, 1267	+		

Remarks.—Forty-five wild flies were fed for four days on an infected goat. Eleven days after their first feed they became infective, and remained so during the experiment. The pooled contents of the 14 remaining flies injected into a goat on the fifty-second day gave rise to infection with *Trypanosoma vivax*.

Experiment 1014.

Date.	Day of experiment.	Procedure.	Results.		Remarks.
			Positive.	Negative.	
1909.					
June 18—22	1—4	Flies fed on infected goat			200 flies used.
„ 23—24	5—6	Flies starved.			July 26, injected infected fly into a goat and monkey, the former of which became infected.
„ 25—July 17	7—29	Flies fed on healthy goat, 1079	+		
July 18	30	Flies starved.			
„ 19—Aug. 3	31—46	Flies fed on healthy goat, 1344	+		Aug. 4, 17 remaining flies dissected; all negative.

Remarks.—Two hundred wild flies were fed for five days on an infected goat. Twenty-one days after their first feed they became infective, and remained so during the experiment. On the 38th day one infected fly was found, which on injection into a goat and a monkey gave rise to *Trypanosoma vivax* infection in the former animal. Seventeen flies remained alive at the end of the experiment, and were killed and dissected. None of them was found to harbour flagellates.

Table VIII.—Number of Flies found infected with Trypanosomes in the Experiments with Lake-shore Flies and *Trypanosoma vivax*.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
997	60	+	2	—	
998	45	+	2	+	14 flies remaining pooled.
1014	200	+	1	+	

Of the above three positive experiments it is seen that five infected flies were found. One of these—Experiment 1014—when injected into a susceptible animal gave rise to *Trypanosoma vivax* infection. In Experiment 998, among the 14 remaining flies, two were found with trypanosomes in their probosces. None of the 14 showed flagellates in the gut. The body-contents of the 14 flies, in addition to the contents of the two probosces, were pooled and injected into a goat, which 12 days afterwards showed *Trypanosoma vivax* in its blood.

F. *The Development of* Trypanosoma vivax *in Laboratory-Bred* Glossina palpalis.

Table IX.—Development of *Trypanosoma vivax* in Laboratory-Bred *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1591	50	4	21	21
1638	25	6	—	—
1698	68	4	35	35
1700	60	4	30	30
1870	50	?	—	—

Five experiments were made with laboratory-bred flies. Three were successful.

Experiment 1591.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 2—4	1—2	Flies fed on infected calf, 1318			50 flies used.
„ 5—6	3—4	Flies starved.			
„ 7	5	Flies fed on infected calf, 1318.			Oct. 4, 35 remaining flies dissected; negative except 3, which had infected probosces.
„ 8—9	6—7	Flies starved.			
„ 10—30	8—28	Flies fed on healthy goat, 1652	+		
Oct. 1—3	29—31	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed for four days on an infected calf; 21 days after their first feed they became infective; 35 flies remained alive on the 32nd day. On these being dissected three were found with infected probosces. These probosces were not injected into animals, so that it is not known if the flagellates were infective by injection.

Experiment 1638.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 6—11	1—5	Flies fed on infected calf			25 flies used.
„ 12—13	6—7	Flies starved.			Sept. 29, 23 remaining flies accidentally killed; negative on dissection.
„ 14—29	8—23	Flies fed on healthy goat, 1682		—	

Remarks.—Twenty-five laboratory-bred flies were fed for six days on a calf infected with *Trypanosoma vivax*, and afterwards for 16 days on a healthy goat. This goat was not infected, and the remaining 23 flies when killed and dissected all proved negative.

Experiment 1698.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 21—24	1—3	Flies fed on infected ox			68 flies used.
„ 25—26	4—5	Flies starved.			Calf 1893 died 29.11.09.
„ 27—Nov. 5	6—45	Flies fed on healthy ox, 425	+		Nov. 12, 53 flies remaining alive dissected; probosces of 5 swarming with flagellates.
Nov. 6—9	46—49	Flies fed on healthy calf, 1893		—	
„ 10—11	50—51	Flies starved.			

Remarks.—Sixty-eight laboratory-bred flies were fed for four days on an ox whose blood contained *Trypanosoma vivax*. About the 35th day the flies became infective. On the 52nd day the 53 flies which remained alive were killed and dissected. The probosces of five, three males and two females, were found to be swarming with flagellates. These were not injected into animals.

Experiment 1700.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 21—24	1—3	Flies fed on infected ox			60 flies used.
„ 25—26	4—5	Flies starved.			Oct. 30, 38 remaining flies dissected; 22 found infected.
„ 27—Oct. 28	6—37	Flies fed on healthy calf, 290	+		

Remarks.—Sixty laboratory-bred flies were fed for four days on an ox whose blood contained *Trypanosoma vivax*. About the 30th day these flies became infective. On the 39th day the 38 remaining flies were killed and dissected. There were 19 males and 19 females; 22 showed infection of the proboscis with crithidia-like flagellates. Only one fly showed flagellates in the gut. Of the 22 flies which had trypanosomes in the proboscis, 9 were males and 13 were females; 10 of the infected probosces were ground up in salt solution and injected into an ox; 12 days afterwards trypanosomes appeared in the blood of this ox.

Experiment 1870.

Remarks.—Fifty laboratory-bred flies were fed on a calf whose blood contained *Trypanosoma vivax*; 10 and 12 days afterwards all the flies were killed and dissected. No flagellates were found in any part of the flies.

Table X.—Number of Flies found Infected with Trypanosomes in the Experiments with Laboratory-Bred Flies and *Trypanosoma vivax*.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
1591	50	+	3	Not injected	Probosces infected.
1698	68	+	5	"	" "
1700	60	+	22	+	" "

In these experiments 178 flies were used, and of these 30, or 17 per cent., became infected. A curious fact is that in all the 30 flies, with the exception of one, the infection was confined to the proboscis. There was a feeling in the minds of the members of the Commission that this growth of flagellates in the proboscis was something quite characteristic of *Trypanosoma vivax*. Only on one occasion was this development of trypanosomes in the proboscis seen after feeding laboratory-bred flies on blood which was known to contain nothing but *Trypanosoma gambiense*.

G. *The Development of* Trypanosoma nanum *in Lake-shore* Glossina palpalis.

Table XI.—The Development of *Trypanosoma nanum* in Lake-shore *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1035	120	3	3	3

Only one experiment was carried out with *Trypanosoma nanum* and Lake-shore *Glossina palpalis*. It is unsatisfactory, as trypanosomes appeared in the first healthy goat a few days after the fly had fed on the infected animal. None of the flies which were dissected showed any flagellates in their alimentary canal.

Experiment 1035.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
June 21—23	1—2	Flies fed on infected goat.			120 flies used.
„ 24—25	3—4	Flies starved.			
„ 26—July 1	5—10	Flies fed on healthy goat, 1171	+		Aug. 3, goat 1257 died; negative.
July 2—4	11—13	Flies starved.			
„ 5—Aug. 2	14—42	Flies fed on healthy goats, 1257 and 1258.			Aug. 25, 9 remaining flies dissected; negative.
Aug. 3—24	43—64	Flies fed on goat 1258		—	

Remarks.—One hundred and twenty wild flies were fed for three days on an infected goat. Five days after their first feed they infected a healthy goat, or, at least, trypanosomes resembling *Trypanosoma nanum* appeared in the blood. They failed to infect other healthy goats, although they were fed up to the 64th day after their first infected feed. Nine remaining flies dissected on the 65th day were negative for flagellates.

H. *The Development of* Trypanosoma nanum *in Laboratory-Bred* Glossina palpalis.

Table XII.—The Development of *Trypanosoma nanum* in Laboratory-Bred *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1738	100	3	—	—

Only one experiment was carried out with laboratory-bred flies. The result was negative.

Experiment 1738.

Remarks.—One hundred laboratory-bred flies were fed for three days on an ox whose blood contained *Trypanosoma nanum*, and then on a healthy goat for 40 days. This goat remained healthy, and all the flies when dissected were found free from flagellates.

A few experiments were made by the Commission on the development of *Trypanosoma brucei* and *Trypanosoma cazalboui* in the Sleeping Sickness tsetse fly, *Glossina palpalis*, but they came to nothing.

Conclusions.

1. That *Trypanosoma gambiense* multiplies in the gut of about one in every 20 *Glossina palpalis* which have fed on an infected animal.

2. That the flies become infective, on an average, 34 days after their first feed.

3. That a fly may remain infective for 75 days.

4. That *Trypanosoma dimorphon*, *Trypanosoma vivax*, and *Trypanosoma nanum* may also multiply in *Glossina palpalis*, which must therefore be looked upon as a possible carrier in these diseases.

5. That multiplication in the tube of the proboscis is characteristic of *Trypanosoma vivax*.

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Further Researches on the Development of Trypanosoma gambiense *in* Glossina palpalis.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C.; and Captain F. P. MACKIE, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

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The object of these experiments was to try to discover if there is any definite cycle of development of the trypanosome of Sleeping Sickness in the tsetse fly, *Glossina palpalis*, and if the late or renewed infectivity of the fly coincides with any phase in this development.

The mode of experimentation was to feed a cageful of *laboratory-bred* tsetse flies on an animal whose blood contained numerous trypanosomes, and at the end of various times to kill the flies and examine their intestinal contents. This was done for periods of one day, two days, three days, and so on, up to 56 days. The microscopical examination of preparations made from the intestinal contents on the various days gave information as to the number and appearance of the trypanosomes.

After the infective feed or feeds the flies were fed every day on a healthy animal, so that by the appearance of trypanosomes in the animal's blood the day on which one or more of the flies became infective could be arrived at.

METHOD USED IN THE EXAMINATION OF THE FLIES.

The flies were killed by exposing them to the vapour of chloroform. After being killed the proboscis and pharynx were removed and examined under a cover-glass with the high and low powers. The terminal segment of the abdomen of the fly was then snipped off, and the whole abdominal viscera gently pressed out. This was moistened with a little normal saline solution, and the gut unravelled without rupturing. The proventriculus and crop were often pulled out intact with the gut. The whole thoracic and abdominal tract could then be laid out in line and examined under a low power.

In taking out the gut it was generally possible to draw out with it the abdominal portion of the salivary glands, which could then be separated without contamination from accidental rupture of the gut. If the salivary glands or proventriculus remained behind they were dissected out after removal of the gut. In every case these organs were thoroughly washed in

three changes of normal saline solution, in order to minimise the chance of their being contaminated by accidental rupture of the intestines.

The stained specimens were examined day after day, and coloured drawings, at a magnification of 2000 diameters, made of all the different forms met with. The drawings of the trypanosomes found in the proboscis, proventriculus, fore-gut, mid-gut, hind-gut, proctodæum, and salivary glands, were kept separate, so that a series of drawings of trypanosomes taken from any one part, from the first day of infection to the 56th day, could be compared.

By arranging these drawings on a wall the horizontal layers would represent the contents of, say, the fore-gut from one day onwards, while the vertical rows would represent the trypanosomes found in the whole length of a fly for one day. More than six hundred drawings were made in this way, so that it seems impossible that any important form can have been left out.

General Considerations regarding the Development of the Trypanosomes in the Fly.

Let us now take a general view of the types of trypanosomes found in the various parts. It is evident that very few of the six hundred drawings can be reproduced; a few types, taken here and there, must suffice. For the first three or four days trypanosomes are found in all the flies, but at the end of six or seven the trypanosomes have disappeared out of many of them. That is to say, it is only in a certain percentage that further development takes place. In one series this was 8 per cent. In 92 per cent., then, of flies which imbibe infected blood, the trypanosomes simply degenerate and die out within the first few days. In 8 per cent., on the other hand, the trypanosomes find conditions more favourable for development, and increase to a marvellous extent, filling the whole of the fore-gut, mid-gut, and hind-gut with countless swarms of trypanosomes.

How long this development continues is unknown. It is considered probable that it continues for the remainder of the fly's life, and this has been proved by experiment up to 96 days.

What the conditions in the intestine of the fly are, which render this development possible, are also unknown. It was thought that it might depend on the reaction of the intestinal fluids. This is, however, found on examination to be faintly acid in all flies, whether development has taken place or not. The presence of bacteria also seems to have no connection with the phenomenon. Sex, moreover, has no influence; development occurs in as many males as females.

Types of Trypanosoma gambiense found in the Alimentary Canal.

It would serve no good purpose to describe separately, day by day, the types found in the various parts of the alimentary canal, as they run into each other in such a way that any classification of them seems impossible.

The following table represents, approximately, the numbers found in the different parts of the alimentary canal at various times after infection. The − sign means that an examination was made and nothing found. ± means few. +, many. + +, very many. + + +, swarming. If no sign, then no examination has been made. (See p. 516.)

The Proboscis.—In our experience *Trypanosoma gambiense* is never found in the proboscis of *Glossina palpalis*, except immediately after an infected feed, when for a short time blood containing trypanosomes may be seen in the lumen of the proboscis. This is very different from what obtains in an infection by *Trypanosoma vivax*, in which case the proboscis is alone infected.

Proventriculus.—As seen from the above table, this part of the alimentary canal is sometimes found empty when the remainder of the gut is swarming.

Fore-, Mid-, and Hind-gut.—It is here that the greatest development of the trypanosomes is found. Among the extraordinary numbers and diversity of type it is difficult or impossible to find one's way. Generally speaking, the trypanosomes found during the first few days are merely degenerating blood forms. After this there appears a type of trypanosome which remains dominant throughout the whole developmental period. 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 trypanosomes.

This seems to be the healthy normal developing type in the intestine of the fly. It is seen in all parts of the intestine and at all times. It forms masses of innumerable individuals alike in size and shape. When a fresh supply of blood is taken in by the fly this 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 host of diverse forms which thus arises beggars description. Some are round or oval in shape, 3 or 4 microns in diameter, with or without a flagellum. From this simple form all shapes and sizes can be seen up to the huge shapeless mass of protoplasm, multi-nucleated and multi-flagellated.

Table I.—Number of Trypanosomes found in the Various Parts of the Alimentary Canal and Salivary Glands.

Time, days.	Proboscis.	Proventriculus.	Crop.	General smear of gut.	Fore-gut.	Mid-gut.	Hind-gut.	Procto-dæum.	Salivary glands.
1	–	–				+ +			–
1	–	–				+	+		–
2	–				+	+	+ +		–
3	–					+ +	+ +		
4	–				+	+	+	–	–
5	–	–	–		–	–	–		–
6	–	–	–	+	+	+			–
7	–	–	–	+					–
8	–	+			+	+ +	+ +		–
8	–				±	±	±		
9	–	–			±	+ +		–	–
9	–				+	+	+		
10	–				+	+ +	+ +		
10	–					+			
11	–	±	–	+ +	–	±	±		–
11	–	±	–	+ +	+ +	±			–
11	–	+ +			+ +	+ +	+ +		–
14	–	+ + +	–		+ +	+ +		–	–
14	–	+ +	–		+ +	+ +	+ +		–
15	–	–			+	+			–
17	–	–	–		±	+ +	+ +		–
17	–	+ +		–	+ +	+ +			–
18	–	–			+ +	+	+ +		–
18	–					+ +	+ +		–
20	–	+ +			+ +	+ +	+ +	±	–
21	–			+ + +					
22	–			+ +					
23	–				+	+	+		
24	–				+ + +	+ + +	+ + +		
25	–	–	–		+	+ + +	+ +		+
28	–			+ +		+ +			+
30	–	+			+ +	+ +			+
30	–	+			+ +	+ +	±		–
31	–	+	+		+ + +	+ +			–
31	–			+					
34	–			+ +	+ +	+ + +	+ + +	–	–
35	–	–			+	+ +	+ +		–
36	–			+ + +	+ + +	+ + +	+		+
36	–	–		+ + +	+ +	+ + +			–
36	–	+ +		+ + +					
37	–			+ +					
40	–			+ +					
40	–			+ +					
42	–	+ +		+	+ +				+
43	–			+ +					+ +
44	–	+			+ +	+ + +			+
44	–				+	+ +	+ +		+
44	–	+			+	+	+		+
46	–	+			+ + +	+ + +	+ + +		+ +
49	–	–	–		+	±			–
51	–		–		+	+	+ +		–
53	–	–			±	±			–
53	–	–			+	+ +			+
53	–		–			±			–
56	–			+ +					+

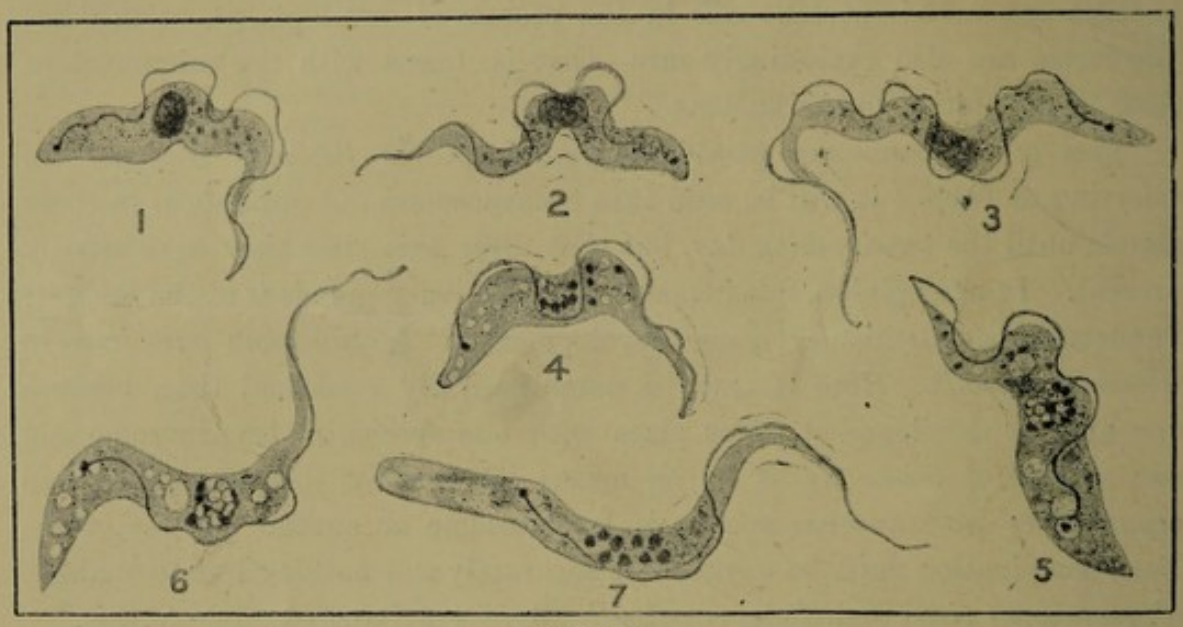
In our experience, the long narrow forms, described by some observers as "male" forms, are exceedingly rare, and it seems impossible to ascribe to to them any very important *rôle* in the process of development. Crithidia-like forms are also exceedingly rare—that is, forms with the micronucleus close to or anterior to the nucleus.

Types of Trypanosoma gambiense *found in the Salivary Glands.*—On referring to Table I it will be seen that trypanosomes did not appear in these glands until the twenty-fifth day, but that after this time they were usually present. In our opinion, this invasion of the salivary glands is of the greatest importance in the history of the development of *Trypanosoma gambiense* in *Glossina palpalis*. Here it may be parenthetically remarked that, because one kind of development takes place with one species of trypanosome and one species of tsetse fly, it by no means follows that the same thing will occur either with another species of trypanosome or another species of fly. Each combination must be worked out separately and nothing left to analogy. *Trypanosoma vivax* and *Glossina palpalis* afford a striking example of this.

In the development of *Trypanosoma gambiense* in *Glossina palpalis* one circumstance, which we think of prime importance, emerges, and that is, that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal blood-type. It must not be imagined, however, that the salivary glands show no other forms but this blood-type. On the contrary, there are many other forms seen; but here only are found trypanosomes apparently identical with the short and stumpy forms found in the blood. What causes or leads up to this reversion to the blood-type in the salivary glands is quite unknown, but, as will be seen later, the *Glossina palpalis* does not become infective by biting until this invasion of the salivary glands takes place.

How the trypanosomes find their way into the salivary glands is also quite unknown. It seems highly improbable that they pass from the alimentary canal by way of the salivary duct, and as they are never found in the body-cavity, it is also difficult to see how they can make their way directly from the intestine to the abdominal portion of the salivary glands.

ILLUSTRATIONS OF VARIOUS MODIFICATIONS IN SHAPE OF TRYPANOSOMA GAMBIENSE IN GLOSSINA PALPALIS.



× 2000.

FIGS. 1—3.—Normal blood parasites (*Trypanosoma gambiense*).
FIG. 4.—24 hours after ingestion by the fly.
FIGS. 5 and 6.—48 hours after ingestion by the fly.
FIG. 7.—96 hours after ingestion by the fly.

Figs. 1—7 represent the trypanosomes as they appear in the intestine of *Glossina palpalis* during the first few days. Figs. 1—3 are ordinary blood forms, as seen immediately after the fly has fed, and before any change has taken place. Figs. 4—7 represent the process of degeneration which takes place during the first four days. The body swells up, the nucleus breaks up, and the cytoplasm becomes vacuolated.

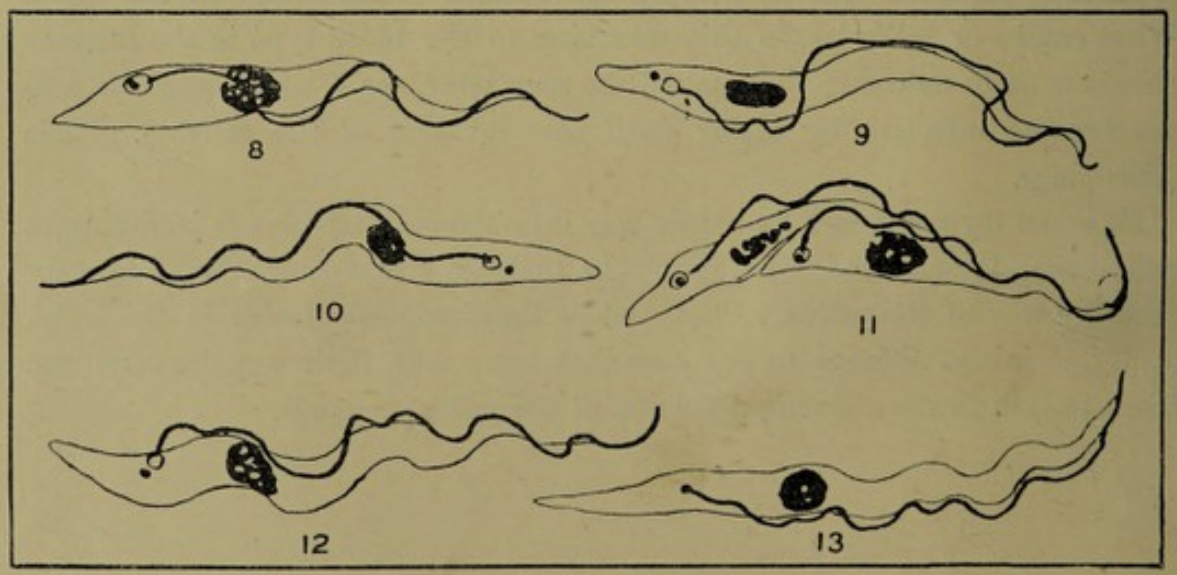


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FIG. 8.—*Trypanosoma gambiense* from fore-gut, 8 days after infected feed.
FIG. 9.—*T. gambiense* from proventriculus, 14 days after infected feed.
FIG. 10.—*T. gambiense* from fore-gut, 18 days after infected feed.
FIG. 11.—*T. gambiense* from mid-gut, 25 days after infected feed.
FIG. 12.—*T. gambiense* from mid-gut, 34 days after infected feed.
FIG. 13.—*T. gambiense* from fore-gut, 44 days after infected feed.

Figs. 8—13 represent what we consider to be the normal reproductive or developing type found throughout the intestine during the whole period of development. It is to be noted that this form is longer and broader than the normal broad form ; the protoplasm is clear, and stains readily and evenly, and this cell looks normal. The nucleus is compact and situated nearer the posterior extremity than the anterior. The micronucleus is small and round, lying at some distance from the elongated posterior extremity. Many dividing forms of this type can be seen. In our opinion, this is the common multiplying form, and from it arises an infinite variety of degenerating forms.

× 2000.

Fig. 14.—Part of a mass of *Trypanosoma gambiense* from the mid-gut.

This figure is given in order to try to convey some idea of the enormous proliferation of trypanosomes which takes place in the intestine of *Glossina palpalis*. Throughout the whole length of the alimentary canal, from the proventriculus to the proctodæum, this condition is frequently seen, and in the living condition presents an extraordinary spectacle. Looked at through the wall of the intestine the matted masses are seen in active movement, swaying about and wriggling in every direction. They do not appear to be attached to the wall of the intestine, but move about freely, and when the intestinal wall is burst pour out in countless numbers. The trypanosomes evidently belong to the type figured in the preceding sketch, called the normal reproductive type.

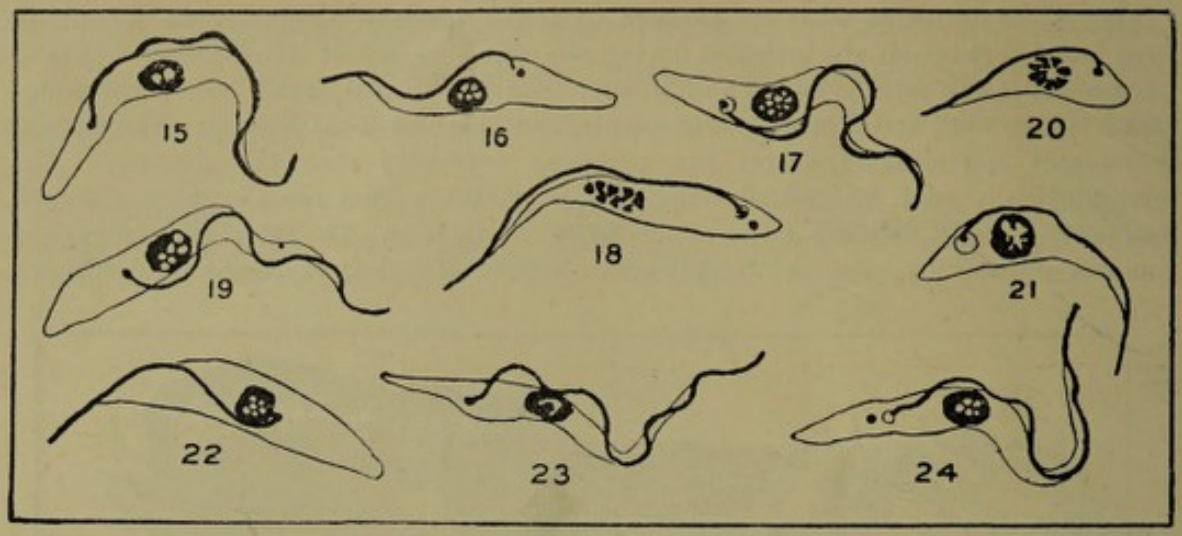

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Fig. 15.—*Trypanosoma gambiense* from hind-gut, 8 days after infected feed.
Figs. 16, 17, and 18.—*T. gambiense* from mid-gut, 14, 20, and 30 days after infected feed.
Fig. 19.—*T. gambiense* from hind-gut, 34 days after infected feed.
Figs. 20 and 21.—*T. gambiense* from proventriculus, 36 days after infected feed.
Fig. 22.—*T. gambiense* from hind-gut, 44 days after infected feed.
Figs. 23 and 24.—*T. Gambiense* from mid-gut, 46 and 53 days after infected feed.

Figs. 15—24 represent smaller forms which are fairly common and occur throughout the intestine and at all times. The examples given above are taken from the eighth day to the fifty-third day. They are called by us "small developmental forms," since they resemble the larger in having clear protoplasm and a compact nucleus. Dividing forms are often seen.

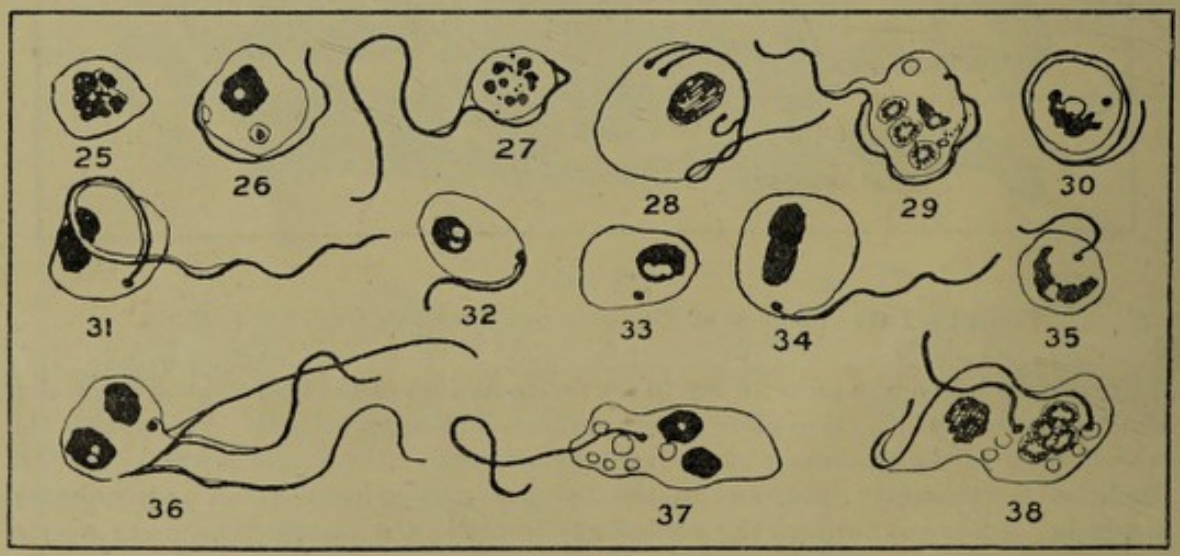

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Fig. 25.—*Trypanosoma gambiense* from mid-gut, 8 days after infected feed.
Fig. 26.—*T. gambiense* from fore-gut, 11 days after infected feed.
Figs. 27 and 28.—*T. gambiense* from mid-gut, 16 and 17 days after infected feed.
Figs. 29, 30, and 31.—*T. gambiense* from fore-gut, 17 and 18 days after infected feed.
Fig. 32.—*T. gambiense* from hind-gut, 20 days after infected feed.
Figs. 33 and 34.—*T. gambiense* from fore-gut, 24 days after infected feed.
Figs. 35, 36, and 37.—*T. gambiense* from mid-gut, 24, 44, and 46 days after infected feed.
Fig. 38.—*T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 25—38 represent round and irregularly-shaped forms of the parasite, taken from the eighth to the forty-sixth day of development. It is impossible to say what is exactly the origin of these forms—whether by the segmentation of large masses, or simply by the division and subdivision of irregular forms. Some of the examples figured are evidently dividing, as they show several nuclei and flagella. Whether the round aflagellar forms correspond to the so-called "latent" forms of various writers it is impossible to say. Those possessing flagella were active during life. In our opinion, they may be looked upon as part of the degenerative processes which are constantly taking place in the intestine of the fly.

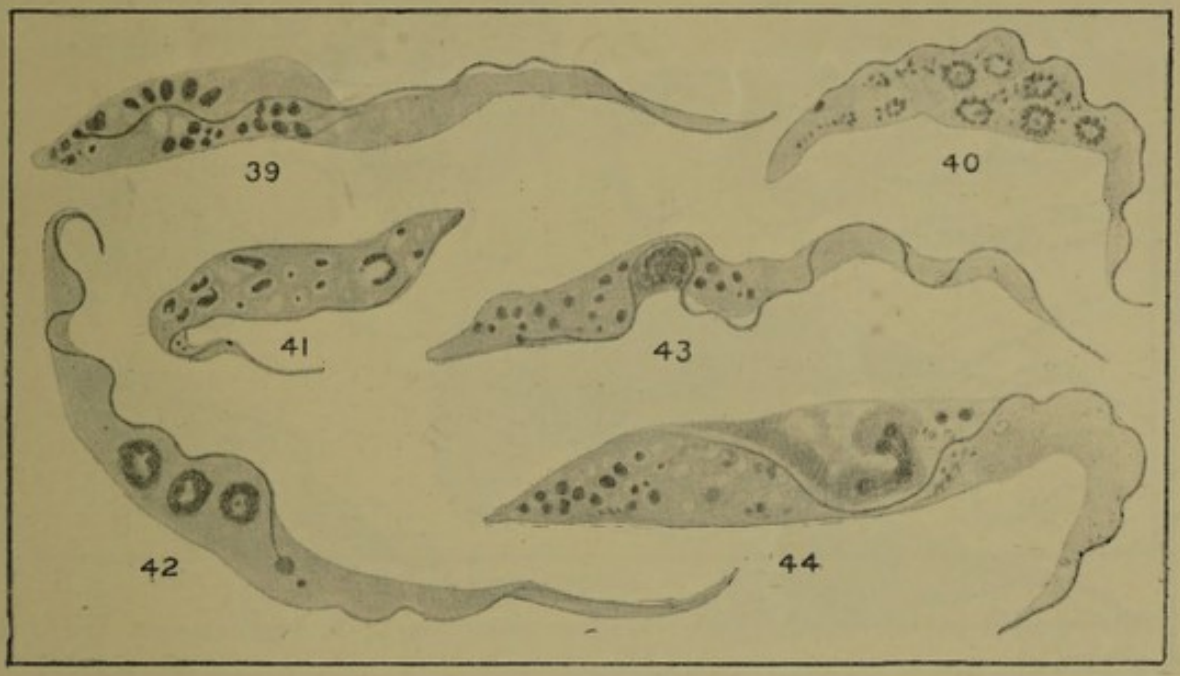

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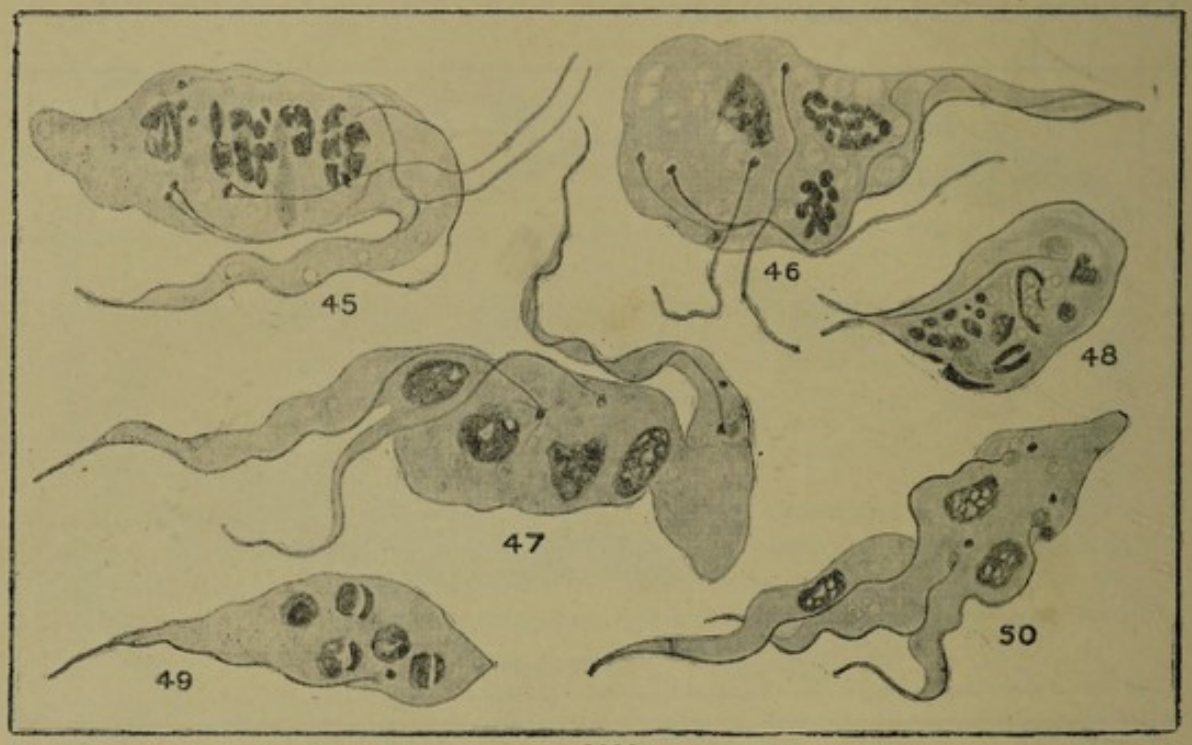
Fig. 39.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 40.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 41.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 42.—*T. gambiense* from hind-gut, 34 days after infected feed.
Fig. 43.—*T. gambiense* from mid-gut, 34 days after infected feed.
Fig. 44.—*T. gambiense* from hind-gut, 46 days after infected feed.

Figs. 39—44 represent what appear to us to be degenerative forms of the "normal reproductive type." They are of all sizes and shapes, and the cell-contents are scattered over with broken-up nuclei, or at least granules of some stainable substance. Figs. 39, 43, and 44 are also vacuolated.



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Figs. 45, 46, and 47.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 48.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 49.—*T. gambiense* from proventriculus, 30 days after infected feed.
Fig. 50.—*T. gambiense* from mid-gut, 46 days after infected feed.

Figs. 45—50 represent some of the more exaggerated types of degenerative forms. As will be seen from the drawings, they are huge, mis-shaped masses of protoplasm, multi-nucleated and, as a rule, multi-flagellated.

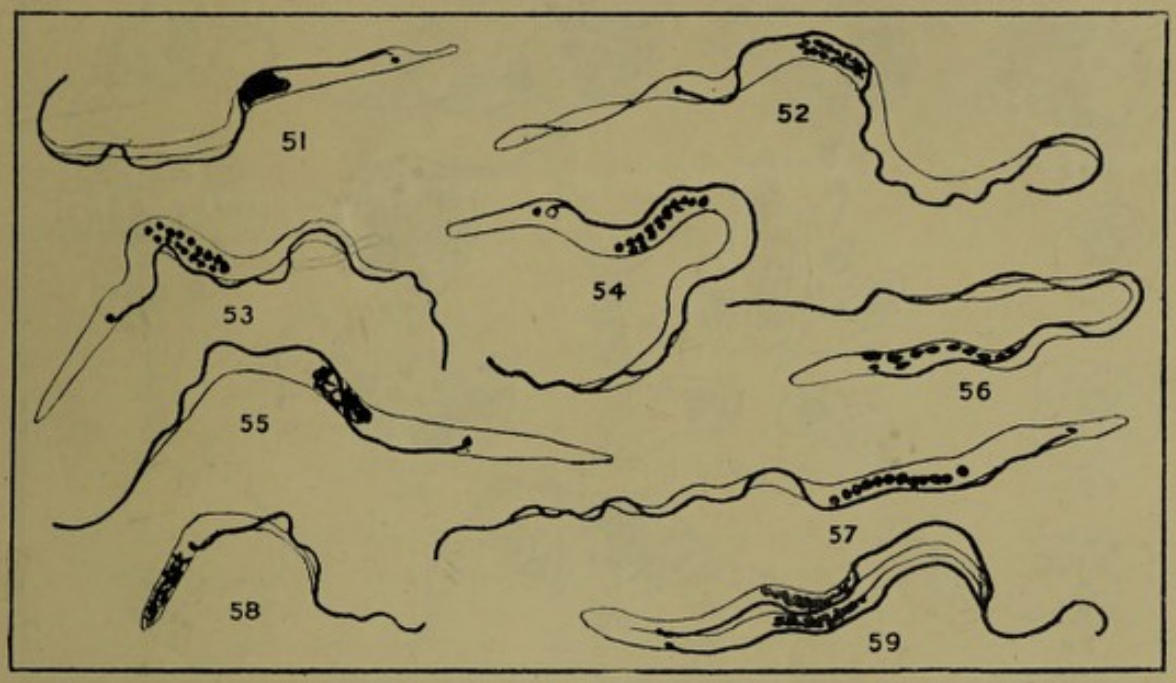

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Fig. 51.—Slender type of *Trypanosoma gambiense* from proventriculus, 17 days after infected feed.

Fig. 52.—Slender type of *T. gambiense* from mid-gut, 17 days after infected feed.

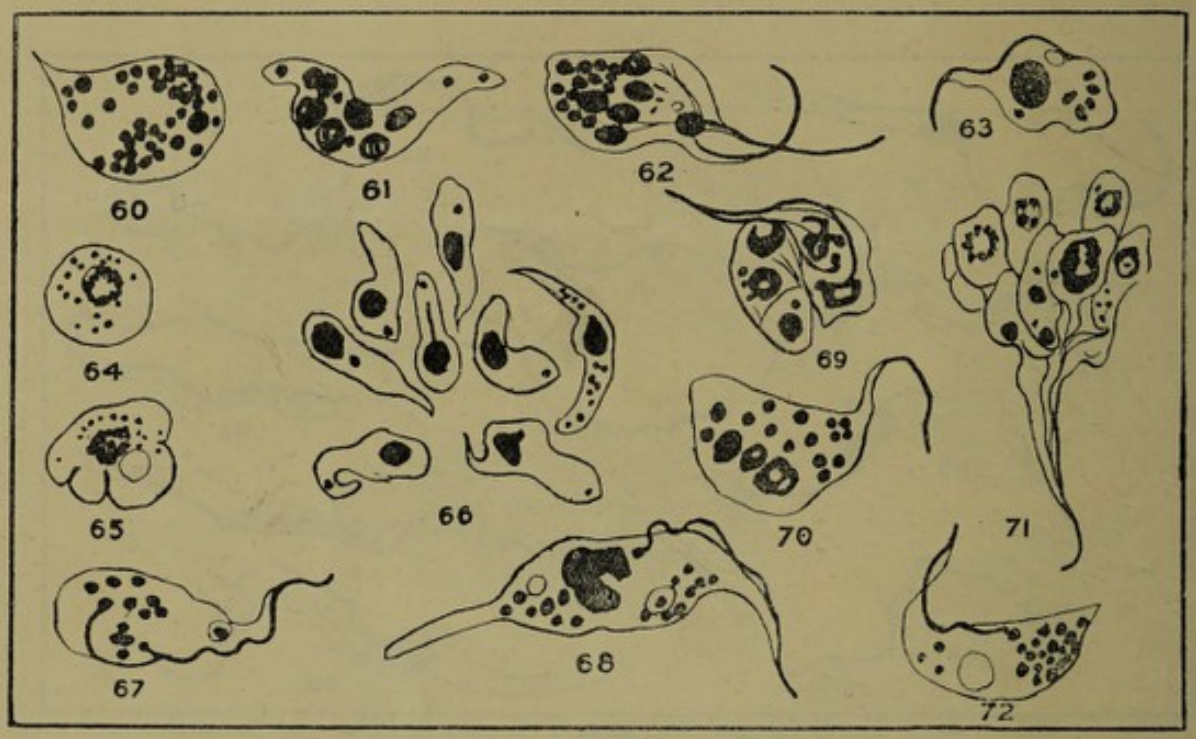
Fig. 53.—Slender type of *T. gambiense* from hind-gut, 20 days after infected feed.

Figs. 54 and 55.—Slender types of *T. gambiense* from fore-gut, 24 and 30 days after infected feed.

Figs. 56 and 57.—Slender types of *T. gambiense* from proventriculus, 44 days after infected feed.

Figs. 58 and 59.—Slender types of *T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 51—59 represent various varieties of the slender type of *Trypanosoma gambiense* found in the intestine of *Glossina palpalis*. Fig. 59 is dividing. Fig. 58 is a Crithidia type. These slender and crithidial types are uncommon, and no special connection between them and the onset of infectivity in the fly has been made out



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Figs. 60 and 61.—*Trypanosoma gambiense* from salivary glands, 25 days after infected feed.

Fig. 62.—*T. gambiense* from salivary glands, 30 days after infected feed.

Fig. 63.—*T. gambiense* from salivary glands, 42 days after infected feed.

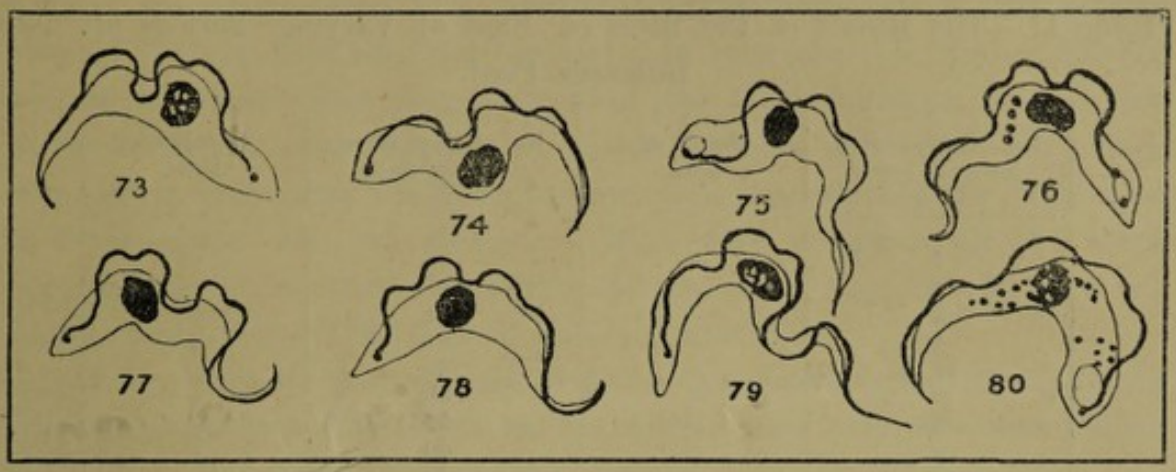
Figs. 64, 65 and 66.—*T. gambiense* from salivary glands, 44 days after infected feed.

Figs. 67 and 68.—*T. gambiense* from salivary glands, 46 days after infected feed.

Figs. 69 and 70.—*T. gambiense* from salivary glands, 53 days after infected feed.

Figs. 71 and 72.—*T. gambiense* from salivary glands, 56 days after infected feed.

Figs. 60—72 represent aberrant forms of *Trypanosoma gambiense* seen in the salivary glands. The first thing to be noted is the fact that the trypanosomes do not appear in the salivary glands until a late date ; in this case the earliest appearance is 25 days after the infected feed. How they reach these glands is up to the present unknown. In what shape they reach the glands is also unknown. Figs. 64, 65, 69 and 71 might be said to point to some process of segmentation, of which the finished product is represented by fig. 66.



× 2000.

Fig. 73.—*Trypanosoma gambiense* from salivary glands, 34 days after infected feed.
Fig. 74.—*T. gambiense* from salivary glands, 42 days after infected feed.
Fig. 75.—*T. gambiense* from salivary glands, 43 days after infected feed.
Figs. 76, 77, and 78.—*T. gambiense* from salivary glands, 46 days after infected feed.
Figs. 79 and 80.—*T. gambiense* from salivary glands, 56 days after infected feed.

When alluding, generally, in a previous part of this paper to the types of *Trypanosoma gambiense* found in the salivary glands, it was said that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal type found in the blood. Figs. 73—80 illustrate this reversion. By comparing them with figs. 1, 2, and 3, which represent normal blood trypanosomes, it will be seen that they are very similar to the short and stumpy form found in the blood. No such forms have ever been seen in any other part of the fly, and we would suggest that the occurrence of these forms in the salivary glands, coinciding as it does with the renewed infectivity of the fly, is more than a mere coincidence.

Infectivity of Trypanosoma gambiense after its Development in Glossina palpalis, as shown by the Bites of the Flies giving rise to the Disease in Healthy Animals.

As has been shown in a previous paper,* the fly ceases to be infective by biting within a short time of its infective feed. From this time on for some 28 days the fly remains non-infective. Table II (p. 526) illustrates this.

From that Table it will be seen that in this series of experiments the flies first became infective 28 days after the infective feed, and that after this time the flies are usually found to be capable of giving rise to the disease by their bites.

It was stated above that the most important discovery made in this research is the connection between the invasion of the salivary glands and the infectivity of the fly. That this appears to be so is shown in Table III.

* 'Roy. Soc. Proc.,' B, 1911, vol. 82, p. 498.

Table II.—The Result of the Bites of Flies at Varying Periods after an Infective Feed.

No. of days after infective feed.	Result of bites.	No. of days after infective feed.	Result of bites.
1	–	28	+
2	–	30	–
3	–	31	–
4	–	34	+
5	–	35	–
6	–	36	–
8	–	37	+
9	–	40	+
11	–	40	+
14	–	42	+
15	–	43	+
17	–	44	+
18	–	46	+
20	–	51	–
23	–	53	+
25	–	56	+

Table III.—To show Correlation between the Invasion of the Salivary Glands of *Glossina palpalis* by *Trypanosoma gambiense* and Infection by the Bite of the Fly.

Experiment No.	No of days after infective feed.	Bites of fly infective or non-infective.	Salivary glands.	Remarks.
1910	1	–	–	
1910	2	–	–	
1910	3	–	–	
1910	4	–	–	
1910	5	–	–	
1910	6	–	–	
1910	7	–	–	
1894	8	–	–	
1894	9	–	–	
2216	10	–	–	
1894	11	–	–	
1871	14	–	–	
1693	15	–	–	
1945	17	–	–	
1945	18	–	–	
1945	20	–	–	
1718	25	–	+	Blood-type not present.
1602	28	+	+	Blood-type present.
1801	30	–	–	
1945	31	–	–	
1760	34	+	+	" "
1769	36	–	–	
1712	42	+	+	" "
2034	43	+	+	" "
1549	44	+	+	" "
2034	44	+	+	" "
1706	51	–	–	
1566	53	+	+	" "
1651	56	+	+	" "

From the above table it is seen that the salivary glands first become invaded 25 days after the infecting feed. In this case the monkey did not become infected. This may have been due to an accident, such as this particular fly not biting the monkey, or the invasion of the salivary glands only taking place after the fly had bitten, or to the fact that the blood-type of trypanosome was not present. With this exception all the other experiments, 12 in number, confirm the hypothesis that the fly does not become infective until the salivary glands are invaded.

Another point which comes out with striking clearness is, that in all the salivary glands from flies which gave a positive result, trypanosomes similar to the short and stumpy blood-type were invariably present.

It is to be hoped that before long the result of this work will be confirmed, and so added to, that the whole story of the development of the trypanosomes within the fly, and their passage into the salivary glands, will be unfolded.

Conclusions.

1. In the course of the development of *Trypanosoma gambiense* in *Glossina palpalis* the proboscis does not become involved, as in the case of some other species.

2. A few days after an infective feed the trypanosomes disappear out of the great majority of the flies, but in a small percentage this initial disappearance is followed by a renewed development.

3. After a very short time the flies which have fed on an infected animal become incapable of conveying the infection by their bites, and this non-infectivity lasts for some 28 days, when a renewed or late infectivity takes place.

4. A fly in which this renewed or late infectivity occurs can remain infective for at least 96 days.

5. An invasion of the salivary glands occurs at the same time as this renewal of infectivity, and without this invasion of the salivary glands there can be no infectivity.

6. The type of trypanosome found in the salivary glands when the fly becomes infective is similar to the short stumpy form found in vertebrate blood, and it is believed that this reversion to the blood-type is a *sine quâ non* in the infective process.

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Further Researches on the Development of Trypanosoma gambiense *in* Glossina palpalis.

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The object of these experiments was to try to discover if there is any definite cycle of development of the trypanosome of Sleeping Sickness in the tsetse fly, *Glossina palpalis*, and if the late or renewed infectivity of the fly coincides with any phase in this development.

The mode of experimentation was to feed a cageful of *laboratory-bred* tsetse flies on an animal whose blood contained numerous trypanosomes, and at the end of various times to kill the flies and examine their intestinal contents. This was done for periods of one day, two days, three days, and so on, up to 56 days. The microscopical examination of preparations made from the intestinal contents on the various days gave information as to the number and appearance of the trypanosomes.

After the infective feed or feeds the flies were fed every day on a healthy animal, so that by the appearance of trypanosomes in the animal's blood the day on which one or more of the flies became infective could be arrived at.

METHOD USED IN THE EXAMINATION OF THE FLIES.

The flies were killed by exposing them to the vapour of chloroform. After being killed the proboscis and pharynx were removed and examined under a cover-glass with the high and low powers. The terminal segment of the abdomen of the fly was then snipped off, and the whole abdominal viscera gently pressed out. This was moistened with a little normal saline solution, and the gut unravelled without rupturing. The proventriculus and crop were often pulled out intact with the gut. The whole thoracic and abdominal tract could then be laid out in line and examined under a low power.

In taking out the gut it was generally possible to draw out with it the abdominal portion of the salivary glands, which could then be separated without contamination from accidental rupture of the gut. If the salivary glands or proventriculus remained behind they were dissected out after removal of the gut. In every case these organs were thoroughly washed in

three changes of normal saline solution, in order to minimise the chance of their being contaminated by accidental rupture of the intestines.

The stained specimens were examined day after day, and coloured drawings, at a magnification of 2000 diameters, made of all the different forms met with. The drawings of the trypanosomes found in the proboscis, proventriculus, fore-gut, mid-gut, hind-gut, proctodæum, and salivary glands, were kept separate, so that a series of drawings of trypanosomes taken from any one part, from the first day of infection to the 56th day, could be compared.

By arranging these drawings on a wall the horizontal layers would represent the contents of, say, the fore-gut from one day onwards, while the vertical rows would represent the trypanosomes found in the whole length of a fly for one day. More than six hundred drawings were made in this way, so that it seems impossible that any important form can have been left out.

General Considerations regarding the Development of the Trypanosomes in the Fly.

Let us now take a general view of the types of trypanosomes found in the various parts. It is evident that very few of the six hundred drawings can be reproduced; a few types, taken here and there, must suffice. For the first three or four days trypanosomes are found in all the flies, but at the end of six or seven the trypanosomes have disappeared out of many of them. That is to say, it is only in a certain percentage that further development takes place. In one series this was 8 per cent. In 92 per cent., then, of flies which imbibe infected blood, the trypanosomes simply degenerate and die out within the first few days. In 8 per cent., on the other hand, the trypanosomes find conditions more favourable for development, and increase to a marvellous extent, filling the whole of the fore-gut, mid-gut, and hind-gut with countless swarms of trypanosomes.

How long this development continues is unknown. It is considered probable that it continues for the remainder of the fly's life, and this has been proved by experiment up to 96 days.

What the conditions in the intestine of the fly are, which render this development possible, are also unknown. It was thought that it might depend on the reaction of the intestinal fluids. This is, however, found on examination to be faintly acid in all flies, whether development has taken place or not. The presence of bacteria also seems to have no connection with the phenomenon. Sex, moreover, has no influence; development occurs in as many males as females.

Types of Trypanosoma gambiense found in the Alimentary Canal.

It would serve no good purpose to describe separately, day by day, the types found in the various parts of the alimentary canal, as they run into each other in such a way that any classification of them seems impossible.

The following table represents, approximately, the numbers found in the different parts of the alimentary canal at various times after infection. The − sign means that an examination was made and nothing found. ± means few. +, many. + +, very many. + + +, swarming. If no sign, then no examination has been made. (See p. 516.)

The Proboscis.—In our experience *Trypanosoma gambiense* is never found in the proboscis of *Glossina palpalis*, except immediately after an infected feed, when for a short time blood containing trypanosomes may be seen in the lumen of the proboscis. This is very different from what obtains in an infection by *Trypanosoma vivax*, in which case the proboscis is alone infected.

Proventriculus.—As seen from the above table, this part of the alimentary canal is sometimes found empty when the remainder of the gut is swarming.

Fore-, Mid-, and Hind-gut.—It is here that the greatest development of the trypanosomes is found. Among the extraordinary numbers and diversity of type it is difficult or impossible to find one's way. Generally speaking, the trypanosomes found during the first few days are merely degenerating blood forms. After this there appears a type of trypanosome which remains dominant throughout the whole developmental period. 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 trypanosomes.

This seems to be the healthy normal developing type in the intestine of the fly. It is seen in all parts of the intestine and at all times. It forms masses of innumerable individuals alike in size and shape. When a fresh supply of blood is taken in by the fly this 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 host of diverse forms which thus arises beggars description. Some are round or oval in shape, 3 or 4 microns in diameter, with or without a flagellum. From this simple form all shapes and sizes can be seen up to the huge shapeless mass of protoplasm, multi-nucleated and multi-flagellated.

Table I.—Number of Trypanosomes found in the Various Parts of the Alimentary Canal and Salivary Glands.

Time, days.	Proboscis.	Proventriculus.	Crop.	General smear of gut.	Fore-gut.	Mid-gut.	Hind-gut.	Procto-dæum.	Salivary glands.
1	−	−				+ +			−
1	−	−				+	+		−
2	−				+	+	+ +		−
3	−					+ +	+ +		
4	−				+	+	+	−	−
5	−	−	−		−	−	−		−
6	−	−	−	+	+	+			−
7	−	−	−	+					−
8	−	+			+	+ +	+ +		−
8	−				±	±	±		
9	−	−			±	+ +		−	−
9	−				+	+	+		
10	−				+	+ +	+ +		
10	−					+			
11	−	±	−	+ +	−	±	±		−
11	−	±	−	+ +	+ +	±			−
11	−	+ +			+ +	+ +	+ +		−
14	−	+ + +	−		+ +	+ +		−	−
14	−	+ +	−		+ +	+ +	+ +		−
15	−	−			+	+			−
17	−	−	−		±	+ +	+ +		−
17	−	+ +		−	+ +	+ +			−
18	−	−			+ +	+	+ +		−
18	−					+ +	+ +		−
20	−	+ +			+ +	+ +	+ +	±	−
21	−			+ + +					
22	−			+ +					
23	−				+	+	+		
24	−				+ + +	+ + +	+ + +		
25	−	−	−		+	+ + +	+ +		+
28	−			+ +		+ +			+
30	−	+			+ +	+ +			+
30	−	+			+ +	+ +	±		−
31	−	+	+		+ + +	+ +			−
31	−			+					
34	−			+ +	+ +	+ + +	+ + +	−	−
35	−	−			+	+ +	+ +		−
36	−			+ + +	+ + +	+ + +	+		+
36	−	−		+ + +	+ +	+ + +			−
36	−	+ +		+ + +					
37	−			+ +					
40	−			+ +					
40	−			+ +					
42	−	+ +		+	+ +				+
43	−			+ +					+ +
44	−	+			+ +	+ + +			+
44	−				+	+ +	+ +		+
44	−	+			+	+	+		+
46	−	+			+ + +	+ + +	+ + +		+ +
49	−	−	−		+	±			−
51	−		−		+	+	+ +		−
53	−	−			±	±			−
53	−	−			+	+ +			+
53	−		−			±			−
56	−			+ +					+

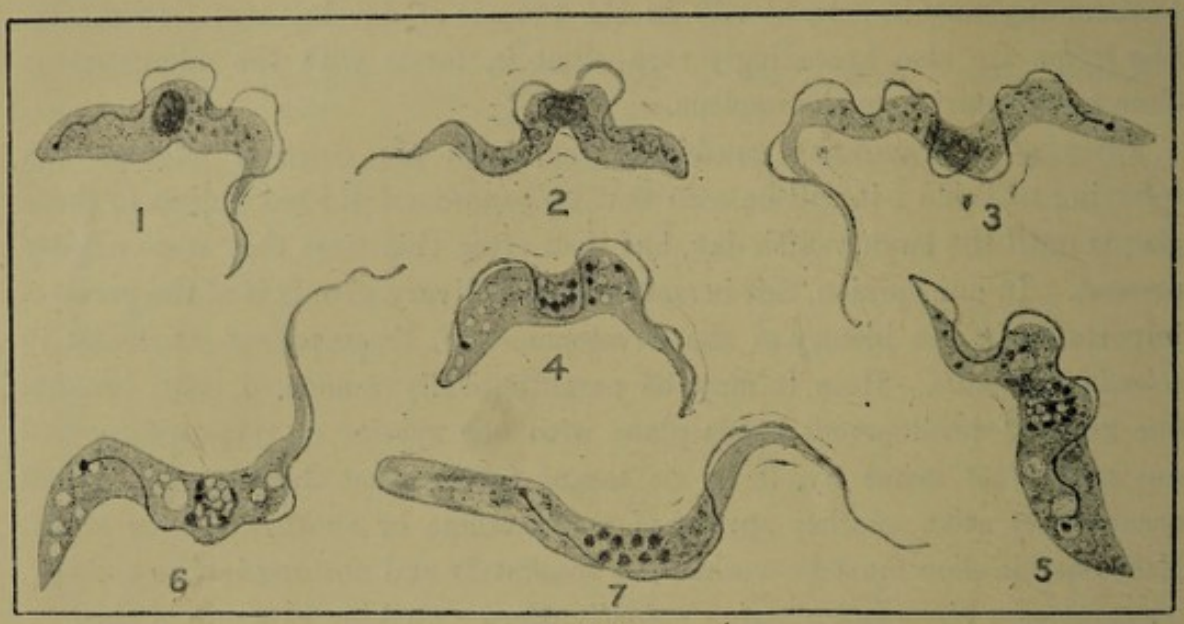
In our experience, the long narrow forms, described by some observers as "male" forms, are exceedingly rare, and it seems impossible to ascribe to to them any very important *rôle* in the process of development. Crithidia-like forms are also exceedingly rare—that is, forms with the micronucleus close to or anterior to the nucleus.

Types of Trypanosoma gambiense *found in the Salivary Glands.*—On referring to Table I it will be seen that trypanosomes did not appear in these glands until the twenty-fifth day, but that after this time they were usually present. In our opinion, this invasion of the salivary glands is of the greatest importance in the history of the development of *Trypanosoma gambiense* in *Glossina palpalis.* Here it may be parenthetically remarked that, because one kind of development takes place with one species of trypanosome and one species of tsetse fly, it by no means follows that the same thing will occur either with another species of trypanosome or another species of fly. Each combination must be worked out separately and nothing left to analogy. *Trypanosoma vivax* and *Glossina palpalis* afford a striking example of this.

In the development of *Trypanosoma gambiense* in *Glossina palpalis* one circumstance, which we think of prime importance, emerges, and that is, that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal blood-type. It must not be imagined, however, that the salivary glands show no other forms but this blood-type. On the contrary, there are many other forms seen; but here only are found trypanosomes apparently identical with the short and stumpy forms found in the blood. What causes or leads up to this reversion to the blood-type in the salivary glands is quite unknown, but, as will be seen later, the *Glossina palpalis* does not become infective by biting until this invasion of the salivary glands takes place.

How the trypanosomes find their way into the salivary glands is also quite unknown. It seems highly improbable that they pass from the alimentary canal by way of the salivary duct, and as they are never found in the body-cavity, it is also difficult to see how they can make their way directly from the intestine to the abdominal portion of the salivary glands.

ILLUSTRATIONS OF VARIOUS MODIFICATIONS IN SHAPE OF TRYPANOSOMA GAMBIENSE IN GLOSSINA PALPALIS.



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FIGS. 1—3.—Normal blood parasites (*Trypanosoma gambiense*).
FIG. 4.—24 hours after ingestion by the fly.
FIGS. 5 and 6.—48 hours after ingestion by the fly.
FIG. 7.—96 hours after ingestion by the fly.

Figs. 1—7 represent the trypanosomes as they appear in the intestine of *Glossina palpalis* during the first few days. Figs. 1—3 are ordinary blood forms, as seen immediately after the fly has fed, and before any change has taken place. Figs. 4—7 represent the process of degeneration which takes place during the first four days. The body swells up, the nucleus breaks up, and the cytoplasm becomes vacuolated.

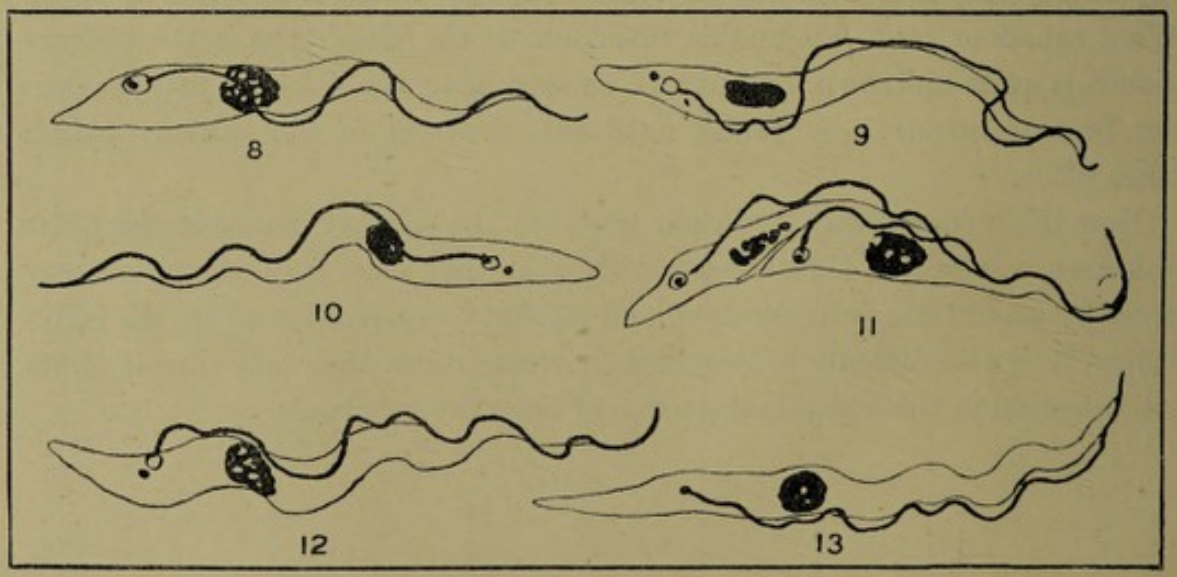

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FIG. 8.—*Trypanosoma gambiense* from fore-gut, 8 days after infected feed.
FIG. 9.—*T. gambiense* from proventriculus, 14 days after infected feed.
FIG. 10.—*T. gambiense* from fore-gut, 18 days after infected feed.
FIG. 11.—*T. gambiense* from mid-gut, 25 days after infected feed.
FIG. 12.—*T. gambiense* from mid-gut, 34 days after infected feed.
FIG. 13.—*T. gambiense* from fore-gut, 44 days after infected feed.

Figs. 8—13 represent what we consider to be the normal reproductive or developing type found throughout the intestine during the whole period of development. It is to be noted that this form is longer and broader than the normal broad form; the protoplasm is clear, and stains readily and evenly, and this cell looks normal. The nucleus is compact and situated nearer the posterior extremity than the anterior. The micronucleus is small and round, lying at some distance from the elongated posterior extremity. Many dividing forms of this type can be seen. In our opinion, this is the common multiplying form, and from it arises an infinite variety of degenerating forms.

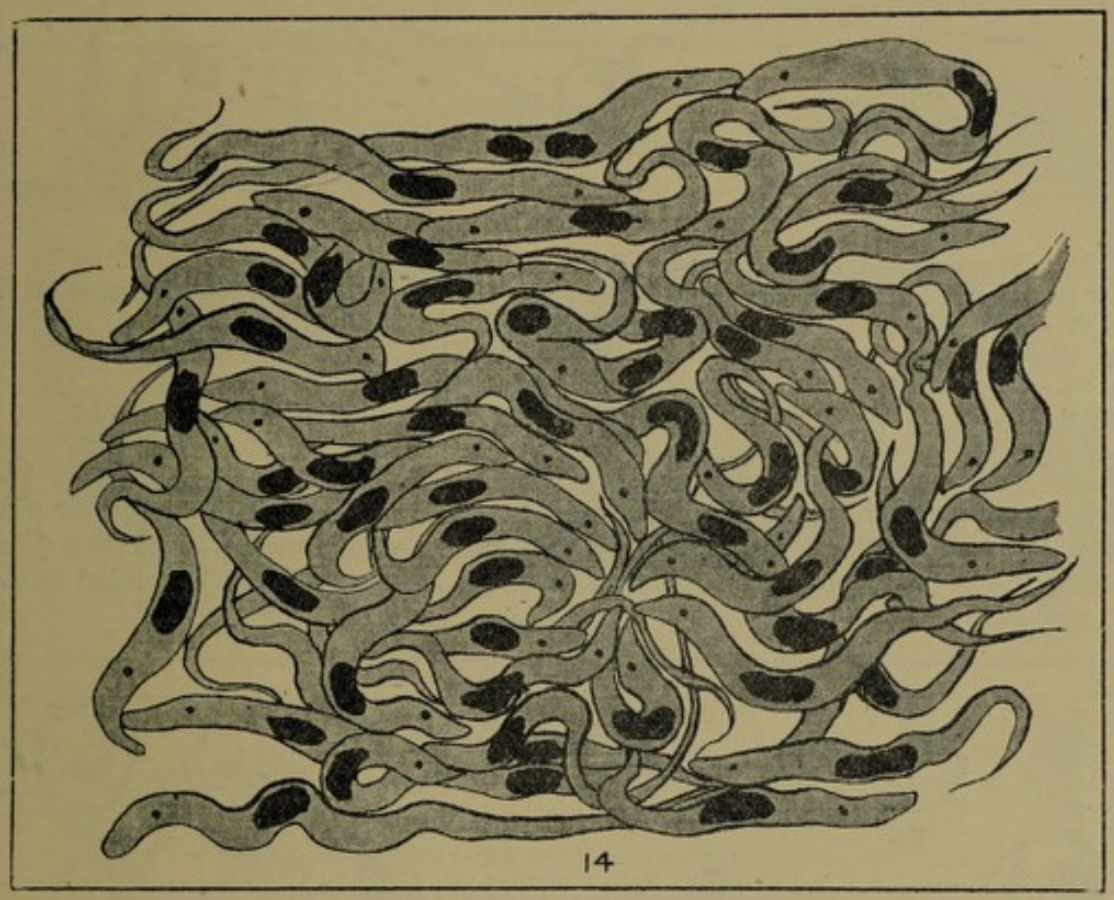

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Fig. 14.—Part of a mass of *Trypanosoma gambiense* from the mid-gut.

This figure is given in order to try to convey some idea of the enormous proliferation of trypanosomes which takes place in the intestine of *Glossina palpalis*. Throughout the whole length of the alimentary canal, from the proventriculus to the proctodæum, this condition is frequently seen, and in the living condition presents an extraordinary spectacle. Looked at through the wall of the intestine the matted masses are seen in active movement, swaying about and wriggling in every direction. They do not appear to be attached to the wall of the intestine, but move about freely, and when the intestinal wall is burst pour out in countless numbers. The trypanosomes evidently belong to the type figured in the preceding sketch, called the normal reproductive type.

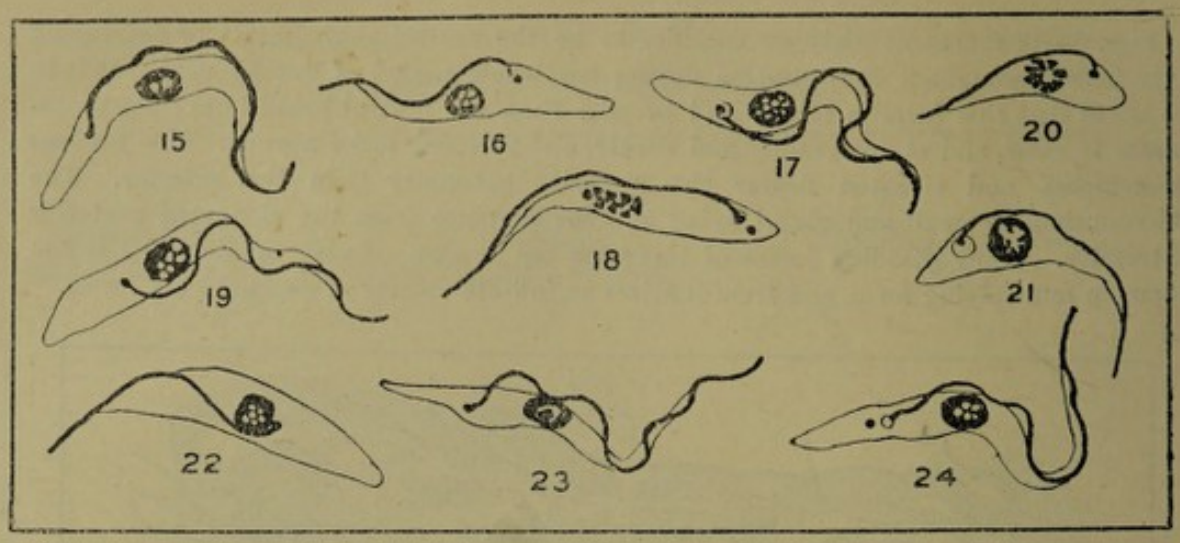

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Fig. 15.—*Trypanosoma gambiense* from hind-gut, 8 days after infected feed.
Figs. 16, 17, and 18.—*T. gambiense* from mid-gut, 14, 20, and 30 days after infected feed.
Fig. 19.—*T. gambiense* from hind-gut, 34 days after infected feed.
Figs. 20 and 21.—*T. gambiense* from proventriculus, 36 days after infected feed.
Fig. 22.—*T. gambiense* from hind-gut, 44 days after infected feed.
Figs. 23 and 24.—*T. gambiense* from mid-gut, 46 and 53 days after infected feed.

Figs. 15—24 represent smaller forms which are fairly common and occur throughout the intestine and at all times. The examples given above are taken from the eighth day to the fifty-third day. They are called by us "small developmental forms," since they resemble the larger in having clear protoplasm and a compact nucleus. Dividing forms are often seen.

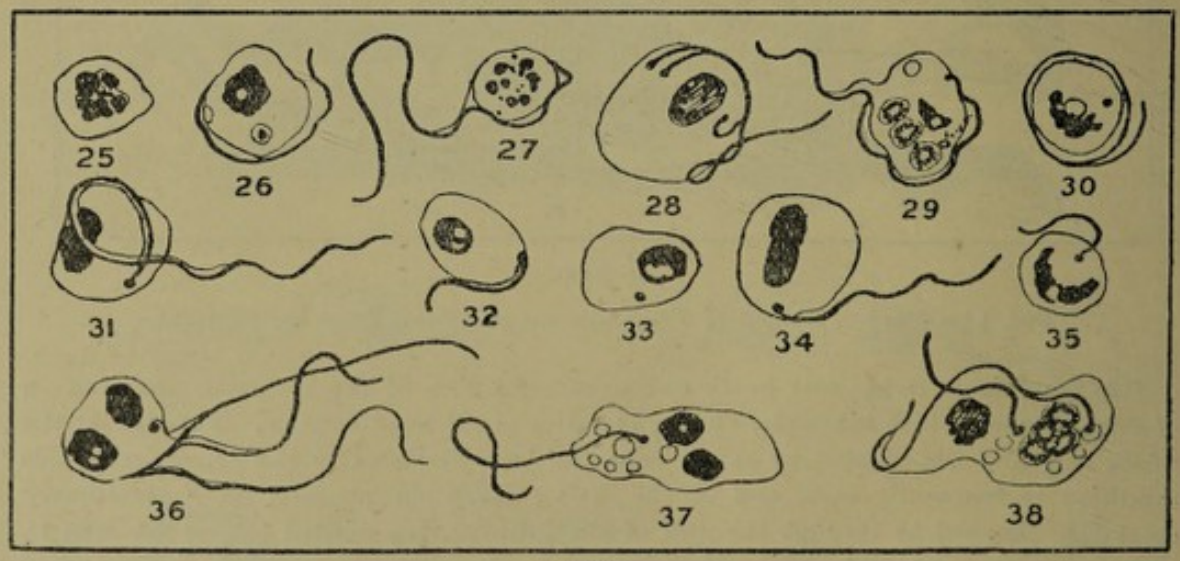

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Fig. 25.—*Trypanosoma gambiense* from mid-gut, 8 days after infected feed.
Fig. 26.—*T. gambiense* from fore-gut, 11 days after infected feed.
Figs. 27 and 28.—*T. gambiense* from mid-gut, 16 and 17 days after infected feed.
Figs. 29, 30, and 31.—*T. gambiense* from fore-gut, 17 and 18 days after infected feed.
Fig. 32.—*T. gambiense* from hind-gut, 20 days after infected feed.
Figs. 33 and 34.—*T. gambiense* from fore-gut, 24 days after infected feed.
Figs. 35, 36, and 37.—*T. gambiense* from mid-gut, 24, 44, and 46 days after infected feed.
Fig. 38.—*T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 25—38 represent round and irregularly-shaped forms of the parasite, taken from the eighth to the forty-sixth day of development. It is impossible to say what is exactly the origin of these forms—whether by the segmentation of large masses, or simply by the division and subdivision of irregular forms. Some of the examples figured are evidently dividing, as they show several nuclei and flagella. Whether the round aflagellar forms correspond to the so-called "latent" forms of various writers it is impossible to say. Those possessing flagella were active during life. In our opinion, they may be looked upon as part of the degenerative processes which are constantly taking place in the intestine of the fly.

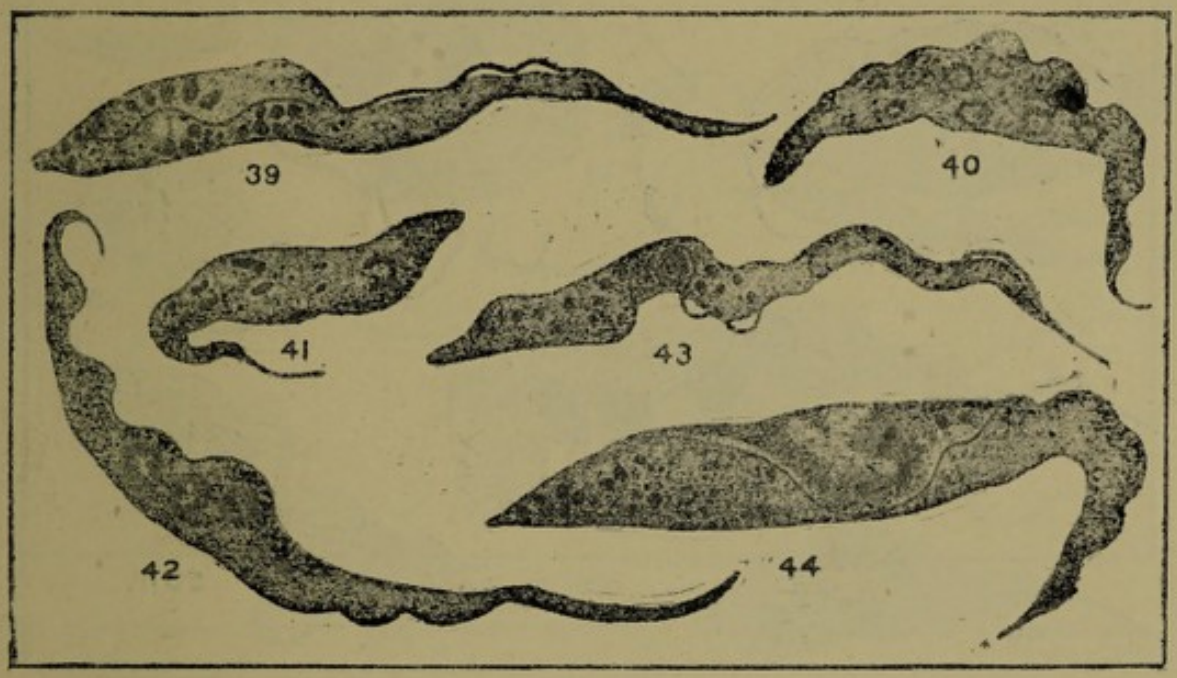

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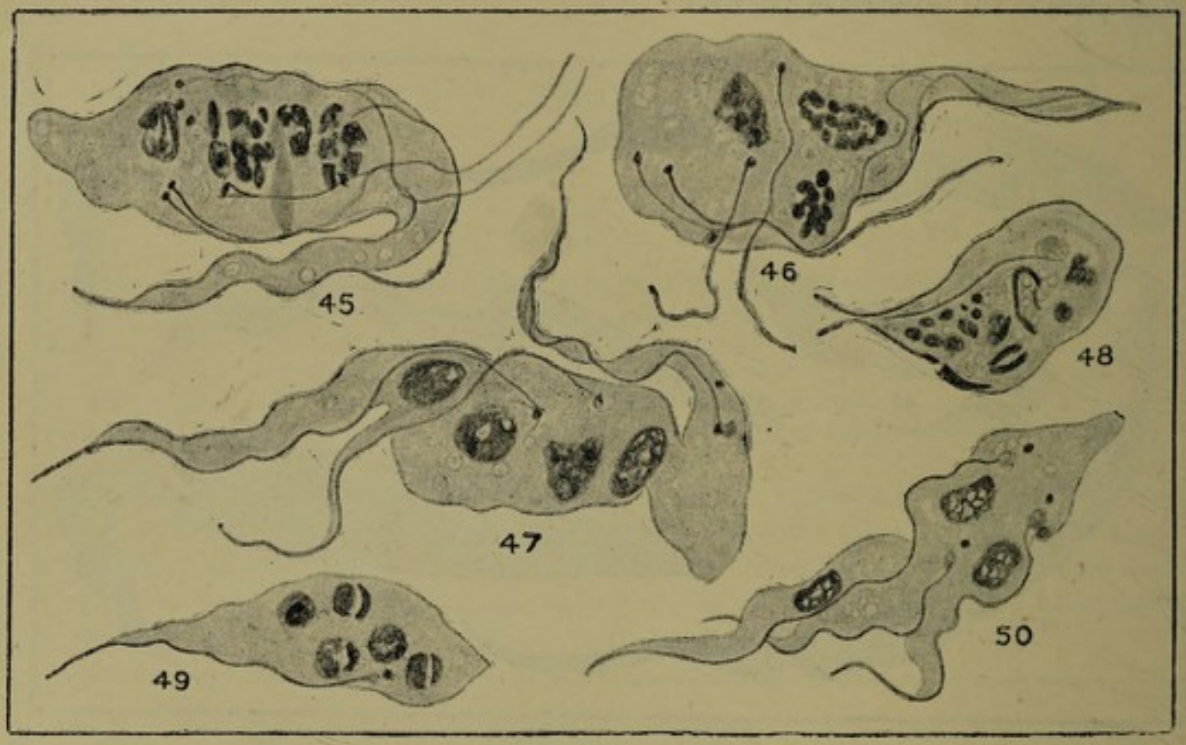
Fig. 39.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 40.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 41.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 42.—*T. gambiense* from hind-gut, 34 days after infected feed.
Fig. 43.—*T. gambiense* from mid-gut, 34 days after infected feed.
Fig. 44.—*T. gambiense* from hind-gut, 46 days after infected feed.

Figs. 39—44 represent what appear to us to be degenerative forms of the "normal reproductive type." They are of all sizes and shapes, and the cell-contents are scattered over with broken-up nuclei, or at least granules of some stainable substance. Figs. 39, 43, and 44 are also vacuolated.



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Figs. 45, 46, and 47.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 48.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 49.—*T. gambiense* from proventriculus, 30 days after infected feed.
Fig. 50.—*T. gambiense* from mid-gut, 46 days after infected feed.

Figs. 45—50 represent some of the more exaggerated types of degenerative forms. As will be seen from the drawings, they are huge, mis-shaped masses of protoplasm, multi-nucleated and, as a rule, multi-flagellated.

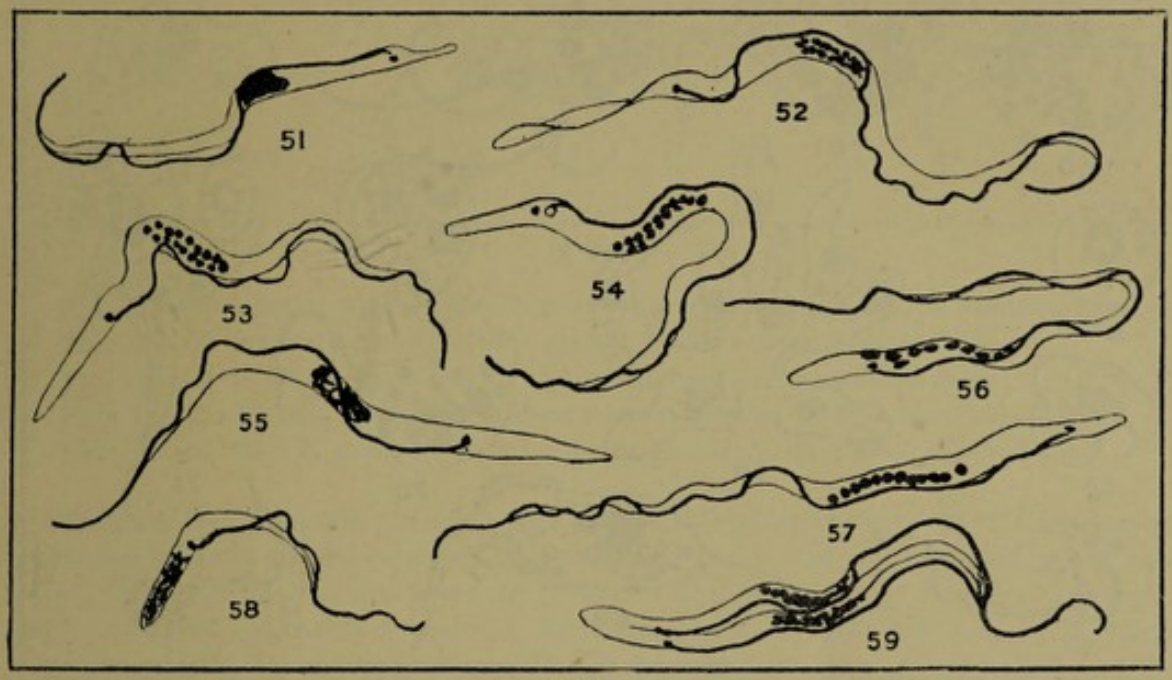

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Fig. 51.—Slender type of *Trypanosoma gambiense* from proventriculus, 17 days after infected feed.

Fig. 52.—Slender type of *T. gambiense* from mid-gut, 17 days after infected feed.

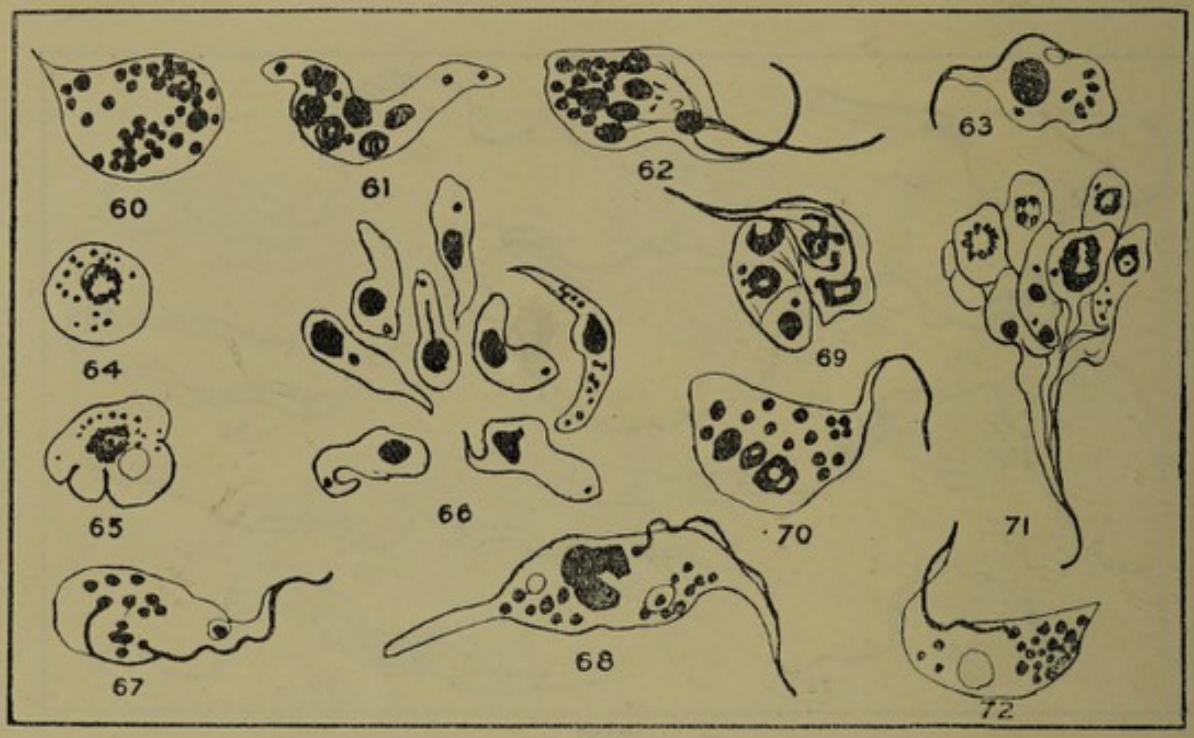
Fig. 53.—Slender type of *T. gambiense* from hind-gut, 20 days after infected feed.

Figs. 54 and 55.—Slender types of *T. gambiense* from fore-gut, 24 and 30 days after infected feed.

Figs. 56 and 57.—Slender types of *T. gambiense* from proventriculus, 44 days after infected feed.

Figs. 58 and 59.—Slender types of *T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 51—59 represent various varieties of the slender type of *Trypanosoma gambiense* found in the intestine of *Glossina palpalis*. Fig. 59 is dividing. Fig. 58 is a Crithidia type. These slender and crithidial types are uncommon, and no special connection between them and the onset of infectivity in the fly has been made out



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Figs. 60 and 61.—*Trypanosoma gambiense* from salivary glands, 25 days after infected feed.

Fig. 62.—*T. gambiense* from salivary glands, 30 days after infected feed.

Fig. 63.—*T. gambiense* from salivary glands, 42 days after infected feed.

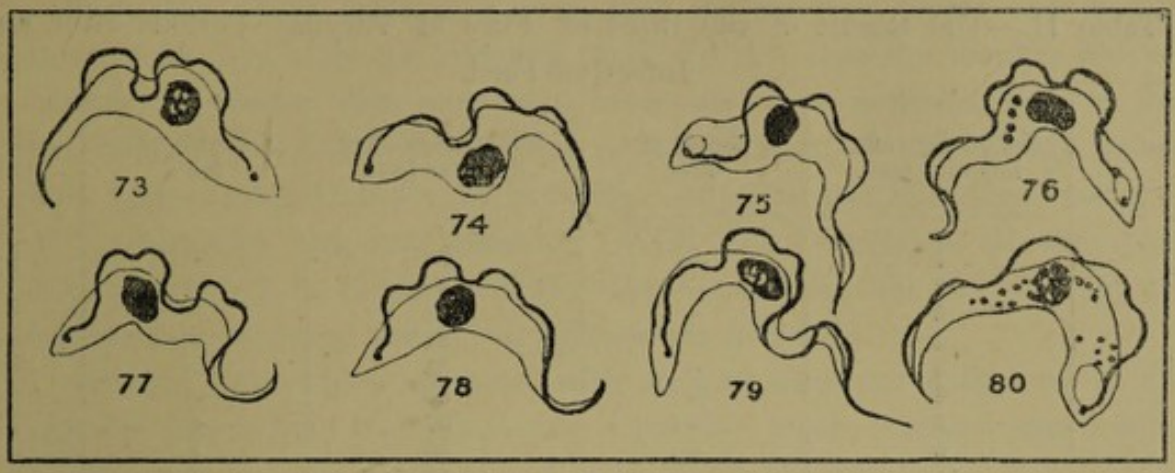
Figs. 64, 65 and 66.—*T. gambiense* from salivary glands, 44 days after infected feed.

Figs. 67 and 68.—*T. gambiense* from salivary glands, 46 days after infected feed.

Figs. 69 and 70.—*T. gambiense* from salivary glands, 53 days after infected feed.

Figs. 71 and 72.—*T. gambiense* from salivary glands, 56 days after infected feed.

Figs. 60—72 represent aberrant forms of *Trypanosoma gambiense* seen in the salivary glands. The first thing to be noted is the fact that the trypanosomes do not appear in the salivary glands until a late date ; in this case the earliest appearance is 25 days after the infected feed. How they reach these glands is up to the present unknown. In what shape they reach the glands is also unknown. Figs. 64, 65, 69 and 71 might be said to point to some process of segmentation, of which the finished product is represented by fig. 66.



× 2000.

Fig. 73.—*Trypanosoma gambiense* from salivary glands, 34 days after infected feed.
Fig. 74.—*T. gambiense* from salivary glands, 42 days after infected feed.
Fig. 75.—*T. gambiense* from salivary glands, 43 days after infected feed.
Figs. 76, 77, and 78.—*T. gambiense* from salivary glands, 46 days after infected feed.
Figs. 79 and 80.—*T. gambiense* from salivary glands, 56 days after infected feed.

When alluding, generally, in a previous part of this paper to the types of *Trypanosoma gambiense* found in the salivary glands, it was said that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal type found in the blood. Figs. 73—80 illustrate this reversion. By comparing them with figs. 1, 2, and 3, which represent normal blood trypanosomes, it will be seen that they are very similar to the short and stumpy form found in the blood. No such forms have ever been seen in any other part of the fly, and we would suggest that the occurrence of these forms in the salivary glands, coinciding as it does with the renewed infectivity of the fly, is more than a mere coincidence.

Infectivity of Trypanosoma gambiense after its Development in Glossina palpalis, as shown by the Bites of the Flies giving rise to the Disease in Healthy Animals.

As has been shown in a previous paper,* the fly ceases to be infective by biting within a short time of its infective feed. From this time on for some 28 days the fly remains non-infective. Table II (p. 526) illustrates this.

From that Table it will be seen that in this series of experiments the flies first became infective 28 days after the infective feed, and that after this time the flies are usually found to be capable of giving rise to the disease by their bites.

It was stated above that the most important discovery made in this research is the connection between the invasion of the salivary glands and the infectivity of the fly. That this appears to be so is shown in Table III.

* 'Roy. Soc. Proc.,' B, 1911, vol. 82, p. 498.

Table II.—The Result of the Bites of Flies at Varying Periods after an Infective Feed.

No. of days after infective feed.	Result of bites.	No. of days after infective feed.	Result of bites.
1	–	28	+
2	–	30	–
3	–	31	–
4	–	34	+
5	–	35	–
6	–	36	–
8	–	37	+
9	–	40	+
11	–	40	+
14	–	42	+
15	–	43	+
17	–	44	+
18	–	46	+
20	–	51	–
23	–	53	+
25	–	56	+

Table III.—To show Correlation between the Invasion of the Salivary Glands of *Glossina palpalis* by *Trypanosoma gambiense* and Infection by the Bite of the Fly.

Experiment No.	No of days after infective feed.	Bites of fly infective or non-infective.	Salivary glands.	Remarks.
1910	1	–	–	
1910	2	–	–	
1910	3	–	–	
1910	4	–	–	
1910	5	–	–	
1910	6	–	–	
1910	7	–	–	
1894	8	–	–	
1894	9	–	–	
2216	10	–	–	
1894	11	–	–	
1871	14	–	–	
1693	15	–	–	
1945	17	–	–	
1945	18	–	–	
1945	20	–	–	
1718	25	–	+	Blood-type not present.
1602	28	+	+	Blood-type present.
1801	30	–	–	
1945	31	–	–	
1760	34	+	+	" "
1769	36	–	–	
1712	42	+	+	" "
2034	43	+	+	" "
1549	44	+	+	" "
2034	44	+	+	" "
1706	51	–	–	
1566	53	+	+	" "
1651	56	+	+	" "

From the above table it is seen that the salivary glands first become invaded 25 days after the infecting feed. In this case the monkey did not become infected. This may have been due to an accident, such as this particular fly not biting the monkey, or the invasion of the salivary glands only taking place after the fly had bitten, or to the fact that the blood-type of trypanosome was not present. With this exception all the other experiments, 12 in number, confirm the hypothesis that the fly does not become infective until the salivary glands are invaded.

Another point which comes out with striking clearness is, that in all the salivary glands from flies which gave a positive result, trypanosomes similar to the short and stumpy blood-type were invariably present.

It is to be hoped that before long the result of this work will be confirmed, and so added to, that the whole story of the development of the trypanosomes within the fly, and their passage into the salivary glands, will be unfolded.

Conclusions.

1. In the course of the development of *Trypanosoma gambiense* in *Glossina palpalis* the proboscis does not become involved, as in the case of some other species.

2. A few days after an infective feed the trypanosomes disappear out of the great majority of the flies, but in a small percentage this initial disappearance is followed by a renewed development.

3. After a very short time the flies which have fed on an infected animal become incapable of conveying the infection by their bites, and this non-infectivity lasts for some 28 days, when a renewed or late infectivity takes place.

4. A fly in which this renewed or late infectivity occurs can remain infective for at least 96 days.

5. An invasion of the salivary glands occurs at the same time as this renewal of infectivity, and without this invasion of the salivary glands there can be no infectivity.

6. The type of trypanosome found in the salivary glands when the fly becomes infective is similar to the short stumpy form found in vertebrate blood, and it is believed that this reversion to the blood-type is a *sine quâ non* in the infective process.

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4

Experiments to Ascertain if Trypanosoma gambiense *during its Development within* Glossina palpalis *is Infective.*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C.; and Captain F. P. MACKIE, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

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It will be remembered that the injection of chopped-up tsetse flies (*Glossina morsitans*) a short time after feeding on an infected animal, did not give rise to nagana. As this was thought to be an interesting fact, and difficult of explanation, experiments were carried out on the same lines with *Trypanosoma gambiense* and *Glossina palpalis.*

If tsetse flies (*Glossina palpalis*) are fed upon an animal whose blood contains *Trypanosoma gambiense*, the trypanosomes can be found living within the intestines of some of the flies for several days after they were ingested. In a small percentage (0·5 to 2·0 per cent.) of flies so infected, active trypanosomes may be found swarming in their intestines on any day between the seventh and the fiftieth day, or even longer, after they have been fed upon an infected animal.

A series of experiments were undertaken, to ascertain if *Trypanosoma gambiense* retained its power of causing Sleeping Sickness when inoculated, subcutaneously, into monkeys, throughout its period of multiplication within the fly, especially during the interval of some 20 days in which the bites of infected flies are harmless. In some of these experiments Lake-shore flies were used; in others, laboratory-bred flies.

The flies were fed upon a monkey whose blood contained many trypanosomes. After a pre-determined time had elapsed, the wings and legs of the infected flies were cut off, and the bodies were either chopped up and brayed in a mortar with saline solution (0·8 per cent.), or the alimentary canal alone was removed. In many instances the gut was proved by microscopical examination to be heavily infected with trypanosomes before it was inoculated into a monkey. In flies thus found to be infected, the salivary glands also were carefully removed and washed thoroughly in several changes of normal saline solution. They were then broken up and injected, by means of a sterile syringe, into the subcutaneous tissue of the groin of a monkey.

The following table shows the result of the individual experiments as carried out with Lake-shore flies:—

Table I.—*Glossina palpalis* and *Trypanosoma gambiense.*

Date.	Expt. No.	No. of hours after infected feed.	No. of flies injected.	Result.	Incubation period in monkey.
1909.					
Mar. 10	521	½ hour	2	+	8 days.
Jan. 6	420	¾ „	1	−	
„ 6	421	1 „	2	−	
Feb. 18	518	1 „	2	−	
Mar. 19	604	1 „	2	+	6 „
„ 19	605	1½ hours	2	+	6 „
Feb. 18	519	2 „	2	−	
June 22	1038	3 „	1	+	6 „
„ 22	1043	3 „	1	+	6 „
Mar. 29	622	4 „	8	+	7 „
June 11	984	4 „	5	+	8 „
„ 16	1009	4 „	5	+	6 „
„ 22	1039	6 „	1	+	6 „
„ 22	1044	6 „	1	+	6 „
„ 22	1045	7 „	1	+	9 „
„ 22	1041	8 „	1	+	9 „
„ 22	1046	8 „	1	+	10 „
„ 22	1042	9 „	1	+	9 „
„ 22	1047	9 „	1	+	9 „
„ 26	1154	16 „	1	−	
„ 26	1155	16 „	1	−	
„ 26	1156	17 „	1	+	13 „
„ 26	1157	17 „	1	−	
„ 26	1158	18 „	1	+	9 „
„ 26	1159	18 „	1	+	13 „
Mar. 20	600	19 „	5	−	
June 26	1161	19 „	1	−	
Mar. 4	524	23 „	6	−	
„ 5	585	44 „	6	−	

Remarks.—Twenty-nine experiments were carried out with Lake-shore flies and *Trypanosoma gambiense.* Eighteen of these experiments yielded a positive result, and were almost uniformly successful up to 18 hours after the infected feed. It seemed to be immaterial whether the fly was introduced whole or whether the alimentary canal alone was injected. In several instances where the gut of the fly was examined in the fresh state, swarms of flagellates natural to the fly, *i.e. Trypanosoma grayi*, were found, but their presence did not appear to modify the virulence of *Trypanosoma gambiense.*

The following table shows the result of the experiments as carried out with laboratory-bred flies:—

Table II.—*Glossina palpalis* and *Trypanosoma gambiense.*

Date.	Expt. No.	No. of hours or days after infected feed.	No. of flies injected.	Result.	Incubation period in monkey.
1909.					
Aug. 24	1545	1 hour	1	+	7 days
„ 24	1546	2 hours	1	+	7 „
Feb. 18	520	4 „	2	−	
Aug. 24	1547	4 „	1	+	7 „
Sept. 14	1681	1 day	3	−	
„ 23	1756	2 days	3	+	12 „
„ 26	1723	3 „	15	−	
Oct. 2	1713	4 „	10	−	
„ 3	1783	5 „	11	−	
„ 4	1752	6 „	10	−	
*Sept. 28	1721	7 „	1	−	
*Nov. 16	1906	8 „	2	−	
Oct. 7	1788	9 „	10	−	
*Nov. 17	1909	9 „	1	−	
Oct. 8	1795	10 „	8	−	
„ 9	1799	10 „	8	−	
*Nov. 18	1911	10 „	1	−	
* „ 19	1913	11 „	2	−	
* „ 29	1923	11 „	1	−	
Oct. 11	1807	13 „	5	−	
Nov. 26	1864	14 „	30	−	
*Nov. 15	1905	14 „	2	−	
*Oct. 3	1781	15 „	1	−	
„ 27	1865	15 „	12	−	
„ 19	1838	16 „	34	−	
„ 29	1872	17 „	12	−	
*Dec. 13	1939	17 „	1	−	
Oct. 30	1873	18 „	12	−	
*Dec. 14	1940	18 „	2	−	
Nov. 1	1877	20 „	12	−	
* „ 16	1951	20 „	1	−	
Nov. 2	1879	21 „	20	−	
„ 4	1886	23 „	15	−	
Dec. 5	1887	24 „	10	−	
* „ 20	1958	24 „	1	+	8 „
„ 6	1889	25 „	22	−	
*Sept. 28	1749	28 „	1	−	
* „ 29	1759	28 „	1	+	11 „
*Nov. 9	1866	30 „	1	+	12 „
„ 4	1884	34 „	1	−	
* „ 11	1897	36 „	2	+	5 „
* „ 16	1907	37 „	1	−	
* „ 4	1885	42 „	1	−	
*Oct. 8	1791	46 „	2	+	9 „
*Nov. 3	1881	49 „	1	−	
* „ 11	1898	51 „	1	−	
* „ 4	1856	53 „	1	−	

Remarks.—In these experiments the intestines only of the infected flies were inoculated. In the experiments marked with an asterisk the gut was first proved, by microscopical examination, to contain swarms of living *Trypanosoma gambiense*, and then immediately injected into a monkey. It will be seen there are 47 experiments in which a total of 296 laboratory-bred flies were inoculated, subcutaneously, into monkeys at various intervals after the flies had fed upon blood infected with *Trypanosoma gambiense*. The injection into a monkey of a single fly, one, two, and four hours after an infected feed, caused Sleeping Sickness. Three flies inoculated two days after their infected feed produced a like result. Between the 2nd and the 24th days

after the flies had fed on infected blood, 249 of them were inoculated; the result was negative in every case, although 15 of these flies proved, by microscopical examination, to be swarming with living *Trypanosoma gambiense* at the time of inoculation. In 13 experiments, 14 flies proved, microscopically, to be infected, were inoculated, between the 24th and 53rd days after they had fed upon an infected animal, and in five of these a positive result was obtained.

The following table shows the result of subcutaneous injections of washed salivary glands dissected from laboratory-bred flies whose intestines contained swarms of *Trypanosoma gambiense*:—

Table III.—*Glossina palpalis* (Salivary Glands) and *Trypanosoma gambiense.*

Date.	Expt. No.	No. of days after infected feed.	No. of flies from which glands injected.	Result.	Incubation period in monkey.
1910.					
Apr. 14	2376	2 days	3	–	
„ 15	2376	3 „	1	–	
„ 17	2376	6 „	2	–	
„ 18	2376	7 „	3	–	
„ 22	2394	8 „	2	–	
„ 25	2394	11 „	2	–	
Jan. 26	2099	14 „	2	–	
„ 31	2114	21 „	1	–	
Feb. 2	2114	23 „	1	–	
„ 7	2148	28 „	1	–	
„ 9	2148	30 „	1	–	
„ 13	2177	36 „	4	+	8 days.

Remarks.—The results of the microscopical examination of the salivary glands show that trypanosomes do not appear in the glands until about the 30th day. Out of 12 experiments 11 were negative, and one was positive. The glands from 19 infected flies were injected subcutaneously, between the 2nd and 30th days after the flies had fed upon an infected animal; the results were all negative. In the positive experiment the salivary glands from 4 infected flies were injected into a healthy monkey 36 days after the flies had fed on a monkey suffering from Sleeping Sickness, with the result that the former animal contracted the disease.

Conclusions.

1. *Trypanosoma gambiense* may retain their virulence, as ascertained by direct inoculation into susceptible animals, for a period of two days after they are ingested by *Glossina palpalis.*

2. After the trypanosomes have been within the gut of the fly for two days the power of infecting animals with Sleeping Sickness, when inoculated subcutaneously, is lost for a period of 22 days.

3. *Trypanosoma gambiense* regains the power of infecting by direct inoculation after it has been about 24 days within the intestine of the fly.

4. The number of days, during which the virulence of the trypanosomes contained in the fly is lost, roughly coincides with the time that the infected fly is incapable of transmitting Sleeping Sickness by biting susceptible animals.

5. There is some evidence that the salivary glands of the fly are invaded by virulent forms of the parasite 36 days after the fly has fed upon infected blood.

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Experiments to Investigate the Infectivity of Glossina palpalis *Fed on Sleeping Sickness Patients under Treatment.*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society Uganda, 1908–10); and Dr. R. VAN SOMEREN, Uganda Medical Staff.

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Introduction.

It is well known that there are cases of Sleeping Sickness in man which, under treatment, may enjoy long periods of apparent good health. In these cases the individual is often able to live an active life, and insists on being at liberty to go about as he pleases. In Uganda care is taken to prevent such cases entering the fly-areas; but the difficulty in maintaining a supervision strict enough to check occasional visits to these areas is obviously great, and in other countries may be insuperable, so that the question as to how drug treatment can influence the infectivity of these patients to the fly may be considered to be of importance.

Other points arise from the consideration of this question, viz.:—Can one or more doses of a trypanocidal drug render a Sleeping Sickness patient non-infective to *Glossina palpalis?* Does prolonged treatment during any stage of the disease render cases of Sleeping Sickness innocuous to the fly? If it be proved that the treatment of Sleeping Sickness patients by certain drugs does not prevent them infecting *Glossina palpalis*, then what percentage of cases give a positive result? and what percentage of flies are infected from these cases, as compared with the number of flies infected from untreated cases?

To answer these questions it is necessary to classify under various headings the patients who are the subjects of this paper; to state whether treated or untreated; if the latter, to give details concerning the nature of the treatment, the duration of treatment, and the total quantity of drugs received. The presence or absence of *Trypanosoma gambiense* in the blood or lymph glands of each patient should also be noted.

The classification of patients, according to the stage of the disease in which they present themselves, is that adopted by Dr. A. D. P. Hodges, C.M.G., Principal Medical Officer, Uganda, in the "Progress Report on the Uganda Sleeping Sickness Camps." from December, 1906, to November 30, 1908.

Class A.—Those who are apparently in good health: gland enlargement with trypanosomes present in the glands.

Class B.—Those in whom there are early signs from which the existence of Sleeping Sickness may be reasonably suspected.

*Class C.**—Those in whom clinical signs are well marked.

*Class D.**—Advanced cases.

These experiments were carried out from January to March, 1910. Of the 33 patients under observation,

6	belonged	to Class	A.
19	„	„	B.
7	„	„	C.
1	„	„	D.

The methods adopted to test the degree of infectivity of patients to the fly in different stages of the disease were: (1) By mechanical transmission from Sleeping Sickness patient to normal monkey, and (2) by transmission of the disease from Sleeping Sickness patient to normal monkey after the parasite had completed its development in the fly.

1. *By Mechanical Transmission from Sleeping Sickness Patient to Normal Monkey.*

The procedure in testing the first method was as follows:—A cage of laboratory-bred flies was first placed for two minutes upon the bare shoulders of a patient suffering from Sleeping Sickness, which was time enough to allow the majority of the flies to draw some blood, but not enough to satisfy them. After an interval of five minutes the cage was transferred to the abdomen of a normal monkey, where it remained for two minutes, by which time nearly all the flies had completed their feed. This procedure was repeated daily for six days.

Ten of the patients under experiment had been treated with soamin and perchloride of mercury administered by subcutaneous injection. Two patients, who had been under treatment for one year, had received several doses of atoxyl, in addition to soamin and mercury.

Trypanosomes had been found at one time in the glands of all the patients, but no trypanosomes were found in their blood during the time the flies were being fed upon them.

* Classes C and D are not now differentiated.

Table I.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients to Healthy Monkeys by Interrupted Feeding.

No. of experiment.	Class of patient.	If treated.	Duration of treatment.	No. of doses.	No. of flies fed.	No. of times flies fed.	Result.
2014	A	Yes	7 days	2	75	6	–
2137	B	,,	7 ,,	2	65	7	–
2144	C	,,	7 ,,	2	75	6	–
2146	B	,,	7 ,,	2	65	6	–
2013	A	,,	30 ,,	4	75	6	–
2027	B	,,	30 ,,	4	75	6	–
2029	B	,,	30 ,,	4	75	6	–
2150	C	,,	30 ,,	4	70	7	–
2152	C	,,	30 ,,	4	50	7	–
2158	B	,,	30 ,,	4	60	6	–
2139	B	,,	1 year	?	70	6	–
2154	B	,,	1 ,,	?	50	6	–
2012	B	No	Nil	Nil	75	6	–
2281	B	,,	,,	,,	100	6	–
2284	B	,,	,,	,,	50	6	–

Remarks.—These experiments are all negative—a not unexpected result now that it is known that the mechanical transmission of Sleeping Sickness may be a somewhat rare event in Nature. The question as to whether the medicinal treatment of Sleeping Sickness patients has any preventive influence on this method of transmission remains unanswered. It may, however, be noted that in the two untreated cases, viz., Experiment 2281, Class B, and Experiment 2284, Class B, the disease was not transmitted mechanically, but, as will be seen later (Table III, Experiments 2280 and 2298), was conveyed after a period of development of the parasite within the fly.

2. *By Transmission of the Disease from Sleeping Sickness Patient to Normal Monkey after the Parasite had Completed its Development in the Fly.*

The procedure adopted in the second method for transmitting the disease from a Sleeping Sickness patient to a monkey was as follows:—A cage of laboratory-bred flies was fed daily for five or six days upon a patient suffering from Sleeping Sickness. They were then starved for 48 hours, and subsequently fed daily for 50 days upon a normal monkey.

In most of these experiments the flies were dissected and examined for flagellates after the period of feeding upon the normal monkey. The monkey's blood was subsequently examined twice weekly for one month after the last date on which the flies were fed upon it.

Trypanosomes had been found at one time in the glands of all these patients, but no trypanosomes were found in their blood during the time the flies were being fed upon them.

In the following tables all the details bearing on each class of patient are given :—

Table II.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class A, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
1941	7 days	2	125	6	50	—	Monkey remained healthy.
1973	7 „	2	100	6	50	—	„
1969	1 month	4	100	7	50	—	„
1995	1 „	4	130	4	50	—	„
2240	2½ years	?	75	4	50	—	„
2252	2½ „	?	80	4	50	—	„

Remarks.—Reference to Table II will show that six patients in Class A stage of the disease were under treatment. Two of these had been under treatment for one week, and had each received two injections of soamin, 0·01 gramme, combined with perchloride of mercury, 0·02 gramme. Two had been under treatment for one month, and had received four injections of soamin, 0·01 gramme, and perchloride of mercury, 0·02 gramme, per dose. Two cases had been under treatment for two and a-half years: the total quantity of drugs received being, in one case, atoxyl, 16·2 grammes, perchloride of mercury, 2·4 grammes, and orpiment, 8·6 grammes; the other case had received 18·8 grammes of atoxyl, 2·4 grammes of perchloride of mercury, and 8·6 grammes of orpiment.

Sleeping Sickness was not transmitted from any of these patients to monkeys by the flies.

Two hundred and seventy-three flies were dissected between the 4th and 51st days after feeding upon infected patients, but no flies were found infected with flagellates.

Only one patient belonging to Class D was available for these experiments. This patient had been under treatment for one and a-half years, and had been treated with a total quantity of 13·4 grammes atoxyl, 0·4 gramme perchloride of mercury, and 6·5 grammes orpiment.

Flies were not infected from this case, and the disease was therefore not transmitted to a normal monkey.

Table III.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class B, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
1993	7 days	2	100	4	50	–	Monkey remained healthy.
2091	7 „	2	50	5	50	–	„
2094	7 „	2	60	5	50	–	„
2170	7 „	2	60	6	50	–	„
1975	30 „	4	100	5	50	–	„
1987	30 „	4	65	5	50	–	„
1991	30 „	4	90	4	50	–	„
2068	30 „	4	65	6	50	–	„
2070	30 „	4	80	6	50	–	„
2107	30 „	4	65	6	50	–	„
2195	30 „	4	50	6	50	–	„
2248	30 „	4	65	4	50	–	„
2096	1 year	8	60	5	50	–	„
2109	1 „	9	65	5	50	–	„
2172	1 „	8	60	5	50	–	„
2193	1 „	9	60	6	50	–	„
2254	2¼ years	?	80	4	50	–	„
2256	2¼ „	?	60	4	50	–	„
2258	3½ „	?	60	4	50	–	„
1943	Nil	Nil	65	6	50	–	„
1957	„	„	100	4	50	–	„
1967	„	„	110	6	50	–	„
2048	„	„	50	6	50	–	„
2280	„	„	130	4	50	+	*T. gambiense* appeared in blood.
2298	„	„	80	3	50	+	„

Remarks.—In Table III, Class B, 25 experiments from 19 different patients are recorded. Six of these were from untreated cases.

Sleeping Sickness was transferred from two untreated patients to normal monkeys. The flies belonging to these experiments were not dissected.

The remaining four experiments from untreated cases were negative, both as regards infection of the flies (190 dissected) and the conveyance of the disease to monkeys.

Of the 19 negative experiments from cases under treatment, four were from two patients who had been under treatment for one week. They had each received two injections of soamin in 1-gramme doses, combined with 0·02 gramme of perchloride of mercury. Eight experiments were from patients who had been one month under treatment. They had each received four injections (soamin 1 gramme, and perchloride of mercury 0·02 gramme, per dose). Four experiments were from two cases who had been under treatment for 11 months. One of these had received injections amounting in all to soamin 8 grammes, $HgCl_2$ 0·10 gramme, and orpiment 2 grammes. The other patient had received a total amount of soamin 9 grammes, $HgCl_2$ 0·10 gramme, and orpiment (?). Two experiments were from patients who had been under treatment for two and a-quarter years. They had each received a total quantity of drugs amounting to atoxyl 19·7 grammes, $HgCl_2$ 0·06 gramme, orpiment 4·60 grammes, and soamin 4 grammes. One experiment was from a patient who had been three and a-half years under treatment, and had received during the last 18 months atoxyl 9·5 grammes, soamin 7·5 grammes, and orpiment 4·6 grammes.

From these 19 negative experiments 509 flies were dissected between the 4th and 52nd days after their last infected feed. No flagellates were found in any of them.

Table IV.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class C, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
2103	7 days	2	60	6	50	–	Monkey remained healthy.
2168	7 „	2	63	6	50	–	„
2250	7 „	1	100	4	50	–	„
2034	30 „	4	100	3	50	+	*T. gambiense* appeared in blood.
2098	30 „	4	50	5	50	–	Monkey remained healthy.
2105	30 „	4	65	4	50	–	„
2189	30 „	4	60	7	50	–	„
2191	30 „	4	65	7	50	–	„
2244	2½ years	?	120	4	50	–	„
2242	Nil	Nil	85	4	50	–	„

Remarks.—In Table IV, Class C, 10 experiments are recorded. One of these was from an untreated case with large numbers of trypanosomes in the cervical glands, but none detectable in the blood.

The monkey was not infected by flies fed upon this patient, and so the flies were not dissected.

Of the 9 experiments from treated cases, Class C, three were from two patients who had been under treatment for one week, of whom one had been given a single dose of soamin 2 grammes, and $HgCl_2$ 0·04 gramme. The other patient had received two doses.

The experiments performed from these patients were negative as regards infection of both monkeys and flies.

Three patients had been under treatment for one month. Each of these patients had been given injections of soamin and perchloride of mercury, amounting to 2 grammes of the former drug and 0·08 gramme of the latter. One of these experiments was positive, both as regards infecting the flies and transmitting the disease to monkeys.

Seventy-five flies were dissected from the positive experiment, 2034. Of these, four flies were found to be infected with *Trypanosoma gambiense* on the 28th, 39th, 40th, and 46th day respectively after their last infected feed. The four flies found to be infected with trypanosomes were injected into a susceptible animal, which subsequently developed Sleeping Sickness.

It is obvious that the results obtained from these experiments, though giving us some information, are insufficient to warrant a decisive answer to the questions before us.

With regard to the first query: Can treatment of Sleeping Sickness patients by the drugs named influence the infectivity of the fly? Omitting the mechanical transmission experiments, we may say that out of the 35 experiments only one produced a positive result, *i.e.* flies were infected and the disease transmitted from a Sleeping Sickness patient under treatment to a normal monkey. The case referred to above will also entitle us to express an opinion as to whether one or more doses of a certain trypanocidal

drug can render a patient non-infective to the fly. It will be seen that this case had been under treatment for one month, and had been injected with four doses of trypanocidal drugs. The tsetse flies were infected 25 days after the last of the four injections had been administered.

Therefore, it may be said that to treat Sleeping Sickness patients with injections of soamin and perchloride of mercury, as specified, does not necessarily cause their blood to be incapable of infecting *Glossina palpalis* with *Trypanosoma gambiense*, and that flies so infected can transmit the disease to monkeys.

The percentage of flies infected from untreated cases was not ascertained, as only 190 flies were dissected, at various intervals, after feeding on their patients, and none were found infected.

A total of 857 flies were dissected, at different periods, after having fed upon treated cases, and four of these were found to be infected, *i.e.* a percentage of 0·46. These four flies all came from a cage containing 100 *Glossina palpalis* which had been fed on three occasions upon the only treated case that proved infective to the flies, *vide* Table IV, Experiment 2034. This patient therefore infected 4 per cent. of flies—an interesting and important fact.

It was hoped that patients under the influence of trypanocidal drugs would be incapable of infecting tsetse flies. Unfortunately, this experiment (2034) is sufficient to show that Sleeping Sickness patients under treatment with arsenical compounds, etc., are not always innocuous to the fly, and should not be allowed their freedom to wander about the country during intervals of comparative good health, but must be kept completely and permanently isolated from all possible contact with the fly.

Conclusions.

1. *Glossina palpalis* fed on natives suffering from Sleeping Sickness, and untreated by drugs, may become infected and be capable of transferring the disease to healthy animals.

2. *Glossina palpalis* fed on natives suffering from Sleeping Sickness, and treated by arsenic and other drugs, may also become infected and be capable of transferring the disease to healthy animals.

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Original Communications.

SLEEPING SICKNESS IN UGANDA.—DURATION OF THE INFECTIVITY OF THE *GLOSSINA PALPALIS* AFTER THE REMOVAL OF THE LAKE-SHORE POPULATION.[1]

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AND
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(*Sleeping Sickness Commission of the Royal Society*, 1908-09.)

DURING the last two years the policy of clearing the shores and islands of Lake Victoria of their inhabitants has been carried out by the Uganda Administration, with a view to the stamping out of sleeping sickness.

It will be remembered that the area of distribution of sleeping sickness and of the *Glossina palpalis* in Uganda is the same, and is limited to a narrow belt along the Lake-shore and islands. For the past two years no native has been allowed to live or work within two miles of the Lake-shore, except at a few cleared landing-places, and within the last few months all the islands have been emptied.

Until recently it was believed that the fly only retained its infectivity for forty-eight hours, and that it would, theoretically, be possible with safety to clear an island of its infected population one day and restock it with healthy natives a few days later. Recent work, however, has shown this to be wrong, since it has been found by experiment that the fly can retain its infectivity

[1] Printed by permission of the Royal Society.

up to eighty days. It is probable that after a fly has become infected it will harbour the trypanosomes for the rest of its life; but what the duration of this is, under natural conditions, is unknown.

From an administrative point of view, therefore, it is most important to find out how long the flies on the Lake-shore remain infective after the native population has been removed. Until this is known it will not be safe to allow the Lake-shore and islands to be re-inhabited.

As soon as the Sleeping Sickness Commission of the Royal Society reached Uganda experiments were begun to test this point. At first the flies were collected at Kibanga, a cleared landing-place in Buka Bay, 6 miles from the laboratory. This landing-place was used as a market, where the inhabitants of the Island of Buvuma came once a week to trade with the natives on the mainland. In November, 1908, Kibanga had become somewhat overgrown, and tsetse-flies were present in some numbers. As the Buvuma islanders were highly infected with sleeping sickness, this constituted a danger to the healthy natives of the mainland, who had come to the market from outside the sleeping sickness area. Steps were at once taken to have the landing thoroughly cleared of undergrowth, with the result that in a short time the flies disappeared. The following experiment shows the result:—

Experiment 52.—*Monkey.*

To ascertain if *Glossina palpalis* caught at Kibanga market-place are capable of giving rise to sleeping sickness in a healthy monkey:—

Date	No. of flies		Trypano-somes	Malaria	Date	No. of flies		Trypano-somes	Malaria
	Put on	Fed				Put on	Fed		
1908					1908				
Nov. 3..	–	–	–	+	Dec. 6..	–	–	–	+
,, 6..	–	–	–	+	,, 7..	–	–	–	+
,, 14..	15	12	–	+	,, 15..	–	–	–	+
,, 16..	17	17	–	+	,, 17..	1	1	..	..
,, 17..	7	7	–	+	,, 18..	–	–	–	+
,, 18..	4	1	–	+	,, 23..	–	–	–	+
,, 19..	7	4	–	+	,, 26..	–	–	–	+
,, 20..	–	–	–	+	,, 30..	–	–	–	+
,, 22..	50	34	–	+					
,, 23..	–	–	–	+	1909				
,, 24..	–	–	–	+	Jan. 4..	–	–	–	+
,, 25..	–	–	–	+	,, 9..	–	–	–	+
,, 27..	–	–	–	+	,, 18..	–	–	–	+
,, 29..	–	–	–	+	,, 20..	–	–	–	+
,, 30..	–	–	–	+	,, 26..	–	–	–	+
Dec. 2..	10	7	–	+	,, 28..	–	–	–	+
,, 3..	12	5	–	+	Feb. 6..	–	–	–	+
,, 4..	5	3	–	+	Mar. 1..	–	–	–	+

Remarks.—The result of this experiment is negative. The number of flies caught is small, and they soon disappeared as the clearing of the place proceeded.

The other experiments were all made with freshly caught flies from uninhabited places on the Lake-shore. The Lake-shore, as stated above, had been cleared of its inhabitants in December, 1907, and had, therefore, been deserted for nearly a year when these experiments began. It was anticipated that the flies would be found non-infective, as, in the absence of sleeping sickness cases, it was difficult to understand where they could obtain the necessary trypanosomes, and at this time the long period of infectivity of the fly was unknown. The following experiments give the result :—

Experiment 214.—*Monkey.*

To ascertain if *Glossina palpalis*, caught on the Lake-shore, where there are no natives, are capable of giving rise to sleeping sickness in healthy monkeys :—

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1908					1909				
Nov. 23..	21	10	..	..	Jan. 4..	–	–	–	+
,, 24..	25	20	..	..	,, 9..	–	–	–	+
,, 25..	50	26	–	+	,, 15..	–	–	–	–
,, 26..	30	17	–	+	,, 25..	43	24	..	..
,, 27..	12	8	..	..	,, 26..	35	29	–	+
,, 28..	96	23	–	+	,, 28..	–	–	–	+
,, 30..	125	41	–	+	Feb. 2..	100	65	–	+
Dec. 1..	150	60	–	+	,, 3..	105	105	..	..
,, 2..	–	–	–	+	,, 4..	100	90	..	..
,, 3..	–	–	–	+	,, 5..	–	–	–	+
,, 4..	–	–	–	+	,, 6..	100	85	..	..
,, 5..	60	23	–	+	,, 8..	100	82	..	..
,, 7..	47	26	–	+	,, 9..	200	165	..	..
,, 12..	60	49	–	+	,, 10..	200	146	–	+
,, 14..	83	37	..	..	,, 15..	200	135	–	+
,, 15..	78	32	–	+	,, 16..	200	120	..	..
,, 17..	14	6	..	..	,, 17..	170	126	..	..
,, 18..	–	–	–	+	,, 18..	200	134	..	..
,, 23..	–	–	–	+	,, 19..	200	110	–	+
,, 28..	80	35	–	+	,, 20..	200	124	..	..
,, 30..	70	32	–	+	,, 22..	130	98	..	..
					,, 23..	200	140	..	..
					,, 24..	200	142	..	..
					,, 25..	200	135	..	..
					,, 26..	–	–	–	+
					Mar. 1..	–	–	+	+

Remarks.—2,500 flies were fed on this monkey for ninety-eight days before a positive result was obtained.

Experiment 571.—*Monkey.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
Mar. 2..	200	152	..	..	Mar. 15..	200	152	..	..
,, 3..	200	156	..	..	,, 16..	100	78	..	..
,, 4..	100	78	..	..	,, 17..	100	74	..	..
,, 6..	150	110	..	..	,, 18..	100	58	–	+
,, 9..	200	120	–	–	,, 20..	200	112	..	..
,, 10..	200	110	..	..	,, 22..	–	–	+	+
,, 11..	200	124	..	..					

Remarks.—Result positive. Infection probably took place on March 15th. This means that 1,002 flies fed on this monkey before infection took place.

Experiment 612.—*Monkey.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
Mar. 25..	340	185	..	..	Mar. 29..	100	76	..	..
,, 26..	250	124	..	..	,, 30..	200	115	–	+
,, 27..	200	115	..	..	April 6..	–	–	+	+

Remarks.—Results positive. Infection, probably March 30th; 615 flies.

Experiment 674.—*Monkey.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
April 8..	250	160	..	..	April 23..	270	180	..	..
,, 9..	500	240	..	..	,, 26..	200	160	–	–
,, 10..	500	220	..	..	,, 28..	400	240	..	..
,, 12..	500	245	–	+	,, 30..	400	160	..	..
,, 15..	500	340	..	..	May 1..	500	290	..	..
,, 19..	–	–	–	–	,, 3..	–	–	–	+
,, 20..	250	180	..	..	,, 7..	–	–	+ +	+
,, 22..	300	190	–	–					

Remarks.—Result positive. Infection, April 30th; 2,315 flies.

Experiment 758.—*Monkey.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
May 8..	270	210	..	..	May 22..	–	–	–	+
,, 11..	250	170	..	..	,, 28..	200	130	–	+
,, 14..	200	120	..	..	June 2..	–	–	–	+
,, 17..	–	–	–	+	,, 7..	–	–	+	+

Remarks.—Result positive. Infection, May 28th; 630 flies.

Experiment 976.—*Monkey.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
June 9..	800	260	..	..	June 18..	200	90	–	+
,, 10..	450	180	..	..	,, 20..	520	230	..	..
,, 17..	550	190	..	..	,, 21..	–	–	+ +	+

Remarks.—Result positive. Infection, June 10th; 440 flies.

Experiment 1,117.—*Monkey.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
June 24..	200	120	..	..	June 30..	7	4	..	..
,, 25..	300	160	..	..	July 1..	–	–	–	+
,, 26..	150	80	..	..	,, 3..	500	130	..	..
,, 28..	380	165	..	..	,, 5..	500	220	+	+
,, 29..	500	210	..	..					

Remarks.—Result positive. Infection, June 28th; 525 flies.

Experiment 1,276.—*Monkey.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
July 9..	110	70	..	..	July 19..	–	–	–	+
,, 12..	500	230	..	..	,, 20..	300	180	..	..
,, 15..	–	–	–	+	,, 22..	–	–	+	+

Remarks.—Result positive. Infection, July 12th; 300 flies.

Experiment 1,462.—*Ox.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
Aug. 16..	120	75	–	..	Aug. 20..	170	80	..	..
,, 17..	410	250	..	..	,, 24..	350	120	–	..
,, 19..	320	180	–	..	,, 26..	–	–	+	..

Remarks.—Result positive. Infection, August 19th; 505 flies.

Experiment 1,465.—*Ox.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
Aug. 27..	150	90	..	..	Sept. 7..	–	–	–	..
,, 28..	60	35	..	..	,, 9..	30	19	..	..
Sept. 4..	230	170	..	..	,, 10..	–	–	+ +	..

Remarks.—Result positive. Infection, September 4th; 295 flies.

Experiment 982.—*Ox.*

Date	No. of flies		Trypanosomes	Malaria	Date	No. of flies		Trypanosomes	Malaria
	Put on	Fed				Put on	Fed		
1909					1909				
Sept. 11..	45	36	..	..	Sept. 20..	–	–	–	..
,, 12..	65	50	..	..	,, 21..	115	85	–	..
,, 14..	110	75	..	..	,, 22..	180	145	..	..
,, 15..	125	95	..	..	,, 23..	410	380	..	..
,, 16..	420	160	–	..	,, 24..	300	240	–	..
,, 19..	55	40	..	..	,, 27..	370	230	+ +	..

Remarks.—Result positive. Infection, September 19th; 456 flies.

The table on p. 139 summarises these results.

It must therefore be concluded that the Glossina palpalis on the uninhabited shores of Victoria Nyanza can retain their infectivity for a period of at least two years after the native population has been removed. How much longer they will remain infective it is impossible to say, but it is obvious that these experiments should be continued, in order to answer this important question.

With the facts at our disposal it is not possible to account for

this continued infectivity. It may be due to the duration of the life of these flies being more than two years—that flies which became infected before the natives left are still alive. Or, it is possible that the flies have lately fed on natives suffering from sleeping sickness, who have been passing in canoes from the islands to the mainland, or on natives who still frequent the Lake-shore in spite of the prohibition. Thirdly, it might be explained, if any of our canoe-men or fly-boys had trypanosomes in their blood. Or, lastly, it is possible that the mammals and birds along the Lake-shore have become infected, and so act as a reservoir of the disease.

Experiment	Place	Number of flies fed	Number of days before infection took place	Result	Percentage of infected flies*
52	Kibanga	91	—	—	—
214	Uninhabited Lake-shore	2,500	98	+	0·04
571	,, ,,	1,002	20	+	0·10
612	,, ,,	615	12	+	0·16
674	,, ,,	2,315	29	+	0·04
758	,, ,,	630	30	+	0·16
976	,, ,,	440	12	+	0·23
1,117	,, ,,	525	11	+	0·19
1,276	,, ,,	300	13	+	0·33
1,462	,, ,,	505	10	+	0·19
1,465	,, ,,	295	14	+	0·34
982	,, ,,	456	16	+	0·22

* This is calculated on the assumption that there is only one infected fly in each batch of flies used in an experiment.

To these speculations it may be answered that it is not at all likely that these flies have the opportunity of becoming infected from passing canoes, which, during the last two years, have been few and far between, or to natives still frequenting the Lake-shore. Our canoe-men and fly-boys have been kept under careful supervision during the whole of the time, their blood constantly examined, and once a month blood from each of them injected into a healthy monkey. There remain, then, the two theories—long duration of life of the fly, and a local reservoir. The former cannot at present be answered, and there is no experimental proof of the latter, since the injection of the blood of the Lake-shore birds and mammals into susceptible animals has always, up to the present, given negative results.

SAND-FLY FEVER IN INDIA.

By Lieutenant-Colonel C. BIRT.
Royal Army Medical Corps.

Newcomers to districts where the sand-fly, *Phlebotomus papatasii*, abounds, suffer from short febrile illnesses during the summer, when the fly makes its appearance. The chief symptoms are severe frontal headache, flushed face, suffused conjunctivæ, half-closed eyelids, tender eyeballs, pain on moving the head, eyes, or limbs, aching and stiffness in the back and legs, furred tongue, anorexia, sometimes vomiting; constipation, though diarrhœa is not infrequent; the temperature rises suddenly to 103° or 104° F., and falls gradually about the third day. The pulse remains slow. No parasites are found in the blood, but the leucocytes are diminished in number. Recovery always ensues. Second attacks are uncommon. In Herzegovina and Malta it has been proved by experiment that the virus which causes this fever is conveyed by the *P. papatasii*. It will be shown that epidemics of a similar febrile ailment prevail every summer in many parts of India. As the *P. papatasii* is widely distributed there, it is probable that destruction of this insect would be a sanitary measure of no little importance to the health of our Indian Army.

The significant increase in the admissions recorded under the headings "Influenza," "Simple Continued Fever," and "Pyrexia of Uncertain Origin," which has occurred in India during the last decade, makes it clear that many short febrile attacks, formerly classed in the ague group, are now regarded to be non-malarial in origin. The following table shows the number of cases among the British troops, 1900-1908:—

	1900	1901	1902	1903	1904	1905	1906	1907	1908
"Influenza"	237	539	107	215	349	1,014	804	864	432
"Simple continued fever" and "Pyrexia of uncertain origin"	1,479	1,486	846	1,300	1,684	3,415	3,917	2,553	5,077
Days of sickness	27,980	31,310	18,652	24,540	30,150	53,380	57,426	37,580	66,070

The disability which has arisen from these diseases has become so large that a critical study of the subject can be delayed no longer. Influenza is a short-range infection, conveyed in the

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Experiments to Ascertain if Certain Tabanidæ Act as the Carriers of Trypanosoma pecorum.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S., and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, 1908–10).

(Received December 20, 1910,—Read March 2, 1911.)

[PLATE 16.]

INTRODUCTION.

The study of the trypanosome diseases of domestic animals in Uganda was a branch, and an important one, of the work undertaken by the Commission. Among other points arising in this investigation, the finding, if possible, of a carrier of the various trypanosome diseases was necessary. In the 'Proceedings'* of the Royal Society, the Commission published a paper entitled "Trypanosome Diseases of Domestic Animals in Uganda. I.—*Trypanosoma Pecorum*." This paper gives full details as to the definition, morphological and cultural characteristics, and distribution in Africa, of *Trypanosoma pecorum*. A few experiments and experiences of the Commission were also given, which led to the belief that possibly Tabanidæ were the common carriers in Africa of the disease caused in cattle by this trypanosome.

With the object of gaining further knowledge on this important problem, the Commission worked in Uganda from January to July, 1910, on the following lines :—

1. An investigation of the biting flies occurring in the vicinity of the laboratory at Mpumu, Chagwe, Uganda.
2. A study of the natural history of these biting flies.
3. Transmission experiments with these flies.
4. A study of the flagellates, if any, natural to these flies.

1. AN INVESTIGATION OF THE BITING FLIES OCCURRING IN THE VICINITY OF THE LABORATORY AT MPUMU, CHAGWE, UGANDA.

No systematic search was made for blood-sucking flies in this neighbourhood till January, 1910. Up to this date the Commission had identified some four different species in the vicinity of Mpumu.

An outbreak of trypanosome disease in cattle having occurred in

* B, 1910, vol. 82, p. 468.

September, 1909,* in the valleys surrounding Mpumu hill, it was decided to make a prolonged and careful search for biting flies in these valleys. Boys were employed whose sole duty it was to hunt daily for these flies, cattle accompanying the boys to act as a bait for the flies. This more systematic search produced unexpected results.

In the place of only three or four varieties of biting flies, no less than 12 species of Tabanidæ, five species of Hæmatopota, two different Chrysops, a new and very handsome Rhinomyza, and *Glossina palpalis*, were captured within a 3-mile radius of the laboratory. These flies were kindly identified for us by Mr. E. E. Austen, F.L.S., to whom specimens were sent, and for whose help and courtesy the Commission is much indebted. The full list is here given, as it will probably be of value to future workers in the Mpumu laboratory, and of interest to entomologists.

Tabanidæ.

Tabanus secedens (Walk.), very numerous.
Tabanus fuscomarginatus (Ricardo), very numerous.
Tabanus thoracinus (Pal. de Beauv.), very numerous.
Tabanus par (Walk.), fairly numerous.
Tabanus tæniola (Pal. de Beauv.), fairly numerous.
Tabanus socialis (Walk.), fairly numerous.
Tabanus variatus (Walk.), fairly numerous.
Tabanus fasciatus (Fabr.), sub. sp. *niloticus* (Austen).
Tabanus insignis (Lw.), scarce.
Tabanus variabilis (Lw.), scarce.
Tabanus obscurissimus (?), scarce.
Tabanus irroratus (Surcouf), rare.

Hæmatopota.

Hæmatopota ugandæ (Ricardo). *H. vittata* (Lw.), syn. *pulchrithorax* (Austen). *H. fusca* (Austen). *H. brunnescens* (Ricardo). *H. similis* (Ricardo).

(All numerous.)

Chrysops.

Chrysops funebris (Austen). *C. silacea* (Austen).

Glossina.

Glossina palpalis (Rob.-Desv.).

Rhinomyza.

Rhinomyza perpulcra (Austen).

* 'Roy. Soc. Proc.,' B, 1910, vol. 82, pp. 476—478.

This part of the investigation made two facts clear to the Commission. Firstly, that experiments to ascertain a carrier of any of the trypanosome diseases of domestic animals in Africa must necessarily be both prolonged and difficult. Secondly, that the distribution of the different species of biting flies in any country cannot be mapped out by a travelling entomologist. Accurate maps can only be made by the whole-hearted co-operation of those officials and settlers who are resident in the country for long periods. The observations should, where possible, be made daily, and continued for at least one year in the same locality.

2. A Study of the Natural History of the Biting Flies Found in the Neighbourhood of Mpumu.

It should be clearly understood that whatever observations are given below refer to the natural history of these flies as observed in this locality only. Elsewhere difference in climatic and other conditions may give rise to variations in the habits and life-history of these flies.

Glossina palpalis.

The habits of the Glossina in this neighbourhood do not vary from the same species on the Lake-shore. Their presence was, however, both interesting and disquieting, as the Kasala stream, where they were captured, is at least 6 miles from the nearest point of Lake Victoria. Further, the stream has no connection whatever with this Lake. Rising in a narrow belt of forest at the foot of Mpumu hill, it flows through forest along the north-west face of the hill and then joins the Sezibwa River, which trends northwards, running parallel with the Nile, to empty into Lake Chioga. The Glossina were very scarce, three or four only being caught monthly between January and July, 1910. They were all caught at one spot—a ford of the stream, situated a mile or so from the laboratory. No tsetse-flies were seen in any other part of the stream. It is possible these flies had found their way down from the hill, since it was by no means an uncommon circumstance for some flies to escape from their cages when brought up by the fly-boys. Escaped flies were found on several occasions on different parts of the hill.

The Tabanidæ.

Habitat.—Narrow belts of dense tropical forest, which commonly mark the windings of a flowing stream. The two essential factors appear to be deep shade and water, not necessarily running water. These flies are not found on grass-covered hill-tops.

Habits.—Emerging from the upper branches of the forest trees, on which they presumably shelter during the night, these flies commence feeding about 7 A.M. if the morning be fine with plenty of sunshine. They cease to feed about 6 P.M. Their most active hours are from 11 A.M. to 1 P.M., viz., the hottest part of the day. From 6 A.M. to 8 A.M., and again from 4 P.M. to 6 P.M., they are sluggish and do not feed readily. In wet, cold, or dull weather no flies are to be seen as a rule. They bite cattle and man freely, but much prefer the former, and will rarely attack man in the presence of cattle. Their bite is like the stab of a sharp lancet, and draws a free trickle of blood from both oxen and man. They have followed cattle a distance of 3 miles to the laboratory, but invariably left the hill-top soon after their arrival, or died in sheltered spots about the houses a few days after their arrival.

Variation in Incidence.—This phenomenon is the most striking characteristic of the Tabanidæ. A forested stream known to be free from a certain species of Tabanus one day will be infested with that species during the next two or three days. This eruption, if it may be so called, appears to be simultaneous over a given area, and may be explained by the flies all arriving at maturity about the same day. This tends to show that the females all lay their batches of eggs about the same time. Further, it is not unreasonable to date the commencement of the fly's winged life from this time, and so, watching carefully for their final disappearance, we arrive, probably with some degree of accuracy, at the length of life of the fully-developed fly. The disappearance of the fly is almost as sudden as their eruption; in some three days a locality previously swarming with a certain species of Tabanus will be quite free from this species. In this manner the Commission have observed carefully three species of Tabanidæ, viz., *Tabanus secedens, Tabanus fuscomarginatus,* and *Tabanus thoracinus.* In these cases, on the above computation, the limit of life of these flies is as follows:—*Tabanus secedens,* four months; *Tabanus fuscomarginatus,* two months; *Tabanus thoracinus,* four months. At least two such eruptions of the same fly may occur in the same locality in the same year.

The Tabanidæ.

Sex.—During the seven months (January–July, 1910) these experiments were taking place, many thousand Tabanidæ were captured, but no male of any of the 12 different species was ever seen or taken.

The Hæmatopota.

Habitat.—The Hæmatopota have a more extensive range than the Tabanidæ. They are to be found in the same localities as the Tabanidæ,

and also, in large numbers, in open swampy areas where few or no trees grow. The rank grass, which grows waist-high in these swamps, appears to satisfy what need they may have for shade. Another very favourite locality for these flies is a road or ford crossing streams and swamps. The fly is absent from dry, open, grass-covered hill tops.

Habits.—The hours of greatest activity, and the feeding times of this genus, correspond to those given above for the Tabanidæ. They have a sluggish flight, and attack man more readily than do the Tabanidæ. When they settle on their victim they rarely bite at once, but usually walk about slowly for a few seconds before inserting their proboscis. They will follow cattle or man for a mile or two.

Sex.—No male fly was ever captured by the Commission during the time these observations were taking place.

The Chrysops and the Rhinomyza.

These flies were comparatively scanty in the neighbourhood, and no accurate observations were made of their natural history. All were caught at the ford on the Kasala stream, at the foot of Mpumu hill. *Rhinomyza perpulcra* appeared to be very localised in its distribution, as it was never seen elsewhere in the vicinity. It was usually captured whilst sunning itself on stones in the stream, or whilst it was biting natives washing clothes at this ford. Its habitat appeared to be the densely forested banks of the ford. Mr. Austen informs us that it is a new African species.

3. Experiments to Ascertain if Tabanus Secedens (Walk.), Tabanus Fuscomarginatus (Ricardo), or Tabanus Thoracinus (Pal. de Beauv.), are Capable of Transmitting Trypanosoma Pecorum from Sick to Healthy Oxen.

The experiments were carried out between March and August, 1910. The above-named species were used, as they were the most common among the Tabanidæ during this period. Only wild flies were employed, as the Commission never succeeded in finding the eggs or larvæ of these flies. The species of Tabanus used in any experiment, and the duration of the experiment, were thus entirely dependent on the seasonal incidence of the fly in the neighbourhood.

Endeavours were at first made to carry out the experiments in the laboratory on Mpumu hill. Cages, some 12 inches square, were made, with sides of wood, the top and bottom consisting of wide-meshed mosquito netting. The captured flies were placed in these cases—about 50 flies per cage—the cages being kept over water in the laboratory when the flies were

not being fed. The daily temperature of the laboratory during the days this method was tried was very constant, being 70°·2 F. (21°·2 C.) in the morning at 8, and 80°·2 F. (26°·8 C.) in the afternoon at 4. The flies were placed on oxen and monkeys for 20 minutes once daily, to give them ample opportunity to feed.

This method was not successful, the flies—*Tabanus secedens* (Walk.)—all dying within four days of being caged, the majority within 48 hours. When placed on an ox, within 12 hours of capture, a few flies fed greedily. After 24 hours in the cages no fly was observed to bite. Monkeys were never bitten by the flies, though they would feed on man as readily as on the ox when freshly caught. If shaded from direct sunlight, the flies refused to feed.

It was decided to build a fly-proof kraal in the natural haunts of the fly, in the hope that the Tabanidæ would live longer and feed more readily. The Kasala stream at the foot of Mpumu hill was chosen as the site of the kraal. The kraal was divided into two compartments, which were made fly-proof from each other and from the outside. In one compartment no flies of any species had access. Into the other compartment the species of Tabanus under experiment were liberated—50 flies per day, on an average. In the kraal four calves were kept; three normal and one calf infected with *Trypanosoma pecorum*. The infected calf and two of the normal calves were taken from the fly-free compartment into the compartment containing the Tabanidæ for three hours daily. The hours chosen were 11 A.M. to 1 P.M., and 3 P.M. to 4 P.M., when the sun was hot and shining on the kraal. Every opportunity was thus given to the flies to feed on both the infected and the healthy calves. The other normal calf was utilised as a control, being kept continuously in the fly-free compartment and so protected absolutely from the bites of blood-sucking flies.

No effort was made to rid any of the calves of ticks, body lice, etc., therefore, if the normal calves which daily entered the fly-compartment with the infected calf contracted *Trypanosoma pecorum* infection whilst the control calf remained healthy, then the Tabanus under experiment was in all probability the transmitting agent. If the control calf also contracted the disease, then some agency other than Tabanidæ, such as ticks, etc., would probably be responsible. Lastly, if all the normal calves escaped infection, then presumably the Tabanus under experiment did not transmit the disease.

The Tabanidæ did not appear to live more than three or four days, in spite of the presence of running water, shrubs and foliage in the fly-compartment. The majority died within the first 48 hours. A few fed greedily on the calves for 12 to 18 hours after capture.

The normal calves and the control calf were examined bi-weekly for trypanosomes throughout the experiment, and for one month after its completion. The infected calf was also examined bi-weekly; *Trypanosoma pecorum* were always seen in its blood; sometimes they were present in large numbers, sometimes scanty.

Table I gives the result of these experiments at a glance.

Table I.

Species of Tabanus used.	Experiment No. of calves.	No. of days Tabanidæ fed on calves.	Approximate No. of flies used.	Result.
Tabanus secedens (Walk.)	2304 & 2305	30	1500	Negative; calves remained healthy.
Tabanus fuscomarginatus (Ricardo)	2400 & 2401	43	2150	,, ,,
Tabanus thoracinus (Pal. de Beauv.)	2542	55	2650	,, ,,

Remarks.—It is seen that the Tabanidæ had ample opportunity to feed indiscriminately on the infected and healthy calves over a considerable period of time. Large numbers of flies were used, yet no normal calf contracted the disease. The fact that the Tabanidæ died within 48 hours of being put in the kraal does away with the possibility of a development of *Trypanosoma pecorum* in the flies. These experiments, therefore, only prove that these flies do not transmit the trypanosome mechanically.

4. A Study of the Flagellates found in Tabanus Secedens, Tabanus Fuscomarginatus and Tabanus Thoracinus.

In this investigation two series of dissections were made. In the first wild flies caught in the neighbourhood of the laboratory were dissected. In the second series dissections were made of wild flies caught in the neighbourhood and introduced into the fly-compartment of the kraal, where they were used in the transmission experiments detailed above.

As wild flies were used throughout, it was necessary to carefully study the flagellates which may infect these flies, in order that parasites natural to the flies, such as Crithidia or Herpetomonas, should not be mistaken for developmental forms of pathogenic trypanosomes in the fly.

It should be noted here that the cattle which grazed in the neighbourhood where the flies were caught were healthy during the period that the investigations were being carried out.

In the first series of dissections 138 *Tabanus secedens* and 49 *Tabanus thoracinus* were dissected. Of the *Tabanus secedens* 7 (5 per cent.) showed a heavy infection of flagellates in the hind-gut, rectum, and proctodæum. The mouth parts and remainder of the alimentary tract contained no

flagellates. Of the *Tabanus thoracinus* dissected, flagellates were found in the hind-gut, rectum and proctodæum of 13 (26·5 per cent.). The mouth parts and remainder of the gut were negative for flagellates. No *Tabanus fuscomarginatus* were dissected in this series.

The flagellates found in the hindermost portions of the intestines of both these species were similar. Two main types predominated.

Type 1, *Oval, Flagellated Parasites.*

A. *Living, Unstained.*

This type is very active, and darts rapidly across the field when free. More commonly large masses of these flagellates are seen to be irregularly grouped together, the flagella of the outermost ones rapidly vibrating. This type divides by longitudinal fission, and forms undergoing this process are commonly seen. Bright points can often be made out in the body-substance of these parasites (chromatin granules); no definite vacuoles could be seen. Masses of these flagellates sometimes occupied a whole field.

B. *Fixed and Stained.*

These flagellates contain a distinct macro- and micro-nucleus. The macro-nucleus is large, roughly circular and sometimes diffused. It lies about the centre of the body of the parasite, often occupying its whole width. The micro-nucleus is circular or rod-shaped, is placed on the flagellar side of and close to the macro-nucleus. The flagellum arises from the micro-nucleus, it is short and projects from the body of the parasite, the protoplasm of the body being often prolonged for a short distance along the flagellum. There is no undulating membrane. Chromatin granules, irregular in number and size, are often to be seen in the vicinity of the macro-nucleus (Plate 16, figs. 1 to 6).

Type 2, *Circular, Non-Flagellated Parasites.*

These parasites contained macro- and micro-nuclei. The drawing clearly indicates their structure (Plate 16, figs. 7 to 14). In the fresh preparations they were motionless.

In the second series of dissections of the Tabanidæ which had been liberated in the fly-compartment of the kraal, 50 *Tabanus secedens*, 24 *Tabanus thoracinus*, and 37 *Tabanus fuscomarginatus* were dissected. Of the 50 *Tabanus secedens*, 2 were infected with flagellates (4 per cent.). Of the 24 *Tabanus thoracinus*, 1 was infected (4 per cent.). No *Tabanus*

fuscomarginatus contained parasites. The three infected flies all contained flagellates in the hind-gut, rectum, and proctodæum, which resembled Types 1 and 2 mentioned above.

Type 3, Elongated, Flagellated Parasites.

One fly, a *Tabanus secedens*, contained many flagellates, of a different variety, in the fore-gut, mid-gut, and fore-part of hind-gut. In the fresh preparation this third type of flagellate was motile, and possessed a poorly developed undulating membrane, which was thrown into short folds. The movements of the parasite were active, but not translatory to any great extent. The accompanying drawing (Plate 16, figs. 15 to 24) so clearly show the structure of this type in the stained state that further description is unnecessary.

Type 3 occurs principally in the thick-walled mid-gut of the fly and, to a lesser extent, in the fore-gut and fore-part of the hind-gut. Types 1 and 2 occur only in the hind-gut, rectum, and proctodæum of the fly.

Inoculation Experiments carried out with these Parasites.

Expt. 2404.

April 23, 1910.—White rat inoculated, intra-peritoneally, with contents of hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had been in fly-compartment of kraal; it was heavily infected with Type 1 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Expt. 2405.

April 23, 1910.—White rat inoculated, intra-peritoneally, with contents of fore-gut and mid-gut of a wild *Tabanus secedens*. This fly had been in kraal, and its fore-gut and mid-gut were swarming with Type 3 of the parasite in an active state.

Result.—Negative. Rat examined for one month after the injection.

Expt. 2411.

April 27, 1910.—White rat inoculated, intra-peritoneally, with hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had never been in kraal. It was heavily infected with Type 2 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Expt. 2412.

April 27, 1910.—White rat inoculated, intra-peritoneally, with hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had never been in kraal; it was heavily infected with Type 1 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Expt. 2412B.

July 4, 1910.—White rat inoculated, intra-peritoneally, with the pooled citrated contents of the hind-gut, rectum, and proctodæum of two wild *Tabanus thoracinus*. These flies had never been in kraal and were heavily infected with Types 1 and 2 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Conclusions.

1. *Tabanus secedens*, *Tabanus thoracinus*, and *Tabanus fuscomarginatus* appeared to be unable to transmit *Trypanosoma pecorum* from infected to healthy cattle by the mechanical method of transmission.

2. Owing to the short life of these tabanids in captivity it is impossible, from the above experiments, to state whether they can convey the disease (*Trypanosoma pecorum*) after a period of development of the trypanosome in the fly.

3. We believe the three types of flagellates found in *Tabanus secedens* and *Tabanus thoracinus* to be various stages in the development of a harmless Crithidium in these flies.

DESCRIPTION OF PLATE 16.

Smear preparation of hind-gut, rectum and proctodæum of a *Tabanus secedens*, stained Giemsa, × 2000.

Figs. 1—6, Type 1, oval, flagellated parasites. Fig. 4 is a dividing form of this type. Fig. 1 shows the most common form of this type. Fig. 6 represents an irregular mass of the parasites; these masses often contain very large numbers of the parasites. The flagella are poorly stained in these preparations, and so do not show up well (Expt. 2412).

Figs. 7—14, Type 2, circular, non-flagellated parasites. Made from smear preparations of the rectum and proctodæum of a *Tabanus secedens* (Expt. 2411).

Figs. 15—24, Type 3, elongated, flagellated parasites. Figs. 17 and 18 show the most typical forms of this type. Fig. 19 is a stout atypical form, which was not common. Fig. 15 represents a form which would appear to be reverting to Type 1. Fig. 16 is a dividing form. The parasites figured in Figs. 15—24 were obtained from a smear made of the mid-gut of a wild *Tabanus secedens* which had been in the fly-compartment of the cattle kraal (Expt. 2405).

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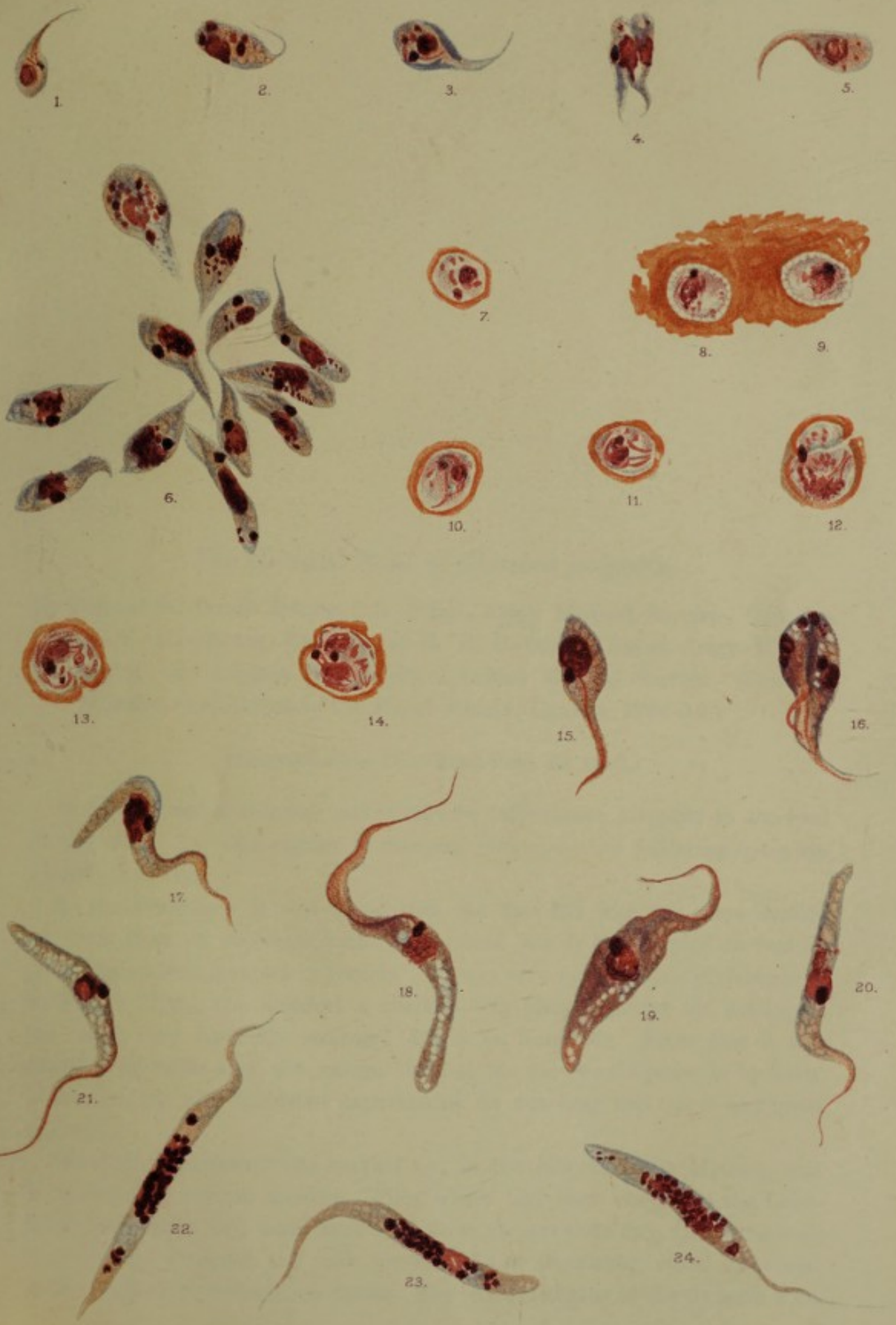

M. E. Bruce, del.

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The Natural Food of Glossina palpalis.

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As a good deal of interest, and it may be importance, attaches to the food of the tsetse fly—the carrier of Sleeping Sickness—the following notes are placed on record:—

In the laboratory it was found that the flies fed with far more avidity on birds than on monkeys, while they could hardly be tempted to feed on young crocodiles, iguanas, or lizards. It was very marked, this preference of theirs for birds; the moment a chicken was placed against the netting of the cage, they instantly swarmed on it in hundreds. From this it was thought probable that the natural food of the flies would prove to be birds' blood, but the two following experiments do not bear this out to any great extent:—

The first experiment was carried out in the laboratory at Mpumu, and extended over several months. Flies which had been caught on the Lake-shore and which had been kept over from the previous day, and sometimes longer, were dissected, and each portion of the alimentary canal examined in the fresh state under a low power. The various organs of the fly were then

smeared separately, and after staining (with one of the modifications of the Romanowsky stain) were examined in the usual way.

The dissections were carried out as follows:—The pharynx and proboscis were removed with a needle, and having been placed under a cover-glass and slightly opened out by pressure were examined in the fresh state. The tube of the proboscis was in this way admirably displayed, and the presence of red blood corpuscles or of flagellates could readily be determined. The alimentary canal was then removed from the abdomen, and the salivary glands detached from the fat-body. The alimentary canal was then unravelled and laid out in its full length on a slide. The whole of the tube could then be passed in review, from the junction of the thoracic intestine with the fore-gut down to the rectum, and the contents at various levels determined, as far as possible, with medium power magnifications. The main portions of the alimentary canal were then noted, and these were separated from one another by cross cuts with a sharp-edged dissecting needle. In this way the fore-gut, mid-gut, hind-gut, and proctodæum were separately removed and smeared on a slide. The smears (whilst still moist) were exposed to the vapour of osmic acid for a few seconds, and then passed through alcohol before washing and staining. The proventriculus can, with a little practice, be removed intact from the ventral aspect of the thorax. If necessary, the thoracic portions of the salivary ducts can also be recovered.

Speaking in general terms, the contents of the various portions of the alimentary canal could be determined with considerable accuracy from the naked-eye appearance during dissection. If the fly has fed very recently, the blood oozes from its proboscis when it is handled. The proventriculus, the crop or sucking stomach, as well as the fore-gut, are greatly distended with red blood. When the fed fly is put aside the blood-cells generally disappear from the proboscis in a few hours, though they may exceptionally be found as long as 24 hours after a feed. Similarly, the proventriculus and thoracic intestine quickly empty themselves, and the crop discharges its contents into the upper alimentary canal within 24 hours.

The blood travels slowly down the alimentary canal, changing in appearance as it goes. The outline of the red cells can sometimes be distinguished three days after a feed, but only when they have been retained in the fore-gut. When the blood reaches the mid-gut it begins to disintegrate and becomes homogeneous and somewhat translucent; when it reaches the lower part of the mid-gut it first becomes dark and eventually black and tarry. When in this state all cell elements are lost, and the colouring matter of the red cells is recognisable in the form of amorphous black granules. When

the contents pass posterior to the Malpighian tubes, that is, into the hind-gut, they at once become fæcal in character, and turn into a dirty, yellowish-brown material, which is microscopically composed of fine granules. It is passed from the fly in this form.

The figures given in the subjoined table refer to flies which were caught at various parts of the Lake-shore, and were generally kept for about 24 hours before dissection. The majority of the dissections were done during October, November, and December, 1908.

Table I.—The Contents of the Alimentary Canal of 220 *Glossinæ palpalis.*

Total flies examined.	Intestine empty.	Intestine contained blood.	Character of Blood.		
			Mammalian.	Non-mammalian.	Non-recognisable.
220	160	60	20	9	31
Percentages...	72·7	27·3	9·1	4·1	14·1
			27·3		

Remarks.—In two cases blood corpuscles were recognised as being derived from monkeys, as the characteristic parasites of monkey malaria were found in them.

The second experiment to ascertain the food supply of *Glossina palpalis* was modified as follows:—

A journey was made to a small peninsula, hereafter referred to as "Crocodile Point." This place was distant about two and a-half to three hours by canoe from the Kibanga landing-place, and lay in a sheltered bay far out of the beaten track of the canoes which come backwards and forwards between the islands of Kome, Damba, and Buvuma, and the weekly market at Kibanga clearing. It was chosen, therefore, partly because it was isolated from human influences, and also because of the large number of flies which lived there and the number of crocodiles and birds which frequented it.

When it was first visited, a large crocodile was disturbed from where she was lying outstretched on a spit of sand. The canoe-men at once set to work, and unearthed 58 crocodile eggs lying in layers about 18 inches below the surface, over which the "form" of the parent was clearly defined in the soil.

The peninsula was pointed in shape, and not more than 60 yards long, and was closed on the land side by the dense wall of forest which fringes

the Lake-shore. It was scattered with light undergrowth and fringed by ambatch trees, on which flocks of divers and cormorants sat with outstretched wings drying in the sun. A barrier of bare rocks and boulders projected on one side, and a small school of hippopotami was generally to be seen floating near and basking in the sun. Many small crocodiles were disturbed from the undergrowth as the point was explored, and various kinds of small land birds frequented the reeds and shrubs. The soil was sandy loam, and shaded by the light foliage. *Glossina palpalis* swarmed. The place has been described at some length, so that the exact conditions may be realised.

Subsequent to this experiment, the daily catch of Lake-shore flies was obtained from this place, and incidentally it may be added that the flies caught here were regularly found to be infective to monkeys.

Table II.—The Naked-Eye Appearances of the Contents of the Alimentary Canal of 183 Tsetse Flies, with their Sex, and the Presence or Absence of a Larva.

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
1	♂	–	–	–	34	♂	–	–	–
2	♂♂	–	–	–	35	♀♀	–	+	–
3	♂♂	–	–	–	36	♀♀	–	–	+
4	♀♀	+	–	–	37	♀♀	+	–	–
5	♀♀	–	–	–	38	♂♂	–	+	–
6	♂♂	–	–	–	39	♂♀	–	–	+
7	♂♂	–	–	–	40	♀♀	–	–	+
8	♀♀	+	–	–	41	♂♂	–	–	+
9	♂♂	–	–	–	42	♂♂	–	+	–
10	♂♀	–	–	–	43	♂♂	–	–	+
11	♀♀	–	–	–	44	♂♀	+	–	–
12	♀♀	–	–	–	45	♀♀	–	–	+
13	♀♀	–	–	–	46	♂♂	–	–	+
14	♂♂	–	–	–	47	♂♀	–	–	+
15	♂♂	–	–	–	48	♂♀	–	–	+
16	♂♂	–	–	–	49	♂♂	–	–	+
17	♂♀	–	–	+	50	♂♂	–	–	–
18	♀♀	–	–	+	51	♂♂	–	–	+
19	♂♂	–	+	–	52	♀♀	+	–	–
20	♂♀	–	–	+	53	♂♂	–	–	–
21	♂♂	–	+	–	54	♂♀	–	+	+
22	♂♂	–	–	+	55	♂♂	–	–	–
23	♀♀	–	–	+	56	♂♀	–	–	+
24	♀♀	–	–	+	57	♂♀	–	–	–
25	♂♂	–	+	–	58	♂♀	+	–	–
26	♂♂	–	–	+	59	♀♀	–	–	+
27	♂♂	–	+	+	60	♀♀	–	–	+
28	♂♀	+	–	+	61	♂♂	–	–	+
29	♂♂	–	–	+	62	♂♂	–	–	+
30	♂♂	–	–	–	63	♂♂	–	–	+
31	♀♀	–	–	+	64	♀♀	–	–	–
32	♀♀	+	+	–	65	♂♂	–	–	–
33	♀♀	–	–	+	66	♂♂	–	–	+

Table II—*continued.*

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
67	♂	−	−	−	126	♂	−	−	+
68	♂♂	−	−	−	127	♀	−	−	−
69	♂♂	−	−	−	128	♂	−	−	+
70	♂♀	−	−	−	129	♀	−	−	+
71	♂♀	−	+	−	130	♀	−	−	+
72	♂♀	−	−	+	131	♂	−	+	+
73	♂♀	−	−	−	132	♂	−	+	+
74	♀♀	+	+	+	133	♂	−	+	+
75	♂♂	−	+	+	134	♀	−	+	+
76	♂♂	−	−	+	135	♂	−	−	−
77	♂♂	−	+	+	136	♂	−	−	−
78	♂♀	−	+	−	137	♀	+	−	+
79	♂♂	−	−	−	138	♀	−	−	+
80	♂♂	−	−	−	139	♂	−	−	−
81	♂♀	−	−	−	140	♂	−	−	+
82	♂♀	−	+	+	141	♂	−	−	+
83	♀♀	+	−	−	142	♂	−	−	+
84	♀♀	+	−	−	143	♂	−	+	+
85	♂♀	−	−	−	144	♂	−	−	−
86	♂♀	−	−	+	145	♂	−	−	+
87	♂♀	−	−	−	146	♂	−	−	−
88	♂♀	−	−	−	147	♂	−	+	+
89	♂♂	−	−	−	148	♀	+	−	+
90	♂♂	−	−	+	149	♀	+	−	+
91	♀♀	+	+	+	150	♀	−	−	+
92	♀♀	+	−	−	151	♀	−	−	+
93	♂♂	−	−	−	152	♀	−	−	+
94	♂♂	−	−	+	153	♀	+	−	+
95	♂♂	−	−	+	154	♂	−	−	+
96	♂♂	−	−	+	155	♂	−	−	−
97	♂♂	−	+	+	156	♂	−	−	−
98	♂♀	−	+	+	157	♂	−	+	+
99	♂♂	−	−	−	158	♂	−	−	−
100	♂♂	−	−	+	159	♀	−	−	+
101	♀♀	+	−	+	160	♂	−	−	−
102	♀♀	+	−	+	161	♀	+	−	+
103	♂♂	−	−	−	162	♂	−	−	−
104	♂♀	+	−	−	163	♂	−	−	+
105	♂♀	−	−	−	164	♂	−	−	−
106	♀♂	+	−	+	165	♂	−	−	−
107	♂♂	−	−	−	166	♂	−	−	−
108	♂♂	−	−	+	167	♀	+	−	+
109	♂♂	−	−	−	168	♀	+	−	+
110	♂♂	−	−	−	169	♀	−	+	−
111	♀♂	+	−	−	170	♀	+	−	+
112	♂♀	−	−	+	171	♂	−	−	+
113	♂♀	−	−	+	172	♂	−	−	+
114	♂♂	−	−	+	173	♀	+	−	+
115	♂♂	−	−	−	174	♂	−	−	−
116	♂♂	−	+	+	175	♂	−	−	+
117	♂♀	−	−	+	176	♂	−	+	+
118	♂♀	−	−	−	177	♀	+	+	+
119	♀♀	+	−	+	178	♀	+	−	−
120	♂♂	−	+	+	179	♀	+	−	−
121	♂♀	−	+	+	180	♀	+	−	+
122	♀♀	−	−	−	181	♀	−	−	+
123	♀♀	−	+	+	182	♂	−	−	−
124	♂♂	−	+	+	183	♂	−	−	−
125	♂	−	−	−					

In the present experiment, the observer landed by canoe and made the paddlers sit round him; the fly-boys moved about amongst them and caught the flies as they came to feed. They were handed at once to the observer, who snipped off their heads, noted their sex, and roughly dissected out the alimentary canal, and, when it contained food-stuff, smeared it at full length on a slide. Notes were made as to the naked-eye contents of the canal and the question of pregnancy. The smears were fixed in the usual way and brought to the laboratory, where they were stained and examined minutely.

The total number of flies examined was 183, of which 104 (57 per cent.) were males and 79 (43 per cent.) were females; of the total number, 108 (59 per cent.) contained blood in a more or less digested state, and 75 (41 per cent.) contained no food-stuff. Out of the 79 females, 32 (40 per cent.) contained nearly fully-developed larvæ.

Table III.—Shows the Nature of the Blood in the Interior of the Flies, and also the Number of Flies which contained Parasites.

No.	Mammalian blood.	Non-mammalian blood.	Trypanosomes.	Halteridia.	No.	Mammalian blood.	Non-mammalian blood.	Trypanosomes.	Halteridia.
1	−	+	−	−	27	−	+	−	−
2	−	+	−	+	28	−	+	−	−
3	−	+	+	−	29	−	+	−	−
4	−	+	−	−	30	−	+	+	−
5	−	+	−	−	31	−	+	−	−
6	−	+	−	−	32	+	−	−	−
7	−	+	−	−	33	−	+	+	−
8	−	+	−	−	34	−	+	−	−
9	−	+	−	−	35	−	+	−	−
10	−	−	+	−	36	−	−	+	−
11	+	−	−	−	37	−	+	−	−
12	−	+	−	−	38	−	−	+	−
13	−	−	+	−	39	−	+	−	−
14	−	+	−	+	40	+	−	−	−
15	−	−	+	−	41	−	−	+	−
16	−	+	−	−	42	−	+	−	−
17	−	+	−	+	43	−	+	−	+
18	−	−	+	−	44	−	+	−	−
19	−	+	−	−	45	−	+	−	−
20	−	−	+	−	46	+	−	−	−
21	+	+	−	−	47	−	+	−	−
22	−	+	−	−	48	−	−	+	−
23	−	+	−	−	49	−	−	+	−
24	−	+	Trypano-plasma	−	50	−	+	−	−
					51	+	−	−	−
25	−	+	−	+	52	+	−	−	−
26	−	−	+	−					

The above table shows that out of the original 183 flies examined, 52 (28 per cent.) contained blood which was recent enough to show the red corpuscles. Out of these 52, 7 (13 per cent.) were from mammalian blood, whilst non-mammalian blood was present in 35 flies (67 per cent.). Trypanosomes were present in 14 flies (27 per cent.) and Halteridia in 5 (nearly 10 per cent.). One contained a trypanoplasma, derived, probably, from bird's blood.

An attempt was made to separate the nucleated red blood corpuscles into avian and reptilian. The distinction was made by size only, it being found from the measurement of corpuscles from birds and reptiles that the average normal length was :—

Standard amphibian (crocodile), 15·4 microns.
Standard avian (horn-bill), 13·1 microns.

In examining the smears from the flies, the average was taken of 10 to 20 red cells, which seemed as natural as possible, and the average obtained in

Table IV.—Shows the Average Measurements of the Nucleated Red Corpuscles and their Probable Origin.

No.	Average length of red cells, in microns.	Probably reptilian or amphibian.	Probably avian.
1	15·3	+	−
2	14·2	+	−
3	14·1	+	−
4	13·3	−	+
5	14·4	+	−
6	14·0	+	−
7	14·0	+	−
8	14·4	+	−
9	14·5	+	−
10	13·0	−	+
11	14·8	+	−
12	14·4	+	−
13	10·6	−	+
14	13·4	−	+
15	14·6	+	−
16	13·2	−	+
17	14·0	+	−
18	15·2	+	−
19	13·2	−	+
20	13·3	−	+
	Totals.....	13	7

Remarks.—Only 20 of the flies contained nucleated blood which was recent enough to justify any deduction as to its origin. Out of these 20 flies, 13 had probably fed on a reptile or amphibian, and 7 on a bird.

this way compared with the standard measurements. No allowance could be made for alterations due to digestive changes: these were presumed to be similar in all cases.

Table V.—Showing the Result of the Examination of *Glossina palpalis* from "Crocodile Point."

No. of flies.	♂.	♀.	♀ containing larvæ.	Intestine empty.	Intestine containing blood.	Mammalian blood.	Non-mammalian blood.	Non-mammalian blood.		Flagellates.	Halteridia.
								Avian.	Reptilian.		
183	104	79	32	75	108	7	35	7	13	14	5
Percentages...	57	43	17·4	41	59	3·8	19·1	3·8	7	7·6	2·7

Conclusions.

Two hundred and twenty *Glossinæ palpalis* were caught on various parts of the Lake-shore, and at intervals extending over several months; they were examined about 24 hours after capture. The examination of their intestinal contents revealed the fact that about 27 per cent. contained the remains of blood, the majority of which was of mammalian origin.

In the second experiment, 183 *Glossinæ palpalis* were caught at one spot where the food supply was abundant—birds and crocodiles—and the flies were examined at once. A much higher percentage (nearly 60 per cent.) contained the remains of a blood meal. The blood in the majority of the flies had been obtained from birds or reptiles, and of these the reptilian blood was twice as frequent as the blood of birds.

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Mechanical Transmission of Sleeping Sickness by the Tsetse Fly.

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(Received June 22,—Read June 30, 1910.)

Up to the beginning of 1909 it was believed that the spread of trypanosome diseases, such as Sleeping Sickness and Nagana, was effected by the mechanical transmission of the parasite by the tsetse fly. The proboscis was supposed to be contaminated by being dipped in the infected blood, and some of the trypanosomes were pictured as remaining for some time within the tube and capable of being injected into a fresh animal at the next feed of the fly. Successful experiments were described which seemed to prove that the tsetse fly was capable of remaining infective for 48 hours, but not longer; and it was thought that any given Sleeping Sickness area would be free from danger a few days after the infected population had been removed from it.

Dr. Kleine, however, at the end of 1908, showed that the tsetse fly remains infective for a much longer period, and that a period of non-infectivity of 20 days or more elapses before this power of passing on the parasite is gained. In other words, that the *Trypanosoma gambiense* undergoes some process of development in the fly before it is able to infect a fresh animal. It was evident, then, that the mechanical theory had to be modified.

It was now held that, in addition to the mechanical method, a mode involving a developmental phase within the fly must be reckoned with. But it was still considered likely that the mechanical was by far the more common mode of infection, and that for every case due to a developmentally-infected fly, a hundred would be due to recent contamination. At the same time, it was quite evident, from an examination of the old feeding experiments after 8, 12, 24, and 48 hours, that the successful results recorded might have to be credited to late-infectivity rather than to mechanical transmission. The experiments lasted so long that there was enough time for the unsuspected late development of the parasite in the fly to have caused the infection.

The following experiments were therefore made to put this matter to the proof. The flies, after having had their infective feed, were, as a rule, not allowed to feed on a healthy animal for more than 12 days, in this way

preventing the chance of the infection being transmitted by flies in which the development of *Trypanosoma gambiense* had taken place.

A. *Mechanical Transmission of* Trypanosoma gambiense. *Interrupted Feeding.*

In interrupted feeding, the cage containing the flies is first placed on the infected animal for some little time, then suddenly transferred to the healthy animal, and so backwards and forwards for 10 to 15 minutes. This is meant to imitate the conditions in Nature, when sick and healthy natives are sitting together on the Lake-shore, and the tsetse flies are continually flitting from one to the other.

Experiment 1550. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. From infected to healthy monkey.

Date.	Day.	Procedure.	Remarks.
1909.			
Aug. 30—31	1	Cage changed from infected to non-infected monkey every 15 seconds. This continued for 10 minutes each day.	65 flies used.
Sept. 1.....	2		
„ 2.....	3		
„ 3.....	4		
„ 4.....	5		Sept. 6, healthy monkey showed *Trypanosoma gambiense*.
„ 5.....	6		
„ 6.....	7		

Result.—Positive. The infection probably took place on the first day (August 30), as it usually takes seven days before the trypanosomes appear in the blood.

Experiment 1316. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. Infected ox to healthy monkey.

The two oxen used for this experiment were known to be infective, as their blood, when injected into a clean monkey, gave rise to a *gambiense* infection.

Date.	Day.	Flies fed on infected oxen and healthy monkey.	Alternate feeds.	Remarks.
1909.				
July 15—16	1	Flies fed daily	20	5 flies used.
„ 17—31	2—16	„ „	20	July 16, 4 flies added.
Aug. 1.....	17	„ „	20	Aug. 1, 20 flies added.
„ 2—Sept. 7 ...	18—54	„ „	20	Sept. 7, 10 flies left; dissected; all negative. Monkey remained healthy.

Result.—Negative. The trypanosomes are usually very few and far between in the blood of cattle, which probably accounts for the negative result.

Experiment 1543. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. Infected monkey to healthy goat.

Date.	Day.	Feedings from infected monkey to healthy goat.	Remarks.
1909.			
Aug. 28	—	20 alternate feeds.	50 flies used.
„ 29—30	1	25 „ „	
Sept. 9.....	2—12	Continued daily for 12 days.	

Result.—Positive. The goat, Experiment 1495, was examined daily for trypanosomes until October 16, but none was seen. On October 1, 21 days after the last feed of infected *Glossinæ palpalis*, 4 c.c. of the blood of this goat were injected into monkey, Experiment 1777. This monkey showed trypanosomes on October 16, proving that the goat, Experiment 1495, had become infected from the monkey by interrupted feeding, though trypanosomes failed to appear in its blood.

Experiment 1565. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. Infected ox to monkey.

Date.	Day.	Interrupted feeding on infected ox and healthy monkey.	Remarks.
1909.			
Aug. 29—Sept. 9	1—11	25 passages to each animal, the whole lasting about half an hour. This continued daily for 11 days.	30 flies used. The monkey, examined for 30 days, failed to show trypanosomes.

Result.—Negative. Blood of ox injected into monkey on August 28. This monkey became infected with *Trypanosoma gambiense* six days later. The negative result may be due to the small numbers of trypanosomes found at any time in oxen.

Experiment 1705. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. Infected monkey to fowl.

Date.	Day.	Interrupted feeding on infected monkey and healthy fowl.	Remarks.
1909.			
Sept. 28—Oct. 4	1—6	30 passages from monkey to fowl, and continued daily for 6 days.	60 flies used.

Result.—Negative. October 7, 1 c.c. of blood of fowl injected into healthy monkey. November 2, 2 c.c. of blood of fowl injected into healthy monkey. Both monkeys remained healthy.

Table I.—Summary of Results of Interrupted Feeding Experiments. Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense.*

Experiment.	Infected animal.	Healthy animal.	No. of days flies fed.	Result.		Remarks.
				Positive.	Negative.	
1550	Monkey ...	Monkey ...	7	+		65 flies used.
1316	Ox	„ ...	54		–	5 to 29 flies used.
1543	Monkey ...	Goat	12	+		50 flies used.
1565	Ox	Monkey ...	11		–	30 flies used.
1705	Monkey ...	Fowl	6		–	60 flies used.

B. *Mechanical Transmission of* Trypanosoma gambiense *by Laboratory-Bred* Glossina palpalis, *with an Interval between the Feeding of the Flies on the Infected and Healthy Animals.*

The following table gives a summary of the results :—

Table II.

Experiment.	No. of days flies fed.	No. of flies fed.	Infected animal.	Healthy animal.	Interval between feedings.	Result.	
						Positive.	Negative.
1712	12	50	Monkey...	Monkey...	½ hour		–
1233	12	14	„ ...	„ ...	1 „		–
1664	12	50	„ ...	„ ...	1 „		–
1665	12	40	„ ...	„ ...	2 hours		–
1524	11	35	„ ...	„ ...	6 „		–
1080	12	11	„ ...	„ ...	8 „		–
1801	13	70	„ ...	„ ...	24 „		–
1802	13	75	„ ...	„ ...	24 „		–
1803	13	120	„ ...	„ ...	24 „		–
1274	15	7	„ ...	„ ...	48 „		–

Conclusions.

1. The mechanical transmission of Sleeping Sickness by means of *Glossina palpalis* can take place if the transference of the flies from the infected to the healthy animal is instantaneous—that is, by interrupted feeding.

2. This mechanical transmission does not take place if an interval of time comes between the feedings.

3. Mechanical transmission plays a much smaller part, if any, in the spread of Sleeping Sickness than has been supposed.

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Experiments to Ascertain if Antelope may Act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

(Received November 9, 1910,—Read January 19, 1911.)

Introduction.

The question of a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals, is of the utmost importance.

Now man and the domestic animals have been removed from the Lake-shore of the mainland for some two and a half years, and from the islands since September, 1909. The effect of this depopulation has been to make a two mile area along the northern shores of the Lake virtually a game reserve, in which water-buck, bush-buck, reed-buck, Speke's *Tragelaphus*, hippopotami, wild pig, and other large game abound. The game water freely at the Lake-shore, and small herds of antelope may frequently be seen grazing on the grassy hillsides overlooking the Lake.

Notwithstanding the removal of man and his domestic animals, the Lake-shore *Glossina palpalis* continued to infect susceptible animals with Sleeping Sickness up to the end of March, 1910. The Commission, therefore, endeavoured to find answers to the following questions:—

1. Can antelope be infected with Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis?*

2. If antelope can be infected with the virus of Sleeping Sickness, can they transmit the infection to laboratory-bred *Glossina palpalis* when these flies are allowed to feed upon them? Further, if these *Glossina palpalis* become infected, can they transmit the virus to susceptible animals?

3. If these *Glossina palpalis* become infected with the virus of Sleeping Sickness, what percentage are so infected?

4. How does Sleeping Sickness affect the health of the antelope?

5. Lastly, are antelope living in the fly-area naturally infected with Sleeping Sickness?

Should all these questions be answered in the affirmative, the importance of the observation is patent. The continued infectivity of the flies on the Lake-shore would be explained. Whilst the movements of man and the domestic animals can, to some extent, be controlled by administrative measures, the movements of antelope in search of new grazing grounds

would be almost impossible to check, owing to the difficult nature of the forested and elephant-grass country which in so many parts borders the Uganda shores of the Lake. Game laws would require modification, and the destruction of the game in the neighbourhood of the lakes and *palpalis*-frequented rivers would become a factor in the control of Sleeping Sickness.

To answer the above queries the following experiments were devised and carried out:—

1. *Can Antelope be Infected with Sleeping Sickness by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis?*

Eleven buck in all were employed in this experiment. Four bush-buck (*Tragelaphus sylvatica?*), six reed-buck (*Cervicapra arundinacea*), and one water-buck (*Cobus defassa*). Other buck, such as "oribi" (*Cephalophus grimmi*) and "entalaganya" (*Cephalophus equatorialis*), were obtained by the Commission, but did not survive long in captivity.

As each buck arrived its blood was usually injected, subcutaneously, into monkeys or rats, to ascertain if the blood was naturally infected with trypanosomes. The method then adopted in each case to infect the antelope with Sleeping Sickness was as follows: A cage of clean* laboratory-bred *Glossina palpalis* was fed on a monkey known to be infected with a *human strain* of *Trypanosoma gambiense*. This cage of artificially-infected flies was then fed on healthy animals until one of the animals (monkeys) became infected with Sleeping Sickness. Having proved that the flies were infective, the cage of flies was then fed, about five minutes daily, for several days, on one or other of the buck. The buck's blood was then examined daily for trypanosomes, and was further tested for Sleeping Sickness by its inoculation into monkeys or rats.

The experiments follow in full detail.

Experiment 2328. Bush-buck.

This bush-buck was fed on for five days (March 24, 25, 26, 28, and 29, 1910) by laboratory-bred *Glossina palpalis* which were known to be infected with a *human strain* of *Trypanosoma gambiense*.

On April 8, 15 days after the first feed of the infected flies on this buck, 5 c.c. of the buck's blood were injected, subcutaneously, into a healthy monkey.

April 19, monkey showed *Trypanosoma gambiense* in its blood. The trypanosomes were verified by examination in fresh and stained preparations of the blood.

Result.—Positive.

Remarks.—The preliminary inoculation of the blood into susceptible animals, to

* The word "clean," applied here and throughout this paper to laboratory-bred *Glossina palpalis*, means flies which have newly hatched out from pupæ in the laboratory and have never fed.

ascertain if the buck's blood naturally harboured trypanosomes, was not made in this case. Though examined almost daily from March 29 to August 5, 1910, *Trypanosoma gambiense* was never seen in the blood of this antelope. Fresh and stained blood films were made and examined. On one occasion only—April 20—*Trypanosoma ingens* was seen in a fresh preparation. Monkeys are not inoculable with this parasite.

Experiment 2357. Reed-buck.

April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy, no trypanosomes appearing in its blood.

Buck was fed on for 12 days, between April 7 and 20, 1910, by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*. On the 13th day after the first feed of these flies, 3 c.c. of the buck's blood were inoculated into a normal monkey. Examined bi-weekly for a month this monkey remained healthy.

Trypanosoma gambiense having appeared in the buck's blood on May 2, a further inoculation of 5 c.c. of its blood was made into a normal monkey on this date. This monkey showed *Trypanosoma gambiense* in its blood on May 10.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in the buck's blood in scanty numbers on May 2, 1910, 25 days after the first feed of the supposed infective *Glossina palpalis*. On May 3 and 4, the trypanosomes were fairly numerous in the blood, and on the 5th and 6th, scanty numbers only were seen. After May 6, *Trypanosoma gambiense* was never again seen in the blood, though examined for almost daily up to August 5, 1910. The long period of 25 days which elapsed between the first feed of the supposed infected *Glossina* and the appearance of the trypanosomes in the buck's blood may be accounted for by the supposition that the infected fly or flies in the cage which was fed on the buck from April 7 to April 19 had died before they had fed on the buck. On April 20, a fresh cage of *Glossina palpalis*, known to be infected with a *human strain* of *Trypanosoma gambiense* was fed. once only, on this buck, and on the 12th day after this feed, on May 2, trypanosomes appeared in the buck's blood. This supposition is probably correct, and further accounts for the failure of the inoculation on April 20.

Experiment 2359. Reed-buck.

On April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for six days (April 25—30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*.

On May 6, the 11th day after flies' first feed, *Trypanosoma gambiense* appeared in buck's blood in fair numbers, and 5 minims were injected, subcutaneously, into normal white rat. On May 11 this rat showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in its blood in fair numbers on May 6, 1910, again on 7th, not examined for on 8th, scanty on 9th, absent on 10th, scanty on 11th, very numerous on 12th, and never seen again, though examined for almost daily, till August 5.

Experiment 2371. Bush-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for eight days (April 22 and 23, and from April 25 to 30, 1910, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 4, the 12th day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in the buck's blood in fair numbers.

On May 5, trypanosomes were numerous, and 2 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey. On May 13 this monkey showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. *Trypanosoma gambiense* appeared in buck's blood for the first time, in scanty numbers, on May 3, 1910, and again in fair numbers on May 4 ; thereafter no trypanosomes were seen up to August 5, 1910, though the blood was almost daily examined.

Experiment 2378. Water-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if the buck naturally harboured trypanosomes in its blood. This monkey was examined bi-weekly for one month ; monkey remained healthy.

Buck was fed on for eight days (April 22, 23, 25 to 30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense. Trypanosoma gambiense* was never seen in this buck's blood, though examined for almost daily from April 22 to August 5, 1910.

On May 5, 5 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey, an interval of 13 days having elapsed since first feed of the infected flies. This monkey showed *Trypanosoma gambiense* in its blood on May 13, 1910.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. Though *Trypanosoma gambiense* never appeared in the buck's blood, yet a positive result was obtained on its inoculation into a monkey, and, as will be seen below (Table II), clean laboratory-fed flies fed on this buck for several days became infected with *Trypanosoma gambiense.*

Experiment 2427. Reed-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat, examined bi-weekly, for one month, remained healthy.

Buck was fed on for six days (May 2 to 7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 9, the seventh day after infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. One cubic centimetre of the blood was then injected, subcutaneously, into a normal white rat. On May 16, *Trypanosoma gambiense* appeared in this rat's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. A few *Trypanosoma gambiense* appeared for the first time on May 9, 1910, in buck's blood ; they were present in fair numbers on the 10th, scanty

again on the 11th, and were seen for the last time, in fair numbers, on May 12. Almost daily blood examinations were made, with negative results, up to August 5, 1910.

Experiment 2428. Bush-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat remained healthy, its blood being examined for one month after the injection.

Buck was fed on for 13 days (May 2—16, inclusive, May 9 and 15 being excepted) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*. *Trypanosoma gambiense* was never seen in this animal's blood, though examined for almost daily from May 4 to August 5, 1910.

On May 16, 14 days after infected flies' first feed on the buck, a few drops of the buck's blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 23. No trypanosomes were ever seen in buck's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. No trypanosomes were ever seen in the buck's blood, nevertheless its blood was infective on inoculation, and, as will be seen later (Table II), capable of infecting clean laboratory-bred *Glossina palpalis*.

Experiment 2429. Reed-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey. This monkey remained healthy, its blood being examined bi-weekly for a month after the injection. On May 3, 1910, 1 c.c. of the buck's blood was injected, subcutaneously, into a normal rat. This rat remained healthy, its blood also being examined bi-weekly for a month after the injection.

The buck was fed on for eight days (May 2—7, and 9 and 10, inclusive) by *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*.

On May 11, the ninth day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of the blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 17.

On July 2, 1910, this buck accidentally broke its leg and had to be killed, 4 c.c. of its heart's blood being injected into a normal monkey. This monkey showed *Trypanosoma gambiense* in its blood on July 12.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into monkeys or rats. *Trypanosoma gambiense* appeared in its blood for three days —on May 11 for the first time in scanty numbers, on May 12, many, and on May 13, very many—thereafter no trypanosomes were seen, though almost daily examinations were made up to July 2, 1910. It will be noted that the buck's blood was still infected with *Trypanosoma gambiense* on July 2—that is, 50 days after the last date (May 13) that trypanosomes were seen in it.

Experiment 2431. Reed-buck.

On May 3, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. Rat remained healthy, its blood being examined bi-weekly for a month after the injection.

The buck was fed on for six days (May 2—7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 12, 1910, the tenth day after the infected flies' first feed, *Trypanosoma gambiense*, in scanty numbers, appeared for the first time in the blood of the buck. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of its blood were injected, subcutaneously, into a normal white rat. This rat showed numerous *Trypanosoma gambiense* in its blood on May 20.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into rats. *Trypanosoma gambiense* appeared in the buck's blood, in scanty numbers, for the first time on May 12, and were present in large numbers on May 13 and 14.

We have now to record one of the most important and suggestive observations in this series of experiments. *From May* 14 *to June* 27 *the blood was examined almost daily, and on the latter date—June* 27—44 *days after trypanosomes were last seen*, Trypanosoma gambiense *reappeared for one day in this buck's blood in fair numbers.* Their identity was established by careful examination in wet and stained preparations of the blood.

(N.B.—Two mechanical transmission experiments were carried out with this buck. In the first, 50 flies were used and were fed on the buck for the three days the trypanosomes were seen in its blood. *Result.*—Negative. In the second, 100 flies were used, and were fed for four days on the buck. No trypanosomes were seen in the buck's blood during these days. *Result.*—Negative. In the first experiment four hours, and in the second one hour, elapsed between the feed on the buck and the feed on the healthy monkey.)

Experiment 2445. Reed-buck.

No preliminary inoculation of this buck's blood was made on its arrival at the laboratory.

The buck was fed on for seven days (May 6 and 7, and 9—13, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 14, 1910, the eighth day after the infected flies' first feed, *Trypanosoma gambiense* appeared in the blood of the buck for the first time. On May 20 the buck died, and a small quantity of its heart's blood was injected, subcutaneously, into a normal white rat. On June 2 rat showed *Trypanosoma gambiense* in its blood. (This rat was overlooked from May 24 to June 2, and its blood was not examined between these dates.)

Result.—Positive.

Remarks.—The blood of this buck was (with the exception of May 8) examined daily from May 6 to 13 with negative results for trypanosomes. On May 14, *Trypanosoma gambiense* appeared for the first time in its blood in scanty numbers ; on the 16th, 17th, and 18th they were very numerous ; on the 19th they were again scanty, and on the day of death, May 20, 1910, no trypanosomes could be found, the peripheral and heart's blood being examined.

Table I gives results of feeding infected *Glossina palpalis* on healthy antelope.

Table I.

No. of experiment.	Species of antelope.	No. of days infected flies fed.	No. of days before trypanosomes appeared.	Result.		Remarks.
				Positive.	Negative.	
2328	Bush-buck	5	—	+		Trypanosomes never seen.
2357	Reed-buck	12	25	+		
2359	„	6	11	+		
2371	Bush-buck	8	12	+		
2372	„	6	8	+		
2378	Water-buck	8	—	+		Trypanosomes never seen.
2427	Reed-buck	6	7	+		
2428	Bush-buck	13	—	+		Trypanosomes never seen.
2429	Reed-buck	8	9	+		
2431	„	6	10	+		
2445	„	7	8	+		

From these experiments it is shown that antelope may be readily infected with Sleeping Sickness by the bites of artificially-infected tsetse flies. Eleven antelope were used, and in every case a positive result was obtained. It will be remembered that in similar experiments made with cattle the same result was obtained.

2. *If Antelope can be Infected with the Virus of Sleeping Sickness, can they Transmit the Infection to Clean Laboratory-bred Glossina palpalis when these Tsetse Flies are allowed to Feed upon Them? Further, if these Glossina palpalis become infected, can they transmit the Virus to Susceptible Animals?*

It has now been proved that water-buck, reed-buck, and bush-buck can be infected with the virus of Sleeping Sickness, with what would seem to be unfailing regularity. Should, however, these antelope be incapable of infecting the *Glossina palpalis* with *Trypanosoma gambiense*, the fact is of academic importance only. On the other hand, should the results of feeding clean laboratory-bred *Glossina palpalis* on these infected buck give positive results when these flies are subsequently fed on normal susceptible animals, a further step has been made in the search for a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals.

The method adopted to test this second query was carried out as follows:— Clean, laboratory-bred *Glossina palpalis* were fed for several days on an infected buck. After an interval of starvation of 24 hours or more the flies were transferred to healthy animals and fed daily. When the healthy animal showed *Trypanosoma gambiense* in its blood the experiment was stopped, and the surviving flies were dissected as soon as possible.

The result of 24 experiments carried out on these lines is given in the following table:—

Table II.—Giving the Result of Feeding Laboratory-Bred Flies on Antelope Infected with Sleeping Sickness.

No. of experiment.	No. of clean flies used.	Species of antelope flies fed on.	No. of days flies fed on antelope.	No. of days before flies became infective.	Result.		Remarks.
					Positive.	Negative.	
2346	160	Bush-buck 2328	12	29	+		Buck 2328 never showed *T. gambiense* in blood. In spite of this, flies fed on it became infected 55 days after the buck's infection.
2384	100	,,	8	28	+		
2414	70	,,	6	29	+		
2501	100	,,	8	39	+		
2351	100	Reed-buck 2357	7	41	+		Buck 2357 showed *T. gambiense* in its blood for 5 days only.
2500	100	,,	8	—		−	
2510	100	,,	5	—		−	
2507	200	Reed-buck 2359	6	44	+		Buck 2359 showed *T. gambiense* in its blood for 7 days only.
2421	50	Bush-buck 2371	6	—		−	Buck 2371 showed *T. gambiense* in its blood for 3 days only.
2477	60	,,	6	29	+		
2499	100	Bush-buck 2372	8	—		−	Buck 2372 showed *T. gambiense* in its blood for 2 days only.
2451	95	Water-buck 2378	6	30	+		Buck 2378 never showed *T. gambiense* in its blood.
2478	60	,,	6	—		−	
2559	50	,,	4	—		−	
2454	110	Reed-buck 2427	6	24	+		Buck 2427 showed *T. gambiense* in its blood for 4 days only.
2456	60	,,	4	33	+		
2508	100	,,	6	30	+		
2485	50	Bush-buck 2428	7	28	+		Buck 2428 never showed *T. gambiense* in its blood.
2460	50	Reed-buck 2429	4	27	+		Buck 2429 showed *T. gambiense* in its blood for 3 days only.
2543	100	,,	6	49	+		
2464	55	Reed-buck 2431	3	28	+		Buck 2431 showed *T. gambiense* in its blood for 4 days only. In spite of this, flies fed on it became infected 81 days after its infection.
2544	90	,,	6	36	+		
2592	100	,,	5	43	+		
2476	50	Reed-buck 2445	4	—		−	Buck 2445 showed *T. gambiense* in its blood for 6 days only.

On glancing at Table II it will be seen that 24 experiments in all were carried out. Of these, 17 were positive (70·84 per cent.), and seven negative (29·16 per cent.). The shortest time which elapsed before the flies became infective was 24 days, the longest 49 days, and the average 33·35 days.

Compare these results with those detailed in the 'Proceedings' of the Royal Society, B, 1910, vol. 82, p. 374, Table III. Of the 42 experiments there described, only 8 (19 per cent.) were positive. The clean laboratory-bred flies were fed on *Trypanosoma gambiense*-infected monkeys in 36 of those experiments, in one case on a Sleeping Sickness patient, and in five cases on oxen infected with the virus of Sleeping Sickness.

Positive results were obtained from all the buck on at least one occasion, with the exception of bush-buck, Experiment 2372, and reed-buck, Experiment 2445. Only two experiments were carried out from these buck, one from each, viz., Experiments 2499 and 2476. In Experiment 2499 the flies were fed on the antelope 19 days after the trypanosomes had disappeared from its blood, as far as microscopical examination went. In Experiment 2476 the flies were non-infective to monkeys up to the 45th day after their first feed on the infected buck. This latter experiment was proceeding when the Commission left Uganda, and a positive result may yet have to be recorded.

The most significant of the above observations is the one in which it is shown that 55 days after the last feed of infected *Glossina palpalis* on bush-buck, Experiment 2328, the blood of this buck was capable of infecting clean laboratory-bred flies, though *Trypanosoma gambiense* had never been seen in its blood.

To illustrate how these experiments were carried out, full details of two are given. They are typical of the methods adopted. One positive and one negative experiment have been chosen. (See next page.)

These experiments show that antelope of the water-buck, reed-buck, and bush-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*. The infected antelope's blood was, in one case, infective to *Glossina palpalis* for at least 81 days, and in another for at least 55 days. These experiments further show that the flies, when infected by the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

Experiment 2501.—To ascertain if Laboratory-Bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
May 23—28 ...	1—5	Flies fed 5 minutes daily on Bush-buck 2328.		100 flies used.
„ 29	6	Flies starved.		
„ 30—31 ...	7—8	Flies again fed 5 minutes daily on Buck 2328.		
June 1.....	9	Flies starved.		
„ 2—July 7	10—45	Flies fed 5 minutes daily on normal Monkey 2517.		July 8, Monkey 2517 shows *T. gambiense* in its blood to-day. Allowing 7 days for incubation of *T. gambiense* in monkey's blood, then the *G. palpalis* became infective on the 39th day after their first infected feed on Buck 2328.
July 8	46	Flies starved, as Monkey 2517 shows *T. gambiense* in its blood.	+	
„ 9	47	The 57 surviving *G. palpalis* dissected; 20·3 per cent. of these flies showed heavy intestinal infection with flagellates (*T. gambiense*).		

Remarks.—This is the experiment referred to above, where the blood of a buck was capable of infecting clean laboratory-bred flies 55 days after last feed of infected flies on the buck.

Experiment 2499.—To ascertain if Laboratory-Bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
May 23—28	1—5	Flies fed 5 minutes daily on Bush-buck 2372.		100 flies used.
„ 29	6	Flies starved.		
„ 30—31	7—8	Flies again fed 5 minutes daily on Buck 2372.		
June 1	9	Flies starved.		Monkey 2552 was examined bi-weekly from June 13 to August 12. It remained healthy. The surviving *G. palpalis* were not dissected as experiment was negative.
„ 2—11	10—19	Flies fed on Cock 2518.		
„ 12	20	Flies starved.		
„ 13—July 12	21—50	Flies fed 5 minutes daily on clean Monkey 2552.	—	
July 13 and following days.	51	Flies allowed to die. Not dissected as experiment negative.		

3. *If Glossina palpalis can be infected with the Virus of Sleeping Sickness by Feeding on the Blood of Trypanosoma gambiense-infected Antelope, what Percentage are found to be so Infected?*

It has been shown now that antelope can be infected with the virus of Sleeping Sickness, that when so infected they can infect the fly, and the fly in its turn can convey the disease to susceptible animals.

These facts form a serious sequence of events, which constitute a danger not formerly appreciated in the administrative measures adopted to check the spread of the disease. What is the extent of the danger? A part of this large and important query can be answered if one can give an idea of the percentage of *Glossina palpalis* that become infected with the virus of Sleeping Sickness after they have fed on the infected antelope.

Throughout all these experiments only clean laboratory-bred flies were employed. The fact that there is no hereditary transmission of trypanosomes in *Glossina palpalis* is considered to have been so conclusively proved that two of the members of the Commission have allowed several hundreds of clean laboratory-bred flies to bite them. Further, no evidence has ever been obtained by the Commission that these flies became infected with any flagellate by contact with other flies or fouled cages. Thus, any flagellates found in the laboratory-bred *Glossina palpalis* in these experiments must be considered to be derived from the infected antelope.

In some of the experiments the flies were fed, for varying periods, upon fowls. As will be shown in a further paper, the Commission found an avian trypanosome in some of the fowls obtained for experimental purposes. It would, therefore, be a fair criticism to state that a percentage of the flagellates found on dissection of the *Glossina* were avian in origin, were it not for the fact that negative experiments went to prove that this fowl trypanosome did not develop in the *Glossina*. It is also true that on one occasion the Commission thought they had succeeded in infecting a fowl with *Trypanosoma gambiense*; it may, therefore, be argued that the fowls fed upon in some of these experiments were naturally infected with *Trypanosoma gambiense*, and that the *Glossina* obtained their infection from such naturally-infected fowls and not from the antelope. Though many experiments were devised and carried out to try and confirm this one positive result, all efforts to infect fowls with Sleeping Sickness were so uniformly negative that the Commission must consider the one "positive" result to be an error.

A reference to Table III will show that *Glossina palpalis* were infected by antelope blood where no fowls were ever fed on; in fact, it will be noticed

Table III.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards only on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Remarks.
2346	Bush-buck 2328	160	122	21	17·2	The gut-contents of 6 infected flies injected into a rat gave it Sleeping Sickness.
2384	„ 2328	100	91	10	11·0	No injection of infected flies.
2414	„ 2328	70	70	9	12·8	Five infected flies injected into a rat gave it Sleeping Sickness.
2501	„ 2328	100	57	12	21·0	No injection of infected flies.
2351	Reed-buck 2357	100	84	9	10·7	„ „ „
2507	„ 2359	200	80	2	2·5	„ „ „
2477	Bush-buck 2371	60	47	4	8·5	„ „ „
2478	Water-buck 2378	60	—	—	—	Negative experiment. Flies not dissected.
2559	„ 2378	60	—	—	—	„ „ „
2454	Reed-buck 2427	110	92	13	14·2	One infected fly injected into a rat gave it Sleeping Sickness.
2485	Bush-buck 2428	50	26	3	11·5	No injection of infected flies.
2460	Reed-buck 2429	50	38	5	13·1	„ „ „
2543	„ 2429	100	—	—	—	Negative experiment. Flies not dissected.
2544	„ 2431	90	53	5	16·6	No injection of infected flies.
2592	„ 2431	100	78	1	1·3	„ „ „
2476	„ 2445	50	—	—	—	Negative experiment. Flies not dissected.

Remarks.—Of these 16 experiments, 12 were positive and 4 negative. In the positive experiments, 838 flies were dissected. Of these, 94 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense*, viz., 11·2 per cent. Of these infected flies, 43 were males and 51 females. If to these 838 flies be added all the *Glossina palpalis* of the negative experiments, we get a total of 1108 flies, and a percentage of 8·5 infected. In only 3 of the positive experiments was an injection made into susceptible animals of the citrated gut-contents of the infected flies. All three gave positive results.

that a higher percentage of negative results were obtained where fowls were introduced into the experiments. In some of the experiments the flies were dissected as they died throughout the whole experiment, and in others the flies were dissected only when the experiment became positive. If the experiment was a negative one the flies were not dissected. In order to avoid over estimations of the percentage of infected flies, no fly was called infected unless its gut was *swarming* with trypanosomes, and all the flies dissected during the earlier days of the experiments have been included.

No fly was found to be infected with trypanosomes before the 19th day after its first infected feed on a buck. The tables otherwise explain themselves.

The methods of procedure adopted in the experiments detailed in the following table were precisely the same as those of Table III, with this one exception: The flies were, for varying periods, fed upon fowls as well as upon monkeys.

Table IV.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards on fowls, before being fed on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Remarks.
2500	Reed-buck 2357	100	—	—	—	Negative experiment. Flies not dissected.
2510	,, 2357	100	—	—	—	,, ,,
2421	Bush-buck 2371	50	—	—	—	,, ,,
2499	,, 2372	100	—	—	—	,, ,,
2451	Water-buck 2378	95	93	2	2·1	No injection of infected flies.
2456	Reed-buck 2427	60	50	9	18·0	,, ,,
2508	,, 2427	100	68	6	8·8	Three infected flies injected into a rat gave it Sleeping Sickness.
2464	,, 2431	55	53	8	15·0	No injection of infected flies.

Remarks.—It will be seen that when the flies were fed on fowls and monkeys in these eight experiments, four were positive and four negative. In the positive experiments 264 flies survived for dissection: of these 25 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense, i.e.* 9·47 per cent. Of the infected flies 11 were males and 14 females. If to these 264 flies be added all the *Glossina palpalis* of the four negative experiments, we get a total of 614 flies and a percentage of 4·0 infected. In the only experiment where an injection was made into a susceptible animal of the pooled citrated gut-contents of infected flies, the result was positive.

An analysis of Tables III and IV brings out the following interesting points:—

In Experiment 2501, Table III, it is seen that 21 per cent. of the *Glossina palpalis* were infected, out of the 57 flies that survived for dissection on the 47th day of the experiment. These flies were infected by bush-buck, Experiment 2328, which had never shown *Trypanosoma gambiense* in its blood; and 55 days had elapsed since any infected *Glossina* had fed on this buck.

Sixteen out of the twenty-four experiments were positive. If all the *Glossina palpalis* dissected in these positive experiments be grouped

together, it is seen that a total of 1,102 flies were examined. Of these, 119 flies—54 male and 65 female—were infected with developmental forms of *Trypanosoma gambiense*—that is, 10·8 flies in every hundred became infected, the sexes being about equally implicated. The highest percentage of infected flies in any one of the positive experiments was 21 per cent., in Experiment 2501, and the lowest was 1·3 per cent., in Experiment 2592. If to the total of 1,102 flies dissected in the positive experiments be added *all* the *Glossina* used in *all* the negative experiments, we get a total of 1,722 flies employed, and 6·9 flies in every hundred infected.

It is perhaps worth noting that a diet for the fly of antelope, fowl, and monkey blood gave a higher percentage of *negative* results and a lower percentage of flies infected than a diet of antelope and monkey blood only. This was quite unexpected, for the Commission, as a result of many experiments and considerable experience, were of the opinion that fowl's blood assisted the development of *Trypanosoma gambiense* in *Glossina palpalis*.

4. *How does Sleeping Sickness Affect the Health of Antelope?*

This point is of considerable importance. If the disease killed the antelope within a short time of infection, or even if it seriously affected their health so as to render them incapable or unwilling to move about freely, the facts detailed above would lose some part of their practical value.

The word "health" is not used here in a technical sense—that is to say, the health was not estimated by a series of blood counts and temperature charts. Interesting as such observations would have been, the Commission regret they were too short-handed and too much pressed by other work to carry them out.

Careful observations were made daily to answer the following questions:—Did the infected antelope during the time they were under observation appear sick? Did they become emaciated? Was there loss of health and strength? Were there corneal opacities, œdematous swellings, conjunctival discharges, or staring coats? These questions may at once be answered in the negative, except in the case of Reed-buck 2445, which will be referred to later below.

When the antelope were brought to the laboratory by the native hunters they invariably suffered from exhaustion, due probably to a combination of causes, such as fright, confinement for two to four days in cages too small to allow free movement, insufficient water and food, and the rough usage undergone when being caught. As a result of these unfavourable con-

ditions, each antelope was kept under observation for a week or two before any experiments were undertaken. During this time they were well fed and comfortably housed in reed kraals erected in a fly-proof house. Some of the antelope, especially the "oribi" and "entalaganya," died during the first fortnight. The 11 survivors were the subjects of these experiments.

Nine of these buck were kept under daily observation for four months after becoming infected with *Trypanosoma gambiense*. They remained apparently in perfect health.

The remaining two antelope were reed-buck. One, Experiment 2429, lived, and appeared very healthy, for 93 days after its infection. It then accidentally broke its leg and had to be killed. A *post-mortem* examination was made, and no evidence of trypanosomiasis was found. The other buck, Experiment 2445, arrived at the laboratory in a poor state of health, and died 12 days after its infection. There was no sign of trypanosomiasis at the *post-mortem* examination.

It is therefore evident that antelope infected with the virus of Sleeping Sickness may live in apparently perfect health for at least four months, and this, though they be kept under conditions less favourable than would occur in nature, the constant handling and fly feedings to which these buck were subject being borne in mind.

5. *Are Antelope Living in the Fly-Area Naturally Infected with Sleeping Sickness?*

Positive evidence on the last query would complete the chain of evidence that antelope living in the fly-areas may act as a reservoir of the virus of Sleeping Sickness. So far it has only been proved that they are "potential" hosts.

The only method by which this query can be investigated is by capturing game in the fly-area and then—

1. Injecting its blood into animals susceptible to *Trypanosoma gambiense* infection.

2. Feeding cages of clean laboratory-bred *Glossina palpalis* on the newly-killed buck and subsequently endeavouring to infect animals susceptible to *Trypanosoma gambiense* with these flies. (The Commission know by observation that *Glossina palpalis* will feed readily on animals newly killed.)

Those who know the local conditions will appreciate the difficulty of carrying out these methods of investigation. It would be necessary for large drives of the buck to be organised in the fly-areas. Several hundred

natives would be required. Large numbers of clean laboratory-bred *Glossina* must be available, many normal susceptible animals must be in readiness, and, at the same time, be so situated that they cannot be bitten by the possibly infected wild flies in the neighbourhood. The laboratory work in hand may have to be abandoned for the time being, and some 50 or 60 buck must be captured or killed before reliable information is forthcoming. The sun is hot, the country very difficult, and the exposure to the bites of the fly very great.

It may be said at once that the Commission were only able to shoot two buck on the Lake-shore. The blood of these gave negative results for *Trypanosoma gambiense* when injected into susceptible animals. One of them, however, gave a positive result for *Trypanosoma vivax*. This trypanosome was proved by the Commission* to be not uncommonly carried by wild Lake-shore *Glossina palpalis*. It is therefore not unreasonable to suppose that at least one of the buck shot had been fed on by the Lake-shore fly.

It was recognised at length that occasional week-end shoots by a member of the Commission were quite inadequate. The assistance of the Acting-Governor of Uganda, S. C. Tomkins, Esq., C.M.G., the Acting Principal Medical Officer, Dr. C. Wiggins, of the Uganda Medical Staff, the Provincial Commissioners of Kampala, F. A. Knowles, Esq., C.M.G., and L. H. Cubitt, Esq., and of Sir Apolo Kagwa, K.C.M.G., the Prime Minister, was then sought to aid in organising a series of large drives of wild game in the fly-area in the neighbourhood of the laboratory. We are greatly indebted to these gentlemen for their ready efforts on our behalf, which resulted in a large drive being organised. Unfortunately, the Commission were ordered to leave the country before the drive could take place. The work on these lines, however, is being continued by Dr. R. van Someren, of the Uganda Medical Staff, and Captain A. D. Fraser, Royal Army Medical Corps, who were instructed to take over the laboratory work.

This very difficult question, therefore, still awaits its answer.

Conclusions.

1. Water-buck, bush-buck, and reed-buck can readily be infected with a *human strain* of the trypanosome of Sleeping Sickness by the bites of infected *Glossina palpalis*.

2. One exposure to the bites of infected flies is sufficient to infect an antelope with the virus of Sleeping Sickness.

3. Though the blood of an antelope may be proved to be infected with

* *Vide* 'Roy. Soc. Proc.,' B, 1910, vol. 82, pp. 63—66.

Trypanosoma gambiense, careful and continued examinations over prolonged periods may fail to reveal the presence of the parasite in the blood.

4. The incubation of the disease (Sleeping Sickness) in antelope is probably seven days.

5. Antelope of the water-buck, bush-buck, and reed-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*.

6. This transmission of the infection to clean laboratory-bred flies may occur at least 81 days after the last feed of the infected flies on a buck.

7. *Glossina palpalis*, when infected with the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

8. An appreciable percentage of *Glossina palpalis* will become infected with the virus of Sleeping Sickness should these flies feed on antelope suffering from this disease.

9. It follows, from the above conclusions, that antelope living in the fly-areas are "potential" reservoirs of the virus of Sleeping Sickness.

10. No antelope up to the present has been found naturally infected with *Trypanosoma gambiense*.

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[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 83]

Experiments to Ascertain if the Domestic Fowl of Uganda may Act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

(Received November 12, 1910,—Read January 19, 1911.)

Introduction.

Birds of various species are very numerous on the shores of Lake Victoria. Of these, cormorants, darters, herons (African grey and purple, and other species), ibises (glossy and sacred), fish-eagles, weaver birds (various species), terns (various species), gulls, geese (Egyptian and spur-winged), plovers (various species), pratincoles, storks, kingfishers and gallinules are the most common. These birds all inhabit areas where the *Glossina palpalis* are numerous, and some evidence is forthcoming that in Nature the fly feeds on avian blood.*

In view of the continued infectivity of the fly in the depopulated areas of the Lake-shore, it is clear that every effort should be made to ascertain the source of such infectivity. Search for a reservoir of the virus of Sleeping Sickness at once suggests itself, and to those acquainted with the fauna of the Lake-shore, an avian host would be included amongst the various species requiring investigation.

Domestic fowls were employed in these experiments. They are readily obtained, easily handled, and the flies feed greedily on them. One species of trypanosome may in Nature only affect one species of avian host; therefore, it follows that whatever the result of this series of experiments may be, that result is only applicable to the fowl. The Commission trust that no wider significance will be attached to the conclusions set forth at the end of this paper: in other words, because a Uganda fowl is or is not a reservoir of *Trypanosoma gambiense*, it does not follow that a cormorant, or other bird, is or is not a reservoir. It would have been better for this reason to have experimented with one or other of the various species of wild Lake-shore birds. The difficulties were, however, too great at the time these experiments were carried out. It is hoped that this paper will stimulate rather than discourage other workers to carry on this line of investigation.

* 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 496, Table IV.

In order to discover whether Uganda fowls can act as a reservoir of the virus of Sleeping Sickness, the Commission asked themselves the following questions:—

1. Can fowls be infected with the virus of Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis?*

2. Can fowls transmit the virus of Sleeping Sickness (*Trypanosoma gambiense*) to clean laboratory-bred *Glossina palpalis?* If this is possible, can the flies so infected convey the virus to normal susceptible animals?

An answer in the affirmative to those two questions would throw suspicion on a possible avian host. To decide these points, the following experiments were devised and carried out:—

Can Fowls be Infected with the Virus of Sleeping Sickness (Trypanosoma gambiense) by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis?

The method adopted to infect the fowls was carried out as follows:—Clean *Glossina palpalis*, hatched out in the laboratory, were given their first feeds on a monkey whose blood contained many *Trypanosoma gambiense*. An interval of at least 24 hours was then allowed to elapse before the infected flies were first fed on the fowl. The fowl was then fed on, for varying periods, by these flies. To make certain the flies were infective, their infectivity was usually tested by their being allowed to bite normal susceptible animals during the experiment. Finally, to discover if the fowl had become infected with *Trypanosoma gambiense* by the bites of the *Glossina palpalis*:—

(*a*) The fowl's blood was examined frequently throughout the experiment for *Trypanosoma gambiense*.

(*b*) The fowl was killed and its centrifuged heart or jugular vein blood was carefully examined microscopically for *Trypanosoma gambiense*.

(*c*) Two or more cubic centimetres of the centrifuged blood were injected into a normal susceptible animal on one or more occasions during the experiment.

Two typical experiments are given in detail.

Experiment 1915 *a*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1909.				
Nov. 22—25	1—3	Flies fed on *T. gambiense*-infected monkey.		250 laboratory-bred *G. palpalis* used.
„ 26—Dec. 31	4—39	Flies fed on Cock 1926.	—	*T. gambiense*-infected flies found on Dec. 13, 14, 16, 17, 20, and 27.
1910.				
Jan. 1—10	40—49	Flies fed on normal monkey, Experiment 1999, to test their infectivity.		100 flies of this experiment survived to 50th day and were used for Experiment 1915*b*.
„ 11	50	Monkey, Experiment 1999, examined; blood showed *T. gambiense*.	+	Allowing 7 days for the incubation of *T. gambiense* in this monkey, it is seen that the flies infected it on Jan. 4—that is, 4 days after feeding on fowl.

Remarks.—On December 17, 1909, 1 c.c., and on December 22, 3 c.c. of the blood of cock, Experiment 1926, were injected into a normal monkey, Experiment 1954. This monkey remained healthy. On January 10, 1910, the cock was killed, 10 c.c. of its heart's blood were centrifuged, and the "buffy layer" carefully examined for *Trypanosoma gambiense*, with negative results. The whole of the 10 c.c. of blood obtained were then injected into two monkeys: 5 c.c. into monkey, Experiment 1954, and 5 c.c. into monkey, Experiment 2054. The blood of these two animals was examined frequently for a month after these injections. No trypanosomes were ever found and the monkeys remained healthy.

Experiment 2081.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
Jan. 9—14 ...	1—5	Flies fed on *T. gambiense*-infected monkey.		120 laboratory-bred *G. palpalis* used.
„ 15—Feb. 2	6—24	Flies fed on cock, Experiment 2074.	—	Feb. 15, monkey, Experiment 2135, showed *T. gambiense* in its blood.
Feb. 3—7	25—29	Flies fed on normal monkey, Experiment 2135, to see if flies are infective.	+	Feb. 22, monkey, Experiment 2178, showed *T. gambiense* in its blood.
„ 8—14 ...	30—36	Flies again fed on cock, Experiment 2074.	—	Fowl, Experiment 2074, has therefore been fed on by infective *G. palpalis*.
„ 15—19 ...	37—41	Flies fed on normal monkey, Experiment 2178, to see if flies still infective.	+	

Remarks.—Cock, Experiment 2074, was killed on March 16, 1910; 10 c.c. of its heart's blood were taken and centrifuged and carefully examined for *Trypanosoma gambiense*, with negative results. The 10 c.c. were then injected, subcutaneously, into normal monkey, Experiment 2301. This monkey was examined bi-weekly up to April 15, 1910. No *Trypanosoma gambiense* were ever seen, and the monkey remained healthy.

The 13 experiments carried out on these lines are now given in tabular form, in Table I below :—

Table I.

No. of experiment.	No. of flies used.	No. of fowl fed on.	No. of infected flies found during experiment.	Result.	Remarks.
1915 *a*	250	Expt. 1926	7	Negative	Proved infective flies fed on this fowl.
1915 *b*	100	„ 2059	1	„	Proved infective flies fed on this fowl.
1915 *c*	50	„ 2182	—	„	Doubtful whether infected flies fed on this fowl.
1918	100	„ 1927	—	„	Proved infective flies fed on this fowl.
2081	120	„ 2074	2	„	Proved infective flies fed on this fowl.
2082	100	„ 2087	12	„	Proved infective flies fed on this fowl.
2208	100	„ 2209	0	„	Proved infective flies fed on fowl, but no flies dissected.
2325	160	„ 2334	2	„	Proved infective flies fed on fowl.
2362	110	„ 2363	7	„	Proved infective flies fed on fowl.
2365	300	„ 2336	0	„	Flies not dissected, but were proved infective.
2366	150	„ 2364	0	„	Flies not dissected, but were proved infective.
2390	180	„ 2367	7	„	Proved infective flies fed on fowl.
2395	100	„ 2396	1	„	Proved infective flies fed on fowl.

Remarks.—In these experiments it is seen that 1820 laboratory-bred and laboratory-infected *Glossina palpalis* were fed on 13 fowls, with uniformly negative results. With one exception, Experiment 1915 *c*, the flies were proved to be infective by feeding them on animals susceptible to *Trypanosoma gambiense* infection. That the fowls had not become infected with *Trypanosoma gambiense* was proved by carrying out the three tests given above, under headings *a*, *b*, and *c*. In Experiment 2325, the liver, spleen and bone-marrow of Fowl 2334 were also examined for trypanosomes, and 10 c.c. of the pooled pulp injected into a normal monkey. This monkey remained healthy.

A further series of experiments bearing on this question was carried out. These experiments are not so complete. The procedure was as follows:—

A varying number of laboratory-bred *Glossina palpalis* were fed for several days on *Trypanosoma gambiense*-infected antelope. The infected flies were then fed for varying periods on fowls. The fowls were then killed and 2 c.c. of their blood were injected into a normal monkey. The monkey's blood was examined for *Trypanosoma gambiense* bi-weekly for a month after the injection of the fowl's blood. The experiments are given in tabular form.

Table II.

Date.	Days of "cycle" the flies fed on fowl.	Experiment No. of fowl.	No. of flies used.	Did flies subsequently prove to be infective?	Result of inoculation of fowl's blood into normal monkeys.
1910.					
May 9—21	7—19th	Cock 2455	50	No	Negative
„ 13—31	4—22nd	„ 2471	60	Yes (18 p. c.)	„
„ 16—June 1	7—23rd	„ 2479	95	„ (2·1 p. c.)	„
„ 16— „ 1	4—20th	„ 2480	55	„ (15 p. c.)	„
June 2—11.....	10—19th	„ 2518	100	No	„
„ 2—14.....	5—17th	„ 2519	100	„	„
„ 2—17.....	8—23rd	„ 2519	100	Yes (8·8 p. c.)	„
„ 2—11.....	11—20th	„ 2519	100	No	„

Remarks.—The chief criticism against these experiments is the fact that the flies fed on the fowls during the earlier days of the experiments, when the *Glossina palpalis* are usually not infective. However, as results have been recorded by the Commission where *Glossina palpalis* have infected susceptible animals as early as the 18th day of an experiment, it has been decided to publish these experiments.

Can Fowls Transmit the Virus of Sleeping Sickness to clean Laboratory-bred Glossina palpalis? If this is possible, can the Flies so Infected convey the Virus to Normal Susceptible Animals?

In view of the results obtained by the first series of experiments, it would seem somewhat unnecessary to follow this question further.

It has so far been proved that the blood of fowls which have been fed upon by infected *Glossina palpalis* is non-infective when injected into susceptible animals. It has now to be ascertained if the blood of such fowls is also incapable of infecting the fly. This would constitute additional evidence against the fact that fowls act as a reservoir of *Trypanosoma gambiense*.

Cages of laboratory-bred and laboratory-infected *Glossina palpalis*, which were known to be infective, were fed on fowls for a varying number of days. Next, clean laboratory-bred flies were fed on these fowls for several days. The flies were then fed on normal susceptible animals (monkeys), in the endeavour to infect such animals with *Trypanosoma gambiense*, and so prove that the blood of the fowl was infective. The full details of three experiments are given below:—

Experiment 2018.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Jan. 4—10.....	1—6	Flies fed on Fowl 1927.		100 laboratory-bred *G. palpalis* used. Fowl, Experiment 1927, has been fed on for 29 days by *T. gambiense*-infected *G. palpalis*.
,, 11	7	Flies starved.		
,, 12—Feb. 23	8—50	Flies fed on Monkey 2061.	—	87 *G. palpalis* dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1927. All were negative for flagellates. Monkey 2061 examined, with negative result, till March 7.

Experiment 2019.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Jan. 4—10.....	1—6	Flies fed on Fowl 1926.		100 laboratory-bred *G. palpalis* used. Fowl, Experiment 1926, has been fed on for 29 days by *T. gambiense*-infected *G. palpalis*.
,, 11	7	Flies starved.		
,, 12—Feb. 23	8—50	Flies fed on Monkey 2062.	—	80 *G. palpalis* dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1926. All were negative for flagellates. Monkey 2062 was examined bi-weekly for *T. gambiense* until March 22; no trypanosomes were seen. Monkey remained healthy.

Experiment 2276.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
Mar. 7—12.....	1—5	Flies fed on Fowl 2087.		200 laboratory-bred flies used. Fowl, Experiment 2087, has been fed on for 18 days by *T. gambiense*-infected *G. palpalis*.
Mar. 13	6	Flies starved.		
„ 14—Apr. 16	7—40	Flies fed on Monkey 2344.	–	116 *G. palpalis* dissected throughout this experiment, between the 23rd and 42nd days after flies' first feed on Fowl 2087. All were negative for flagellates. Monkey 2344 was examined bi-weekly for *T. gambiense* till May 10; no trypanosomes ever seen. Monkey died of broken neck later.

Remarks.—It will be seen that 400 laboratory-bred *Glossina palpalis* fed on the three fowls, Experiments 1927, 1926, and 2087, subsequently failed to infect monkeys with the virus of Sleeping Sickness, and that all the 283 *Glossina* dissected throughout these experiments were negative for flagellates. This absence of flagellates in the flies is interesting, because Fowl 1927 and Fowl 1926 were both found to be naturally infected with a large avian trypanosome. It would thus seem that this fowl trypanosome does not undergo development in *Glossina palpalis*.

Conclusion.

The Uganda fowl cannot act as a reservoir of the virus of Sleeping Sickness.

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Original Communications.

EXPERIMENTS TO ASCERTAIN IF CATTLE MAY ACT AS A RESERVOIR OF THE VIRUS OF SLEEPING SICKNESS (*TRYPANOSOMA GAMBIENSE*).[1]

BY COLONEL SIR DAVID BRUCE, C.B., F.R.S.,
CAPTAINS A. E. HAMERTON, D.S.O., AND H. R. BATEMAN,
Royal Army Medical Corps;
AND
CAPTAIN F. P. MACKIE,
Indian Medical Service.

THE question as to whether cattle can act as a reservoir of the virus of sleeping sickness is an important one. It was usually believed until lately that man was the main reservoir, and that the other animals might be ignored. But in view of the fact that the flies on the Lake-shore have remained infective for some two years after the native population have been removed, it is necessary to inquire if it is not possible that other animals may act as well.

In this regard cattle have been, perhaps, the most important, as on the once thickly populated Lake-shore and islands they were numerous, and in many cases grazed and watered in the fly-area. Another reason of their importance is, that if they can act as a reservoir, then the same will probably be true of the different species of antelope which inhabit the Lake-shore. It may be presumed that these will greatly increase now that the natives and domestic animals have been removed, and that they will take the

[1] Reprinted from the *Proceedings of the Royal Society*, B., vol. lxxxii., 1910.

place of the cattle in keeping up the infectivity of the *Glossina palpalis*.

The Commission, therefore, thought it would be well to inquire into the question, and the result is given in this paper.

Answers to the following questions were sought: Are cattle capable of being infected with sleeping sickness by the subcutaneous injection of blood containing *Trypanosoma gambiense*? Can cattle be infected with sleeping sickness by the bites of artificially infected tsetse-flies? Can cattle be infected with sleeping sickness by the bites of the naturally infected flies caught on the Lake-shore? Is it possible to infect tsetse-flies by feeding them on cattle infected with sleeping sickness, and afterwards to transmit the disease by means of these flies to healthy animals? Finally, if these questions are answered in the affirmative, will it be possible to find that cattle which have lived in the fly-area are naturally infected with sleeping sickness?

I. *Are Cattle capable of being Infected with Sleeping Sickness by the Subcutaneous Injection of Blood containing* Trypanosoma gambiense?

Experiment 869. Bull.

September 10th, 1909.—A bull was inoculated with 5 cc. of blood containing large numbers of *T. gambiense* from an infected monkey.

Its blood was examined daily, and 18 days after injection the bull was found to be infected with *T. gambiense*. The identity of the trypanosome was established by injecting a monkey with some blood from the ox. This monkey showed *T. gambiense* on the sixth day.

Conclusion.—From this experiment it is seen that oxen are capable of being infected with sleeping sickness by the injection of blood containing *T. gambiense*. The trypanosome appears in small numbers in the blood, and the blood, when injected into susceptible animals such as monkeys, gives rise to a fatal form of the disease.

II.—*Can Cattle be Infected with Sleeping Sickness by the Bites of Artificially Infected* Glossina palpalis?

The two following experiments were carried out by feeding *G. palpalis* first on an infected monkey, and immediately afterwards on a healthy ox. Wild flies from the Lake-shore were used.

Experiment 890. Ox.

May 20th, 1909.—The ox was thrown and a monkey heavily infected with sleeping sickness was laid across its flank. Two cages of *G. palpalis*, containing 100 and 150 flies respectively, were allowed to feed for a few seconds on the monkey and then on the ox. The flies were allowed from 30 to 35 interrupted feeds on each animal every day. This was continued for 38 days, during which time 561 flies were estimated to have fed on one or other animal.

July 17th.—Fifty-eight days after the first infected feed *Trypanosoma gambiense* appeared in the blood of the ox.

The identity of the trypanosome was established by injection of the ox's blood into two monkeys. The first monkey was injected with blood from the ox 76 days, and the second monkey 181 days after the flies had first fed on the ox. Both monkeys developed sleeping sickness, the first 7 days and the second 11 days after injection of the blood.

Experiment 891. Calf.

The details of this experiment were similar to those of the last. *T. gambiense* appeared in the blood of the calf 57 days after the flies had been first fed upon it.

Three cubic centimetres of the blood of the calf were injected into a monkey, and the monkey developed sleeping sickness after an incubation period of 8 days.

Conclusion.—These two experiments show that when artificially infected *G. palpalis* are allowed to feed on healthy cattle, these animals develop sleeping sickness, and that the blood of the cattle is capable of giving rise to infection of *T. gambiense* in monkeys when injected into them.

III. *Can Cattle be Infected with Sleeping Sickness by the Bites of the Naturally Infected Flies caught on the Lake-shore?*

In the next three experiments freshly caught *G. palpalis* brought up to the laboratory from the Lake-shore were allowed to feed straightway on healthy cattle. By this means it will be shown whether *G. palpalis* in their wild state are capable of giving sleeping sickness to healthy cattle.

Experiment 982. Bull.

2,195 freshly captured *G. palpalis* were applied to a bull, and of these 1,536 were estimated to have fed. This feeding of the flies

extended over a period of 16 days, at the end of which time *T. gambiense* appeared in the blood of the bull.

To help in the identification of this trypanosome, 3 cc. of the blood of the bull were injected into a monkey. The monkey developed sleeping sickness 18 days later. 5 cc. of the blood of the bull were also injected into a goat. *T. gambiense* appeared in the blood of the goat after an incubation period of 38 days.

Experiment 1,462. Bull.

The details of this experiment were similar to those of the last one. Over a period of 8 days 1,370 wild flies from the Lake-shore were applied to the bull, of which 705 fed. Ten days from the first application of flies *T. gambiense* appeared in the blood of the bull.

Two animals, a monkey and a goat, each received 1 cc. of the blood of the bull by injection under their skin. The monkey developed sleeping sickness seven days later, but the goat died in 16 days without showing any infection.

Experiment 1,465. Bull.

During a period of 13 days, 459 freshly caught Lake-shore *G. palpalis* were applied to a bull, and of these 314 fed. On the 14th day after the flies were first fed the bull developed an infection of *T. gambiense.*

Some blood from this bull was injected into a monkey and into a goat. Neither animal became infected.

Conclusion.—These experiments prove that *G. palpalis*, when captured in their natural state on the Lake-shore, are capable of transmitting the virus of sleeping sickness to cattle, and that the blood of these cattle gives rise to a fatal form of the disease in monkeys and in goats when it is injected into them.

IV. *Is it possible to Infect Tsetse-flies by Feeding them on Cattle Infected with Sleeping Sickness, and afterwards to Transmit the Disease by means of these Flies to Healthy Animals?*

Five experiments under this heading were carried out. Laboratory-bred flies were used in all of them. Three were negative and two positive. The three negative experiments will be shortly summarised first.

Experiment 1,451.

Ninety laboratory-bred *G. palpalis* were fed for 10 successive days on a calf whose blood contained *T. gambiense.* The flies were starved for 72 hours. They were then fed on a clean monkey daily

for 45 successive days. The monkey failed to develop sleeping sickness.

When the remainder of the flies were dissected, one contained flagellates, but when the contents of this fly were injected into a goat the animal failed to show any infection of *T. gambiense*.

Result.—Negative.

Experiment 1,269.

The details of this experiment were similar to those of the last. After the *G. palpalis* had been fed on two oxen whose blood contained *T. gambiense*, they were applied daily to a monkey. They were fed on this monkey for 35 consecutive days and were then transferred to a second monkey. Both the monkeys remained healthy.

Two of the flies were found on dissection to contain flagellates, but when these were injected into a monkey and a goat no development of sleeping sickness took place in these animals.

Result.—Negative.

Experiment 1,672.

Here again the technique was similar to the last. The *G. palpalis* were fed on alternate days for a lengthened period, on a clean monkey and a clean goat. Both animals remained healthy.

Some infected flies were found on dissection, but when introduced under the skin of a goat and of a monkey did not give rise to sleeping sickness.

Result.—Negative.

The next two experiments, which were carried out in the same way as the two preceding ones, were positive.

Experiment 1,566.

The *G. palpalis* were fed on an infected ox, and after a starve of 72 hours were fed on a clean monkey for 45 successive days. Sixty-eight days after the flies had taken their first infected feed this monkey developed sleeping sickness.

When the flies came to be dissected nine of them showed flagellates either in the proboscis or in the alimentary tract. Some of these were injected into goats and into a monkey, but with negative results.

Result.—Positive.

Experiment 1,602.

Fifty laboratory-bred flies were fed for four successive days on an ox whose blood contained *T. gambiense*. After a period of starvation they were applied to a monkey and to a goat on alternate days.

The monkey died before it could have become infected, but the goat developed sleeping sickness 20 days after the flies had their first infected feed.

The remainder of the flies, 32 in all, were dissected, and five were found to contain flagellates. The alimentary contents of one of these flies were injected into a monkey, and after an incubation period of 13 days *T. gambiense* appeared in its blood.

Result.—Positive.

Conclusion.—Laboratory-bred tsetse-flies can be infected by feeding them on cattle infected with sleeping sickness, and afterwards the disease can be transmitted to healthy animals by means of these flies.

V. *Do Cattle, when Living in the Fly-area, actually carry the Virus of Sleeping Sickness?*

About seventeen cattle from various sources were examined with this point in view. Not all these cattle could be proved to have been exposed to the bites of *G. palpalis*, but most of them came from places where these flies are plentiful. One was positive.

Experiment 1,633.

This cow came from the island of Kome, in Lake Victoria, where human sleeping sickness is prevalent and where *G. palpalis* abound.

T. gambiense was found in its blood by microscopical examination, and when 3 cc. of the blood were injected under the skin of a monkey the animal developed sleeping sickness after an incubation period of seven days.

Conclusion.—This experiment proves that cattle in their natural state, and apparently in good health, may harbour the virus of sleeping sickness.

General Conclusions.

It has been proved by experiment that cattle may act as a reservoir of the virus of sleeping sickness, and that healthy animals may be infected from them by means of *G. palpalis*.

It has also been proved that cattle in the fly-area do naturally harbour *G. gambiense*.

It is, therefore, possible that the cattle and antelope living in the fly-area may act as a reservoir, and so keep up the infectivity of the *G. palpalis* for an indefinite period, but there is no proof up to the present that this actually takes place in Nature.

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13

Trypanosome Diseases of Domestic Animals in Uganda.
I. Trypanosoma Pecorum.

By Colonel SIR DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–9.)

(Received May 13,—Read June 16, 1910.)

[PLATES 11 AND 12.]

SYNONYMS.

Trypanosoma dimorphon (Dutton and Todd).
Trypanosoma congolense (Broden).
Trypanosoma confusum (Montgomery and Kinghorn).
Dr. Edington's trypanosome from Zanzibar (Bruce, Hamerton, and Bateman).
Trypanosome from Chai-Chai, Zambesi, Zululand (Theiler).
Trypanosome from Southern Rhodesia (Bevan).

INTRODUCTION.

As might be expected from the tropical nature of the country, Uganda suffers much from protozoal diseases, and as the wealth of the natives consists principally in cattle, goats, and sheep—the King and chiefs having huge herds—there is much loss.

To give an idea of the enormous mortality which must take place among the herds of cattle in Uganda, Sir Apolo Kagwa, K.C.M.G., the Prime Minister, may be quoted. He informed the Commission that during 1908 he had 1396 cows, which had 2021 calves, and of these calves 709 died—35 per cent. Further, of cows, bulls, and full-grown bullocks, there had died 351. This makes a total of 1060 deaths in one year. The number of the whole herd is not given, but may be put down at 4000. This means a yearly death-roll of about 25 per cent. During the same year the Government Transport Department in Entebbe lost 156 oxen between June and November.

During 1909 the Commission had the opportunity of investigating several outbreaks of trypanosome disease among domestic animals, one among the transport cattle at Entebbe (lat. 0° 3′ N., long. 32° 30′ E.); another at Kampala (lat. 0° 18′ N., long. 32° 35′ E.); a third at the Uganda Company's estate at Namukekera (lat. 0° 40′ N., long. 32° 17′ E.); a fourth at Mr. Walsh's farm at Kabula Muliro, a few miles west of Namukekera; and a fifth at the Mabira Rubber Estate (lat. 1° 50′ N., long. 32° 40′ E.).

The commonest trypanosome disease among cattle in Uganda is caused by a trypanosome of the *dimorphon* type, which it is proposed to call *Trypanosoma pecorum*. This species is probably the same as that which has been known by the name *Trypanosoma dimorphon* (Dutton and Todd), and is either identical with, or very similar to, Broden's *Trypanosoma congolense*. The name *dimorphon* is a misleading one, and can only be accounted for by Dutton and Todd describing under one name two species of trypanosomes occurring in the same animal. No one, so far as we are aware, has re-discovered Dutton and Todd's *Trypanosoma dimorphon*, although many attempts to reconcile later observations with theirs have been made. Certainly the strain kept at the Liverpool School of Tropical Medicine under that name does not agree with the original description. The term *Trypanosoma dimorphon* must therefore disappear, since it was born of a misapprehension. But it will at once be said, if the name *Trypanosoma dimorphon* must go, why not call the species *Trypanosoma congolense*? The only reasons that can be given are, that *Trypanosoma congolense* is a local name and, therefore, not very suitable, and secondly, that if it comes to strict priority, then *Trypanosoma dimorphon* holds the field in spite of its misleading character and the error its authors fell into, because it cannot be denied that there is a strong feeling that *Trypanosoma dimorphon* and *Trypanosoma congolense* are one. At the same time it must also be granted that there are others who are strongly of opinion that *Trypanosoma dimorphon* and *Trypanosoma congolense* are distinct species. Under these circumstances it seems best to unite the old names under a new one, and *Trypanosoma pecorum* seems appropriate enough, as this trypanosome disease is peculiarly one of herds. Montgomery and Kinghorn have lately proposed the name *confusum*, in order to get out of the difficulty; but this name has been used for a trypanosome already, and it is, moreover, an awkward term.

It must then be understood that in the species *Trypanosoma pecorum* we include *Trypanosoma dimorphon*, *Trypanosoma congolense*, the trypanosome discovered in Zanzibar by Edington and described in the 'Proceedings,'* that from Portuguese East Africa and Zululand described by Theiler, and the species found in Northern Rhodesia by Montgomery and Kinghorn, and in Southern Rhodesia by Bevan.

The other species of trypanosomes found in the blood of cattle in Uganda were *Trypanosoma gambiense*, *Trypanosoma brucei*, *Trypanosoma vivax*, *Trypanosoma cazalboui*, and *Trypanosoma nanum*. These names, however, may require to be reconsidered.

* B, vol. 81, p. 14.

Distribution in Uganda.

Cattle suffering from *Trypanosoma pecorum* were sent to the Sleeping Sickness Commission's laboratory at Mpumu from Entebbe, Kampala, Namukekera, Kabula Muliro, and Mabira. It is probably widely distributed throughout Uganda. It was also found in a horse which had arrived in Nairobi, British East Africa, from Abyssinia. The Commission is indebted to Mr. Stordy, Principal Veterinary Officer, for the opportunity of studying this trypanosome. It is not known where the horse became infected, but it must have been at some point between Nairobi and the Abyssinian border.

Morphology of Trypanosoma pecorum.

A. *Living, Unstained.*

This trypanosome, when observed in a preparation of fresh blood, is seen to remain at or near the same spot in the field, that is to say, it is non-translatory. It is, however, active and restless, the body quivering rapidly, and the undulating membrane and flagellum keeping up a constant vibratory motion. As a rule, it moves with the flagellar end in front. The contents of the cell are homogeneous, except for a vacuole at the posterior extremity.

A marked characteristic of this species is that it exhibits alternating periods of quiescence and activity. When quiescent it is usually invisible, as it has a habit of burying itself under small collections of red blood corpuscles.

B. *Fixed and Stained.*

Method of Staining.—Giemsa's method, as described in a former paper,* was used.

Length.—The same method of measuring was used as described in the same paper (p. 17).

Breadth.—Without the undulating membrane the average is about 2 microns, with the membrane about 3 microns.

Shape.—This trypanosome when stained is short and stout in form. The posterior extremity is blunt, or rounded, or pointed and angular. The anterior end is narrower. The undulating membrane is fairly well developed, more so, perhaps, than in *Trypanosoma nanum*. The flagellum arises near the micronucleus, and passes along the edge of the undulating membrane. There is no free flagellum.

Contents of Cell.—Generally homogeneous. Sometimes granules are seen which take on a chromatin stain, and are situated anterior to the nucleus.

Nucleus.—Is oval in shape, and situated about the middle of the body.

* 'Roy. Soc. Proc.,' B, vol. 81, p. 16.

Table I.—*Trypanosoma pecorum.*

No. of experiment.	Animal.	Day of disease.	Method of fixing and staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
82	Ox	—	Osmic vapour and Giemsa	11·7	14·0	10·0
505	,,	42	,,	12·3	15·0	10·0
,,	,,	—	,,	13·2	15·0	11·0
593	Sheep	50	,,	14·5	17·0	10·0
44	Monkey	31	,,	12·2	14·0	10·0
559	,,	14	,,	12·7	16·0	11·0
461	Dog	44	,,	15·3	18·0	13·0
543	,,	10	,,	14·3	16·0	11·0
1406	,,	31	,,	13·7	16·0	11·0
551	Rat	19	,,	12·6	18·0	8·0
626	,,	—	,,	12·8	15·0	10·0
398	Mouse	28	,,	14·6	16·0	13·0
398	,,	36	,,	13·1	17·0	10·0
			Average	13·3	16·0	10·6

Micronucleus.—Small and round, and situated near to, but not at, the posterior extremity.

Undulating Membrane.—Is simple, but fairly well developed.

Flagellum.—There is no free flagellum. In very rare cases, where there is an appearance of a free flagellum, the trypanosome will be found to be dividing.

As it is difficult to gain an idea of the general appearance of a trypanosome, a series of coloured drawings by Lady Bruce, R.R.C., is also given to supplement the written description (Plate 11). The trypanosomes have been stained by Giemsa's method, and drawn at a magnification of 2000. By referring to them, the shape, disposition of the micronucleus, and other parts of the structure of *Trypanosoma pecorum*, will be more readily understood.

As will be seen from this tabulated statement, the disease set up in domestic animals by *Trypanosoma pecorum* is a serious and fatal one.

Cattle, goats, sheep, monkeys, dogs, rats, and mice are susceptible. Guinea-pigs, on the other hand, are refractory. Horses, mules, donkeys, and rabbits were not available at Mpumu, so that, unfortunately, it is not possible to say whether they are inoculable or not. In regard to guinea-pigs it would be interesting to know whether a series of inoculations into rats or rabbits would make the *Trypanosoma pecorum* also pathogenic for them.

Animals Susceptible to Trypanosoma pecorum.

Table II.

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.	
82	Natural infection	?	2	Died 2 days after arrival.
110	,,	?	243	Treated with lithium antimonyl tartrate.
230	,,	?	4	Died 4 days after arrival.
357	,,	?	3	,, 3 ,,
358	,,	?	1	,, 1 day after arrival.
359	,,	?	60	Treated with arsacetin.
360	,,	?	14	Died of *Trypanosoma pecorum*.
391	,,	?	313	,, ,,
475	,,	?	9	Died 9 days after arrival.
476	,,	?	2	,, 2 ,,
477	,,	?	13	,, 13 ,,
482	,,	?	5	,, 5 ,,
483	,,	?	34	,, 34 ,,
505	,,	?	265	Killed.
550	,,	?	—	Treated with arsenic; still alive after 254 days.
1459	,,	?	43	Died 43 days after arrival.
1560	,,	?	46	,, 46 ,,
1731	,,	?	79	Killed.
1733	,,	?	76	Died 76 days after arrival.
1735	,,	?	79	Killed.
1736	,,	?	79	,,
1737	,,	?	84	Died of *Trypanosoma pecorum*.
97	Ox	9	46	,, ,,
447	,,	8	287	Killed.
1463	,,	7	35	Died of *Trypanosoma pecorum*.
1464	,,	7	14	,, ,,
1225	Monkey	—	—	Died 8 days after inoculation; bitten by snake. Never showed trypanosomes.
1357	Dog	6	15	Died of *Trypanosoma pecorum*.
1358	,,	6	18	,, ,,
1359	,,	6	22	,, ,,
1363	,,	6	15	,, ,,
1364	,,	6	38	,, ,,
1365	,,	6	29	,, ,,
	Average.....	6·7	63	
			Goat.	
1005	Ox	—	—	Never showed trypanosomes; still alive after 72 days.
1006	,,	14	44	Cause of death doubtful.
1404	,,	9	41	,, ,,
1405	,,	—	—	Never showed trypanosomes; under observation 31 days.
633	Monkey	12	51	Cause of death doubtful.
	Average.....	11·6	45	

* Duration includes the days of incubation, it dates from the day of infection.

Table II—*continued.*

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Sheep.	
697	Ox	21	—	Still alive after 170 days.
632	Monkey	8	43	Cause of death doubtful.
593	Dog	19	168	Died of *Trypanosoma pecorum.*
	Average.....	16	105	
			Monkey.	
44	Ox	14	35	Died of *Trypanosoma pecorum.*
49	,,	10	16	,, ,,
376	,,	11	56	,, ,,
683	,,	10	61	,, ,,
1740	,,	11	—	Still alive after 45 days.
719	Sheep	21	—	,, 216 ,,
350	Monkey	10	181	Died of *Trypanosoma pecorum.*
459	,,	6	80	,, ,,
1000	,,	16	45	,, ,,
460	Dog	11	75	Killed for cultivation experiments.
559	,,	12	39	Died of *Trypanosoma pecorum.*
560	,,	14	32	,, ,,
581	Rat	13	86	,, ,,
	Average.....	12·3	64	
			Dog.	
543	Horse	9	26	Died of *Trypanosoma pecorum.*
148	Ox	10	46	,, ,,
349	,,	22	98	,, ,,
552	,,	—	—	Re-injected after 17 days.
1007	,,	—	—	Found dead after 14 days.
1406	,,	21	29	Killed.
1407	,,	—	—	Died; under observation 31 days.
1193	Monkey	8	16	Died of *Trypanosoma pecorum.*
433	Dog	7	44	,, ,,
434	,,	9	58	,, ,,
461	,,	6	40	,, ,,
1544	,,	10	21	,, ,,
552	Rat	—	—	Still alive after 49 days.
	Average.....	11·3	42	
			Guinea-pig.	
685	Ox	—	—	Still alive after 96 days.
1162	,,	—	—	,, 36 ,,
1163	,,	—	—	,, 36 ,,
1164	,,	—	—	,, 36 ,,
1647	,,	—	—	Died; under observation 29 days.
628	Monkey	—	—	Still alive after 104 days.
1002	,,	—	—	,, 50 ,,
566	Dog	—	—	,, 78 ,,

* Duration includes the days of incubation, it dates from the day of infection.

Table II—*continued.*

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		White rat.		
397	Ox	17	39	Died of *Trypanosoma pecorum.*
551	,,	16	18	Killed for cultivation experiments.
684	,,	16	32	Died of *Trypanosoma pecorum.*
699	,,	—	—	Experiment stopped after 57 days.
1646	,,	12	16	Died of *Trypanosoma pecorum.*
626	Monkey	12	22	Killed for cultivation experiments.
1001	,,	13	23	Died of *Trypanosoma pecorum.*
455	Rat	9	12	,, ,,
729	,,	11	21	,, ,,
1708	,,	8	12	,, ,,
	Average.....	12·6	21	
		Mouse.		
686	Ox	—	—	Experiment stopped after 59 days.
398	Monkey	26	41	Died of *Trypanosoma pecorum.*
627	,,	12	26	,, ,,
454	Mouse	6	12	,, ,,
	Average.....	14·7	26	

* Duration includes the days of incubation, it dates from the day of infection.

Theiler describes a trypanosome from Chai-Chai, near the mouth of the Limpopo, in Portuguese East Africa; from the mouth of the Zambesi; and also from Zululand, which resembles the one under discussion, in not infecting guinea-pigs; and considers that this one fact is sufficient for the creation of a new species. We cannot agree with him in this, as there is no practical importance, except in the laboratory, in the fact that the guinea-pig is insusceptible; and, moreover, until more experiments have been made, we cannot be sure that under certain conditions of dosage or passage through the smaller animals the guinea-pig will remain refractory.

The important facts in regard to this species are, that man is not susceptible, but that the valuable domestic animals are, and that in these animals the disease is, as a rule, a fatal one.

As long as our knowledge of trypanosomes is limited it seems better to group them under as few names as possible. As knowledge grows, and as fundamental differences emerge, then it will be time to define them more strictly. As far as our present knowledge goes, the morphology of

Trypanosoma dimorphon, *Trypanosoma congolense*, the Uganda, Zanzibar, Chai-Chai, Zambesi, Zululand, and Rhodesian strains is identical; these trypanosomes affect the same important domestic animals; the carrier is probably or may be the same, though this is not known, and therefore these various forms should, for the present, be grouped under one name; and for certain reasons given above we propose the name *Trypanosoma pecorum* for this group.

Disease set up in Cattle by Trypanosoma pecorum.

It is unnecessary in this paper to describe in detail the symptoms which can be noted during life, or the pathological changes set up in the organs of cattle by this trypanosome. It will be sufficient to say that the main symptoms are emaciation, anæmia, and progressive weakness, and that the principal *post-mortem* appearances are those due to anæmia.

Incubation.—As is probably true of most trypanosome diseases in susceptible animals, the period of time which elapses between the infection of the animal and the appearance of the trypanosomes in sufficient numbers in the peripheral blood to be seen by the microscope, is a short one: in this case, an average of 6·7 days.

Duration.—Of the course and duration of this disease in cattle little, unfortunately, is known. Most of the cattle which came under observation at the laboratory of the Commission at Mpumu were already sick when they arrived, and it was, as a rule, impossible to know when they had been infected. By referring to the table it will be seen that 22 naturally-infected cattle were under observation. Four of them lived, on an average, nine months. Of these four, one, treated with arsenic, was still alive and apparently healthy in December, 1909, one was killed, one had been treated with lithium antimonyl tartrate, and one died without treatment at the end of 313 days. It is therefore impossible to say from the insufficient data at our disposal whether spontaneous cure ever takes place in this disease in cattle.

When we turn to the cases of cattle which were inoculated on the hill, and were therefore under observation from the beginning, we are struck by the rapid course of the disease. One animal certainly lived 287 days, but the remainder died, on an average, in 26 days from the date of infection. Most of these oxen were inoculated with a strain of this trypanosome which had caused a rapidly fatal epidemic among a herd of milch cattle belonging to Mr. Walsh, at Kabula Muliro. In the short space of one month 24 of Mr. Walsh's cattle died, and in two months 34 had died out of a herd of about 300 head.

It may therefore be concluded that *Trypanosoma pecorum* sets up a rapid and fatal disease in cattle.

Disease set up in Goats and Sheep by Trypanosoma pecorum.

The number of cases of this disease in goats and sheep which came under observation is too small to draw any conclusions from. At Mpumu the goats and sheep were not satisfactory experimental animals, as many of them died from some unknown cause. It was thought that as these animals usually lived in the valleys, and were often housed in their owner's hut during the night, the exposure on the top of the hill had a bad influence. One sheep was still alive after 168 days, and it is probable that most of the goats and sheep would have lived much longer if they had been kept under more favourable conditions.

Disease set up in the Smaller Laboratory Animals.

It is not necessary to describe in detail the action of *Trypanosoma pecorum* on the monkey, dog, and smaller laboratory animals, as a reference to the table will show the average periods of incubation and duration. It will be seen that this is a fairly rapid and fatal disease in the dog, white rat, and mouse. In the monkey the average duration is about two and a-half months.

THE CARRIER OF TRYPANOSOMA PECORUM.

Glossinæ.—From experiments made in the laboratory at Mpumu it seems probable that *Glossina palpalis* is capable of acting as a carrier of this trypanosome. Four experiments were made with ordinary wild Lake-shore flies, and of these one was successful. Four were also made with laboratory-bred flies, and one again came off. The latter experiment, however, with laboratory-bred flies was not free from doubt; but from the other, which seemed free from doubt, it appears that *Trypanosoma pecorum* can develop in *Glossina palpalis* and infect a healthy animal after a period of 21 days. More observations are required. It may be noted that in no instance did *Trypanosoma pecorum* appear in the blood of animals upon which freshly-caught Lake-shore tsetse flies had been fed. These flies were found to be naturally infected with *Trypanosoma gambiense* and *Trypanosoma vivax*. This is an argument, though a small one, that *Glossina palpalis* is not the common or chief carrier of *Trypanosoma pecorum*.

Tabanidæ.—There is some circumstantial evidence available to show that *Trypanosoma pecorum* is carried by the *Tabanidæ*. In the valleys round Mpumu Hill, so far as we are aware, there are no tsetse flies at any time of

the year. As a rule, there are a few *Tabanidæ*. The cattle belonging to the Commission went down to the foot of the hill every morning to graze, and returned to their kraal on the top at sunset. Half of the herd went to the east of the hill and half to the west. On both sides there was a small valley or glen, through which ran a small stream. In these valleys during the year, as a rule, a *Tabanus* or two or a *Hæmatopota* could be seen, but they were in small numbers. Now it is a curious fact that at certain times of the year enormous numbers of *Tabanidæ* will suddenly appear in places where only a few are, as a rule, to be found. For example, Mr. Brown, at Mabira, who was collecting the biting flies of his district, wrote on March 14, 1909, that the *Tabanidæ*, which for months had been scarce, were then swarming everywhere in countless numbers, and he afterwards wrote that this invasion lasted about a month. The particular species which appeared at Mabira at this time was *Tabanus socialis* (Walk.).

So, in the same way, at Mpumu, the *Tabanidæ*, which had been rare, suddenly appeared in swarms. They were first seen in the valley to the west of the hill in September, 1909, and a month later in the valley to the east. Soon after this the cattle which had shown no signs of disease during the previous year were found to be suffering from *Trypanosoma pecorum*. Those which grazed in the valley to the west were the first to be affected, and afterwards those which grazed to the east of the hill. The species of *Tabanidæ* in this case was *Tabanus secedens* (Walk.). In both groups of cattle there were cases of *Trypanosoma pecorum* disease, so that the *Tabanidæ* had a reservoir from which to draw the virus.

Another sudden epidemic of *Trypanosoma pecorum* disease occurred on Mr. Walsh's farm at Kabula Muliro, where, as stated above, 34 milch cattle died within two months in a herd of 300. The evidence is all against this epidemic having been caused by tsetse flies. During February and March, and again later in the year, during August and September, as many as 100 fly-boys were engaged scouring this district for biting flies. *Tabanidæ*, *Hæmatopota*, and *Chrysops* were brought in, but not a single tsetse, although a reward of 5 rupees was offered for each specimen. The commonest *Tabanus* in this district during August was *varietus* (Walk.).

It may, therefore, in our opinion, be concluded that the trypanosome disease caused by *Trypanosoma pecorum* can be carried from sick to healthy animals without the aid of *Glossinæ*, but what other species of fly, if any, acts as carrier is merely a matter of speculation at present.

Stomoxys.—Montgomery and Kinghorn state that they have strong evidence against this genus. At Mpumu several experiments were made to attempt to settle this question, but although they were persevered in for

months, they remained negative. *Stomoxys* are so numerous in every part of the country all the year round that it seems inconceivable that they can act as carriers. From October, 1908, until the following September, although numerous cases of cattle with *Trypanosoma pecorum* in their blood grazed all day long with healthy cattle, yet not a single case of infection took place. The *Stomoxys* were exceedingly numerous all this time, forming a small cloud of flies round the cattle, and passing constantly from one animal to another, being driven hither and thither by the rapidly-swishing tails. This is a natural experiment on a large scale.

It will therefore require very convincing proof to bring this Commission to the belief that *Stomoxys* are carriers of this disease.

The subject of the carrier of *Trypanosoma pecorum* must remain in this unsatisfactory state for the present, but it is hoped that experiments which are at present being carried out at Mpumu may throw some light on this important part of the subject.

Cultivation of Trypanosoma Pecorum.

One difficulty experienced at Mpumu in attempts to cultivate the various trypanosomes was that rabbits were not available to supply the blood for Novy and MacNeal's medium. The blood of rats, goats, and oxen was used; but in making the cultivation of trypanosomes a factor in their diagnosis uniformity must be of the first importance.

Another difficulty was the frequency of contamination of the tubes. This was, perhaps, to be expected in a laboratory on the top of a hill in the tropics, with very free ventilation.

Trypanosoma pecorum grows fairly readily on blood-agar medium. At the end of 24 hours clumps may be seen containing many trypanosomes, with their flagellar ends directed outwards and in active motion (Plate 12, fig. 1). The individual flagellates have irregularly-shaped granules of chromatin scattered through their body substance, and also many brightly-refractile vacuoles. After 48 hours' culture every field contains many active trypanosomes, and also small clumps composed of 10, 20, or more members (Plate 12, fig. 2).

After six days the trypanosomes are still very active; they vary much in size and shape, from the plasmodial to the elongated, flagellated, highly active trypanosome. After this they seem to degenerate, and in a few days living trypanosomes can no longer be found in the tube.

This description approaches to some extent that of the cultural characters of Dr. Edington's trypanosome from Zanzibar and *Trypanosoma dimorphon*, but there is not that extraordinary growth which was described as covering

several fields of the microscope. Whether this was due to the difference in the composition of the blood medium, or to the higher temperature at Mpumu, it is impossible at present to say.

By comparing the coloured plates of this trypanosome and the one from Zanzibar, a certain resemblance will be seen, but how far this should be taken as a factor in the diagnosis is difficult to say.

Conclusions.

1. *Trypanosoma pecorum* is an important trypanosome disease of domestic animals in Uganda.

2. It is similar in morphology, action on animals, and cultural characters, to the *Trypanosoma dimorphon* described by Laveran and Mesnil, and to Dr. Edington's trypanosome from Zanzibar, described in the 'Proceedings,'* except that *Trypanosoma pecorum* is not pathogenic to guinea-pigs.

3. The carrier is unknown, but is probably a *Tabanus*, and not *Stomoxys*.

DESCRIPTION OF PLATES.

Plate 11.

This plate represents the general appearance of *Trypanosoma pecorum* in stained preparations. Note the rounded, blunt, or angular shape of the posterior extremity; the small, round micronucleus and vacuole placed near this end; the oval nucleus; and the absence of a free flagellum, except in dividing forms. Stained Giemsa, × 2000.

Plate 12.

Fig. 1.—An aggregation, or clump, of *Trypanosoma pecorum* after 24 hours' growth in blood-agar. Stained Giemsa, × 2000.

Fig. 2.—The same after 48 hours. Stained Giemsa, × 2000.

Figs. 3, 4, and 5.—*Trypanosoma pecorum* after 3 days' growth. Stained Giemsa, × 2000.

Figs. 6 and 7.—*Trypanosoma pecorum* after 4 days' growth. Stained Giemsa, × 2000.

Figs. 8 and 9.—*Trypanosoma pecorum* after 8 days' growth. Stained Giemsa, × 2000.

Figs. 10, 11, and 12.—*Trypanosoma pecorum* after 10 days' growth. Stained Giemsa × 2000.

* B, vol. 81, p. 14.

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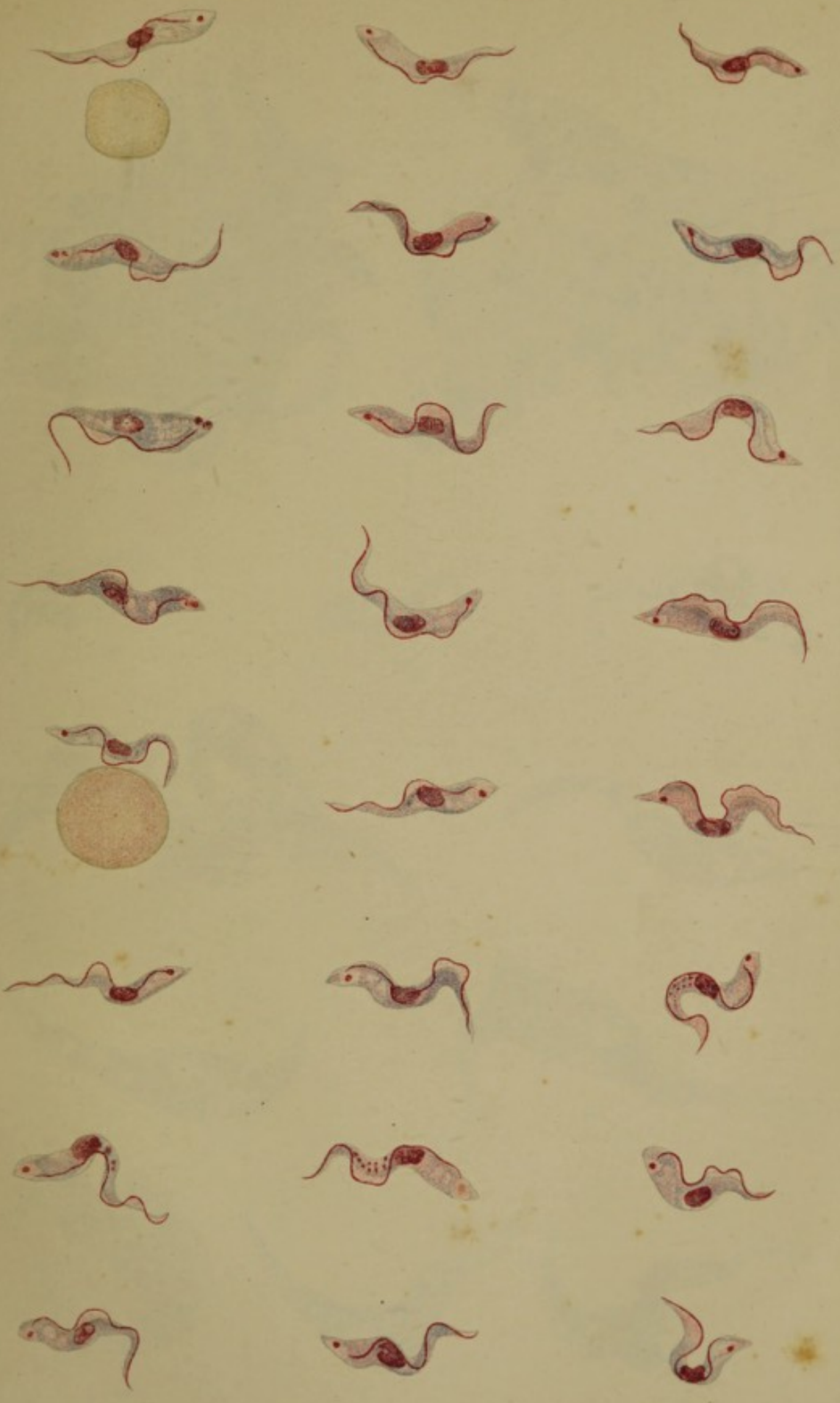


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*Trypanosome Diseases of Domestic Animals in Uganda.**

II.—*Trypanosoma brucei* (Plimmer and Bradford).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C.; and Captain F. P. MACKIE, I.M.S.
(Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10.)

(Received July 15, 1910.)

[PLATES 1 AND 2.]

Synonym : "Jinja trypanosome," Sleeping Sickness Commission, Royal Society, 1903.

INTRODUCTION.

This species was only met with on one occasion during the work of the Commission in 1909. This was in the blood of an ox from the Mabira Rubber Estate (latitude 0° 30′ N., longitude 32° 55′ E.). The manager wrote that the animal came from the Bukedi District, about 100 miles to the north (latitude 1° 50′ N., longitude 32° 40′ E.). Not much is known of this district, as it has only recently come under administration, and therefore it is impossible to say whether the ox was infected in Bukedi or on the journey south.

This is the species of trypanosome which was first discovered by Bruce, in 1894, in Zululand, to be the cause of Nagana, or tsetse-fly disease. During the work of the Sleeping Sickness Commission of the Royal Society in 1903, it was also met with in a herd of cattle from the same district of Bukedi, and then described as the "Jinja trypanosome."† It is impossible to name with any certainty the trypanosome seen in 1903, which affected the horses, camels, and dogs of the Abyssinian Boundary Commission. This was described as the "Abyssinian trypanosome." Its morphology, as given

* Continued from 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 479.

† 'Reports of the Sleeping Sickness Commission of the Royal Society,' No VI, p. 112.

in the coloured plate,* shows it to be similar to *Trypanosoma brucei*, so that in all likelihood it was either this species or the closely related *Trypanosoma evansi*. As camels were infected, it was more probably the latter.

In regard to the third kind of trypanosome found in 1903, and referred to as the "Mule trypanosome," no opinion can, at present, be given.

As to the remaining trypanosome seen in 1903, and known as "Pordage's ox trypanosome,"† an examination of the old specimens at once disclosed its identity. It is *Trypanosoma vivax* (Ziemann); and this accounts for the fact that the blood of the ox injected into a monkey and a dog had no effect, these animals being refractory to *Trypanosoma vivax*.

Morphology of Trypanosoma brucei, Uganda, 1909.

This species is interesting on account of its well-marked dimorphism, in which *Trypanosoma gambiense* resembles it. There are two varieties—the long and slender with free flagellum, and the short and stumpy without free flagellum. The shortest are only 13, and the longest 35 microns in length.

In this paper the long and slender are considered to be those which measure from 25 to 35 microns; the short and stumpy from 13 to 21 microns. Those trypanosomes which measure 22, 23 or 24 microns are called intermediate. The general character of these forms may be given, shortly, as follows:—The long and slender have a narrow extended posterior extremity, an elongated oval nucleus, a well-developed undulating membrane thrown into many waves, folds or plications, and a long free flagellum (see Plate 2, figs. 1 to 5). The short and stumpy, on the other hand, have a rounded posterior extremity, somewhat like the head of a turtle, a round or oval nucleus lying transversely to the long axis of the trypanosome, a well-developed undulating membrane thrown into bold folds, and no free flagellum (see Plate 2, figs. 11 to 16). The intermediate have a short free flagellum, a posterior extremity resembling that of the short and stumpy form but narrower, an oval nucleus, and a well-developed undulating membrane (see Plate 2, figs. 6 to 10).

A. *Living, Unstained.*

No observations were made in Uganda on this species of trypanosome in the living, unstained condition.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as described in the 'Proceedings.'‡

* *Ibid.*, No. VI, plate 2, p. 113.

† *Ibid.*, No. IV, p. 48.

‡ B, 1909, vol. 81, pp. 16 and 17.

Length.—The following table gives the length of this species as found in Uganda, in the ox, monkey, dog, guinea-pig, and rat. Only one trypanosome was found in the blood of the original Mabira ox. It measured 24 microns. In the monkey only 11 trypanosomes could be found. In all the others, 20 trypanosomes have been drawn and measured. These were taken as they came, except that dividing forms were passed by.

Table I.—Measurements of *Trypanosoma brucei*, Uganda strain, 1909.

Date.	No. of expt.	Animal.	Day of disease.	Method of fixing and staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1909.							
Aug. 12	1461	Ox	Not known	Osmic acid and Giemsa	24·0	24·0	24·0
Sept. 30	1627	Monkey	26th	,, ,,	24·8	30·0	19·0
Aug. 26	1479	Dog	41st	,, ,,	26·5	32·0	22·0
,, 30	1479	,,	45th	,, ,,	22·0	29·0	16·0
Sept. 30	1479	,,	76th	,, ,,	23·5	34·0	20·0
Oct. 11	1644	Guinea-pig	33rd	,, ,,	20·7	34·0	15·0
,, 18	1644	,,	40th	,, ,,	27·1	34·0	17·0
Sept. 9	1482	Rat	5th	,, ,,	24·7	30·0	21·0
,, 13	1482	,,	9th	,, ,,	23·5	31·5	19·0
,, 16	1643	,,	8th	,, ,,	19·6	21·0	18·0
					23·6	34·0	15·0

It will be seen from the above table that *Trypanosoma brucei*, Uganda strain, 1909, varies in length between 15 and 34 microns. Doubtless, individuals could be found slightly longer or shorter than this if they were hunted for. In Table I, as already mentioned, the 20 trypanosomes drawn are the first met with, except that dividing forms are left out, and this rule is adhered to throughout.

The average length of the 172 trypanosomes dealt with in Table I is 23·6 microns; but, as this does not give much information, the following table has been prepared. This represents by means of dots the length of each of 160 trypanosomes, in eight preparations of 20 each. Although *Trypanosoma brucei* is described as a dimorphic species, it must not be supposed that there is any strict line of demarcation between the long and slender and short and stumpy forms. There are the intermediate forms. This table is also useful in showing at a glance the distribution of the various lengths.

Table II.—Represents the Distribution in respect to Length of *Trypanosoma brucei*, Uganda strain, 1909.

Expt. No.	Short and stumpy.							Intermediate.			Long and slender.											Average, in microns.
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	
1479								●●●		●	●●	●●●	●●	●●●	●	●●		●				26·5
1479		●	●		●	●●	●●	●●	●●●	●●●				●	●●							22·0
1479						●●●	●●●	●●●		●	●●●					●			●●	●		23·5
1644	●	●	●●	●●●	●	●	●●●	●●	●●	●				●						●		20·7
1644			●		●	●			●●●		●●●	●	●●	●	●●●	●●●	●		●	●		27·1
1482							●	●●	●●●	●●●	●●●	●	●	●●	●	●						24·7

1482					●●●	●●●●	●●●	●	●		●		●	●●●	●●			●				23·5
1643				●●	●●●●	●●●●●●●●●●●●●	●															19·6
Totals......	1	2	4	6	10	26	14	14	12	9	12	6	6	12	10	7	1	2	3	3		
Percentage	0·6	1·3	2·5	3·8	6·2	16·2	8·7	8·7	7·5	5·6	7·5	3·8	3·8	7·5	6·2	4·4	0·6	1·3	1·9	1·9		

This division of *Trypanosoma brucei* into long, intermediate, and short forms is, of course, quite artificial, as the one form passes into the other by insensible gradations. It may, however, prove of some use in the identification of this species. In the same way a series of measurements of the various parts of the trypanosome body may also prove useful. The following table gives the distance between the posterior extremity and the micronucleus, the micronucleus and the nucleus, the size of the nucleus, the distance between the nucleus and the anterior extremity, and the length of the free flagellum.

Breadth.—The long and slender forms average 1·5 micron; the short and stumpy 2·5 microns.

Shape.—As already mentioned, this is a markedly dimorphic species, composed of long, slender forms with free flagella, and short, stout forms without free flagella. It is curious that the long forms may preponderate in the blood one day and the short forms another. For example, as will be seen from Table I, the trypanosomes in the guinea-pig's blood on October 11 were mostly short forms, giving an average of 20·7 microns, while a week later they were mostly long forms, giving an average of 27·1 microns. Note also Rat 1643, with an average of 19·6 microns, and Rat 1482, with an average of 24·7 microns.

This species may be compared with Dutton and Todd's *Trypanosoma dimorphon*, and in truth the coloured drawings given by them in their original plate* (figs. VII, IX, and X) are very similar, if not identical, in shape and size with *Trypanosoma brucei*. Fig. X, the long, slender form with free flagellum, is 30·5 microns in length. Fig. VII, the short, stumpy form without free flagellum, is 16 microns in length. As to fig. VI, on the same plate, stated to represent the "tadpole form," one would suspect from its general shape and appearance that it had no connection with the others. But in regard to figs. VII and X, anyone comparing Dutton and Todd's plate with the coloured plate accompanying this paper must be struck with the close resemblance between these two forms of *Trypanosoma dimorphon* and *Trypanosoma brucei*. Is it possible that Dutton and Todd were dealing with *Trypanosoma brucei* when they described *Trypanosoma dimorphon*?

Contents of Cell.—The protoplasm which is stained a pale blue is often dotted over with chromatin granules, especially in the anterior half (Plate 2). In well-stained preparations the distribution and number of these granules is sufficient, according to McFadyean, to differentiate this species from *Trypanosoma evansi*. This was also pointed out by Laveran

* 'First Report of the Trypanosomiasis Expedition to Senegambia (1902),' University Press of Liverpool, 1903.

Table III.—Measurements of the various Parts of *Trypanosoma brucei*, Uganda strain, 1909.

Experiment.	Posterior extremity to micronucleus.	Micronucleus to nucleus.	Nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
			Long and Slender Forms.			
1644	4	5	3·5	11·5	10	34
1644	3	7	4	10	10	34
1644	3	6·5	3·5	13	7	33
1644	2·5	7·5	3	10	8	31
1644	2	7	3·5	12·5	5	30
1644	2	7	3	11	7	30
1644	2	7	3	8	10	30
1482	1	7	3	11	8	30
1627	2	6	2·5	10·5	9	30
1627	3	7·5	2·5	13	4	30
1644	2	6	3	12	6	29
1644	3·5	5·5	3·5	9·5	7	29
1644	2	6·5	3	12·5	5	29
1644	2·5	7	3	6·5	10	29
1482	2	6	3·5	9·5	8	29
1644	3	6	3	8	8	28
1644	2	7	3	16	—	28
1482	1·5	6·5	2·5	13·5	4	28
1482	1·5	8	2·5	8	8	28
1627	1	7·5	2·5	11	6	28
1627	1	7·5	2·5	8	9	28
1644	2	7	3	10	5	27
1644	2	6	3·5	8·5	7	27
1627	1	7	3	9	7	27
1482	2	7·5	2	11·5	4	27
1644	2	6	2·5	10·5	5	26
1482	2	7	3	9·5	4·5	26
1644	2	6	3	7	7	25
1644	2	7	3·5	8·5	4	25
1644	1·5	5·5	3	10	5	25
1482	2	5·5	2·5	10	5	25
1482	1	6	3	11	4	25
1482	2	6·5	3	8·5	5	25
1627	2	6	2·5	8·5	6	25
Average...	2·1	6·6	3·0	10·2	6·4	—
			Intermediate Forms.			
1644	1	6	2·5	14·5	—	24
1482	2	6·5	3·5	8	4	24
1482	1	6	3	10	4	24
1482	1·5	6	3	9·5	4	24
1644	1	5	2·5	14·5	—	23
1644	1	7·5	2·5	12	—	23
1482	1	6	3	8·5	4·5	23
1482	2	5	2·5	8·5	5	23
1482	2	5	4	9	3	23
1482	1	5·5	3	9	4·5	23
1482	2	5	3	10	3	23
1627	1·5	7	3·5	4	7	23
1644	2	6·5	3	5·5	5	22
1644	1	7	3·5	6·5	4	22
1482	2	5·5	2	12·5	—	22
1482	1·5	6	2·5	7	5	22
1627	1	6	2	7	6	22
Average...	1·4	6·0	2·9	9·2	3·5	—

Table III—*continued.*

Experiment.	Posterior extremity to micronucleus.	Micronucleus to nucleus.	Nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
			Short and Stumpy Forms.			
1644	1	6	3	11	—	21
1644	1	5·5	2·5	12	—	21
1644	1	5·5	2·5	12	—	21
1482	2	5	3	11	—	21
1643	1	4·5	2·5	13	—	21
1627	1·5	6·5	3	7·5	2·5	21
1644	2	3	3	12	—	20
1644	1	5	2	12	—	20
1643	1	4·5	2	12·5	—	20
1643	1	4	2·5	12·5	—	20
1643	1	6	2·5	10·5	—	20
1643	1	5	2·5	11·5	—	20
1643	1	5·5	2	11·5	—	20
1643	1	5	3	11	—	20
1643	1	5·5	2·5	11	—	20
1643	1	4·5	2·5	12	—	20
1643	1	4	2·5	12·5	—	20
1643	1·5	5·5	2	11	—	20
1643	1	4	2·5	12·5	—	20
1643	1	5	2	12	—	20
1643	1	4·5	2	12·5	—	20
1627	1	6	2	11	—	20
1644	1	5	2	11	—	19
1644	1	3·5	2	12·5	—	19
1643	1	5	2·5	10·5	—	19
1643	1	5	2	11	—	19
1643	1	4·5	2	10·5	—	19
1643	1	5·5	3	10·5	—	19
1627	1	7	3	8	—	19
1644	1	4	2·5	10·5	—	18
1644	1	4	2·5	10·5	—	18
1644	1	3·5	3	10·5	—	18
1644	1	6	2·5	8·5	—	18
1643	1	4·5	2·5	10	—	18
1643	2	4	2·5	9·5	—	18
1644	1	5	3	8	—	17
1644	1	4·5	2·5	9	—	17
1644	1	6	2·5	7·5	—	17
1644	1	5·5	2	7·5	—	16
1644	1	5	2	7	—	15
Average ...	1·1	4·9	2·4	10·7	—	—
Average of three forms	1·5	5·8	2·8	10·0	3·3	—

and Mesnil some years ago, when they wrote: "Avec un peu d'habitude on arrive au contraire à distinguer les deux Trypanosomes, quand on dispose de préparations bien colorées et riches en parasites."*

Nucleus.—Is oval or elongated in shape in the long forms, a short oval in the intermediate, round or oval in the short, stumpy forms, and situated about the middle of the body.

* 'Trypanosomes et Trypanosomiases,' 1904.

Micronucleus.—Small and round, situated, on an average, 2·1 microns from the posterior extremity in the long and slender forms, 1·4 microns in the intermediate, and 1·1 microns in the short and stumpy.

Undulating Membrane.—This is well developed in this species and thrown into many folds and undulations. In this it differs markedly from *Trypanosoma vivax.*

Flagellum.—The flagellum in the long and slender forms is free and, on an average, 6·4 microns in length. In the short and stumpy forms there is no free flagellum.

Comparison of Trypanosoma brucei, Uganda, 1909, with Trypanosoma brucei, Zululand, 1894 (see Plate 1).

When *Trypanosoma brucei* was discovered in Zululand, in 1894, it was naturally thought to be the one and only trypanosome in Africa, and a detailed description seemed unnecessary. Now, however, since the number of species in Africa has increased to such an alarming extent, it is necessary to add to the old description by more detailed measurements. Luckily, many of the old Zululand preparations are still extant, so that it has been possible to do this. It must, however, be borne in mind that the Zululand preparations are some 15 years old, and were stained with carbol-fuchsin and not by Giemsa as in the case of the Uganda strain. They may, therefore, have shrunk to a greater extent than the more recently stained specimens.

Table IV.—Measurements of the Original Strain of *Trypanosoma brucei*, discovered in Zululand in 1894.

Trypanosoma brucei, Zululand, 1894.

Date.	No. of expt.	Animal.	Method of fixing and staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1/11/95	212	Horse	Osmic acid; carbol-fuchsin	22·4	32·0	14·5
1/11/95	212	,,	,, ,,	20·6	32·5	14·0
7/11/95	219	Donkey	,, ,,	23·0	35·0	14·5
23/11/95	214	Ox	,, ,,	18·0	25·5	13·0
4/7/97	—	Monkey	,, ,,	20·5	32·0	15·0
6/1/95	190	Dog	,, ,,	25·0	35·0	17·0
10/12/95	—	,,	,, ,,	24·0	32·0	17·0
14/1/96	233	,,	,, ,,	27·8	34·0	18·0
25/1/96	244	,,	,, ,,	20·6	33·0	15·0
7/2/96	229	,,	,, ,,	28·7	35·0	19·0
9/7/97	433	,,	,, ,,	19·7	29·0	17·0
				22·8	35·0	13·0

Table V.—Represents the Distribution in respect to Length of *Trypanosoma brucei*, Zululand strain, 1894.

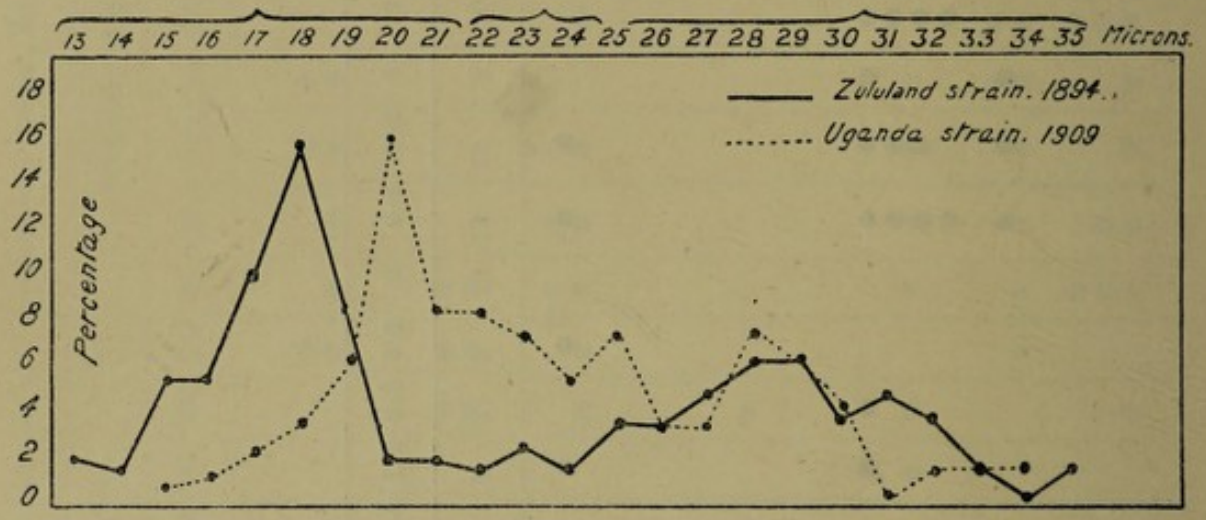
Expt. No.	Short and stumpy.									Intermediate.			Long and slender.										Average, in microns.
	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	
212			●	●●	●●	●●●	●			●	●			●●	●	●	●●	●●		●				22·4
212		●	●●●	●●	●●	●●●	●						●●	●●	●●					●				20·6
219			●●●	●●	●	●	●						●	●	●●	●●	●●●		●				●	23·0
214	●●●	●●	●	●	●●●	●		●	●●	●	●		●											18·0
—	●		●●	●●	●●●	●	●●					●	●		●	●	●	●		●				20·5
—					●●	●●●	●	●	●	●	●●			●	●	●	●	●●	●●	●				24·0
233						●●	●				●		●		●●●	●●	●	●	●●●	●●	●●	●		27·8

244			●	●		●●●●●●●	●●●●●	●●								●	●	●			●			20·6
229							●●					●	●			●●●●	●●●	●	●●●●	●●			●●	28·7
433					●●●	●●●●●●●●●●●	●●●					●		●		●	●							19·7
Totals............	4	3	11	11	20	32	17	4	4	3	5	3	7	7	10	13	13	8	10	8	3	1	3	
Percentage......	2	1·5	5·5	5·5	10	16	8·5	2	2	1·5	2·5	1·5	3·5	3·5	5	6·5	6·5	4	5	4	1·5	0·5	1·5	

The average length of the *Trypanosoma brucei*, Uganda, 1909, was 23·6 microns, the maximum 34 microns, and the minimum 15 microns. The measurements of the two strains therefore correspond very closely.

Tables II and V can be compared in the following chart, which gives the curves of the distribution in respect to length of *Trypanosoma brucei*, Uganda, 1909, and *Trypanosoma brucei*, Zululand, 1894.

Chart giving Curves of the Distribution, by percentages, in respect of Length, made up from Tables II and V.



From these curves it will be seen that there is a marked resemblance between the two strains when represented in this way.

Animals Susceptible to Trypanosoma brucei.

Table VI shows the effect of inoculating various animals with *Trypanosoma brucei*, Uganda strain, 1909.

The number of experiments carried out on the various animals is too small for comparative purposes, but sufficient to show that cattle and the smaller laboratory animals are susceptible to this trypanosome.

Cultivation of Trypanosoma brucei, Uganda Strain, 1909.

No attempt was made in Uganda to cultivate this trypanosome.

Carrier of Trypanosoma brucei.

No experiments were made in the laboratory at Mpumu with *Glossina palpalis* as a carrier of this trypanosome, and no evidence is to hand as to how it is conveyed from sick to healthy animals in the district of Bukedi.

Table VI.

Date.	Expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
			Cattle.		
1909. Aug. 11 ...	1461	Nat. infec.	?	—	Killed on 79th day.
			Goat.		
Aug. 16 ...	1478	Ox 1461	—	—	Never showed trypanosomes; under observation 37 days.
			Monkey.		
Aug. 16 ...	1480	Ox 1461	—	—	Died 10 days after inoculation; never showed trypanosomes.
Sept. 4 ...	1627	Dog 1479	13	69	Died of *T. brucei*.
			Dog.		
Aug. 16 ...	1479	Ox 1461	8	49	Died of *T. brucei*.
			Rabbit.		
Sept. 8 ...	1645	Rat 1482	26	—	Still alive after 67 days.
			Guinea-pig.		
Aug. 16 ...	1481	Ox 1461	—	—	Never showed trypanosomes; under observation 18 days.
Sept. 3 ...	1481	Dog 1479	5	—	Still alive after 71 days.
„ 8 ...	1644	Rat 1482	26	—	„ „ 67 „
			Rat.		
Aug. 16 ...	1482	Ox 1461	—	—	Never showed trypanosomes; under observation 18 days.
Sept. 4 ...	1482	Dog 1479	5	23	Died of *T. brucei*.
„ 8 ...	1643	Rat 1482	8	22	„ „
			Mouse.		
Aug. 16 ...	1483	Ox 1461	—	—	Accidentally killed on 9th day; never showed trypanosomes.

Conclusion.

With the evidence available, the Commission consider themselves justified in considering the trypanosome recovered from the Uganda ox to be identical with *Trypanosoma brucei*, the cause of Nagana in Zululand and other parts of South Africa.

DESCRIPTION OF PLATES.

Plate 1.

(1) *Trypanosoma brucei*, Zululand, 1894, stained carbol-fuchsin. ×2000.

Figs. 1 and 2.—Long and slender forms.
Figs. 3 and 4.—Intermediate forms.
Figs. 5, 6 and 7.—Short and stumpy forms.

(2) *Trypanosoma brucei*, Uganda, 1909, stained Giemsa. ×2000.

Figs. 8 and 9.—Long and slender forms.
Fig. 10.—Intermediate form.
Figs. 11, 12 and 13.—Short and stumpy forms.

(3) *Trypanosoma dimorphon* (?), Khartoum. ×2000.

Figs. 14 and 15.—Long and slender forms.
Fig. 16.—Intermediate form.
Figs. 17, 18 and 19.—Short and stumpy forms.

Plate 2.

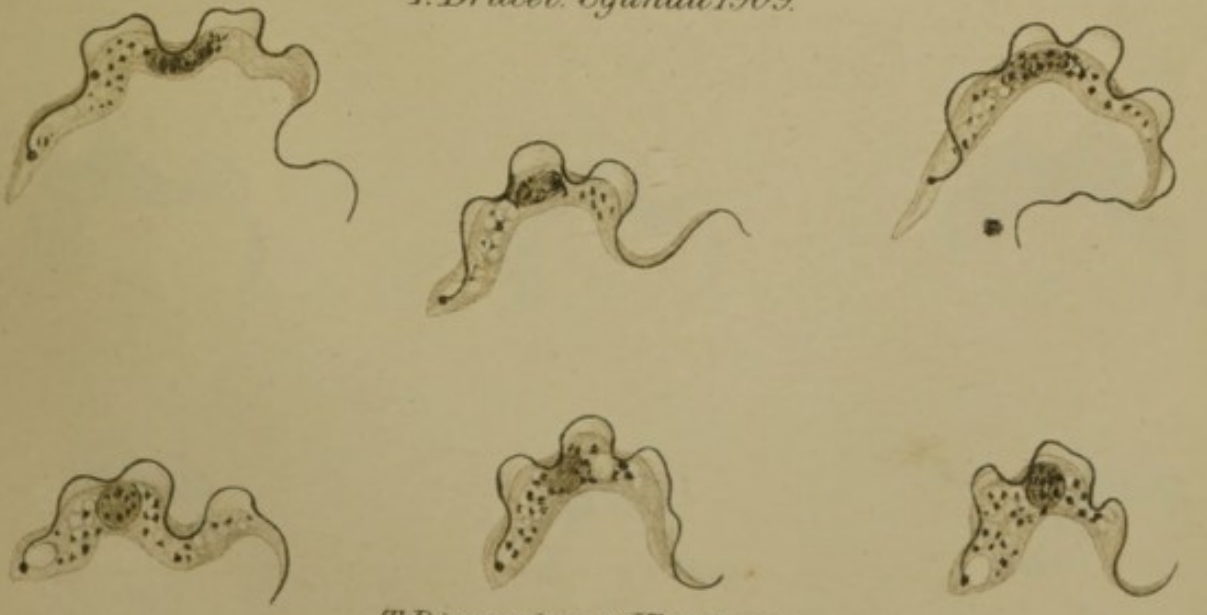
Trypanosoma brucei, Uganda, 1909, stained Giemsa. ×2000.

Figs. 1–5.—Long and slender forms.
Figs. 6–10.—Intermediate forms.
Figs. 11–16.—Short and stumpy forms.

Harrison and Sons, Printers in Ordinary to His late Majesty, St. Martin's Lane.

T. Brucei. Zululand 1894.

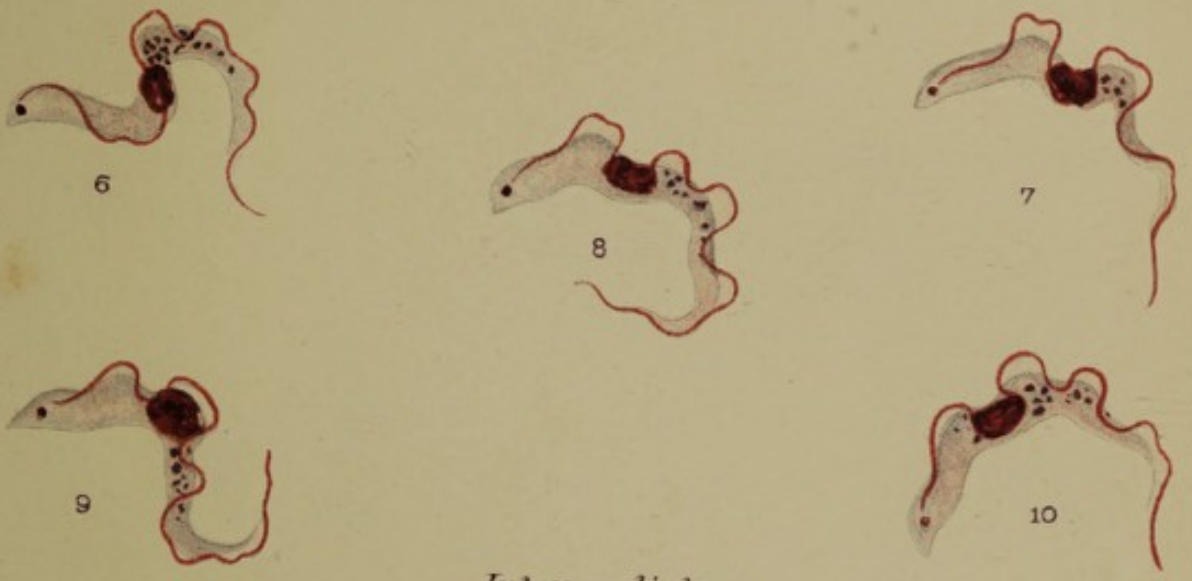
T. Brucei. Uganda 1909.



T. Dimorphon? Khartoum.

M E. Bruce, del.

Long & Slender.



Intermediate.

Short & Stumpy.

M. E. Bruce, del.

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15

*Trypanosome Diseases of Domestic Animals in Uganda.**
III.—*Trypanosoma vivax* (Ziemann).

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(Received July 22, 1910.)

[PLATES 3—5.]

Synonyms. { Trypanosome of Pordage's ox, 1903 (Sleeping Sickness Commission of the Royal Society, 1903).
Trypanosoma cazalboui (souma). Laveran.

INTRODUCTION.

This interesting species of trypanosome appears to be widely distributed in Uganda. It was first discovered by the Commission in two cattle which came from Kavirondo, the district lying to the north-east of Victoria Nyanza. These oxen were driven to Kampala round the north end of the Lake, and probably became infected on the way.

Then the Government Transport Department lost many of their oxen from this trypanosome. They were worked between Kampala, the native capital, and Luzira, the port on the Lake-shore, which lies about seven miles to the south-east. When the epidemic broke out these cattle were kraaled near the Lake-shore, along which they were allowed to graze, and where tsetse-flies are numerous. Afterwards, at the suggestion of the Commission, they were kraaled at Kampala, when the epidemic stopped, and no more deaths from *Trypanosoma vivax* occurred among them.

Cases also occurred among the transport cattle belonging to the German Company, "Victoria Nyanza Agentur." These cattle were employed carrying goods between Lake Victoria and Lake Albert.

Lastly, the *Glossina palpalis* on the Lake-shore near Mpumu, where the Sleeping Sickness Commission laboratory was placed, were found to be naturally infected with *Trypanosoma vivax*, and this trypanosome was found in the blood of a bush-buck, shot at the same place at which the flies were collected.

* Continued from preceding article.

The evidence, therefore, at present points to the *Glossina palpalis* being the carrier of this disease, and that the wild animals living in the fly area act as a reservoir of the virus.

This species of trypanosome is similar to *Trypanosoma nanum*, in that it is only pathogenic to equines and bovines, and has no effect on the smaller laboratory animals. There was no opportunity in Uganda of inoculating it into horses, mules, or donkeys.

Nine cattle came under observation suffering from a natural infection of the disease. Seven of these died after being at Mpumu, on an average, 103 days. Five cattle were successfully inoculated, and these died, on an average, in 69 days. The disease is, therefore, a fairly rapid and fatal one in cattle. It may be noted that two of the cattle (Experiments 493 and 583), after living for 268 and 237 days respectively, died at last from an accidental infection of *Trypanosoma pecorum*. It is possible that these two oxen had recovered, and if not, it is certain that trypanosomes were either absent or very scanty in their blood, as inoculations from them failed to infect goats and sheep. It may also be noted that none of the four sheep which were inoculated ever showed signs of the disease. This is probably due to the scarcity or absence of the trypanosomes in the blood used in the experiment for inoculation, and not to any natural immunity.

Morphology of Trypanosoma vivax.

This species of pathogenic trypanosome can at once be recognised among all the others by its shape alone. There is no tendency to dimorphism, as in *Trypanosoma brucei* and *Trypanosoma gambiense*. On the contrary, the individuals tend to run persistently to one type. This type has an average length of 24 microns. The body posterior to the nucleus is swollen, and contains clear protoplasm, in which an alveolar structure can be made out; the posterior end is rounded, as a rule, and close to it is the large round micronucleus; the anterior part of the body rapidly narrows and terminates in a free flagellum; the nucleus is elongated and situated in the narrowed part, is often broken up and diffused, and seems to fill up the part of the body in which it lies; the undulating membrane is narrow and simple, and can, as a rule, only be made out by the deeply-stained flagellum running along its border. An examination of the plates which illustrate this paper will, however, give a clearer idea of the general appearance of this species than any written description.

A. *Living, Unstained.*

This species of trypanosome is extremely active in its movements. It dashes across the field of the microscope with such rapidity that it is impossible to follow its movements, cyclone-like leaving a clear path, the corpuscles in its track having been flung on either side. If it remains at the same spot for a time, as it sometimes does, it has an appearance of great energy and power, throwing the surrounding red blood corpuscles about in wild confusion.

B. *Fixed and Stained.*

The blood-films were fixed, stained and measured as described in the 'Proceedings.'*

Length.—The following table gives the average length of this trypanosome in the ox and goat. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given.

Table I.—*Trypanosoma vivax*, Uganda, 1909.

No. of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
290	Ox	Osmic acid	Giemsa	23·7	28·0	21·0
445	,,	,,	,,	23·8	26·0	20·0
450	,,	,,	,,	23·0	25·0	21·0
450	,,	,,	,,	23·4	25·0	22·0
493	,,	,,	,,	23·7	25·0	21·0
597	,,	,,	,,	25·1	28·0	22·0
598	,,	,,	,,	25·8	28·0	24·0
779	,,	,,	,,	24·6	27·0	21·0
982	,,	,,	,,	23·5	25·0	22·0
1267	,,	,,	,,	24·2	27·0	20·0
1462	,,	,,	,,	23·8	28·0	16·0
864	Goat	,,	,,	24·2	25·0	23·0
864	,,	,,	,,	24·7	26·0	19·0
1036	,,	,,	,,	23·5	29·0	19·0
				24·1	29·0	16·0

As is shown by the above table, this trypanosome varies in length between 16 and 29 microns. Individuals only 16 microns long are rare, and only occur immediately after division. The following table represents the distribution of length among 180 trypanosomes, and it will be seen by it that only three are found to measure less than 19 microns.

* B, 1909, vol. 81, pp. 16, 17.

Table II.—Represents the Distribution in respect to Length of *Trypanosoma vivax*, Uganda strain, 1909.

Expt. No.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	Average, in microns.
445								●		●●	●●●	●●●●●●	●●●●●●	●										23·8
450							●●	●●●	●●●●●	●●●●●	●													23·0
597										●	●	●●●	●●●●●●●●●	●●●	●●	●								25·1
598												●●●●	●	●●●●●●●●●	●●●	●								25·8

779									●	●●	●●	●●	●●●●●●●	●●●	●●●									24·6
1267								●	●		●●●●●	●●●	●●●●	●●●●●	●									24·2
1462				●	●●					●	●	●●●●●	●●●	●●●●	●●	●								23·8
864							●				●●	●●	●●●●●●●●●	●●●●●●										24·7
1036							●	●●	●	●●●●	●●●	●	●●●●●	●			●●							23·5
Totals...........	—	—	—	1	2	—	4	8	10	16	18	27	44	34	11	3	2	—	—	—	—	—	—	
Percentage......	—	—	—	0·6	1·1	—	2·3	4·4	5·5	8·9	10	15	24·4	18·9	6·1	1·7	1·1	—	—	—	—	—	—	

Breadth.—Varies between 2 and 3 microns.

Shape.—This species can be recognised with certainty by its shape alone. The body of the creature lies mostly posterior to the nucleus, and this part is broad and swollen and filled with beautifully clear protoplasm, in which a delicate alveolar structure can be made out. The body narrows at the nucleus and tapers off rapidly to the anterior extremity (Plates 3 and 4).

Contents of Cell.—Clear, with a delicate alveolar structure, and now and then a chromatin-staining granule, especially in the narrow anterior part.

Nucleus.—Long and oval, often diffuse, situated towards the anterior extremity, and in a narrowed or waist-like part of the trypanosome.

Micronucleus.—Large, round and terminal, or sub-terminal.

Undulating Membrane.—Narrow, simple, straight, and little in evidence.

Flagellum.—There is a well-marked flagellum, the free part varying from 3 to 6 microns in length (Plates 3 and 4).

Comparison of Trypanosoma vivax, Uganda, 1909, with Trypanosoma vivax, Togoland, 1903.

Thanks to the courtesy of the Director of the Hamburg Institute of Tropical Medicine, through Dr. Fülleborn, the Commission are enabled to compare the Uganda strain with the original preparations made by Ziemann in 1903.

Table III.—Measurements of the Original Strain of *Trypanosoma vivax* (Ziemann), 1903.

Animal.	Method of fixing and staining.	In microns.		
		Average length.	Maximum length.	Minimum length.
Ox	?	24·8	26·0	23·0
"	?	26·5	31·0	23·0
Sheep	?	21·4	23·0	18·0
"	?	22·1	25·0	20·0
	Average.....	23·7	31·0	18·0

The trypanosomes found in the blood of this sheep were shorter, narrower, and have a more pointed posterior extremity that in the type described. Whether this is the rule or only exceptional it is impossible with our scanty material to say.

Table IV.—Represents the Distribution in respect to Length of *Trypanosoma vivax*, Togoland strain, 1903.

Expt. No.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	Average, in microns.
Ox											●●●	●●●●	●●●●●●●	●●●●●●	●●●●●●●●●									24·8
Ox											●	●	●●●	●●●	●●●●●●●	●●			●					26·5
Sheep						●	●●	●	●●●●	●●●●●	●●●●●													21·4
Sheep								●●●●	●●●	●●●●	●●●●●	●●●	●											22·1
Totals	—	—	—	—	—	1	2	5	8	10	14	8	11	9	9	2	—	—	1	—	—	—	—	
Percentage	—	—	—	—	—	1·2	2·5	6·3	10	12·5	17·5	10	13·7	11·3	11·3	2·5	—	—	1·2	—	—	—	—	

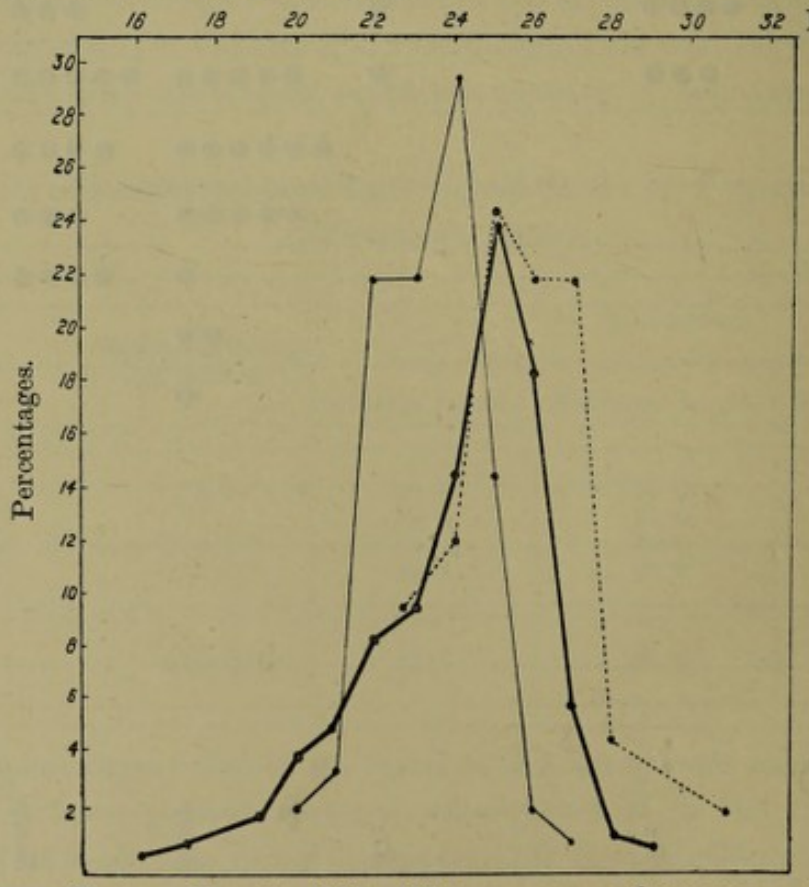
COMPARISON OF TRYPANOSOMA VIVAX, UGANDA, 1909, WITH TRYPANOSOMA VIVAX, 1903 (PORDAGE'S OX).

On examining the preparations made in 1903 from two oxen belonging to the Uganda Transport, the trypanosomes in them were at once recognised as belonging to this species (Plate 3).

Table V.—Measurements of *Trypanosoma vivax*, Uganda, 1903 (Pordage's Ox).

No. of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1	Ox	Osmic acid	Leishman	23·3	25·0	21·0
1	"	"	"	23·1	26·0	20·0
2	"	"	"	23·3	25·0	22·0
2	"	"	"	23·5	27·0	20·0
			Average...	23·3	27·0	20·0

CHART giving curves representing the distribution, by percentages, in respect of length of *Trypanosoma vivax*, Uganda, 1909, *Trypanosoma vivax*, Uganda, 1903 (Pordage's ox), and *Trypanosoma vivax*, Togoland, 1903.



T. vivax, Uganda, 1909; 180 trypanosomes from oxen and goats.
T. vivax, Uganda, 1903 (Pordage's ox); 80 trypanosomes from ox.
T. vivax, Togoland, 1903; 40 trypanosomes from ox.

Table VI.—Represents the Distribution in respect to Length of *Trypanosoma vivax*, Uganda, 1903 (Pordage's Ox).

Expt. No.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	Average, in microns.
1									●	●●●●●	●●●●●●	●●●●●●	●●●●●											23·3
1								●		●●●●●●	●●●●●●	●●●●●●	●●	●										23·1
2										●●●●●●	●●●●●	●●●●●●●	●●●●●											23·3
2								●	●●	●●	●●●	●●●●●●●	●●●	●	●									23·5
Totals	—	—	—	—	—	—	—	2	3	18	18	24	12	2	1	—	—	—	—	—	—	—	—	
Percentage	—	—	—	—	—	—	—	2·5	3·7	22·5	22·5	30	15	2·5	1·3	—	—	—	—	—	—	—	—	

From a comparison of these tables and the figures on Plate 3, there can be little doubt that the trypanosome found in the blood of cattle in Uganda in 1909 is identical with that seen in 1903, and also with that discovered by Ziemann in Togoland, and named by him *Trypanosoma vivax.*

As Dr. Laveran kindly examined the Uganda specimens and pronounced them to be *Trypanosoma cazalboui,* it is probable that *Trypanosoma vivax* and *Trypanosoma cazalboui* are the same species.

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA VIVAX.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
			Cattle.		
1909.					
July 12	431	Nat. infec.	?	—	Alive after 38 days.
Jan. 14	450	,,	?	48	Died of *T. vivax.*
Feb. 2	493	,,	?	268	Also infected by *T. pecorum.*
Mar. 6	583	,,	?	237	,, ,,
,, 14	597	,,	?	49	Died of *T. vivax.*
,, 14	598	,,	?	79	,, ,,
May 16	779	,,	?	6	,, ,,
July 12	1309	,,	?	40	,, ,,
,, 12	1318	,,	?	—	Alive after 30 days.
Sept. 29	290	Ox 1465	30	32	Died of *T. vivax.*
Mar. 3	467	Ox 450	—	—	Experiment stopped after 77 days.
May 19	869	Oxen 493, 583, & 598	—	—	,, ,, 101 ,,
,, 19	870	,,	—	—	,, ,, 101 ,,
July 27	737	Goat 864	15	60	Died of *T. vivax.*
June 21	1030	,,	10	83	,, ,,
July 6	1267	,,	22	55	,, ,,
,, 6	1268	,,	15	114	Killed.
		Average ...	18	89	
			Goat.		
Mar. 31	623	Ox 598	—	—	Experiment stopped after 48 days.
Apr. 2	636	Ox 494	—	—	,, ,, 46 ,,
,, 3	639	Ox 583	—	—	,, ,, 45 ,,
May 19	864	Ox 779	15	148	Died of *T. vivax.*
Aug. 4	1419	Ox 1309	22	—	Still alive after 30 days.
Sept. 10	1652	Ox 1318	21	31	Died of *T. vivax.*
June 22	1036	Goat 864	27	29	,, ,,
—	1037	,,	—	—	Never showed trypanosomes; under observation 66 days.
June 25	1079	,,	21	—	Experiment stopped after 21 days.
July 19	1344	,,	36	—	,, ,, 36 ,,
,, 26	1383	,,	24	—	,, ,, 24 ,,
Aug. 6	1433	,,	12	17	Died of *T. vivax.*
		Average ...	22	56	

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA VIVAX—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
			Sheep.		
1909.					
Mar. 23	610	Ox 598	—	—	Experiment stopped after 56 days.
Apr. 2	637	Ox 493	—	—	„ „ 46 „
May 19	863	Ox 779	—	—	Died 124 days after inoculation.
June 17	1013	Ox 583	—	—	Experiment stopped after 72 days.
			Monkey.		
Feb. 4	487	Ox 450	—	—	Experiment stopped after 57 days.
„ 4	488	„	—	—	„ „ 57 „
Apr. 3	640	Ox 583	—	—	„ „ 45 „
„ 3	643	Ox 493	—	—	„ „ 45 „
May 19	859	Ox 779	—	—	„ „ 40 „
July 9	1304	Ox 1030	—	—	Died 30 days after inoculation.
„ 26	1384	Goat 864	—	—	Experiment stopped after 33 days.
			Dog.		
Feb. 4	489	Ox 450	—	—	Died 30 days after inoculation.
Mar. 3	576	„	—	—	„ 31 „ „
June 18	1021	Ox 583	—	—	„ 28 „ „
„ 30	1197	Goat 864	—	—	Experiment stopped after 43 days.
July 16	1343	Dog 1021	—	—	„ „ 31 „
			Guinea-pig.		
Mar. 27	617	Ox 598	—	—	Experiment stopped after 52 days.
Apr. 3	642	Ox 583	—	—	„ „ 45 „
„ 3	645	Ox 493	—	—	„ „ 45 „
May 19	862	Ox 779	—	—	„ „ 40 „
			Rat.		
Mar. 23	609	Ox 598	—	—	Experiment stopped after 56 days.
Apr. 3	641	Ox 583	—	—	„ „ 45 „
„ 3	644	Ox 493	—	—	„ „ 45 „
May 19	860	Ox 779	—	—	„ „ 40 „
			Mouse.		
Mar. 27	616	Ox 598	—	—	Experiment stopped after 52 „
Apr. 3	646	Ox 583	—	—	„ „ 45 „
„ 3	647	Ox 493	—	—	„ „ 45 „
May 19	861	Ox 779	—	—	„ „ 40 „

CULTIVATION OF TRYPANOSOMA VIVAX.

A. *Living, unstained.*

This species grew readily on blood-agar, the blood used in the preparation of the medium being that of the goat. The history of a tube which had been

inoculated with a drop of blood from the heart of an ox, Experiment 450, dead of *Trypanosoma vivax* disease, is as follows :—

March 3, 1909.—Inoculated six tubes. The trypanosomes are very scarce in the blood.

March 6.—Tube No. 1 has been examined daily for signs of growth. Nothing living has been seen. The tube remains sterile.

March 8.—To-day many very active trypanosomes are seen. They are single or in small groups of ten or twenty or so. The cell-contents are granular. The cells possess flagella, but no obvious undulating membranes. There are many dividing forms to be seen, and division is evidently going on rapidly, as many individual trypanosomes appear to be undergoing fission into three or more at the same time.

March 9.—Since yesterday an immense multiplication has taken place. The trypanosomes are still very active. They appear to have free flagella and long-pointed, finely granular posterior extremities.

March 11.—Many highly active trypanosomes seen. They are elongated, thin, and have a long free flagellum, sometimes as long as the body itself.

The trypanosomes remained alive and active up to 20 or 30 days, when the tubes became contaminated, or dried up, and were thrown away.

Size.—Cultural forms of *Trypanosoma vivax* vary much in size. At first, when they begin to multiply, on or about the sixth day, the individuals forming groups are about 15 microns in length. Next day they are longer and thinner, and later many are seen 50, 60, or more, microns in length.

Shape.—At first, the young forms are oval or elliptical in shape; afterwards they become more attenuated; and later still may be seen as huge trypanosomes with undulating membranes and long free flagella.

Contents of Cell.—In the living unstained condition this appears to be finely granular.

Undulating Membrane.—In the smaller forms composing the groups no obvious undulating membrane can be made out.

Flagellum.—This is free and well developed.

Motion.—The single forms are extremely active.

B. *Fixed and Stained* (Plate 5).

Protoplasm.—Is homogeneous, but contains many irregular granules and vacuoles.

Nucleus.—Is usually broken up and diffuse.

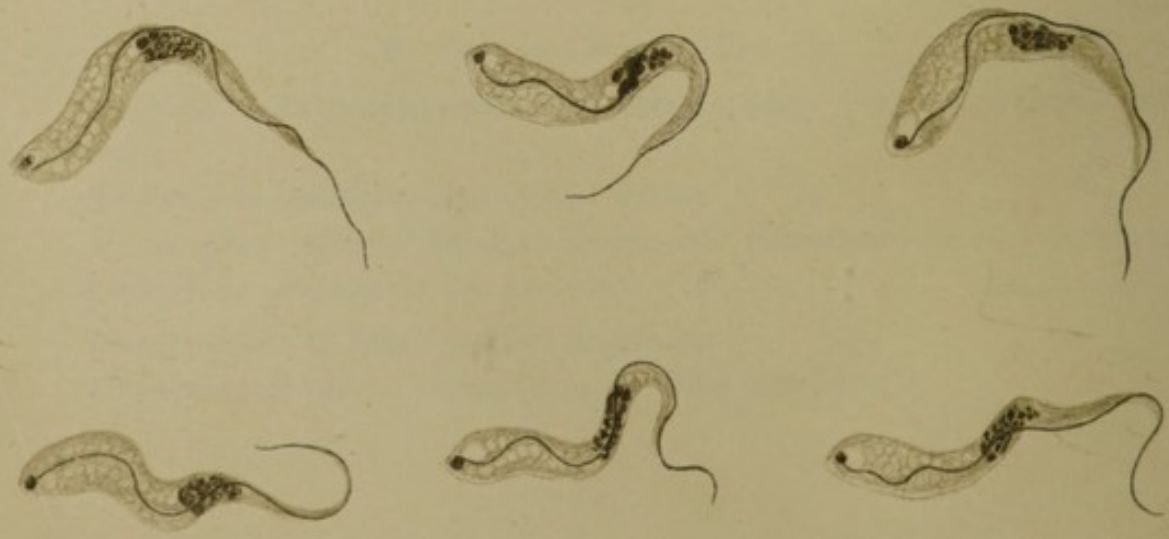
Micronucleus.—Often difficult to distinguish. Sometimes placed anterior, at the side of, or posterior to, the nucleus.

Flagellum.—Well developed free flagellum.

Undulating Membrane.—Present in the older free forms.

T. Vivax. Uganda 1909.

T. Vivax. Pordage's Ox 1903.



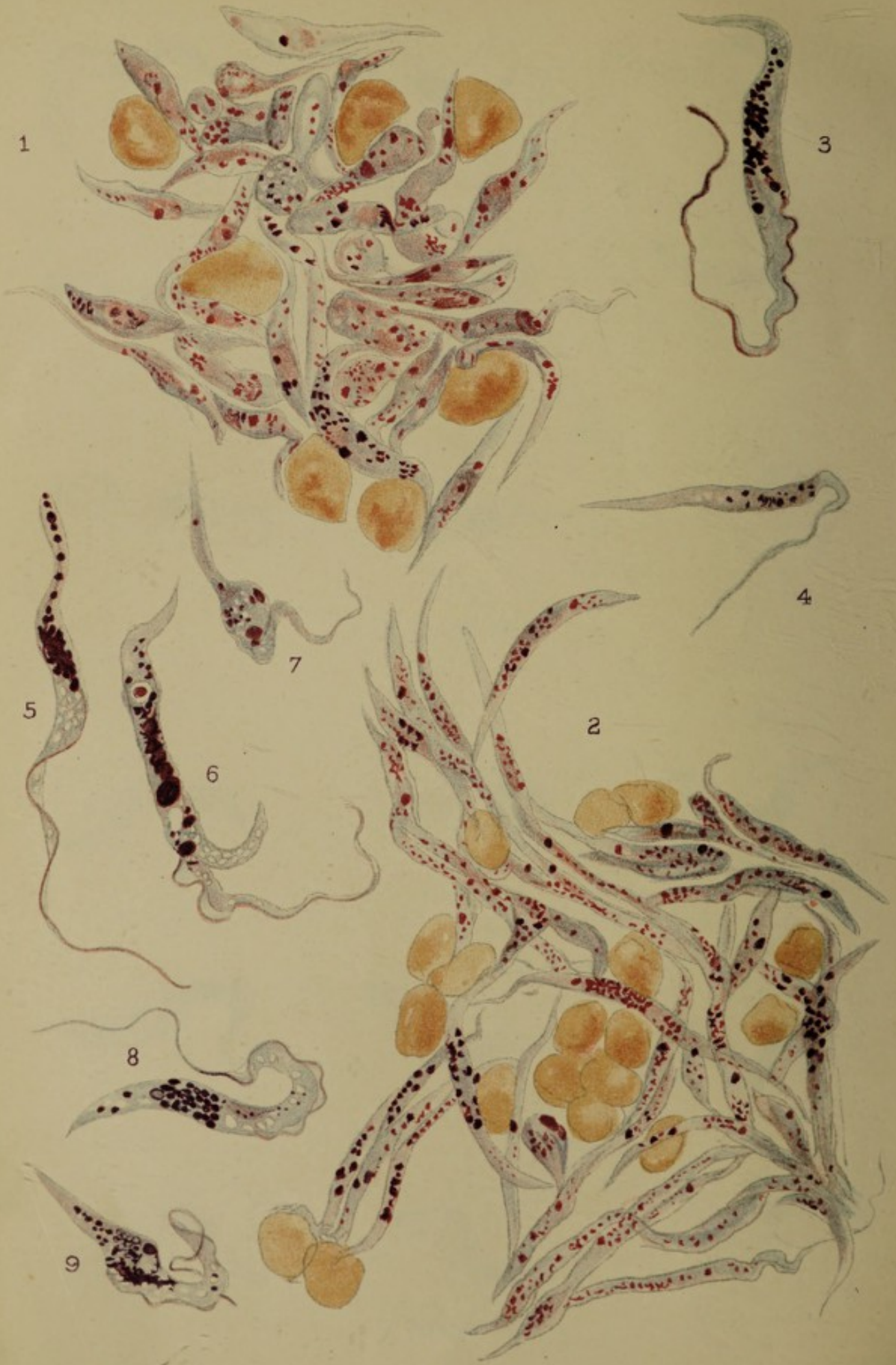
T. Vivax. Ziemann Togoland. 1903.

M. E. Bruce, del.

T. Vivax. Ox.

T. Vivax. Goat.

M. E. Bruce, del.



M.E. Bruce, del.

The Carrier of Trypanosoma vivax.

It was pointed out in a previous paper that, in laboratory experiments, *Trypanosoma vivax* readily develops in *Glossina palpalis*, and that this tsetse fly can convey the infection to healthy animals after a period of from 20 to 30 days.* It was also found that this fly is naturally infected with this trypanosome, and several animals became ill and died of this disease when freshly-caught Lake-shore flies were fed upon them.† The epidemic among the Government Transport oxen at Kampala also points to this fly being the carrier. As stated above, as long as the cattle were kraaled at the Lake-shore and exposed to the bites of *Glossina palpalis*, so long did they suffer from *Trypanosoma vivax* infection. Afterwards, when stabled at Kampala, the fatality from this disease ceased.

Although it must be confessed the evidence is rather scanty, what there is points to the *Glossina palpalis* as being a carrier, if not the chief carrier, of *Trypanosoma vivax*; and there is no evidence at present to incriminate the *Tabanids* or *Stomoxys*.

Conclusions.

1. *Trypanosoma vivax*, an easily recognisable species, gives rise to a fatal disease of cattle in Uganda.

2. The carrier of *Trypanosoma vivax* is probably *Glossina palpalis*, which is found naturally infected on the Lake-shore.

3. The reservoir of the virus is possibly the antelope which frequent the *Glossina palpalis* area.

DESCRIPTION OF PLATES.

Plate 3.

Figures for the comparison of *Trypanosoma vivax*, Uganda, 1909, *Trypanosoma vivax*, Uganda, 1903 (Pordage's ox), and *Trypanosoma vivax*, Togoland, 1903.

Plate 4.

Trypanosoma vivax, fixed osmic acid, stained Giemsa. ×2000. Note the large round terminal micronucleus; the broad, swollen anterior part filled with clear protoplasm, in which a hint of the alveolar structure is given; the nucleus situated in the narrowed part of the body; the slightly developed undulating membrane; and the free flagellum.

Plate 5.

Fig. 1.—A group of *Trypanosoma vivax* after 5 days' growth in blood-agar. Stained Giemsa. ×2000.

Fig. 2.—*Trypanosoma vivax* after 6 days' growth. Stained Giemsa. ×2000.

Figs. 3 and 4.—After 7 days' growth. Stained Giemsa. ×2000.

Figs. 5–9.—After 8 days' growth. Stained Giemsa. ×2000.

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* 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 381.

† 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 63.

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16

Glossina palpalis *as a Carrier of* Trypanosoma vivax *in Uganda.*

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One of the important trypanosome diseases of cattle in Uganda is that caused by *Trypanosoma vivax* (Ziemann). This species of trypanosome appears to be widely distributed in Central Africa. It has been reported from Senegal, the Sudan and Erythrea in the North, to Rhodesia in the South. It is fairly easily recognised on account of its extreme activity during life, its characteristic shape in stained specimens, and the fact that it only affects cattle, goats, and sheep; while monkeys, dogs, rabbits, guinea-pigs, rats, and mice are refractory. Its carriers have usually been reported as tabanus and stomoxys.

This short note is written to place on record that fact, that in Uganda the tsetse flies, *Glossina palpalis*, which are found in large numbers on the Lake-shore, are infected, not only by *Trypanosoma gambiense*, the cause of sleeping sickness, but also by *Trypanosoma vivax*. The first experiment which showed that these tsetse flies are infected with the latter trypanosome was the following:—

Experiment 1318.—Calf.

To ascertain if oxen will become infected by trypanosomes if allowed to feed in the "fly area."

July 12, 1909. A healthy calf was taken down to the Lake-shore at Kibanga and ferried across the bay to Nsonga, where tsetse flies are numerous. The flies were observed to feed on it in numbers. It was then brought back to Kibanga. In future this calf will be taken out every day by the fly-boys to different parts of the Lake-shore, where it will graze while the boys are catching tsetse flies.

August 8. Returned from Lake-shore to Mpumu.

August 11. *Trypanosoma vivax* present in the blood of this calf.

Remarks.—If the incubation period of this disease is assumed to be eight days, then this calf remained 19 days at the Lake-shore before it became infected. The proof that the trypanosome found in this calf's blood was *Trypanosoma vivax* and not *Trypanosoma gambiense* was the shape and appearance of the parasite, the fact that the calf's blood injected under the skin of two monkeys gave negative results, and, lastly, that 50 laboratory-bred flies fed on this calf afterwards infected a goat with *Trypanosoma vivax*.

Experiment 431.—Cow. (Mother of Calf, 1318.)

July 12, 1909. This cow accompanied her calf to Kibanga, and remained with it during the experiment.

August 8. Returned to Mpumu.

August 19. *Trypanosoma vivax* discovered in blood.

The following table shows the dates of examination :—

Date.	Parasites in blood.	
	Piroplasma.	Trypanosoma.
1909.		
January 9	—	—
„ 20	—	—
„ 21	—	—
„ 28	—	—
February 2	—	—
„ 9	—	—
„ 26	—	—
August 9	—	—
„ 14	—	—
„ 19	—	+

Remarks.—It is possible that this cow became infected from her calf, but it is more probable that she became infected in the same way and about the same time as her calf.

The remaining experiments were carried out by bringing freshly-caught *Glossina palpalis* from the Lake-shore to the laboratory at Mpumu and placing them on healthy oxen.

Experiment 1462.—Bull.

To ascertain if freshly-caught *Glossina palpalis*, fed on healthy cattle, will give rise to any trypanosome disease.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
August 16	120	75	—	—
„ 17	410	250		
„ 19	320	180	—	—
„ 20	170	80		
„ 24	350	120	—	—
„ 26			+	—
September 1			—	+
„ 2			—	+
„ 3			—	+
„ 4			—	+
„ 6			+	+
„ 7			+	+
„ 8			+	+
„ 9			—	+

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Experiment 445.—Bull.

Date.	Flies.		Result. *Trypanosoma vivax.*
	Put on.	Fed.	
1909.			
September 28.....	470	220	
„ 29.....	160	95	
„ 30.....	65	45	—
October 1	300	180	
„ 2	400	280	
„ 4	500	300	—
„ 5	190	85	
„ 7	250	170	—
„ 8	300	165	
„ 11	100	70	—
„ 15	450	360	—
„ 16	470	350	
„ 18	—	—	+
„ 19	—	—	+ +

Remarks.—*Trypanosoma vivax* only appeared in the blood of this bull.

Experiment 1465.—Bull.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
August 27	150	90	—	—
,, 28	60	35	—	—
September 4	230	170	—	—
,, 7	—	—	—	—
,, 9	30	19		
,, 10	—	—	—	+
,, 11	—	—	+	—

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Experiment 982.—Bull.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
September 11.....	45	36		
,, 12.....	65	50		
,, 14.....	110	75		
,, 15.....	125	95		
,, 16.....	420	160	—	—
,, 19.....	55	40		
,, 20.....	—	—	—	—
,, 21.....	115	85	—	—
,, 22.....	180	145		
,, 23.....	410	380		
,, 24.....	300	240	—	—
,, 25.....	—	—	—	—
,, 27.....	370	230	+	—
October 14	—	—	—	+

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Conclusions.

1. The *Glossina palpalis* on the shores of Victoria Nyanza are infected, not only by *Trypanosoma gambiense*, but also by *Trypanosoma vivax*.

2. What the reservoir of the virus of *Trypanosoma vivax* is, is unknown, but the buffalo, waterbuck, and other antelope which live on the Lake-shore should be examined.

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[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 83]

*Trypanosome Diseases of Domestic Animals in Uganda.** IV.—Trypanosoma uniforme, sp. nov.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C.; and Captain F. P. MACKIE, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

(Received October 1,—Read December 8, 1910.)

[PLATE 12.]

INTRODUCTION.

The name *Trypanosoma uniforme* has been given to this species on account of the uniformity in shape and general appearance which characterises it.

Only four oxen were found by the Commission to show this trypanosome in their blood. One was received from Sir Apolo Kagwa, K.C.M.G., the Prime Minister, on August 30, 1909, and its blood at once inoculated into a series of animals. The other three came from the Uganda Company's estate at Namukekera.

The ox, goat, and sheep were found to be susceptible, while the monkey, dog, guinea-pig, rat, and mouse proved refractory. This was the case with *Trypanosoma vivax*, and in truth these two species resemble one another very closely. *Trypanosoma uniforme* differs from *T. vivax* in size, and perhaps to a slight extent in shape; but, from the small amount of material at the disposal of the Commission, it would be rash to generalise.

The few facts gathered will, therefore, be put on record, in order to draw the attention of future workers on Uganda trypanosomes to its presence.

MORPHOLOGY OF TRYPANOSOMA UNIFORME.

A. *Living, Unstained.*

This is a small and active trypanosome, which has a marked translatory movement in the field of the microscope. This movement, however, is not to be compared with that of *T. vivax* in point of rapidity or range. The rapid vibratory movement of the body and flagellum sometimes slows down for a perceptible fraction of time; but this trypanosome does not become completely quiescent as is commonly the case with *T. pecorum*. The cell-contents are clear and homogeneous, without any appearance of a vacuole.

B. *Fixed and Stained.*

Length.—This trypanosome is smaller than *T. vivax*. The average is 16·0 as against 23·7 microns. The following table gives the average length of

* Continued from 'Roy. Soc. Proc.,' 1910, B, vol. 83, p. 27.

this trypanosome in the ox, goat, and sheep. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given :—

Table I.—*Trypanosoma uniforme.*

No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1437	Ox	Osmic acid	Giemsa	16·0	17·5	15·0
1442	,,	,,	,,	16·0	18·0	14·0
1442	,,	,,	,,	16·3	19·0	14·0
1581	,,	,,	,,	16·5	19·0	14·0
1732	,,	,,	,,	15·5	17·5	13·5
1734	,,	,,	,,	15·3	17·0	14·0
1689	Goat	,,	,,	16·4	17·5	15·0
1694	,,	,,	,,	17·7	19·0	16·0
1694	,,	,,	,,	14·7	16·0	13·0
1497	Sheep	,,	,,	17·8	19·0	16·0
1497	,,	,,	,,	14·7	16·0	12·0
				16·0	19·0	12·0

The following table represents the distribution in respect to length of 200 individuals of this species of trypanosome :—

Table II.—Distribution in respect to Length of 200 individuals of *Trypanosoma uniforme.*

No. of expt.	Microns.								Average, in microns.
	12.	13.	14.	15.	16.	17.	18.	19.	
1437	—	—	—	5	10	5	—	—	16·0
1442	—	—	3	2	9	4	2	—	16·0
1442	—	—	1	6	5	5	1	2	16·3
1581	—	—	1	3	8	2	4	2	16·5
1734	—	—	4	8	6	2	—	—	15·3
1689	—	—	—	3	6	11	—	—	16·4
1694	—	—	—	—	2	5	9	4	17·7
1694	—	1	8	7	4	—	—	—	14·7
1497	—	—	—	—	4	7	8	1	17·8
1497	1	—	7	6	6	—	—	—	14·7
Totals	1	1	24	40	60	41	24	9	
Percentages	0·5	0·5	12·0	20·0	30·0	20·5	12·0	4·5	

Breadth.—Varies from 1·5 to 2·5 microns.

Shape.—This species of trypanosome seems to differ in shape from *T. vivax* in that there is not the marked narrowing or constriction opposite the nucleus. The posterior extremity is rounded or blunt, and in this resembles *T. vivax* (Plate 12).

Contents of Cell.—Resembles *T. vivax* in showing the appearance of clear protoplasm with fine alveolar structure.

Nucleus.—Oval in shape and compact; not, as a rule, situated in a narrowed part of the body or waist, as in *T. vivax*. It also seems to be placed about the centre of the body, and does not take up the whole width of the cell as in the closely allied species.

Micronucleus.—Resembles *T. vivax* in being large, round, and terminal.

Undulating Membrane.—Narrow and little developed, as in *T. vivax*.

Flagellum.—There is a well-marked flagellum, the free part varying from 2 to 5 microns in length.

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA UNIFORME.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1909.					
Aug. 7	1442	Nat. infec.	?	5	Died of *T. uniforme*.
„ 30	1581	„	?	61	Killed.
„ 11	1732	„	?	79	Died of *T. uniforme*.
„ 11	1734	„	?	80	Killed.
			Goat.		
Sept. 1	1491	Ox 1581	15	18	No *post-mortem*.
„ 16	1689	„	18	34	„ „
„ 18	1694	Goat 1491	9	35	„ „
„ 24	1716	Ox 1581	—	—	Never showed trypanosomes; under observation 40 days.
		Average ...	14	29	
			Sheep.		
Sept. 16	1497	Ox 1581	18	46	Killed.
„ 1	1601	„	—	—	Never showed trypanosomes; died 10 days after inoculation.
„ 24	1717	„	—	—	Never showed trypanosomes; died 13 days after inoculation.
			Monkey.		
Sept. 1	1653	Ox 1581	—	—	Experiment stopped after 34 days.

* Duration includes the days of incubation; it dates from the day of infection.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Dog.		
Sept. 1	1600	Ox 1581	—	—	Experiment stopped after 34 days.
			Guinea-pig.		
Sept. 1	1599	Ox 1581	—	—	Experiment stopped after 34 days.
			Rat.		
Sept. 1	1597	Ox 1581	—	—	Experiment stopped after 34 days.
			Mouse.		
Sept. 1	1598	Ox 1581	—	—	Experiment stopped after 34 days.

* Duration includes the days of incubation; it dates from the day of infection.

Cultivation of T. uniforme.

No attempt was made to cultivate *T. uniforme*.

Carrier of T. uniforme.

No experiments were made in the laboratory with *Glossina palpalis* as a carrier of *T. uniforme*, and no evidence is to hand as to what the carrier is.

Conclusions.

1. *Trypanosoma uniforme* resembles *T. vivax* in shape and general appearance, but differs markedly in size.

2. It also resembles *T. vivax* in not being pathogenic to the smaller laboratory animals.

3. There is no evidence available, as in the case of *T. vivax*, as to what the carrier of *T. uniforme* is.

DESCRIPTION OF PLATE.

Plate 12.

Trypanosoma uniforme in the ox, goat, and sheep. Fixed osmic acid, stained Giesma. ×2000. Note the large round terminal micronucleus, the oval compact nucleus, situated about the centre of the body, the narrow undulating membrane, and the free flagellum.

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Roy. Soc. Proc. B. vol. 83, Pl. 12.

Oxen.

Goats.

Sheep.

M. E. Bruce, del.

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 83]

*Trypanosome Diseases of Domestic Animals in Uganda.** V.—Trypanosoma nanum (*Laveran*).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C.; and Captain F. P. MACKIE, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

(Received October 1,—Read December 8, 1910.)

[PLATES 13 AND 14.]

INTRODUCTION.

Only two cattle (Experiments 503 and 1118) examined by the Commission at Mpumu were found to harbour this trypanosome in their blood. Both came from the Uganda Company's estate at Namukekera, some fifty miles from Lake Victoria, and both had become infected on the estate.

This species differed from *Trypanosoma pecorum*, in that it did not affect the smaller laboratory animals, such as monkeys, dogs, rats, or mice. Guinea-pigs were also refractory to the disease. Horses, mules, donkeys, and rabbits were not available at Mpumu, so that it is impossible to say what would have been the effect of inoculation in them. Oxen and goats were inoculable, but the only sheep experimented on failed to become infected, although inoculated with the same blood which infected a goat.

It seems rash to recognise *T. nanum* as a Uganda species on the insufficient evidence at our disposal; but yet the fact remains that this trypanosome in every case failed to infect the smaller animals, and, moreover, by placing these cases on record it will draw the attention of future workers to its possible presence.

MORPHOLOGY OF TRYPANOSOMA NANUM.

A. *Living, Unstained.*

T. nanum is indistinguishable from *T. pecorum* in the fresh and living condition.

B. *Fixed and Stained.*

Length.—The following table gives the average length of this trypanosome in the ox and goat. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given :—

* Continued from preceding paper.

Table I.—*Trypanosoma nanum.*

No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
503	Ox	Osmic acid	Giemsa	13·3	15·0	11·0
983	„	„	„	13·1	14·0	12·0
698	Goat	„	„	13·9	16·0	13·0
698	„	„	„	15·2	16·0	12·0
882	„	„	„	12·9	15·0	11·0
883	„	„	„	13·3	15·0	11·0
1691	„	„	„	13·7	16·0	12·0
				13·6	16·0	11·0

The average length of *T. nanum* corresponds closely with that of *T. pecorum*, which was 13·3 microns. The question arises as to whether it would not be better for the sake of simplicity to include, for the present at least, this species in that of *T. pecorum*. If the morphology is the same, if the important domestic animals are susceptible to both, if the geographical distribution is the same, and if the carrier is found to be the same, there seems little need of separating the two under different names.

Breadth.—At the widest part from 1·5 to 2·5 microns.

Shape.—Much the same as *T. pecorum*, but perhaps slightly narrower, due to the less development of the undulating membrane (Plate 14).

Contents of Cell.—As a rule homogeneous.

Nucleus.—Oval and situated about the middle of the body.

Micronucleus.—Small and rounded, and situated near the posterior extremity.

Undulating Membrane.—Perhaps a little narrower and simpler than in *T. pecorum*. It was thought to be applied more closely to the body and less thrown into folds than in that species.

Flagellum.—There is no free flagellum.

From a comparison of Tables II and III, Chart 1, and the coloured figures of the two species, it is evident that *T. pecorum* and *T. nanum* resemble each other very closely morphologically.

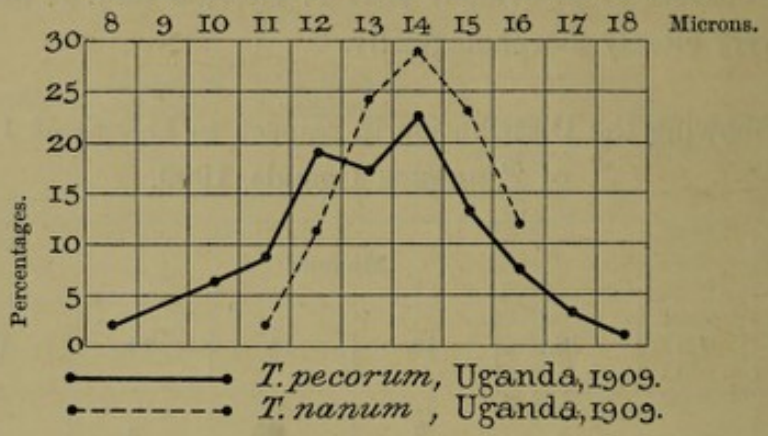
Table II.—Showing the Distribution in respect to Length of 100 Individuals of *T. nanum*, Uganda, 1909.

No. of expt.	Microns.						Average, in microns.
	11.	12.	13.	14.	15.	16.	
689	—	—	7	8	4	1	13·9
698	—	1	—	2	7	10	15·2
882	1	5	10	3	1	—	12·9
883	1	4	5	7	3	—	13·3
1691	—	1	2	8	8	1	13·7
Totals.....	2	11	24	28	23	12	
Percentages	2·0	11·0	24·0	28·0	23·0	12·0	

Table III.—Showing the Distribution in respect to Length of 260 Individuals of *T. pecorum*, Uganda, 1909.

No. of expt.	Microns.											Average, in microns.
	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	
82	—	—	2	6	8	3	1	—	—	—	—	11·7
505	—	—	2	2	9	4	1	2	—	—	—	12·3
—	—	—	—	2	3	6	6	3	—	—	—	13·2
593	—	—	1	—	2	1	3	7	5	1	—	14·5
44	—	—	4	2	5	3	6	—	—	—	—	12·2
559	—	—	—	2	9	4	4	—	1	—	—	12·7
461	—	—	—	—	—	5	4	1	2	6	2	15·3
543	—	—	—	2	—	2	6	6	4	—	—	14·3
1406	—	—	—	1	5	2	5	5	2	—	—	13·7
551	1	—	3	3	3	3	3	2	1	—	1	12·6
626	—	—	3	2	3	2	7	3	—	—	—	12·8
398	—	—	—	—	—	1	9	6	4	—	—	14·6
398	—	—	1	1	3	9	4	—	1	1	—	13·1
Totals ...	1	—	16	23	50	45	59	35	20	8	3	
Percentages	0·4	—	6·1	8·8	19·2	17·3	22·7	13·5	7·7	3·1	1·2	

CHART 1.—Giving Curves representing the Distribution, by Percentages, in respect to Length of *T. pecorum*, Uganda, 1909, and *T. nanum*, Uganda, 1909.



COMPARISON OF T. NANUM, UGANDA, 1909, WITH T. NANUM, SUDAN, 1904.

Thanks to Dr. Andrew Balfour, the Director of the Wellcome Research Laboratories, Khartoum, the Commission is enabled to compare the Uganda strain with the original Sudan strain.

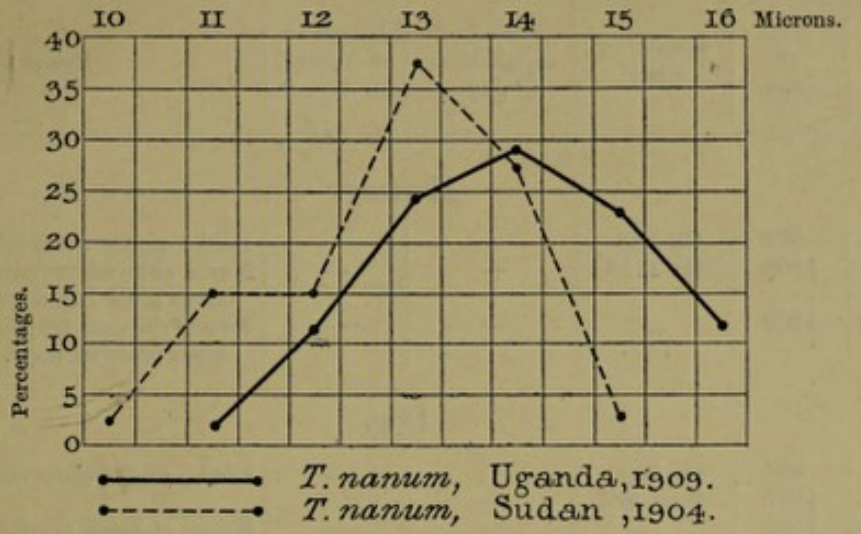
Table IV.—Measurements of Dr. Balfour's Sudan Strain of *T. nanum*, 1904.

Animal.	Method of fixing and staining.	In microns.		
		Average length.	Maximum length.	Minimum length.
Ox	?	12·9	15·0	11·0
„	?	12·7	14·0	10·0

Table V.—Showing the Distribution in respect to Length of 40 Individuals of *T. nanum*, Sudan, 1904.

Animal.	Microns.						Average, in microns.
	10.	11.	12.	13.	14.	15.	
Ox	—	3	4	6	6	1	12·9
Ox	1	3	2	9	5	—	12·7
Totals	1	6	6	15	11	1	
Percentages	2·5	15·0	15·0	37·5	27·5	2·5	

Chart 2.—Giving Curves representing the Distribution, by Percentages, in respect to Length of *T. nanum* Uganda, 1909, and Dr. Balfour's *T. nanum*, Sudan, 1904.



From a comparison of these tables and the figures in Plate 13 there can be little doubt as to the identity as regards morphology of *T. nanum*, Uganda, 1909, and Dr. Balfour's *T. nanum*, discovered in the Sudan in 1904:—

Animals susceptible to T. nanum, Uganda, 1909.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1909. Feb. 5	503	Nat. infec.	?	245	From Namukekera. Died of *T. nanum*.
June 25	1118	„	?	3	From Namukekera. Died of *T. nanum*.
Sept. 29	780	Ox 983	—	—	Never showed trypanosomes; still alive after 47 days.
June 11	982	Ox 503	—	—	Never showed trypanosomes; still alive after 141 days.
„ 11	983	„	20	141	Killed.
			Goat.		
Apr. 12	698	Ox 503	25	137	Died of *T. nanum*.
May 21	882	„	26	91	„ „
„ 21	883	„	6	46	„ „
Sept. 16	1691	Oxen 503 & 982	8	12	„ „
		Average ...	16	71	
			Sheep.		
May 21	880	Ox 503	—	—	Never showed trypanosomes; stil l alive after 82 days.

* Duration includes the days of incubation; it dates from the day of infection.

ANIMALS SUSCEPTIBLE TO T. NANUM, UGANDA, 1909—*continued.*

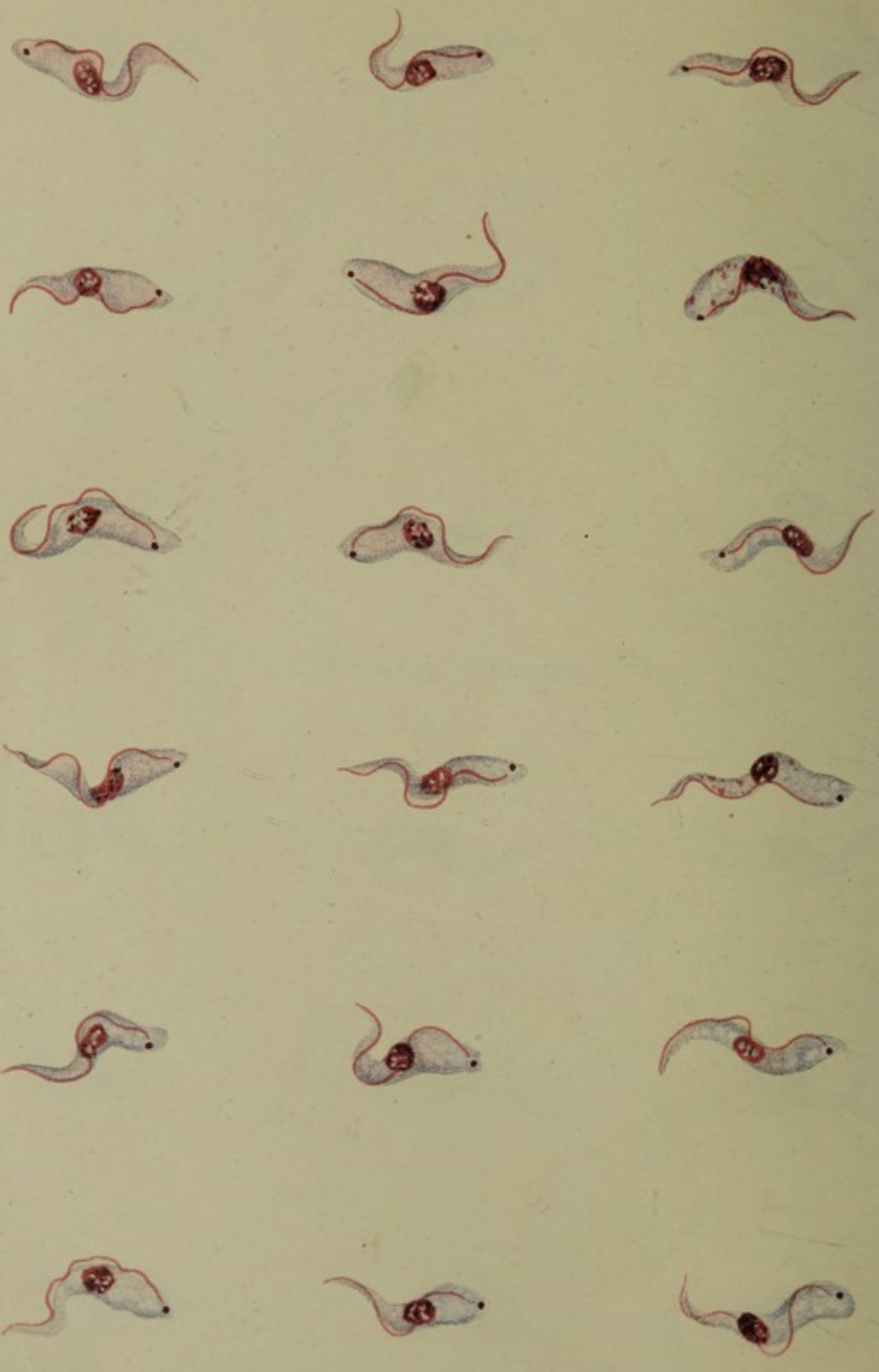
Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Monkey.		
Apr. 10	687	Ox 503	—	—	Died; under observation 23 days.
June 26	1168	Ox 1118	—	—	Never showed trypanosomes; still alive after 141 days.
„ 26	1169	„	—	—	Never showed trypanosomes; still alive after 97 days.
			Dog.		
May 21	884	Ox 503	—	—	Died; under observation 66 days.
June 30	1194	Goat 883	—	—	„ „ 42 „
„ 30	1195	„	—	—	Never showed trypanosomes; still alive after 43 days.
„ 30	1196	„	—	—	Died; under observation 26 days.
			Guinea-pig.		
Apr. 10	689	Ox 503	—	—	Never showed trypanosomes; still alive after 59 days.
June 26	1162	Ox 1118	—	—	Never showed trypanosomes; still alive after 36 days.
„ 26	1163	„	—	—	Never showed trypanosomes; still alive after 36 days.
„ 26	1164	„	—	—	Never showed trypanosomes; still alive after 36 days.
			Rat.		
1909.					
Feb. 9	529	Ox 503	—	—	Experiment stopped; alive after 44 days.
„ 9	530	„	—	—	Experiment stopped; alive after 44 days.
Apr. 10	688	„	—	—	Experiment stopped; alive after 59 days.
„ 12	717	„	—	—	Experiment stopped; alive after 57 days.
June 26	1165	Ox 1118	—	—	Experiment stopped; alive after 57 days.
„ 26	1166	„	—	—	Experiment stopped; alive after 57 days.
„ 26	1167	„	—	—	Experiment stopped; alive after 57 days.
May 21	885	Goat 698	—	—	Experiment stopped; alive after 80 days.
			Mouse.		
Apr. 10	690	Ox 503	—	—	Experiment stopped; alive after 59 days.

* Duration includes the days of incubation; it dates from the day of infection.

T. Nanum, Uganda, 1909.

T. Nanum, Sudan, 1904.

M E. Bruce, del.



M. E. Bruce, del.

Cultivation of T. nanum.

No attempt was made at Mpumu to cultivate *T. nanum*.

The Carrier of T. nanum.

Of the two experiments made at Mpumu with *T. nanum* and *Glossina palpalis*, one was unsatisfactory and the other negative. Since the two oxen supposed to be suffering from *T. nanum* disease came from Namukekera, where it is stated there are no tsetse flies, it is probable that *T. nanum*, like *T. pecorum*, is carried by some species of biting fly other than *Glossina*.

Conclusions.

1. *T. nanum* is indistinguishable from *T. pecorum* either in the living condition or when fixed and stained.

2. It differs from *T. pecorum* in not being pathogenic to the smaller laboratory animals.

3. The carrier of *T. nanum* is probably the same as that of *T. pecorum*, as both diseases occur under the same conditions, but there is no evidence available as to what the carrier is.

DESCRIPTION OF PLATES.

Plate 13.

Figures in black and white for the comparison of *Trypanosoma nanum*, Uganda, 1909, and *T. nanum*, Sudan, 1904. ×2000.

Plate 14.

T. nanum, Uganda, 1909, in the goat. Fixed osmic acid, stained Giesma. ×2000.

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Trypanosoma ingens, n. sp.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service.

(Received April 30,—Read May 20, 1909.)

(Sleeping Sickness Commission of the Royal Society, 1908—09.)

[PLATE 7.]

This is such an extraordinary looking parasite that the Commission thinks it deserves a short preliminary note, a name, and to be figured.

The name is taken from Virgil's description of the Cyclops, *informe, ingens.* It was first discovered in the blood of a reed-buck on February 13, 1909, at Namukekera, Uganda (lat. 0° 40′ N.; long. 32° 15′ E.), the estate of the Uganda Company, Limited; then in a bush-buck, and lastly in an ox. The wild animals and the cattle feed in the same pastures, so that it is not remarkable that the oxen should become infected.

At present it is not known what the carrier is, and this will probably be a difficult thing to determine. Collections of the blood-sucking flies and ticks are being made on the Namukekera Estate, and this may lead in time to the discovery of the carrier. Up to the present the following list includes all the blood-suckers found in this particular district:—

Chrysops distinctipennis, Austen.
Stomoxys calcitrans, Linn.
Stomoxys nigra, Macq.
Tabanus tæniola, Pal. de Beauv.
Hæmatopota unicolor, Ricardo.
Hæmatopota, sp. nov.
Hæmatopota brunnescens, Ricardo.

Trypanosoma ingens, when seen alive in a fresh preparation, moves slowly and deliberately across the field of the microscope, with a fine rippling, or at times a broader undulating movement.

In stained preparations this huge trypanosome may measure as much as 122 microns, and even then it is lying in such a formless huddled-up way among the red blood corpuscles that it looks capable of stretching out to a much greater length. The other specimens figured measure 72, 77, 88, and 82 microns. The breadth is 7 to 10 microns.

The micronucleus is small and round. It measures about a micron in diameter. It lies posterior to, and quite close to, the nucleus. From it, in well-stained specimens, a well-marked, though narrow, undulating membrane arises, which runs to the anterior extremity and ends in a free flagellum.

The nucleus is oval in form, and lies across the body. It is situated

nearer the posterior end than the anterior, and in our specimens has stained a pale pink.

The body substance is markedly granular behind the nucleus, while in front the structure described as myonemes is particularly well marked.

More minute measurements of one of these trypanosomes are as follows:—

	microns.
Posterior end to micronucleus	18
From micronucleus to nucleus	4
Nucleus: long diameter, 8 microns; short diameter	4
Nucleus to anterior end	40
Free flagellum	17
Total	83

It is unnecessary in this preliminary note to go more fully into the structure of this trypanosome, or to describe it at greater length. An examination of the coloured drawings reproduced in Plate 7 will give a more distinct idea of its appearance than any written description.

The drawings were made by Lady Bruce, R.R.C. Figs. 1, 3, and 4 are from reed-buck, fig. 2 from the ox. All are magnified 2000 and stained Giemsa.

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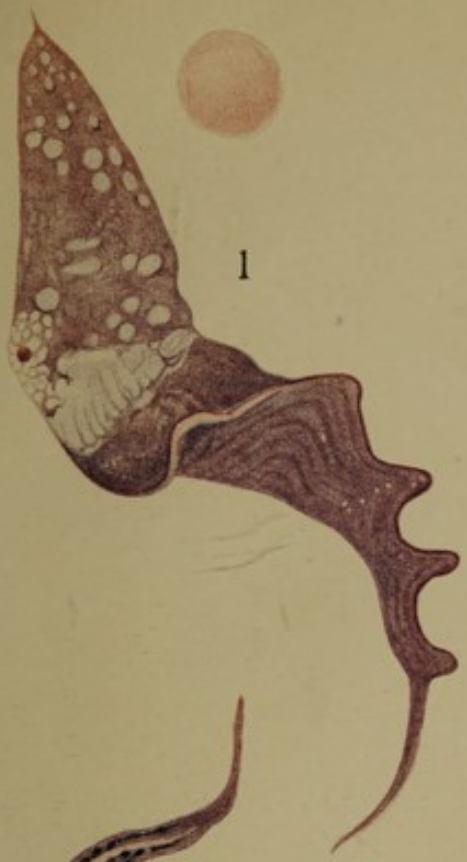
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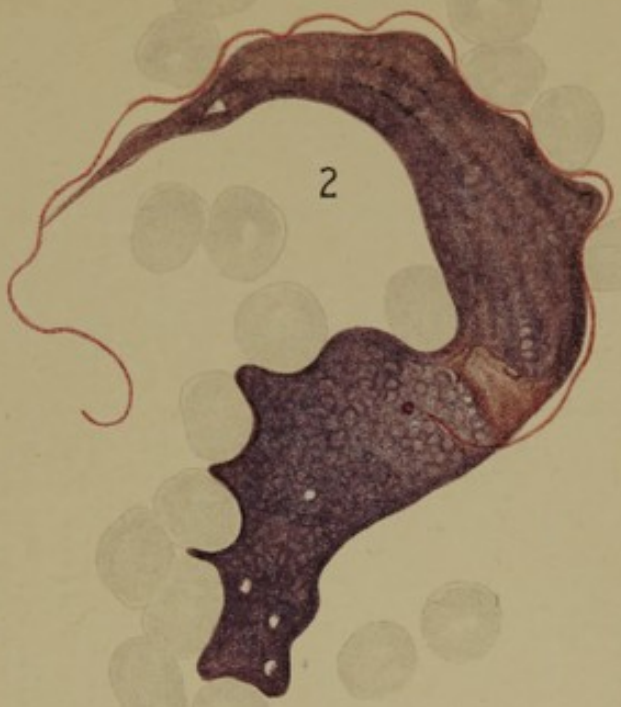
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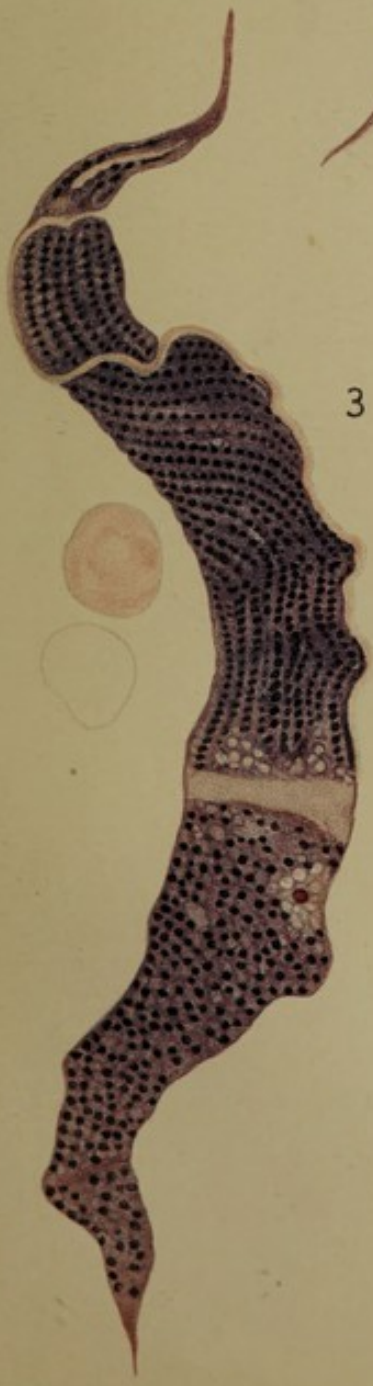
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[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 81]

20

A Note on the Occurrence of a Trypanosome in the African Elephant.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908.)

(Received July 5, 1909.)

[PLATE 12.]

As trypanosomes have never been reported as having been observed in the blood of the African Elephant, the Commission thought it would be of interest to note this observation.

In Laveran and Mesnil's book on trypanosomes, translated by Nabarro, on p. 261 it is stated that "the occurrence of Surra (*Trypanosoma evansi*) in elephants in India and Burmah is practically proved. In this connection we have only the statement of G. H. Evans that, in 1893, 14 out of 32 elephants died of the disease in Burmah." The year 1893 is almost prehistoric for trypanosomes. At that time observers had even failed to distinguish between the common rat trypanosome—*Trypanosoma lewisi*—and that of Surra. It may well be, then, that Evans was mistaken in his diagnosis of the species causing this large mortality in elephants.

The African elephant, in whose blood this trypanosome was found, was

shot by Mr. L. C. Lea-Wilson, of the Uganda Company Limited, at a spot two miles from the eastern shore of Lake Albert, near Ngogole, about 31° 10′ E. lat. and 1° 30′ N. long. It is to be regretted that none of the blood was injected into a dog, donkey, or ox, in order that a fuller study of this trypanosome might have been made. As it is, all the material available are a couple of smears made by Mr. Lea-Wilson and sent to the Commission.

Morphology of the Trypanosome of the Elephant.

Method of Fixing and Staining.—The two slides received from Mr. Lea-Wilson were fixed in osmic acid vapour and alcohol, stained in Giemsa, and decolorised in orange tannin.*

Length.—For method of measurement see the same paper, p. 16. As will be seen from the coloured plate, which was drawn by Sergeant Gibbons, R.A.M.C., this trypanosome is of medium size. The average length of 18 individuals is 18·5 microns: maximum 21, minimum 15.

Breadth.—On an average the breadth at the thickest part is 3 microns.

Shape.—This trypanosome is of the *Trypanosoma brucei* type, inasmuch as it has a well-developed undulatory membrane and free flagellum. As will be seen from the drawing (Plate 12), one noteworthy feature it has is the uniformity in size and shape of the different individuals. The posterior end is blunt, or conical, reminding one somewhat of the head of a seal, with the bulging micronucleus for an eye. The body thickens as far as the middle, when it gradually tapers away to the anterior end.

Contents of Cell.—The protoplasm is clear and particularly free from granules.

Nucleus.—The nucleus is compact and sharply defined from the neighbouring protoplasm. In shape it is round, or oval, and often lies nearer the anterior extremity than the posterior. Its length averages 2 microns.

Micronucleus.—The micronucleus is small, round and distinct. It is situated close to the posterior extremity, and often appears to bulge above the surface.

Undulating Membrane.—The undulating membrane is well developed and thrown into well-marked folds.

Flagellum.—The flagellum stains deeply. It runs from the micronucleus along the edge of the undulating membrane, beyond which it projects as a free flagellum for some 5 or 6 microns.

* *Vide* 'Roy. Soc. Proc.,' Series B, vol. 81, p. 16.

Sergt Gibbons, R.A.M.C., del. West, Newman chr.

Conclusions.

In our present state of knowledge it seems impossible to name trypanosomes from their form alone. We were, however, much surprised, a short time ago, by Sir John McFadyean separating with ease *Trypanosoma brucei* from *Trypanosoma evansi*. If this can be done for such closely related species, surely it should be possible to do it for all. To assist to this end it would be well if observers would adopt one method of fixing, staining, and measuring. In the 'Third Report of the Wellcome Research Laboratories,' Khartoum, facing p. 30, there is a coloured plate of trypanosomes, stated to have a magnification of 1000. On measuring one of them it is found to have a magnification of between 2000 and 3000. Then, again, many of the trypanosomes depicted are dividing forms, which is misleading.

The method of measuring must also make a difference. For example, in Laveran and Mesnil's book the length of *Trypanosoma brucei* in the rat is given as 26 to 27 microns, whereas by our method of measuring the average length of 20 individuals is 22·8 microns: maximum 25, minimum 20.

The trypanosome of the elephant has an average length of 18·5 microns: maximum 21, minimum 15, a well-developed undulatory membrane and free flagellum. The trypanosomes with free flagella are *Trypanosoma brucei*, *cazalboui*, *evansi*, *gambiense*, *pecaudi*, and *soudanense*. It probably is neither *Trypanosoma cazalboui* nor *pecaudi*, on account of its well-developed undulating membrane and uniform size. Under the circumstances it is impossible to decide as to its identity with *Trypanosoma brucei*, *gambiense*, or *soudanense*, but if a guess were hazarded then it would be *Trypanosoma soudanense*.

Until the nature of this species is better known we propose to name it *Trypanosoma elephantis*.

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[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 82]

21

Amakebe: A Disease of Calves in Uganda.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. Sleeping Sickness Commission of the Royal Society, 1908—09.

(Received December 18, 1909,—Read January 20, 1910.)

[PLATE 10.]

Introductory.

Amakebe is the most important disease of cattle in Uganda. It attacks the calves soon after they are born, and destroys more than half of them. Among the native cattle the loss is reported to be as much as 75 per cent., but, with careful nursing and hand-feeding, this mortality may be reduced to between 20 and 30 per cent. This is an enormous toll to pay, and renders the breeding of cattle in Uganda for dairy purposes, or, indeed, for any purpose, very up-hill work.

Little up to the present has been written as to the nature and causation of Amakebe. It has been described as a trypanosome disease, but this evidently on insufficient knowledge.

Distribution in Uganda.

Amakebe appears to occur all over the Kingdoms of Uganda, Unyoro, Ankole, and Busoga. Lieutenant A. D. Fraser, Royal Army Medical Corps, the medical officer lately in charge of the Sleeping Sickness Camp, Sesse, reports, however, the curious fact that it does not occur among the cattle on the Sesse Islands. Mr. C. W. Hattersley also informs the Commission that

cows brought to Mengo from Ankole invariably contract the disease, which would go to show that in some parts of Ankole the disease does not occur. Mr. R. J. Stordy, the chief veterinary officer, British East Africa, states that Amakebe is found at every altitude in that Protectorate. Dr. A. Theiler, C.M.G., the chief veterinary bacteriologist, Transvaal, who lately visited Uganda, writes that Dr. Lichtenfeld, the principal veterinary officer, German East Africa, told him that a disease similar to Amakebe exists in Ruanda, on the western shores of Victoria Nyanza and adjoining Ankole.

It is evident, then, that this disease is widely prevalent in Central Africa, and most disastrous in its effects.

Nomenclature.

In Uganda the disease is known as "kebe," "makebe," or "amakebe," and means calves' swollen glands, or mumps. At Ngora, to the west of Mount Elgon, the natives call the disease "angarwe." In Unyoro, "masugu." In Ankole, "amashuyu" or "amashui."

Symptoms.

The chief symptom of this disease is the swelling of the lymphatic glands, especially those in the region of the ear, in front of the shoulder, and in front of the hip. The glands frequently reach a large size, those in front of the shoulder often being three or four inches in length. They are soft to the touch, giving the impression of an elastic body under the skin. The hair is rough and staring, the head hangs, the ears droop, and there is frequently a watery discharge from the eyes and nose. During the illness the temperature runs high, often reaching 107° F. or more. The calf becomes rapidly emaciated, and often a dry, scabby eruption of the skin is seen. Diarrhœa is frequent, and the dung is often dark in colour, with an evil odour. The urine never shows any trace of blood, as in redwater.

The duration of the disease is usually about a fortnight, but sometimes the calves get over it in three or four days. The fever goes, they pick up condition, and the swelling of the glands subsides. The glands, however, never regain their normal size, but remain permanently enlarged throughout life.

When a calf has recovered from Amakebe it is no longer susceptible to the disease. It is immune for the rest of its life.

The following cases illustrate the course of the disease:—

Experiment 1387.—To study Amakebe in the Calf.

July 26, 1909.—Animal received from Sir Apolo Kagwa, K.C.M.G., Kampala.

July 29.—The prescapular glands are the size of a walnut. The calf looks fairly well, is thin, and hair slightly rough.

Aug. 2.—The lymphatic glands are much more enlarged. The prescapular glands measure 3½ by 2 inches.

„ 12.—This calf is now looking very sick. Conjunctival mucous membrane congested. The hair is falling off in patches, leaving a rough, scabby surface. Diarrhœa.

„ 14.—Discharge from eyes and nose. Diarrhœa.

„ 26.—This calf got steadily worse, and died at 11.30 A.M. to-day.

The following chart represents the course of the temperature:—

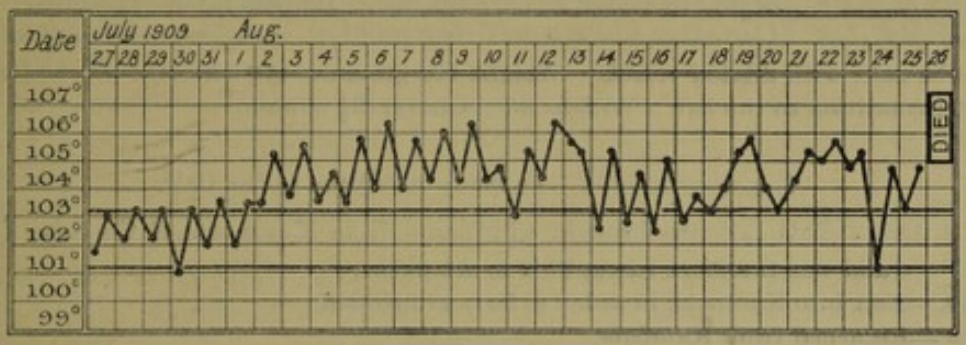

CHART 1.—Experiment 1387. Temperature Curve in a severe Case of Amakebe.

Aug. 26, 1909.—*Post-mortem* immediately after death.

External Appearances.—The body is emaciated. *Rigor mortis* absent. The hair is staring and has a ragged appearance. There are many patches of eruption on the surface of the body, especially on the face and head. These eruptions are, as a rule, about the size of half-a-crown and resemble limpet-shells.

Internal Appearances.—On removing the skin, the flesh is seen to be pale in colour. On opening into the abdomen the spleen is seen to be enlarged. The surface of the stomach and intestines is exceedingly pale and yellowish. There is no fluid in the peritoneal cavity. On opening into the thorax no fluid is found in either pleural cavity. There is about a tablespoonful of bright, chrome-coloured, clear fluid in the pericardium. The anterior mediastinum contains a quantity of bright yellow, jelly-like material. The serous membranes are shining and smooth.

Lymphatic Glands.—The lumbar chain of glands are enlarged, some of them being the size of a small walnut. These enlarged glands on being cut into are found to be very œdematous, but not hæmorrhagic. The prescapular glands are much enlarged, being 8 cm. (3½ inches) in length.

Circulatory System.—Heart.—The fat of the auriculo-ventricular groove is infiltrated with gelatinous material, which is bright yellow in colour. There are no petechiæ under the epicardium. On opening into the left ventricle many minute petechiæ are seen under the endocardium. The colour of the aorta is bright yellow. The substance of the heart is pale in colour and flabby to the touch. Weighs 125 grammes (4½ ounces).

Respiratory System.—Left lung is purplish in colour, with a dark purple patch in the anterior lobe about the size of half-a-crown. This, on being cut into, shows hæmorrhagic infarction. On section the left lung appears to be fairly healthy. Weighs 210 grammes (7½ ounces). *Right lung.*—Anterior lobe and part of the middle lobe are purplish-red in colour and solid. The anterior lobe sinks in water. The surface is purplish-red in colour, and across the surface is a network of yellow-coloured, clear, jelly-like strands, resembling the lung in a case of horse-sickness. The strands in some places are ¼ inch wide. On section the substance is seen to be hepatised and dark purple in colour, and across the cut section the same network of yellow, gelatinous-like material is seen. The

posterior lobe is pale in colour, and on section appears fairly normal. Weighs 345 grammes (12¼ ounces).

Alimentary System.—Spleen is enlarged. 29 cm. in length, 9 cm. broad, and 2·5 cm. in thickness (11″ × 3½″ × 1″). Capsule is purplish in colour. On section the tissue is dark purple in colour and friable. Weighs 245 grammes (8¾ ounces). *Liver* is bright yellow in colour, tinged with red, like bronze. Capsule is smooth. On section the substance is pale, with congested areas. *Gall-bladder* is distended with thick, greenish-yellow bile. Weighs 890 grammes (31½ ounces).

Fourth Stomach.—Is pale in colour. No ulceration. Intestines not examined.

Urinary System.—Left kidney.—Capsule strips readily. On section the cortical part is seen to be pale, with dilated vessels. Weighs 102 grammes (3½ ounces). *Right kidney*, in a similar condition to the left. Weighs 95 grammes (3¼ ounces).

Experiment 1634.—To study Amakebe in the Calf.

Sept. 4, 1909.—This calf was brought to Mpumu from Kome, one of the Sesse Islands. and was therefore susceptible to Amakebe.

„ 14.—Sent into Kampala, in order to become infected.

„ 24.—Returned from Kampala.

Oct. 4.—Lymphatic glands much enlarged. Oct. 18.—Died.

The following chart represents the course of the disease :—

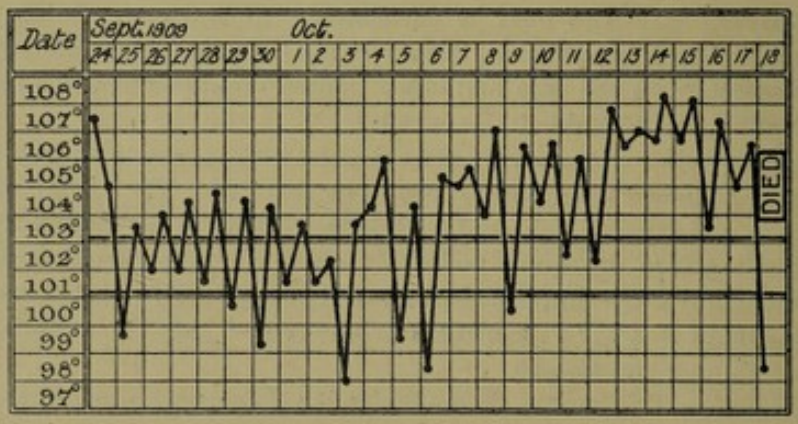

CHART 2.—Experiment 1634. Temperature Curve in a severe and fatal Case of Amakebe.

Oct. 18, 1909.—*Post-mortem* immediately after death.

External Appearances.—Animal about one year old. Preauricular, prescapular, and precrural glands are much enlarged. The prescapular glands measure 3 inches in length and 1½ inches in breadth. On section the glandular tissue is œdematous and, in some places, hæmorrhagic.

Internal Appearances.—On opening into the peritoneal cavity about a gallon of clear, amber-coloured fluid is found. There is a large quantity of yellow, gelatinous infiltration into the omentum. The serous membrane of the omentum is markedly hæmorrhagic, being covered with small petechiæ. The small intestine is dark crimson in colour and intensely congested. The whole of the peritoneal aspect of the diaphragm is covered with small hæmorrhages. On removing the sternum a quantity of yellow, gelatinous material is found in the mediastinum. About 2 ounces of the same clear, amber-coloured fluid are seen in the pleural cavity. The pericardium contains a small quantity of clear, straw-coloured serum.

Circulatory System.—Heart.—A quantity of yellow, gelatinous material is seen at the base. Many small petechiæ both inside and outside the heart.

Respiratory System.—A quantity of white frothy fluid exuded from the nose during the last hours of life. On opening into the *trachea*, however, it is now found to be empty. The *left lung* is partially collapsed and is dark purple in colour. On section the organ is dark crimson in colour and intensely congested. It is, in places, solid in consistence and a portion placed in water sinks. On pressure a white, frothy fluid exudes. *Right lung* is pale in colour, and there are numerous hæmorrhages into the serous membrane. On section it is found to be congested. No part of the lung sinks in water.

Alimentary System.—Spleen is enlarged. Measures 14 inches in length and 4½ inches in breadth. Numerous petechiæ into the capsule. On section the substance is dark in colour, soft, and friable. Weighs 480 grammes (17 ounces). *Liver* is enlarged. On section is seen to be congested. Gall-bladder is distended with dark, olive-green-coloured bile. Weighs 3 lbs. 10 ozs.

Fourth Stomach.—Is reddened, and there are numerous small ulcers in the serous membrane.

Urinary System.—Right Kidney.—Capsule strips readily. There are numerous petechiæ into the capsule. Surface of the organ is injected. On section the kidney is seen to be congested, with many hæmorrhages into the substance. *Left kidney* is in a similar condition to the right.

Experiment 1636.—To study Amakebe in the Calf.

Sept. 4, 1909.—From Kome. Same history as Experiment 1634. Great enlargement of lymphatic glands. Oct. 12.—Died.

The following chart represents the course of the disease :—

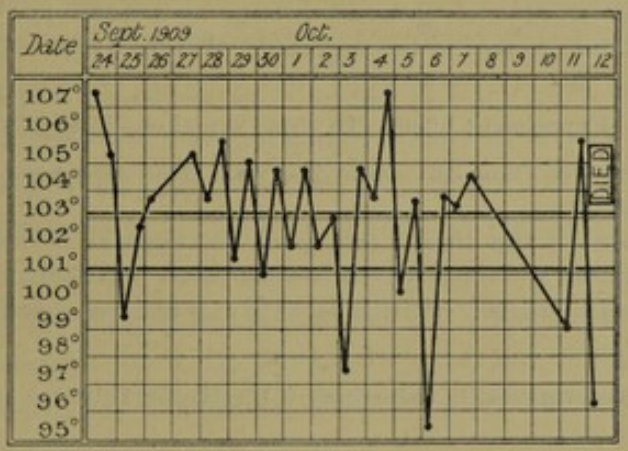

CHART 3.—Experiment 1636. Temperature Curve in a severe and fatal Case of Amakebe.

Oct. 12, 1909.—*Post-mortem* two hours after death.

External Appearances.—This calf has had a running from the nose of clear fluid, which has made a small pool under its head, and at death there was a marked collection of white foam at the nose, like that which occurs in horse-sickness, but not to such an extent.

Internal Appearances.—On removing the skin and opening into the peritoneum, about 4 ounces of clear, straw-coloured fluid is found. The omentum is infiltrated with a yellow jelly-like material. On opening into the thorax, 2 ounces of clear, straw-coloured fluid is seen in the pericardium. About 4 ounces of the same straw-coloured fluid in both pleural cavities. On removing the tongue and trachea a large quantity of jelly-like material is found under the trachea.

Lymphatic Glands.—The prescapular glands are enlarged. One measures 4½ inches in length and 1 inch in thickness. On section it is seen to be dark crimson in colour and hæmorrhagic. The glands generally are enlarged and hæmorrhagic. Some of them show signs of breaking down into pus.

Circulatory System.—Heart.—A large quantity of yellow, jelly-like material at the base is seen. There are numerous small petechiæ into the epicardium. None in the endocardium. Muscular substance is pale. Weighs 1 lb. 5 ozs.

Respiratory System.—Tongue normal. The *trachea* is full of white froth. Glands at the bifurcation of the trachea are much enlarged and hæmorrhagic. *Right lung*, anterior lobe is dark purple in colour and is found to be the seat of a large infarct. Posterior lobe is also the seat of an infarct at the margin, about 3 inches by 2 inches in extent. On section the substance of the lung is extremely œdematous. A large amount of frothy fluid exudes on pressure. Weighs 2 lbs. 10 ozs. *Left lung* is also the seat of numerous infarcts. On section a large amount of frothy fluid exudes on pressure. Weighs 1 lb. 6 ozs.

Alimentary System.—Spleen measures 13 inches in length and 4 inches in breadth. On section the substance is soft and friable. Weighs 13 ounces. *Liver* is enlarged. It is full of flukes. The gall-bladder is distended with dark, chocolate-coloured bile, which contains many flukes. Weighs 8 lbs. 5 ozs.

Fourth Stomach.—The mucous membrane of the fourth stomach is congested. It is dark crimson in colour, and numerous small ulcers are seen scattered throughout.

Urinary System.—Right Kidney.—Capsule strips readily. Under the capsule numerous infarcts are seen, about the size of a pea. On section the substance of the organ is congested. Weighs 8 ounces. *Left Kidney.*—Capsule strips readily. In the pelvis of the organ there is a quantity of yellow, jelly-like material. Under the capsule there are also several small infarcts. One of these is as large as a small marble. On section the substance of the kidney is seen to be congested. Weighs 5 ozs.

Experiment 1386.—To study Amakebe in the Calf.

July 26, 1909.—Received from Sir Apolo Kagwa. Had also been kraaled at Kampala for some days.

Aug. 27.—The course of the disease was much the same as in Experiment 1387. The prescapular and other glands became much enlarged, one of them measuring 4 inches in length. By this date the calf had recovered, and was returned to owner.

The following chart represents the course of the temperature :—

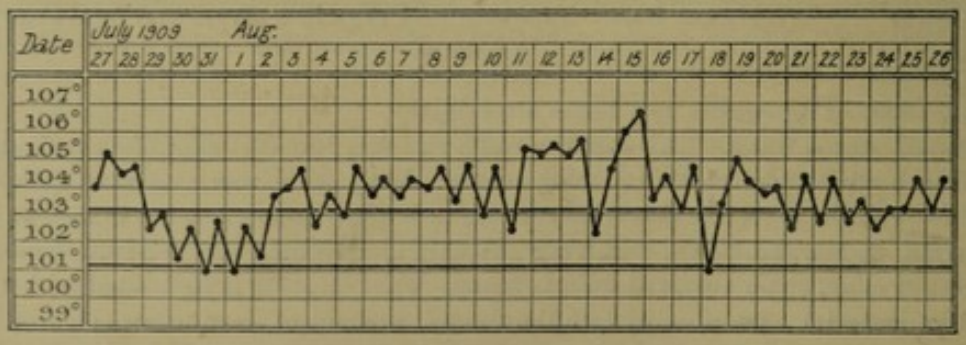

Chart 4.—Experiment 1386. Temperature Curve in a Case of Amakebe ending in Recovery.

Experiment 1635.—To study Amakebe in the Calf.

The following chart represents the course of the temperature —

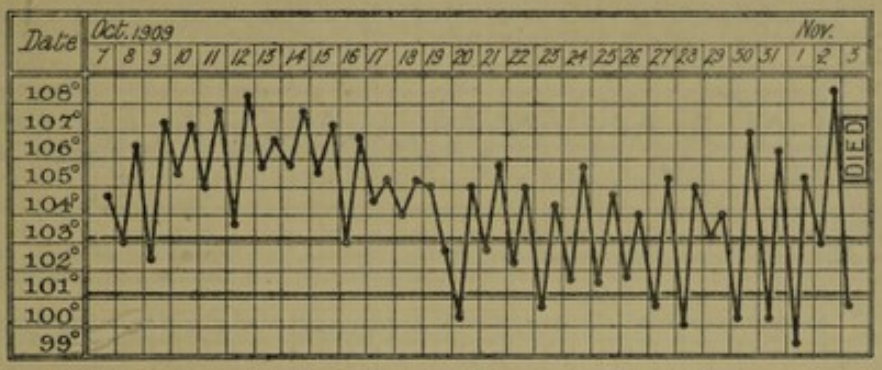

CHART 5.—Experiment 1635. Temperature Curve in a Case of Amakebe ending Fatally.

From these foregoing cases and the *post-mortem* examinations, it will be seen that amakebe is an acute disease of calves, and that the main features of the *post-mortem* are signs of intense anæmia, petechiæ of serous membranes, infiltration of jelly-like material into omentum, anterior mediastinum, base of heart, etc., œdema of lungs, swelling and softening of spleen, hæmorrhagic infarcts into lungs, spleen and kidneys, and sometimes ulceration of the mucous membrane of the stomach.

Piroplasms usually found in the Blood of Uganda Cattle.

When the blood of cattle in Uganda is examined microscopically, two parasites are always to be found, though usually in very small numbers. One of these can readily be recognised as *Piroplasma bigeminum* from its large size and the characteristic appearance of the two pear-shaped bodies (Plate 10, fig. 1). It may, however, also appear as irregularly-shaped, amœboid forms, especially in the spleen (Plate 10, fig. 1). The other parasite is much smaller in size, and is usually seen in the form of a small rod or ring (Plate 10, fig. 2). Both these parasites are inoculable, and appear in the blood of calves without giving rise to any marked disturbance.

Have either of them any connection with Amakebe?

The following experiments go to show that they have not :—

Experiment 556.

To ascertain the effect on a Susceptible Calf of the Injection of Blood containing the Small Rod and Ring-shaped Piroplasm. Will it give rise to Amakebe?

Feb. 22, 1909.—This calf was born last night. To-day the mother was cleared of ticks by hand-picking, and then completely smeared with a mixture of paraffin and cyllin, and mother and calf then placed in a tick-free enclosure.

„ 26.—Injected this calf with 5 c.c. blood from calf, Experiment 430, whose blood contains the small rod and ring-shaped piroplasm.

The following chart gives the result :—

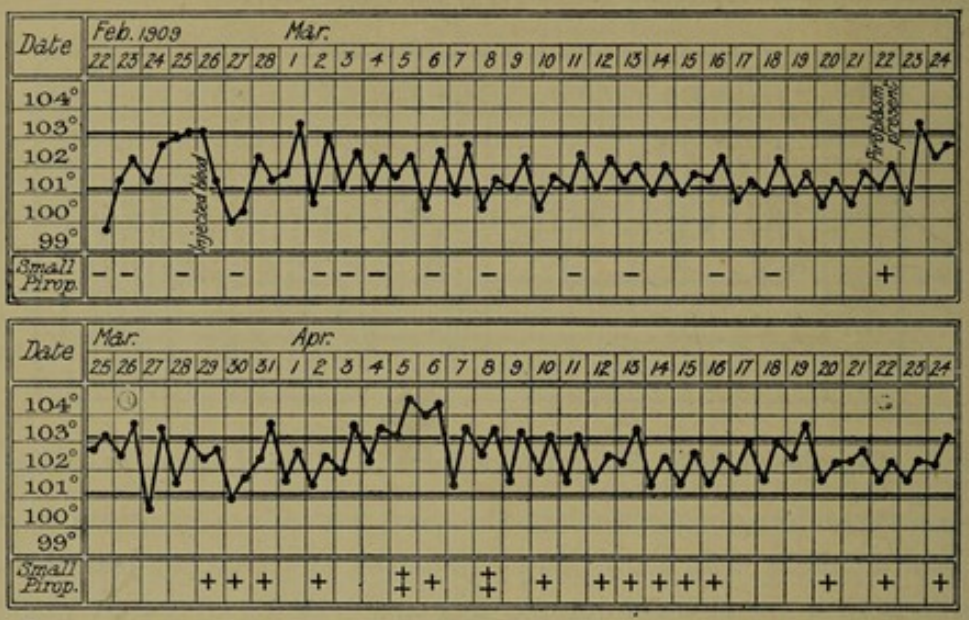

CHART 6.—Experiment 556 represents the Temperature Curve of a Calf which has been Injected with Blood containing the Small Rod and Ring-shaped Piroplasm. The *minus* and *plus* signs show the absence or presence of the small rod and ring-shaped piroplasm in the blood.

Remarks.—Twenty-four days after the injection of the blood containing the small piroplasm, the same rod and ring forms appeared in the blood. The temperature curve hardly shows any response to the invasion of the parasite, and the calf shows no signs of illness. It is evident, then, that the injection of blood containing this small piroplasm gives rise to no symptoms like those seen in Amakebe.

In the same way the injection of blood containing *Piroplasma bigeminum* is followed, after some days, by the appearance of this parasite.

Experiment 1901.

To ascertain if the Injection of Blood containing *Piroplasma bigeminum* will give rise to Symptoms of Amakebe.

Aug. 20, 1909.—Injected 2 c.c. blood containing *Piroplasma bigeminum* into this calf.

The following chart gives the temperature curve :—

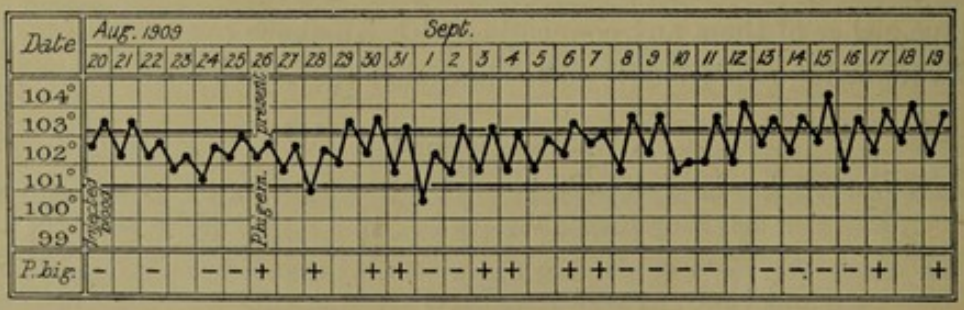

CHART 7.—Experiment 1901 represents the Temperature Curve of a Calf which has been Injected with Blood containing *Piroplasma bigeminum*. The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* in the blood.

Remarks.—Six days after the injection of blood containing the *Piroplasma bigeminum*, this parasite appeared in the blood. The temperature curve is not affected, nor does the calf appear ill. It may, therefore, be concluded that Amakebe is not caused by the injection of blood containing either *Piroplasma bigeminum* or the small rod and ring form.

It is well known that *Piroplasma bigeminum* is carried from affected to susceptible animals by different varieties of the blue tick, as well as other species of ticks. It would seem that the small rod and ring form of piroplasm is carried by the brown tick, as the following two experiments will show.

Experiment 747.

To ascertain if Brown Nymphs which had fed as larvæ on an Animal whose blood contained the Small Rod and Ring Forms, are capable of carrying them to a Susceptible Animal, and if the disease so set up will have the Symptoms of Amakebe.

June 24, 1909.—This calf, like the others, has been brought up in a tick-free shed. It has been under observation since May 10 without showing any small rod and ring forms in its blood. To-day, a large number of brown nymphs which had fed as larvæ on an ox whose blood contained the small rod and ring piroplasm were placed on this calf.

The following chart shows the course of the temperature :—

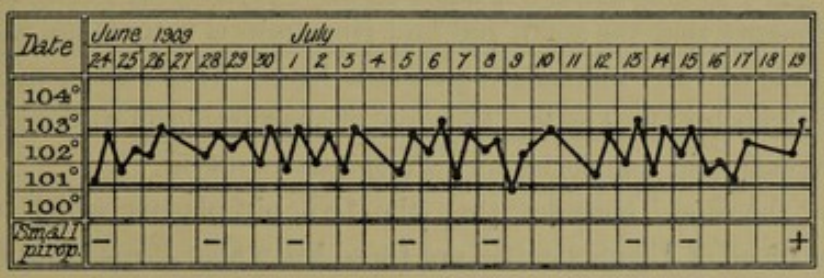

Chart 8.—Experiment 747 represents the Temperature Curve of a Calf upon which Infected Brown Nymphs have been fed. The *minus* and *plus* signs show the absence or presence of the small rod and ring piroplasm in the blood.

Remarks.—Twenty-five days after the infected brown nymphs were fed on this calf the small rod and ring-shaped piroplasm appeared in the blood. The temperature curve is not affected, and the calf shows no signs of Amakebe. It is evident, then, that the small rod and ring-shaped piroplasms transferred to a susceptible calf by means of brown nymphs do not give rise to amakebe.

Experiment 659.

To ascertain if Adult Brown Ticks which had Fed as Nymphs on an Animal whose Blood contained the Small Rod and Ring Forms are capable of carrying them to a Susceptible Animal and setting up the Symptoms of Amakebe.

Aug. 23, 1909.—This calf was born on April 4 in a tick-free shed. It has been examined almost daily since that date, and up to the present has shown no parasites of any kind in its blood. To-day, a large number of adult brown ticks were placed on this calf.

The following chart shows the course of the temperature :—

CHART 9.—Experiment 659 represents the Temperature Curve of a Calf upon which Infected Brown Adult Ticks had been fed. The *minus* and *plus* signs show the absence or presence of the small rod and ring piroplasm in the blood.

Remarks.—Twenty-one days after the infected brown adults had fed on this calf the small piroplasm appeared in the blood. The temperature curve is only slightly affected, and the calf shows no symptoms of Amakebe.

From the foregoing experiments it may be concluded, then, that the appearance of *Piroplasma bigeminum* or of the small rod and ring form of piroplasm in the blood of a susceptible calf, whether introduced by the injection of blood or, in the case of the latter, by the agency of the brown tick, is not accompanied by the symptoms of Amakebe. It also is seen from these experiments that the small rod and ring form is inoculable, is carried by the brown tick, and the incubation period is long. This corresponds with the description given by Dr. Theiler, Pretoria, of the piroplasm discovered by him in the Transvaal, and named by him *Piroplasma mutans.*

We may, therefore, consider that the two piroplasms which constantly occur in the blood of Uganda cattle are those known as *Piroplasma bigeminum* and *Piroplasma mutans*, and that neither is the cause of Amakebe.

Is Amakebe Inoculable?

It has been shown that blood containing either *Piroplasma bigeminum* or *Piroplasma mutans* if injected into susceptible cattle will give rise to these diseases. Is it equally true that Amakebe is inoculable? The following experiments were carried out to obtain an answer to this question :—

Experiment 1902.

To ascertain if Blood taken from an Animal suffering from Amakebe and injected into a susceptible Calf will give rise to the Disease.

Feb. 22, 1909.—This calf was born last night. Placed in tick-free shed.

„ 26.—Injected with a 5 c.c. blood from calf, Experiment 430, suffering from Amakebe.

The following chart shows the result :—

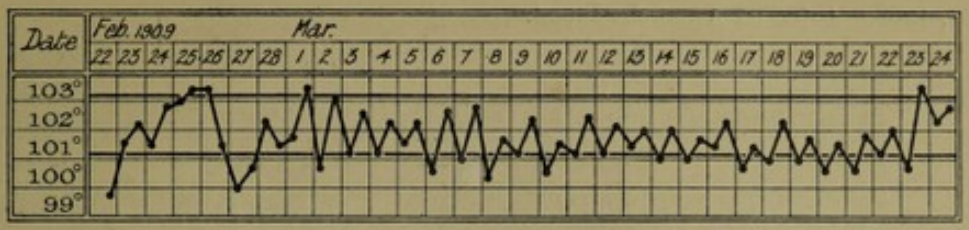

Chart 10.—Experiment 1902, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—The temperature curve is not disturbed by the injection of Amakebe blood, nor is the calf affected in any way.

Experiment 1903. (The above experiment repeated.)

Aug. 20, 1909.—Injected 5 c.c. mixture of blood and gland-juice from calf, Experiment 1387, which is suffering from Amakebe.

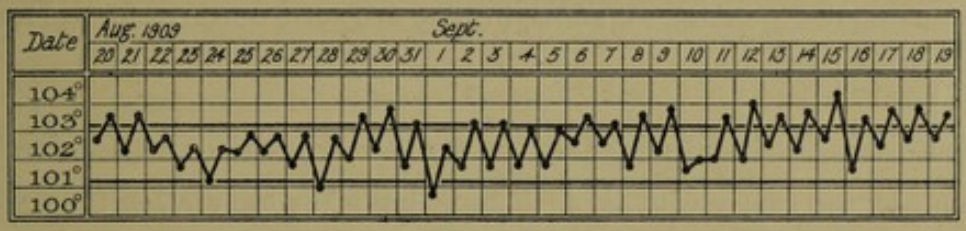

Chart 11.—Experiment 1903, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—The result of the injection of Amakebe blood is again negative.

Experiment 1904. (The above experiment again repeated.)

Aug. 21, 1909.—Injected Amakebe blood.

Chart 12.—Experiment 1904, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—Result negative.

Experiment 1905.

Sept. 24, 1909.—Injected Amakebe blood.

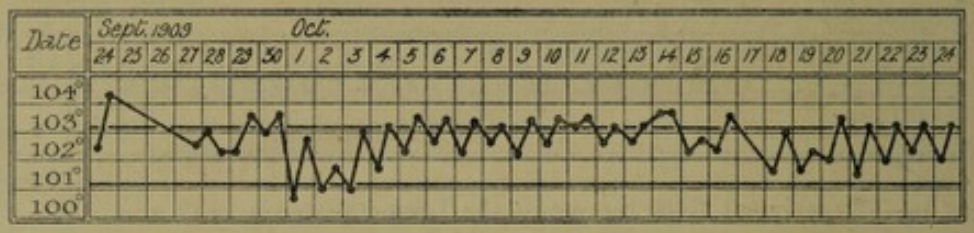

CHART 13.—Experiment 1905, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—Result negative.

On three other occasions (Experiments 659, 1585 and 1586) was this experiment repeated, and always with a negative result.

It may be concluded, then, that Amakebe differs from *Piroplasma bigeminum* and *Piroplasma mutans*, in that it is not inoculable, whereas the latter diseases are.

Result of Exposing Susceptible Calves in a Kraal contaminated by Amakebe.

Kampala, the native capital of Uganda, has a bad reputation for Amakebe. This is probably due to the number of calves stabled in the vicinity. Kampala has a large population of both Europeans and natives, and the milk supply is obtained from private cows kept in the town. The herds of cattle belonging to different individuals are grazed in various parts of the country, but as soon as a cow has calved, she is sent into Kampala to provide milk for her owner. Almost all the calves brought in die of Amakebe, which brings about an unhealthy state of things in the cattle kraals where the calves are kept during the day.

The following experiments will show the effect of exposing susceptible calves for a few days in a Kampala cattle kraal:—

Experiment 1590.

To ascertain the Effect of exposing a susceptible Calf in a Kraal contaminated by Amakebe.

Oct. 11, 1909.—Sent this calf into Kampala. Oct. 17.—Returned from Kampala.

The following chart shows the course of the temperature, and the presence or absence of *Piroplasma bigeminum* or the small rod-and-ring-formed piroplasma in the blood.

Remarks.—The result of exposing this calf to a contaminated kraal is an attack of Amakebe, characterised by high fever, swollen glands, and death.

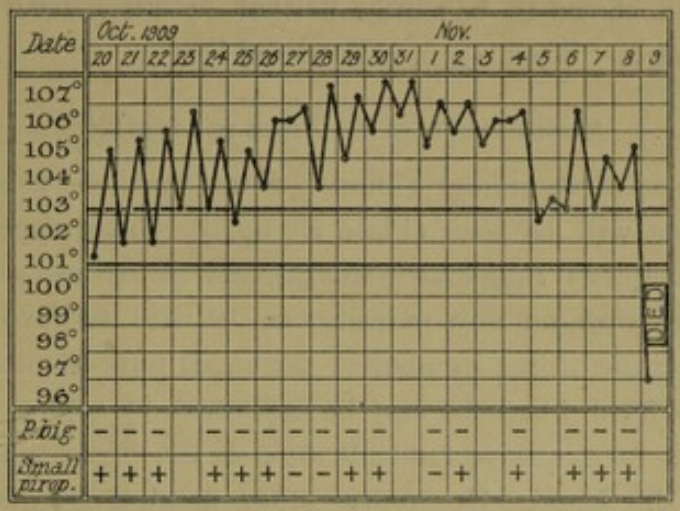

CHART 14.—Experiment 1590, represents the Temperature Curve of a Calf which has been exposed in a Kraal contaminated by Amakebe. The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* or the small rod and ring piroplasm in the blood.

Experiment 1593.

To ascertain the Effect of exposing a susceptible Calf, as in the previous Experiment.

Oct. 11, 1909.—This calf sent into Kampala. Oct. 17.—Returned from Kampala.

The following chart represents the course of the disease, and the presence or absence of *Piroplasma bigeminum* or the small piroplasm in the blood :—

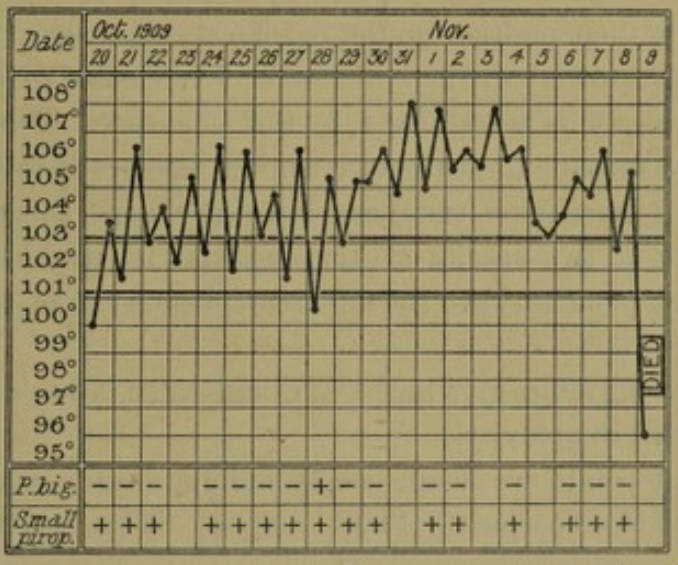

CHART 15.—Experiment 1593, represents the Temperature Curve of a Calf which has been exposed in a Kraal contaminated by Amakebe. The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* and the small rod and ring piroplasm in the blood.

It is evident, then, that the exposure of susceptible calves for a few days in a kraal where Amakebe is common is followed by a serious illness. There is high fever, glandular enlargement, emaciation, and, as a rule, death. This

disease has been shown to be caused neither by *Piroplasma bigeminum* nor *Piroplasma mutans*. What, then, is it caused by?

Examination of the Blood in Amakebe.

When the blood of an animal suffering from Amakebe is examined, many small piroplasms will be seen (Plate 10, fig. 3), which appear to be of the same size and shape as *Piroplasma mutans*, and sometimes a few *Piroplasma bigeminum*; otherwise, no new parasite can be said to have come into the blood. This increase in the number of the small piroplasms in the blood of a calf suffering from Amakebe may be explained by saying that the severe illness has led to an excessive multiplication of the *Piroplasma mutans* which was already in the blood. Or, on the other hand, it may be that another species of piroplasm, similar in size and shape to *Piroplasma mutans*, has appeared in the blood, and that the phenomena of Amakebe are due to it.

Marginal Points.—Besides the large and small piroplasms, another kind of body is found in the red blood corpuscles, which Theiler has called *marginal points*. In a lecture delivered by him in August, 1909, at Nairobi, in British East Africa, and published in the 'Agricultural Journal of British East Africa,' October, 1909, he states: "I have recently come to the conclusion that the disease called gall-sickness, and hitherto looked on as a sequel of redwater, is due to the presence of another parasite, which I have called 'Marginal Points' owing to their position in the red blood corpuscles. Gall-sickness is, therefore, a separate and distinct disease." Dr. Theiler considers it proved that this new disease is transmitted by the blue tick. This all shows how complicated and difficult to distinguish are the diseases of cattle. An ox may have *Piroplasma bigeminum*, small rod-and-ring-shaped piroplasms, marginal points, and one or two species of trypanosome in its blood at the same time. To which parasite have the different phenomena of the disease to be credited?

The two following tables give the blood examination in two cases of Amakebe, and illustrate this complexity.

The marginal points are small, deeply-staining bodies, usually placed near the edge of a red blood corpuscle (Plate 10, fig. 3). If these bodies really constitute a new and undescribed parasite, the discovery will be one of the greatest interest. Bodies similar in every way to these are found, however, in healthy young rats, goats, calves, etc., so that it is difficult to believe at once in their parasitic nature. Rather would they appear to be cell enclosures, due to rapid changes taking place in the blood, such as take place in young animals or in anæmias. In Amakebe they are sometimes very numerous, and it requires no great stretch of the imagination to see in them the youngest

Experiment 1387.—Blood Examination in a Case of Amakebe.

Date.	Parasites in blood.			No. of red blood corpuscles in 1 c. mm. of blood. Normal 10,000,000.
	Piroplasma bigeminum.	Small rod and ring forms.	Marginal points.	
1909.				
July 28	−	−	+	
29	−	+	−	
30	−	−	+	
31	−	−	+	
Aug. 1	−	−	−	
2	−	−	+ +	5,420,000
3	−	+	+ +	5,670,000
4	−	+	−	
5	−	−	+	
6	−	+	+ +	5,680,000
7	−	+	+ +	
8	−	+	+	
9	−	+	+	
10	−	−	+	
11	−	−	+ +	4,420,000
13	−	+	+ + +	
14	−	+	+ + +	
16	−	−	+ + +	4,540,000
19	−	+	+ + +	4,500,000
23				2,890,000
24	+ + +	+	+	2,820,000

Table I, Experiment 1387. The parasites to be found in a case of Amakebe. The *plus* and *minus* signs show the presence or absence of these bodies in the blood. The fourth column gives the number of red blood corpuscles in a cubic millimetre. + present, + + numerous, + + + very numerous.

Experiment 1636.—Blood Examination in a Case of Amakebe.

Date.	Parasites in blood.			
	Piroplasma bigeminum.	Small rod and ring forms.	Marginal points.	*Trypanosoma vivax.*
1909.				
Sept. 24	−	−	−	−
27	+	+	+	−
28	−	+	−	−
29	−	−	−	−
30	−	−	−	−
Oct. 1	−	−	−	+
2	+	+	−	−
4	−	−	−	−
5	−	+	−	−
6	−	+ +	−	−
7	−	+	+	−
8	−	+	−	+
9	−	+	+	+
11	+	+	+	+
12	+	+	+	+

Table II, Experiment 1636. Parasites found in a case of Amakebe. The *plus* and *minus* signs show the presence or absence of these bodies in the blood.

Remarks.—From these two tables it will be seen that small piroplasms and marginal points are commonly found in Amakebe, and that trypanosomes may also be present.

stage of the intra-corpuscular parasite, which from being round becomes wedge-shaped, oval or circular and rod-shaped. It may be that both these views are true—that some of the so-called marginal points are remains of chromatin from some previous nuclear structure, and that others are the earliest stages of an intra-corpuscular parasite. More work is required before any definite conclusion can be arrived at.

Koch's Granules or Blue Bodies.—Another body which may sometimes, though rarely, be seen in the blood of Amakebe calves, is one similar to that first described by Koch, and known as Koch's Granules or Blue Bodies. They are found principally in the spleen, lymphatic glands and liver, where they may be quite numerous. Stained by Giemsa the body appears as a blue-coloured cell, filled with coarse chromatin granules (Plate 10, fig. 5).

The following table gives cases of Amakebe in which these bodies were found :—

Experiment.	Date.	Spleen.	Liver.	Lymphatic glands.	Kidney.	Lung.	Blood.
	1909.						
415	May 10	+					
1392	July 24	+					
1593	Nov. 10	+					
1633	Oct. 5	+ + +	+ + +	+ + +			
1634	18	+ +	+ +	+	+	+	
1635	15						+
1636	12	+	+	+	+	+	
1637	6	+	+	+	+	+	
1638	6	+ +	+	+ +	+	+	+
1833	—	+	+		+		
1888	Nov. 5	+ +					
1891	8	+					
1908	14	+ +					

Table III, showing the presence of blue bodies in cases of Amakebe. + present, + + numerous, + + + very numerous, — absent.

Diagnosis of Amakebe.

What, then, is Amakebe? In the opinion of the Commission it is the disease of cattle discovered by Koch, and named by him East Coast Fever. The chief grounds for this opinion are, the symptoms during life, the appearances after death, the occurrence of a small piroplasm in the blood indistinguishable from *Piroplasma parvum*, and lastly and chiefly, the presence of the blue bodies in the spleen and other organs. These bodies have never been known to occur in any other disease, and the diagnosis of East Coast Fever is made in South Africa if such bodies are found in spleen smears.

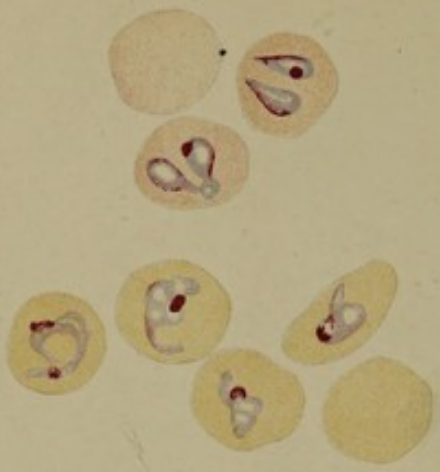

Fig. 1.

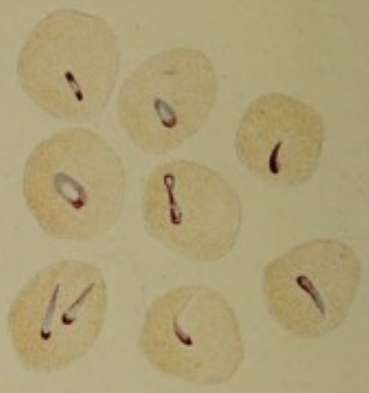

Fig. 2.

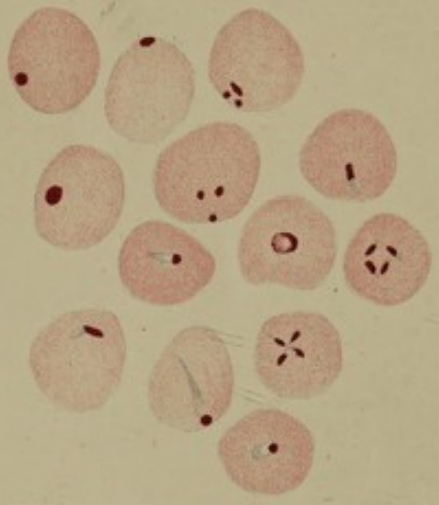

Fig. 3.

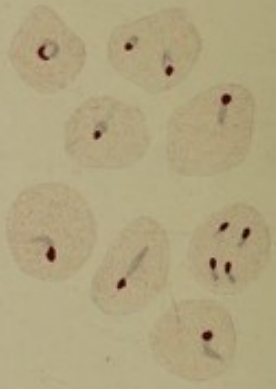

Fig. 4.

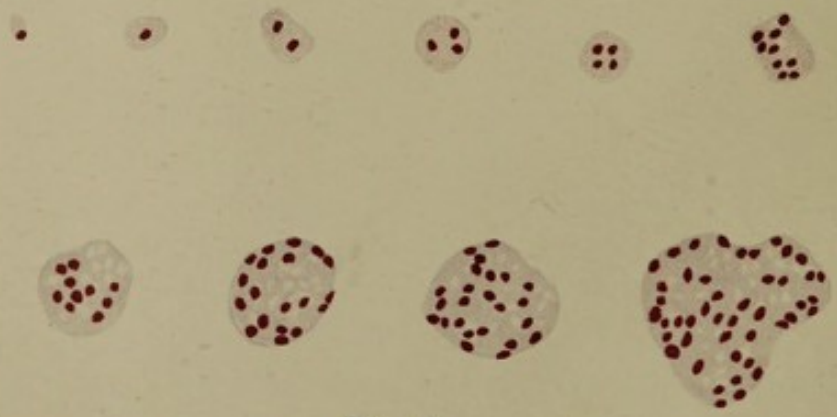

Fig 5.

Conclusions.

1. The blood of cattle in Uganda almost always contains *Piroplasma bigeminum* and *Piroplasma mutans*, and the cattle are therefore immune to these two diseases.

2. The disease of calves called Amakebe is East Coast Fever, so that very many of the cattle in Uganda are almost immune to this disease.

3. Owing to the nature of East Coast Fever, inasmuch as animals recovered from the disease are no longer infective, some calves may escape attack of Amakebe, and so remain susceptible.

4. Thus the calves of the Sesse Islands escape Amakebe, and when as grown-up cattle they are transferred to the mainland, they mostly die of East Coast Fever.

5. The carriers of East Coast Fever—*Rhipicephalus appendiculatus*, or brown tick; *Rhipicephalus evertsi*, or red-legged tick; and *Rhipicephalus simus*—are all common in Uganda.

DESCRIPTION OF PLATE.

Fig. 1.—The two upper corpuscles show the characteristic pear-shaped forms of *Piroplasma bigeminum* as they appear in the blood. The lower amœboid forms are drawn from a preparation of spleen. Stained Giemsa. × 2000.

Fig. 2.—*Piroplasma mutans* in the blood. Stained Giemsa. × 2000.

Fig. 3.—The small rod-and-ring-shaped piroplasm, as seen in the blood of a case of Amakebe. Among them are the deeply-stained bodies known as marginal points. Stained Leishman. × 2000.

Fig. 4.—Red blood corpuscles containing piroplasms from the spleen of a case of Amakebe. Stained Giemsa. × 2000.

Fig. 5.—Koch's granules or blue bodies from the spleen of a case of Amakebe. Stained Giemsa. × 2000.

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"*Muhinyo*," *a Disease of Natives in Uganda.*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–10.)

(Received June 7,—Read June 30, 1910.)

When the Sleeping Sickness Commission passed through Kampala, the native capital of Uganda, at the end of October, 1908, on their way to their camp at Mpumu, they were informed by Sir Apolo Kagwa, K.C.M.G., the Prime Minister, that a new disease had broken out in the province of Ankole, and that many people were sick.

This is probably the same disease which was described by Dr. A. G. Bagshawe in 1906. He gives the history and symptoms of nine cases which he saw in Ankole. He concluded that the disease was beri-beri, and states that at one village 25 per cent. of the inhabitants were suffering from a more or less severe form of the disease.

In the same year Dr. L. D. Lowsley also described "Muhinyo," but was of opinion that it might possibly be dengue with persistent joint pains.

Nothing more seems to have been written about "Muhinyo" until the beginning of 1909, when Dr. A. C. Rendle reported its presence in large numbers in the country round Lake Albert Edward. He says that all classes suffer, and that he has no hesitation in saying that "the disease is closely allied to kala-azar, the black death of India."

Thanks to the kindness of Sir Apolo Kagwa and Chief Saulo Mayanja Lumama, the Commission had an opportunity of seeing a case of "Muhinyo," which was sent to Mpumu from Ankole in January, 1909. This patient, who was said to have been ill for three months, was extremely weak and thin, but otherwise he showed no symptoms which pointed to any special disease.

As no other cases could be sent such a long journey as to Mpumu, it was decided that a member of the Commission should proceed to the district, in order to examine sick natives whom A. H. Watson, Esq., the District Commissioner, had kindly undertaken to have collected there.

On May 23, 1909, Dr. A. D. P. Hodges, the Principal Medical Officer, Uganda Protectorate, accompanied by Colonel Sir David Bruce, Director of the Commission, went to Masaka on the borders of Ankole, where they found some 50 sufferers from this disease awaiting them.

Distribution of "Muhinyo" in Uganda.

The principal focus of the disease is along the eastern shore of Lake Albert Edward, which corresponds nearly to longitude 30° E., in the latitude of the Equator. The most severe cases have been met with at Katwe (Fort George), a settlement on the eastern shore of Lake Albert Edward. It appears to have spread down the eastern shore of this lake, and to have extended in a south-easterly direction into Ankole. Cases have been recorded as far east as the western shore of Lake Victoria, and as far north as the Katonga River, which runs parallel to, and about 10 miles north of, the Equator. The disease is therefore quite limited in its distribution.

There is no evidence to show how it originated.

Epidemiology.

The tribes most effected by the disease are the Bakonjo and the Basongora. The former are morally and socially about the lowest class of people to be met with in Uganda. They are abjectly poor and dirty in their persons and in their habits. They live in rude grass huts, which they share with their domestic animals. The Bakonjo keep goats and, if they can afford them, cattle also. They prefer the milk of the cow, but also drink largely of goats' milk. The Basongora are a higher type of native, and resemble in appearance and customs the Bahima, the aristocracy of Uganda. They are not so poor as the Bakonjo, whom they use as serfs; they keep cattle and goats, and consume the milk of both animals. The flesh of the goat is largely eaten by both classes in a partially cooked state. The milk of sheep is occasionally used in default of that of the other animals.

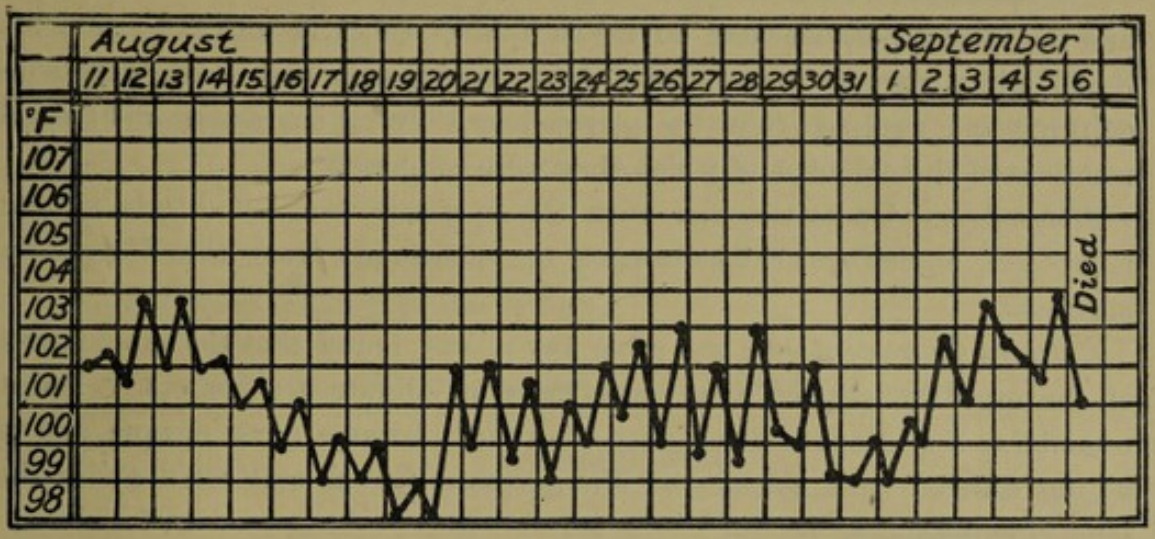
Clinical Symptoms of "Muhinyo."

As the result of the examination of the 50 cases sent into Masaka, it appeared that the principal symptoms of "Muhinyo" are fever, profuse sweating, pains in the joints and along the course of nerves, swelling of the various joints, especially the ankles, and extreme weakness and emaciation. The disease is of long duration; most of the patients had been ill for several months. In 13 cases, taken at random, the average duration was three and a-half months. Another had been ill for two years.

The following temperature chart was the only one which could be obtained.

In most of the cases there was no marked enlargement of the spleen or liver, nor symptoms of paresis or paralysis. The microscopical examination of the blood showed various degrees of anæmia, but no parasites or marked changes in the white blood corpuscles could be detected. Further, the

examination of the splenic pulp, obtained by puncture of the spleen, failed to reveal the presence of the parasites of kala-azar. It was therefore evident that "Muhinyo" was neither kala-azar nor beri-beri, but the long duration of the fever, the joint pains, and the extreme weakness and emaciation suggested a continued fever, such as typhoid or Malta fever.



Examination of the Blood for Agglutinative Phenomena.

The blood of several of these cases was therefore tested with *Bacillus typhosus* and *Micrococcus melitensis* by Widal's method, with the result that no reaction was obtained with the former, but positive results, in fairly high dilutions, were got with the latter.

The following table represents the result of the examination of the blood of "Muhinyo" with a strain of *Micrococcus melitensis* from Malta, from which it will be seen that five out of the seven cases examined gave a positive reaction :—

Table I.

No. of experiment.	Dilution of serum.			Control.
	1 in 50.	1 in 100.	1 in 200.	
927	+	+	−	−
928	+	+	−	−
929	−	−	−	−
930	−	−	−	−
931	+	+	+	−
932	+	+	+	−
933	+	+	−	−

Isolation of the Micrococcus of Malta Fever from the Spleens of Cases of "Muhinyo."

The next thing to be undertaken was the isolation of the *Micrococcus melitensis* from the tissues of patients suffering from "Muhinyo."

The spleens of two cases (925 and 926) were punctured in the usual way, and the splenic pulp smeared on the surface of tubes of nutrient agar-jelly. Small white colonies were grown from both cases, and these were sub-cultured and used to study the morphology, cultural characters, and animal reactions of the organism of which they were composed.

Morphology.—Under the microscope the organisms were found to be minute micrococci, indistinguishable in size or appearance from the *Micrococcus melitensis.*

Cultural Characters.—In the same way it was found that the sub-cultures of the organism showed after some days as minute transparent colonies, resembling tiny drops of dew, which afterwards became more opaque, and in no way differed from colonies of *Micrococcus melitensis* cultivated under the same circumstances.

Animal Reactions.—The sub-cultures were also emulsified in saline solution and injected into a monkey and rabbit. The monkey sickened with fever, and when the agglutinating power of its blood was tested with the strain of *Micrococcus melitensis* from Malta it was found to give a complete reaction in a dilution of 1 in 200. Having thus proved that two animals treated with the "Muhinyo" organism gave a serum capable of agglutinating a known *Micrococcus melitensis* from Malta, the converse experiment was made.

A rabbit was inoculated with the Malta strain, and its serum tested on the "Muhinyo" organism. This rabbit's serum, immunised against Malta fever, agglutinated the "Muhinyo" organism in a dilution of 1 in 200; and thus the proof that the micrococcus obtained from the spleen of "Muhinyo" cases and that obtained from cases of Malta fever were identical, was established.

Examination of Goats from the "Muhinyo" District, to ascertain if they are reservoirs of the virus of Malta Fever.

By Widal's Reaction.—In Malta, the Royal Society Commission discovered, in 1905, that the drinking of goats' milk was the sole mode of infection in Malta fever. Many of the Maltese goats were examined, and 50 per cent of them found to be affected in some way by the disease, while 10 per cent. were actually excreting the *Micrococcus melitensis* in their milk.

It was, therefore, a matter of importance, as well as curiosity, to ascertain if the Ankole goats also suffered from Malta fever, and if the causation of this disease was the same in Central Africa as it had been proved to be on the shores of the Mediterranean, in the Soudan, and in South Africa.

When Sir Apolo Kagwa was approached as to the feasibility of obtaining goats from the most affected districts, he informed the Commission that he would see what could be done. About six weeks later a flock of goats, numbering in all 24, was driven up to the laboratory at Mpumu, and it was stated that these had come from a place where "Muhinyo" was common. They were at once examined, with the result that the blood of three out of their number reacted to the strain of *Micrococcus melitensis* obtained from cases of "Muhinyo," and also to the Malta strain.

The following tables give the details :—

Table II.—*Micrococcus melitensis* ("Muhinyo" strain).

No. of experiment.	Dilutions of serum.					Control.
	1 in 10.	1 in 20.	1 in 50.	1 in 100.	1 in 200.	
1512	+	+	+	−	...	−
1507	+	+	+	+	...	−
1776	+	+	+	+	+	−

Table III.—*Micrococcus melitensis* (Malta strain).

No. of experiment.	Dilutions of serum.				Control.
	1 in 10.	1 in 20.	1 in 50.	1 in 100.	
1512	+	+	+	−	−
1507	+	+	+	+	−

Isolation of the Micrococcus of Malta Fever from the Tissues of the Goats.—After having found that some of the Ankole goats reacted to the agglutination test, an attempt was made to isolate the *Micrococcus melitensis* from their tissues. This proved successful in two cases. The following experiment gives one of these in detail :—

Experiment 1475. Goat.

August 11, 1909.—This goat, which was one of a herd from Ankole, died this morning. The spleen was removed, and small portions of the pulp spread over the surface of agar tubes.

August 16.—A growth consisting of several very small, round, white colonies appeared after three days. A stained preparation from one of these showed that they were composed of organisms resembling *Micrococcus melitensis*. Sub-cultures made.

September 29.—The growth from two agar tubes was made into an emulsion with salt solution, and an agglutination test made with serum from a rabbit immunised against *Micrococcus melitensis*, Malta strain.

The result was that the organism from the goats agglutinated completely in a dilution of 1 in 100, and the proof was complete that the Ankole goats are liable to contract Malta fever, and to act as a reservoir of the virus.

Conclusions.

1. "Muhinyo" is Malta fever.

2. "Muhinyo" is conveyed from the goat to man by the drinking of goats' milk.

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