Conclusion.

The pathogenic action of *T. brucei*, Zululand strain, 1913, on various animals is so similar, not only in regard to the symptoms during life but also in the *post-mortem* appearances and rate of mortality, to that of the trypanosome causing disease in man in Nyasaland, that it affords another proof that these two trypanosomes are identical.

The Trypanosome causing Disease in Man in Nyasaland. Part III.—*Development in* Glossina morsitans.

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S.; Major A. E. Hamerton, D.S.O., and Captain D. P. Watson, R.A.M.C.; and Lady Bruce, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

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[Plate 24.]

Introduction.

In previous papers* the morphology of this trypanosome and the susceptibility of various animals to its pathogenic action have been described. In this is given an account of its development in *Glossina morsitans*.

In Uganda the study of the development of *Trypanosoma gambiense* in *G. palpalis* was much assisted by the circumstance that large numbers of laboratory-bred tsetse flies were available. This was due to the fact that the pupæ of *G. palpalis* could be collected on the lake-shore in practically unlimited numbers. It is quite otherwise with *G. morsitans*. It has been found impossible to find the pupæ of this species in any numbers, so that all laboratory-bred *G. morsitans* have had to be hatched out of pupæ obtained from captive flies, a slow and laborious process. The flies are caught some 20 to 30 miles from the laboratory and brought up to Kasu camp by a native on a bicycle. This kills a large number of the flies. Moreover, the climatic conditions at the camp are not always favourable for breeding and hatching out. This was remedied to some extent by establishing a breeding station down in the low-country, but as this had to be left in the charge of natives the results were not always very satisfactory.

* 'Roy. Soc. Proc.,' B, vol. 85 (1912), and B, vols. 86 and 87 (1913).

The study of the development of this trypanosome in *G. morsitans* has therefore been rendered difficult by the small number of laboratory-bred tsetse flies which could be obtained. Over and above that, flies bred from captive flies are not so strong and healthy as those hatched out from wild pupæ.

An attempt was made to use wild flies by feeding batches of about 20 on healthy animals and picking out those cages which did not give rise to infection. But this is at best a roundabout and clumsy method, as it can never be certain, although every care is taken, that only clean flies are being dealt with.

The Development of the Trypanosome causing Disease in Man in Nyasaland in G. morsitans.

Eleven experiments were carried out with laboratory-bred flies. Three were positive and eight negative.

Five experiments were also carried out with wild flies, as no laboratory-bred flies were available. All were positive.

Tables I and II show these 16 experiments: the number of flies used; the number of infected flies found on dissection; and the number of days which elapsed before the flies became infective. As each fly died it was dissected and the result noted. As will be seen from Table I, several infected flies were found in the negative experiments. This probably means that the flies were only infected, not infective. The number of days before a fly becomes infective is arrived at by deducting seven days from the number of days which elapsed between the first infected feed of the flies and the

Table I.—Laboratory-bred Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1912.						
May 22	563	18	–	0		
June 13	668	22	–	1		
July 15	879	32	–	7		
„ 29	1003	28	+	2	31	84° F. (29° C.)
Aug. 17	1072	27	–	3		
Oct 23	1494	22	–	3		
Nov. 6	1560	19	–	0		
Dec. 13	1686	24	–	2		
„ 23	1710	30	–	0		
„ 30	1723	35	+	3	14	84° F. (29° C.)
1913.						
Aug. 31	2405	30	+	4	23	84° F. (29° C.)

appearance of trypanosomes in the blood of the experimental animal. Seven days is put down as the average number of days between the infection of the animal and the appearance of the trypanosomes in its blood—the incubation period. It is probably a day or two shorter.

The number of flies used in each experiment was small, due to the difficulty of obtaining laboratory-bred flies. They were kept during the experiment in the incubator at a temperature of 84° F. (29° C.).

In Experiment 1723 the number of days which elapsed before the flies became infective is only 14. This number is obtained, as mentioned above, by deducting seven days for the incubation period, but this may have been a day or two less. The flies were kept at an evenly warm temperature, which would tend materially to shorten the period of development. Still, 14 days seems a short time to elapse between the first feed on the infected animal and the appearance of an infective fly in the cage.

Two hundred and eighty-seven laboratory-bred flies were used and 25 infected flies were found—8·7 per cent.

Table II.—Wild Flies.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Temperature at which flies kept.
1912.						
Dec. 11	1680	80	+	8	18	84° F. (29° C.)
„ 13	1688	40	+	6	3	84° F. (29° C.)
„ 18	1705	45	+	7	1	84° F. (29° C.)
1913.						
Jan. 9	1748	70	+	1	25	84° F. (29° C.)
„ 14	1729	20	+	1	30	84° F. (29° C.)

Experiments 1688 and 1705 are evidently cases of infection by naturally-infected wild flies which had escaped detection. They are included in the table as they both show invasion of the salivary glands and so help to throw light on the mode of development of this trypanosome in *G. morsitans*. The other three pass through an interval of 18, 25, and 30 days before the cages became infective. These are probably cases where there was no naturally-infected fly in the cage, and these periods therefore represent the usual length of time required for the cycle of development of this trypanosome to take place in *G. morsitans*. The wild flies were also kept in the incubator at a temperature of 84° F.

Two hundred and fifty-five flies were used and 23 infected flies were found —9 per cent.

Details of the Eight Positive Experiments.

The following table gives the details of the eight positive experiments :—

Table III.

Expt.	Day of expt.	Procedure.	Remarks.
1003	1–2 3 4–41	Flies fed on infected dog. Starved. Fed on clean Monkey 1023.	Trypanosomes appeared in blood of Monkey 1023 on the 38th day.
1723	1–4 5 6–22	Flies fed on infected dog. Starved. Fed on clean Monkey 1733.	Trypanosomes appeared in blood of Monkey 1733 on the 21st day.
2405	1–6 7 8–32	Flies fed on infected monkey. Starved. Fed on clean Monkey 2410.	Trypanosomes appeared in blood of Monkey 2410 on the 30th day.
1680	1–2 3 4–22	Flies fed on infected dog. Starved. Fed on clean Dog 1708.	Trypanosomes appeared in blood of Dog 1708 on the 25th day.
1688	1–2 3 4-12	Flies fed on infected monkey. Starved. Fed on clean Monkey 1699.	Trypanosomes appeared in blood of Monkey 1699 on the 10th day.
1705	1–2 3 4–9	Flies fed on infected monkey. Starved. Fed on clean Monkey 1707.	Trypanosomes appeared in blood of Monkey 1707 on the 8th day.
1748	1–2 3 4–30	Flies fed on infected monkey. Starved. Fed on clean Monkey 1845.	Trypanosomes appeared in blood of Monkey 1845 on the 32nd day.
1729	1–2 3 4–38	Flies fed on infected dog. Starved. Fed on clean Dog 1767.	Trypanosomes appeared in blood of Dog 1767 on the 37th day.

Omitting Experiments 1688 and 1705, it would appear from the remaining six experiments that an average period of 24 days is required to complete the cycle of development of the trypanosome causing disease in man in Nyasaland in *G. morsitans*, the flies being kept at a temperature of 84° F.

Details of the Eight Negative Experiments.

The following table shows the method of procedure in carrying out the eight negative experiments :—

Table IV.

Expt.	Day of expt.	Procedure.	Remarks.
563	1–3	Flies fed on infected monkey.	All flies negative on dissection.
	4	Starved.	
	5–52	Fed on clean Monkey 594.	
668	1–2	Flies fed on infected dog.	One infected fly found on the 42nd day.
	3	Starved.	
	4–63	Fed on clean Dog 699.	
879	1–2	Flies fed on infected monkey.	Seven infected flies found.
	3	Starved.	
	4–32	Fed on clean Monkey 910.	
	33–63	Fed on clean Monkey 1073.	
1072	1–3	Flies fed on infected dog.	Three infected flies found.
	4	Starved.	
	5–54	Fed on clean Dog 1148.	
1494	1–3	Flies fed on infected monkey.	Three infected flies found.
	4–5	Starved.	
	6–44	Fed on clean Monkey 1514.	
1560	1–3	Flies fed on infected monkey.	All flies negative on dissection.
	4	Starved.	
	5–37	Fed on clean Monkey 1581.	
1686	1–4	Flies fed on infected monkey.	Two infected flies found.
	5	Starved.	
	6–43	Fed on clean Monkey 1704.	
1710	1	Flies fed on infected dog.	All flies negative on dissection.
	2	Starved.	
	3–47	Fed on clean Monkey 1718.	

Result of the Dissection of the Infected Flies.

All the flies dying during the progress of these experiments were dissected.

In the three positive experiments with the laboratory-bred flies nine infected flies were found. The following table gives the results of the dissection of these nine flies. The second column gives the number of days which elapsed between the fly's first infected feed and its death and dissection. In the third column the labial cavity and hypopharynx are included under "Proboscis." At the time these experiments were made no attempt was made to distinguish between the two parts, as has been done lately in the case of *T. simiæ*.* When the proboscis is marked positive, as in Table VI, it may be that the trypanosomes are contained in the labial cavity or the hypopharynx, or both.

In the development of *T. gambiense* in *G. palpalis* trypanosomes were never

* 'Roy. Soc. Proc.,' B, vol. 87, p. 59 (1913).

noted as occurring in the proboscis.* In this species they are noted on several occasions as occurring in this position, but only in the wild-fly experiments, not in the laboratory bred. It seems natural to expect that if the salivary glands are swarming with trypanosomes that some of them will sometimes appear in the hypopharynx and, moreover, in the wild flies some of the infections of the proboscis are no doubt due to *T. pecorum*, *T. simiæ* or *T. capræ*, all of which develop in the proboscis.

Table V.—Laboratory-bred Flies. Result of the Dissection of the Infected Flies found in the Positive Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
1003	33	–			+		–
1003	39	–			+		?
1723	30	–	+ +	+ +	+ +	+ +	–
1723	30	–	+ +	+ +	+ +	+ +	–
1723	48	–	–	–	–	–	–
2405	32				+		–
2405	33	–	+ +	+ +	+ +	+ +	+ +
2405	33	–	–	+	+	+	–
2405	33	–	–	+	+	+	–

In Experiment 1003, two infected flies were found. The first had only a gut infection and, unfortunately, it was found impossible to dissect out the salivary glands of the second. Neither had an infection of the proboscis.

In Experiment 1723, three infected flies were found. The first and second had the alimentary tract swarming with flagellates, but none in the salivary glands. The third was found on dissection to be free from trypanosomes throughout. This is curious because this fly had been isolated in a glass tube as an infective fly, and had, when used alone on a rat and rabbit, infected both these animals. The fly remained alive in the tube for 13 days, and the only explanation that can be given is that in this case the trypanosomes disappeared absolutely from the fly some few days before its death. This was the first time this had been observed to take place, and it was thought to be a remarkable phenomenon and difficult to credit, until another example of the same kind was observed. It must, therefore, be held as probable that an infective fly, with presumably both salivary glands and alimentary tract swarming with trypanosomes, can lose all these flagellates and become non-infective.

In Experiment 2405, four infected flies were found. Three of these were infections limited to the gut. The fourth was a good example of a salivary-

* *Ibid.*, B, vol. 82 (1910).

gland infection. The glands were swarming with trypanosomes, and a portion of one of them injected under the skin of Rat 2417 gave rise to infection.

Table VI.—Wild Flies. Result of the Dissection of the Infected Flies found in the Positive Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Fore-gut.	Mid-gut.	Hind-gut.	Salivary glands.
1680	5	−	−		+		−
1680	19				+		−
1680	32	+	+ +	+ +	+ +	+ +	+ +
1680	32	−	−	+	+	+	−
1680	33	−	+ +	+ +	+ +	+ +	−
1680	33	−	−		+		−
1680	33	−	−	+ +	+ +	+ +	−
1680	33	−	−	+	+	−	−
1688	10	−			+		−
1688	10	−	−	−	+		−
1688	11	−	−	−	+		−
1688	13	−			+		−
1688	15	−	+ +	+ +	+ +	+ +	+ +
1688	15	+	−		+		−
1705	8	+	+	+	+		−
1705	8	−	+	+	+		−
1705	10	−		−	+		−
1705	11	−		+			−
1705	12	+	+	+	+	+	+ +
1705	26	−	−	+ +	+ +	+ +	+ +
1705	33	−	+	+ +	+ +	+ +	+ +
1748	31	−	−	+ +	+ +	+ +	+ +
1729	48	+	+	+	+	+	+ +

In Experiment 1680, eight flies were found to be infected. In seven the flagellates were confined to the alimentary tract. The eighth had a well-marked invasion of the salivary glands. In this case trypanosomes were also seen in the proboscis, but whether in the labial cavity or the hypopharynx is not specified.

In Experiment 1688, six flies were found to contain trypanosomes in the alimentary canal. In one of these there was also infection of the salivary glands, which were crowded with trypanosomes. This fly must have been naturally infected when caught, as sufficient time had not elapsed since the infected feed to allow of time for development to take place. The flagellates contained in the salivary glands injected into Rat 1721 gave rise to infection.

In Experiment 1705, seven infected flies were found. Three of these had the salivary glands invaded. One of these, the fifth, must also have been a naturally-infected wild fly.

In Experiment 1748, only one infected fly was found. It had a copious infection of the salivary glands, a portion of which injected into Rat 1852 gave a positive result.

In the last Experiment, 1729, there was also only one infected fly found. The salivary glands were swarming with trypanosomes.

The next table gives the result of the dissection of the infected flies found in the experiments which remained negative.

In the negative Experiments 563, 1560, and 1710, none of the flies were found to be infected with trypanosomes in any part (see Table I). These experiments are therefore omitted from this table.

Table VII.—Laboratory-bred Flies. Result of the Dissection of the Infected Flies found in the Negative Experiments.

Expt.	Time, days.	Proboscis.	Proventriculus.	Fore-gut.	Mid-gut.	Hind-gut.	Procto-dæum.	Salivary glands.
668	42	–		+	+	+		–
879	7	–	–	+	+	+		–
879	8	–		+	+ +			–
879	9	–		+	+	–		–
879	11	–			+			
879	24	–	+	+	+	+	–	–
879	28	–	+ +	+ +	+ +	+ +		–
879	40	–	+ +	+ +	+ +	+		–
1072	7	–	–	+	+	+		–
1072	10	–		+	+	+		
1072	38	–	+ +	+ +	+ +	+ +		+ +
1494	7	–	+ +	–	–	–		–
1494	17	–		+	+	–		–
1494	31	–		+	+			–
1686	8	–	–	+ +	+ +	+		–
1686	26		–	+	+	+		–

From these negative experiments it will be seen that only in one fly did an infection of salivary glands occur. Why this fly did not infect the animal it fed on is impossible to say.

The Methods used in the Examination of the Flies.

The flies were dissected as described in a previous paper.* As each fly in a cage died it was dissected, and the result, as regards the presence of trypanosomes in the alimentary tract and salivary glands, recorded. Fixed and stained preparations were then made from the various parts and numerous drawings of the various types of trypanosomes encountered were made. The method described in a previous paper† of isolating infective flies and inducing them to salivate on clean cover-glasses was also made use of. This is a useful, simple and practical method, as it demonstrates clearly the type of trypanosome thrown out from the tip of the proboscis when the fly feeds.

* 'Roy. Soc. Proc.,' B, vol. 83, p. 513 (1911).

† *Ibid.*, B, vol. 87, p. 63 (1913).

The Trypanosomes Found in the Alimentary Tract.

In this species of trypanosome the developmental changes which take place in the intestine of *G. morsitans* are similar to those already described as occurring in the development of *T. gambiense* in *G. palpalis.** The latter development has also been worked out very fully and completely by others.† It is therefore unnecessary here to do more than refer to these previous descriptions as being equally applicable to the species under consideration.

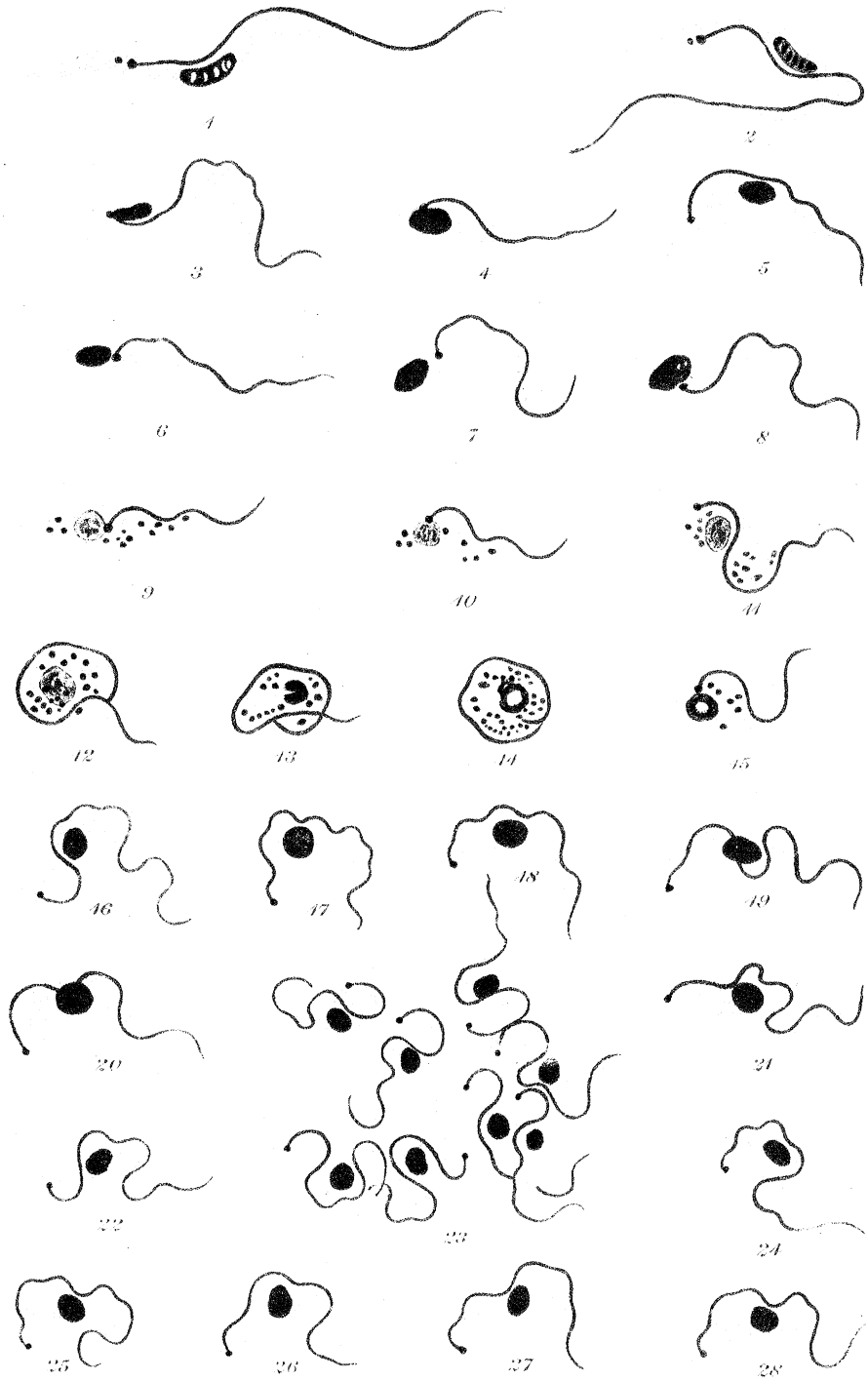
In this species of trypanosome also, as in *T. gambiense,* it is only a small percentage of the flies fed on an infected animal which become infected. In one series of *T. gambiense* this was 8 per cent.‡ In this species the experiments with laboratory-bred flies was 8·7 per cent., with wild flies 9 per cent. Just as in *T. gambiense,* the development takes place in the alimentary tract and salivary glands and not in the proboscis.

The Trypanosomes Found in the Salivary Glands.

In the trypanosome causing disease in man in Nyasaland, as in *T. gambiense,* the crux of the whole matter is the invasion of the salivary glands. After a certain number of days—in this species from 14 to 31—the trypanosomes reach the salivary glands and the fly becomes infective.

Plate 24, figs. 3–28, represent the various stages in the development of this trypanosome in the salivary glands. Figs. 1 and 2 are trypanosomes from the proventriculus; these represent the dominant intestinal type, from which the salivary-gland types arise. It is still a matter of speculation as to how they gain access to the glands, but as described in a former paper,§ there is no doubt they are often thrown forward into the proboscis during or just in the act of feeding, and may, under these conditions, be drawn into the hypopharynx and so reach their destination. These proventricular forms, however, have never been actually seen by the Commission in the hypopharynx. Figs. 3–11 are forms found in the salivary glands. Many of these are crithidial in type and occur in numbers. Figs. 12–14 are what appear to be encysted forms. Figs. 16–21 are "blood forms" and occurred in large numbers in the same preparation as the crithidial type shown in figs. 3–8. Figs. 22–28 are "blood forms" which were thrown out on to a cover-glass by a living infective fly. The preparation was beautifully clear, each individual trypanosome standing out distinctly. Fig. 23 is from the same preparation

* *Ibid.*, B, vol. 83, p. 515 (1911).
† Muriel Robertson, M.A., 'Phil. Trans.,' B, vol. 203 (1913).
‡ 'Roy. Soc. Proc.,' B, vol. 83, p. 514 (1911).
§ *Ibid.*, B, vol. 87, p. 65 (1913).



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and has the appearance of a small bunch or clump of "blood forms" in the act of breaking apart.

Conclusions.

1. The trypanosome causing disease in man in Nyasaland belongs to the same group as *T. gambiense,* the development taking place in the alimentary tract and salivary glands, not in the proboscis, of the fly.

2. The percentage of flies which become *infected* is the same as in *T. gambiense,* 8 per cent.

3. The percentage of flies which become *infective* is about 1 per cent.

4. The length of time which elapses before a fly becomes infective varies from 14 to 31 days, average 23 days.

5. The infective type of trypanosome in the salivary glands—corresponding to the final stage of the cycle of development—is similar to the short and stumpy form found in the blood of the vertebrate host.

DESCRIPTION OF PLATE.

Figs. 1–2.—Trypanosomes from proventriculus. These represent the dominant intestinal type.

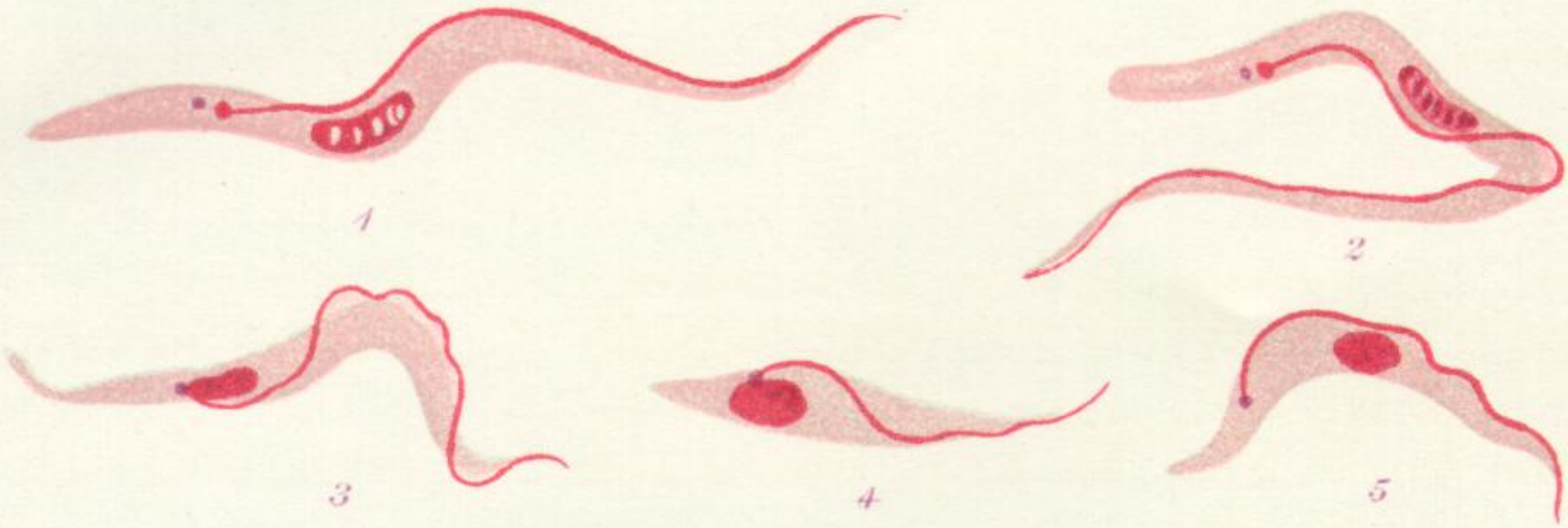
Figs. 3–8.—Trypanosomes taken from a preparation of the salivary gland of an infective fly. Many of these are crithidial in type, *e.g.*, figs. 6, 7, and 8.

Figs. 9–15.—Other forms seen in the salivary glands. Figs. 12–14 have the appearance of being encysted.

Figs. 16–21.—The fully developed "blood forms." Without these the fly is non-infective. These were drawn from the same preparation as figs. 3–8.

Figs. 22–28.—Trypanosomes ejected by a living infective *G. morsitans* on attempting to feed through a cover-glass. Fully developed "blood forms."

Stained Giemsa. × 2000.



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