Glossina palpalis *in its Relation to* Trypanosoma gambiense *and other Trypanosomes* (*Preliminary Report*).

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[Plates 12—14.]

Introductory.

In this paper we propose to give a brief statement of the results obtained by us with reference to the relation of the tsetse-fly (*Glossina palpalis*) to the trypanosome of Sleeping Sickness (*Trypanosoma gambiense*), and to other species of trypanosomes which this fly carries.

Our investigations have consisted of observations and experiments upon (A) Flies fed in the laboratory on animals which had been infected by the inoculation of cerebro-spinal fluid from sleeping sickness patients, and which showed trypanosomes in their blood as the result of such inoculation. (B) Flies caught in various localities which were found on dissection to contain trypanosomes in their digestive tracts.

Of the trypanosomes under the latter heading (B) we have found two distinct types. One of these types has been named by Professor F. G. Novy* *Trypanosoma grayi*, and one of us (Professor E. A. Minchin) proposes to call the other type *Trypanosoma tullochii*.

Type I. *T. tullochii*, n. sp. Minchin.—This type is distinguished by its more rounded nucleus placed near the middle of its body, by a small, usually circular, blepharoplast placed well behind† the nucleus, *i.e.*, at the end furthest from the flagellum (Plate 14, figs. 53—60).

Type II. *T. grayi*, Novy.—This form is characterised by its large nucleus, which may be oval, spherical, or compressed, and which is in all cases situated not far from the posterior end of the body. In many cases the nucleus shows distinctly eight chromosomes. The blepharoplast is large, transversely elongated, and situated close to the nucleus, either at its side

* 'Journal of Infectious Diseases,' vol. 3, No. 3 (May, 1906), pp. 394—411, Plates 15—17. Professor Novy gives some excellent microphotographs of these trypanosomes, taken from preparations sent him by Lieutenant Gray.

† In this memoir we use the terms anterior and posterior purely with reference to the direction of locomotion of the trypanosomes described by us, and without prejudice to the disputed morphological questions involved.

or more usually anterior to it. Sometimes, however, it may be posterior to the nucleus, a point which we discuss further below. The flagellum is often distinctly thickened at the tip. This type varies very greatly both in form and size. We distinguish (1) male forms, very slender, with long free flagellum, with nucleus very compressed, and with the blepharoplast situated in front of it (Plate 13, figs. 21 and 22). Some of the forms reach an extraordinary length (Plate 13, fig. 33). (2) Female forms which are bulky, often thickened at the posterior end and with an oval or rounded nucleus. The blepharoplast is variable in position, and the free flagellum is very short (Plate 13, figs. 23—25 and 34). (3) Young forms (Plate 13, figs. 31 and 32) and indifferent forms, varying greatly in character; among the latter we may particularly note forms which are nearly spherical (Plate 14, figs. 43—51). The very protean character of these forms (see Plate 13, figs. 35—40; Plate 14, figs. 41—52) makes it very uncertain as to whether they are really all of the same species. Since, however, we have noticed a marked difference between trypanosomes from flies which had fed after being caught and those in flies which had not fed (Plate 13, figs. 33 and 34, and Plate 14, fig. 41), we think that these variations of type are to be explained as the result of the conditions of nutrition of the host. The forms from flies which had not been fed were both scarcer and larger than those from flies which had recently sucked blood. In flies dissected soon after feeding it was found that small forms (Plate 13, figs. 31 and 32) largely predominated, and dividing forms were numerous (Plate 13, figs. 27, 28, and 29); on the other hand, in those cases in which flies were found to contain forms of a more indifferent character (Plate 13, figs. 36—40), it was noticed that stages of division were extremely rare, and that aggregations of similar forms into large masses were frequent (Plate 14, fig. 42).

The mode of division in *T. grayi* is noteworthy and characteristic. The two sister individuals which result from it are markedly unequal in size and differ also in the relations of their nucleus and blepharoplast. The smaller of the two has the blepharoplast placed in what may be considered the normal position, that is to say, well in front of the nucleus. On the other hand, the larger individual has the blepharoplast placed behind the nucleus (Plate 13, fig. 28). We consider, therefore, that the forms not infrequently found, in which the blepharoplast is situated behind the nucleus, represent, in many cases at least, the larger of two sister individuals resulting from recent division. Multiplication by division has only been observed by us in individuals of indifferent or female type, never in fully differentiated male forms. Finally, we may draw attention to the numerous chromidia always present in young, indifferent, or female forms.

In their staining reactions the chromidia seem to resemble the blepharoplast more than the nucleus.

(A) *Observations and Experiments with Flies Artificially Infected with* T. gambiense.—We undertook very numerous experiments to determine the exact mode of infection by the fly, particularly with the object of determining whether the fly became infectious at any definite period after having been fed on an infected animal. For instance, a batch of freshly caught flies was fed first on an infected animal, and then fed on successive days on a series of healthy animals, using a fresh animal for each feed, the experiments covering a period of 22 days from the time of the original infection of the flies. All such experiments, however, gave entirely negative results. On the other hand, we obtained positive proof that *G. palpalis* can convey trypanosomes by means of its proboscis from an infected to a healthy animal, if it goes straight from the one to the other. Our method of experimenting was as follows: A single fly was placed in a test-tube and the mouth of the tube covered with gauze. The mouth of the tube was then pressed on to the infected animal and the fly carefully watched. When the fly had about half fed it was removed from the infected animal and placed on a healthy one, on which it was allowed to finish its meal. Infection by trypanosomes was effected by this means in four out of five experiments when *G. palpalis* was used as the transmitting agent, and once out of four experiments when a *Stomoxys* was used in a similar manner. In order to determine further whether in these cases the infection was brought about by contamination with the fly's proboscis only or by the possible regurgitation of already ingested trypanosomes from the digestive tract, a further series of experiments was carried out, in which the fly, after having partially fed on an infected animal, was then allowed to feed on two healthy animals in succession. Five such experiments were carried out, in each of which it was observed that the fly (*Glossina*) had sucked blood from both the infected and the two healthy animals. In every case the *first* of the two healthy animals, and only the first, was infected even when the fly had only been allowed to dip its proboscis for a moment into the first healthy animal and was then immediately transferred to the second healthy animal. This shows, in our opinion, that the infection is conveyed by contamination of the proboscis, and that if the fly be allowed to clean its proboscis by piercing the skin of one animal, it is no longer infectious to a second. In these experiments upon direct transmission the "Jinja" cattle-trypanosome was used by us, because it is abundant in the blood of infected animals (rats) with which we were working, and also on account of the fact that the infection or non-infection of a rat with this trypanosome is a matter of

certainty within a very few days, whereas had we used *T. gambiense* the results of our experiments would have remained uncertain for a very long while.

It has also been proved by the experiments of Bruce and by ourselves that freshly caught specimens of *G. palpalis*, at Entebbe, are capable of infecting animals with the trypanosome of Sleeping Sickness, but in this case all experiments seem to show that the number of fly-bites required to produce infection is a very variable one indeed, since over and over again more than 1000 flies have fed on a susceptible animal without infecting it. The smallest batch with which we ourselves have been successful in producing infection consisted of 134 flies.

Observations on the fate of trypanosomes introduced into the digestive tract of the tsetse-fly by feeding it in the laboratory upon animals infected with *T. gambiense* gave the following results:—The trypanosomes, never very numerous in the ingested blood, show at the end of 24 hours a slight increase in number, and many of the parasites are observed in stages of division. At the same time they have become differentiated into two very distinct forms. The first is a very slender type with cytoplasm free from granules, with the nucleus sometimes rounded but more usually compressed, and with a considerable length of free flagellum (Plate 12, figs. 1 to 6). Many of these slender forms are observed at this stage to be in the act of extruding granules of chromatin from the nucleus (Plate 12, figs. 4 to 6). The second form of parasite is relatively very large and bulky with granular and deeply-staining cytoplasm, with very large spherical nucleus, with short free flagellum, and with the blepharoplast often some distance from the posterior end (Plate 12, figs. 7 to 14). These two forms may be regarded, on the analogy of developmental facts recorded of other trypanosomes, as male and female respectively. In both forms stages of division were observed, but in no case have we succeeded as yet in observing with certainty any process of conjugation. The two forms are easily distinguished in the living condition, the slender males being also characterised by much greater activity than the bulky females.

Male and female forms could also be recognised in the blood of the experimental animals (monkeys), especially in films fixed with osmic vapour. In films dried in the ordinary way the characteristic differences were much less distinct. In either case the differentiation of sexual characters is far less marked than it becomes in the intestine of the fly. Trypanosomes of male character (Plate 12, fig. 16) are common in blood-films, but those of female character (Plate 12, fig. 17) are very scarce, and only two were found, both of which were remarkable for having the nucleus composed of

four distinct masses of chromatin. On the other hand, an abundant form in the blood films is an indifferent type (Plate 12, figs. 18 and 19), characterised usually by very short free flagellum, and it is this form which develops into the female form in the fly. In this connection attention should be drawn to the forms, distinctly of the female type, obtained by two of us (Gray and Tulloch) in a culture (Plate 12, fig. 20, see Appendix II).

It may be pointed out that the sexual forms of *T. gambiense* from the tsetse-fly are very similar to the forms of *T. brucii* described by Koch* from other species of *Glossina*, so far as can be judged from Koch's figures. It is our opinion, however, that many of the forms described by Koch as developmental stages of *T. brucii* are really stages of one or more distinct species of trypanosomes carried by the flies, comparable to, and perhaps identical with, *T. grayi* and *T. tullochii* in *G. palpalis*.

At 48 hours after feeding the trypanosomes are still numerous in the intestine of the fly, and a type of more indifferent character begins to make its appearance (Plate 12, fig. 15). At 72 hours the trypanosomes are usually beginning to become more scanty and difficult to find in the digestive tract of the fly, although in some cases they are still numerous and chiefly of the indifferent type. At 96 hours, in almost every case, not a single trypanosome could be found even after the most careful searching. In one case a single trypanosome was found, and in another case two, on the fourth day, but in all other cases the trypanosomes seemed to have vanished completely at this period, and could never be found at any subsequent time. It would appear as if they died out with the absorption of the blood with which they were ingested, and were unable to pass forward in the digestive tract into the blood taken up by the fly at any subsequent feeding. In this they contrast sharply with the trypanosomes described above, occurring in the fly under natural conditions.

The disappearance of *T. gambiense* from the digestive tract of the tsetse-fly could be interpreted in one of three ways: (1) the trypanosomes may actually die out and be digested; (2) the trypanosomes may pass from the digestive tract into other organs of the fly; (3) the trypanosomes may become, by rapid division, so minute as to escape detection, like the forms of *Spirochæta ziemanni* described by Schaudinn, or like the invisible micro-organism of yellow fever. In order to test the second of these two possibilities, the internal organs of a number of artificially infected flies were carefully examined, but always with negative results, while the experimental results of Bruce and ourselves seem to disprove infectivity of the fly at any period after 48 hours, and, therefore, render improbable the third possibility

* 'Deutsch. Med. Wochenschr.,' 1905, No. 47.

suggested above. So far then as it is possible to draw conclusions from our observations, it would appear that *T. gambiense* does actually die out in the tsetse-fly after the third day. In all cases *T. gambiense* was found only in the mid-gut of the fly, and appeared never to pass either backwards into the proctodæum or forwards into the proventriculus, another point in which they contrast with the "fresh fly trypanosomes."

(B) *Observations and Experiments upon Freshly-caught Tsetse-flies Found to Contain Trypanosomes.*—When freshly-caught tsetse-flies were examined by us in the laboratory, either after having been fed upon a healthy animal or not, a certain percentage of them were found to contain trypanosomes of one or rarely of both types referred to above as *T. grayi* and *T. tullochii*. In such cases the trypanosomes were usually present in enormous numbers, especially if the fly had been previously fed. These trypanosomes, when compared with *T. gambiense* artificially introduced into a fly's intestine, are distinguishable by their appearance and movements. They are far more active than the sluggish *gambiense*, especially the male forms, which often shoot across the field of the microscope with the greatest rapidity. When moving in this way the body of the parasite remains nearly stiff, while the forwardly directed flagellum vibrates with rapid serpentine movements. In a few cases they were found in masses in the proctodæum, but in most cases they occurred in the intestine, swarming and multiplying in the freshly ingested blood. Occasionally they were found passing along the thoracic intestine into the proventriculus. The parasites found in the proventriculus did not differ appreciably either in size or appearance from those found in the digestive tract. By the method suggested by Koch, of compressing the bulb of the proboscis, we succeeded in forcing trypanosomes out from the proboscis, but only in those flies in which the parasites were found in the proventriculus. Of the two types described above, *T. grayi* was the most commonly found, being present in 1·47 per cent. of a total of 3000 flies examined, while *T. tullochii* was found in 0·17 per cent. of flies, and both trypanosomes together in the same fly only three times. When trypanosomes were found in the fly's proventriculus it was more usually *T. tullochii* which was present, while when trypanosomes were found only in the fly's intestine it was more usually *T. grayi* that occurred, but no conclusions can be drawn from this until more flies have been examined.

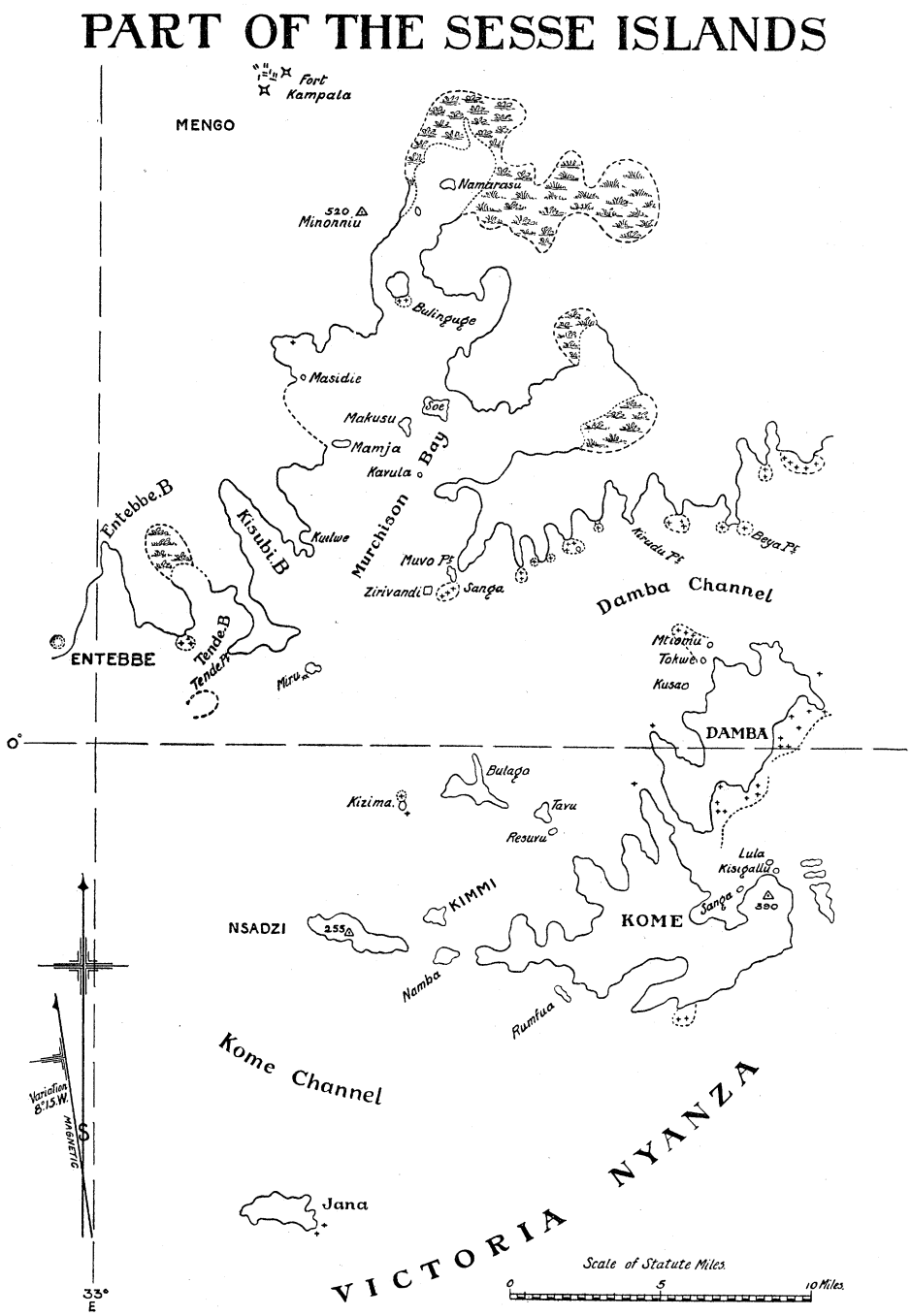
The object of our experiments on these "fresh fly trypanosomes" was to determine whether one or both of the two types found were or were not developmental stages of *T. gambiense*. As it is now beyond all doubt that *G. palpalis* is the agent which conveys the trypanosome of Sleeping Sickness from an infected to a healthy individual, it would seem most probable at

first sight that any trypanosomes found in the bodies of these tsetse-flies caught in a Sleeping Sickness area would be developmental stages of *T. gambiense*. We felt no doubt at the outset of our investigations that these fresh fly trypanosomes were to be identified with *T. gambiense*. Koch* evidently worked on the same assumption, since in his comparison of the supposed developmental stages of *T. brucii* and *T. gambiense* there can be no doubt that he has taken the form which has been called *T. grayi* for a developmental phase of *T. gambiense*. As we proceeded, however, with our investigations we were gradually led to doubt any connection between these "fresh fly trypanosomes" and Sleeping Sickness. In order to determine this point we carried out a number of experiments on flies caught on the island of Kimmi. This island was chosen because it swarmed with these tsetse-flies, of which a high percentage contained trypanosomes, and because it was, and has been for a very long while, quite uninhabited.

Kimmi is a small island of the Sesse group, about two miles long by a mile wide (see map). There is a narrow strip of sandy shore all round it, the remainder of the island being covered with thick undergrowth and forest. On the foreshore are many ambatch trees, where cormorants, other diving birds, and weaver birds are very plentiful. This island is a regular feeding ground for hippopotami and is crossed in all directions by their tracks. Crocodiles are also very numerous. Kimmi is situated about 15 miles from Entebbe and is two miles from Nsadzi Island in the one direction and from Kome Island in the other. For more than a year this island has been quite uninhabited and natives now never visit it. The whole island swarms with tsetse-fly (*G. palpalis*). In spite of the total absence of human beings on Kimmi Island, we found that more than 7 per cent. of the tsetse-flies caught there contained trypanosomes of one or other of the two types mentioned, while only 1·7 per cent. of the flies caught on the main land near Entebbe, a place with a numerous population, among whom Sleeping Sickness is common, contained similar parasites.

Our method of experimenting with these flies was as follows:—Our camp with our apparatus and experimental animals was placed on the neighbouring healthy island of Nsadzi, in a region free from fly and where there is no Sleeping Sickness. A steam-launch was placed at our disposal by the authorities and by means of it batches of flies were brought back from Kimmi, so that we were not obliged to take possibly infected native canoemen, a class among whom Sleeping Sickness is very common, to this island. These Kimmi flies were divided into batches and each batch assigned to a particular animal (monkey, rat, guinea-pig, or hen) on which the batch

* 'S.-B. k. pr. Akad. Wiss. Berlin, 1905, pp. 958—962.


PART OF THE SESSE ISLANDS
Fort Kampala
MENGO
Namarasu
520
Minonniu
Bulingugé
Masidie
Soe
Makusu
Mamja
Karula
Bay
Entebbe B.
Kisubi B.
Kulwe
Murchison
Muvo Pt.
Zirivandi
Sanga
Damba Channel
Aruudu Pt.
Beya Pt.
Mtiomu
Tokwe
Kusao
DAMBA
ENTEBBE
Tende B.
Tende Pt.
Miru
0°
Bulago
Kizima
Tavu
Resuru
Lula
Kisigallu
390
KIMMI
NSADZI
255
KOME
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Namba
Rumfua
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Variation 8°15 W.
MAGNETIC
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Scale of Statute Miles
0
5
10 Miles

was fed at once, and again repeatedly on successive days. After 12 days or a fortnight of such daily feeding, the flies of each batch were dissected and examined for the trypanosomes which they might contain. In practically every case one or more flies of each batch were found to contain trypanosomes, so that every experimental animal was definitely known to have been fed upon repeatedly by at least one fly containing trypanosomes. Had these trypanosomes therefore been identical with *T. gambiense*, it might have been expected that at least some of these susceptible animals (such as monkeys, guinea-pigs, and rats) would have become infected, *but this did not occur in a single instance.* We thought that *T. grayi* might possibly be a bird-trypanosome, but the negative results of feeding flies containing it on fowls did not bear out this supposition.

In addition to these feeding experiments, we inoculated other experimental animals of the same kinds with the contents of the various parts of the digestive tracts of flies containing these trypanosomes, some from the proventriculus, some from the intestine and some from the proctodæum, *but again in every case the results were negative.*

We are, therefore, now convinced from the results of these numerous experiments, of which a list is given on p. 253, *that the trypanosomes found in the freshly caught tsetse-flies, and referred to by us as* T. grayi *and* T. tullochii, *have nothing to do with Sleeping Sickness and are not developmental stages of* T. gambiense.

It is a matter of regret to us that we have not been able to establish on what vertebrate host, if any, these trypanosomes are parasitic. It seemed at least probable that *T. grayi*, some forms of which greatly resemble *T. johnstoni*, Dutton and Todd* from *Estrelda estrelda*, was taken up by the fly from some of the numerous water birds that haunt the lake-shore. On the other hand, *T. tullochii*, which is very similar in its morphological characters to *T. gambiense*, might similarly be derived from a mammalian host. We may draw attention in this connection to the remarkable manner in which this tsetse-fly haunts the lake-shore. There is nothing in the breeding habits of the fly which should oblige it to frequent the vicinity of water, as in the case of the mosquito. Our experience of flies kept in the laboratory convinced us that a certain amount of moisture is necessary for them, since they died much faster in their cages if not kept over water. It may be supposed, however, that one attraction that the lake-shore exerts upon this voracious blood-sucker is that of food-supply. Along the shores of the lake and on all the small islands are vast numbers of cormorants and other fish-eating birds perched with their wings extended,

* Liverpool School of Tropical Medicine, Mem. XI, Pl. 2, fig. 1.

drying themselves in the sun on the trees, and especially on the ambatch trees, where the flies are found in swarms. These birds might furnish one constant and important source of food. We found in the laboratory that tsetse-flies fed very rapidly on captive fowls, creeping under their wings to bite the poorly protected parts of the skin. On the other hand, when a heap of recently shot water-birds, some of which were hardly dead, were lying on the lake-shore at Kimmi Island, the swarms of tsetse-flies did not attempt to settle on them, although freely biting us and our servants. A second possible source of food supply is furnished by the aquatic animals of the lake-shore, such as the hippopotamus, the otter, the crocodile and the python. We have definite evidence that the fly feeds on the hippopotamus and on the crocodile. Flies were caught in the act of biting a hippopotamus just recently shot, settling chiefly on the ears and nose. We, therefore, made blood-films and had blood-films sent us of as many aquatic birds and animals as possible, including five or six hippopotami. Only in a single case did we find a trypanosome, namely, in a not very well preserved film of crocodile's blood; beyond its large size and general resemblance to other reptilian

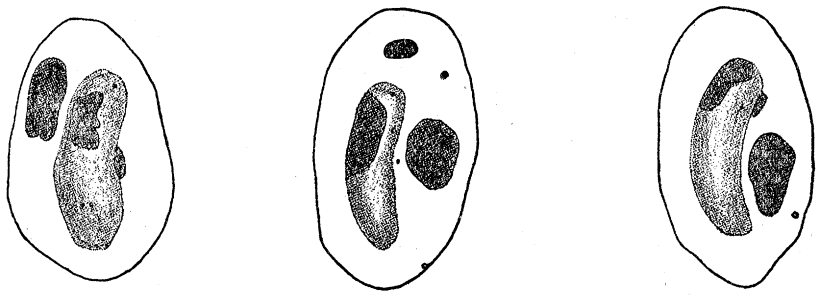


Fig. 1.—Hæmogregarine in the red blood-corpuscles of the crocodile. × 2000.

trypanosomes, it was not possible to make out any details of structure in this parasite. We may mention, however, that the blood of many of the birds contained Halteridia, and that a Hæmogregarine was quite common in the blood of the crocodiles (fig. 1). We also observed that flies in captivity sucked the blood of lizards, chameleons and snakes very freely.

There are, therefore, two possible sources for the trypanosomes in the freshly caught tsetse-flies. Either they are taken up from some of the numerous animals upon which the fly feeds, or they may be parasites of the fly itself, like *Herpetomonas muscæ-domesticæ* in the house-fly. In this respect it is interesting to note that a small percentage of another common blood-sucking fly in Uganda (*Stomoxys* sp.) contain a species of *Herpetomonas* very similar to that of the common house-fly in Europe. With regard to

G. palpalis we were never able to obtain any definite proof that it fed on anything but blood. It is therefore difficult to understand how a parasite of the tsetse-fly itself could be conveyed from one fly to another except by the hereditary method. We have a single instance to record which certainly suggests hereditary transmission of these trypanosomes. A tsetse-fly was bred in the laboratory in August and was fed for two months on fowls, which were unfortunately also used for feeding our stock of tsetse-flies in our breeding cages. On October 9, the fly was fed on a monkey showing very scanty trypanosomes (*T. gambiense*) in its blood. The next day, 21 hours later, this fly was dissected and found to contain a few scanty *T. gambiense*, one of which is figured on Plate 12, fig. 14, and vast swarms of *T. grayi* (Plate 13, figs. 23 and 28). It is obvious, therefore, that this fly was either infected with *T. grayi* when it emerged from its pupa or that it became infected from one of the fowls which had possibly been infected in its turn by the fresh flies which fed on it. It may be mentioned in this connection that experiments directed towards obtaining flies infected with *T. gambiense* by the hereditary method, that is to say, by breeding from flies fed continually on infected animals, gave no result.

In conclusion, one remarkable experiment of ours may be mentioned. At our camp on Nsadzi, referred to above, we fed a large number of freshly caught Kimmi flies on a goat which we obtained from natives on the island. We then dissected these flies and, to our astonishment, could not find trypanosomes in a single one of some 500 flies which had so fed, whereas in other Kimmi flies, caught at the same time, which had fed on our other experimental animals (monkeys, etc.), trypanosomes were present in the usual proportion. We then prepared some goat's serum and added a drop of it to the contents of a fly's intestine teased out on a slide, which contained *T. grayi* in large numbers. Another drop of this same goat's serum was added to a preparation of *T. gambiense* obtained from an infected rat and the two preparations watched. It was found that in the preparation of *T. grayi* the trypanosomes rapidly became immobile and died off, while the *T. gambiense* remained active. We then tried the same two experiments over again, using human serum instead of the goat's serum, and then found that the trypanosomes were not affected in either case. This result seems to us to furnish an additional means of distinguishing between *T. gambiense* and *T. grayi*.

Appendix I.

Table I.—List of Animals on which Tsetse-flies known to contain Trypanosomes of the two types mentioned have fed. All these animals remained uninfected by this feeding.

Animal.	Number of flies which had fed found to contain trypanosomes.	Class of trypanosome present in fly.	Presence or absence of trypanosomes from fly's proventriculus.
Monkey No. 370	2	*T. grayi.*	
„ No. 391	3	„	
„ No. 369	2	„	
„ No. 397	1	„	Present.
„ No. 335	3	*T. grayi* in two flies. *T. tullochii* in one fly.	Present in one of the former.
„ No. 474	2	*T. grayi* in one and *T. tullochii* in the other.	Present in both.
„ No. 499	4	*T. grayi.*	Absent in all.
„ No. 525	5	„	„
„ No. 553	1	„	Absent.
„ No. 554	4	„	Present in one fly.
„ No. 473	2	*T. grayi* in one and *T. tullochii* in the other.	Present in both.
„ No. 498	4	*T. grayi.*	Absent in all.
„ No. 555	1	„	Absent.
„ No. 556	3	„	Present in two.
„ No. 557	1	*T. grayi* and *T. tullochii* together.	Present.
Guinea-pig, F. F.....	8	*T. grayi* in 7. *T. tullochii* in 1.	Present in two.
„ No. 528 ...	5	*T. grayi.*	Absent.
Rat (white), No. 533 ...	1	*T. tullochii.*	Present.
Hen No. 505.....	4	*T. grayi.*	Present in two.
Hen No. 506	6	*T. grayi* in 5. *T. tullochi* in 1.	Present in three.

Appendix II.—*An Experiment on the Cultivation of* T. gambiense.

By Lieutenant A. C. H. Gray, R.A.M.C., and the late Lieutenant F. M. G. Tulloch, R.A.M.C. (Sleeping Sickness Commission).

Our numerous failures in this direction have been attended by one partial success.

The following method was employed. A tube of agar, prepared according to the formula of McNeal and Novy, was melted and cooled to 60° C. Three times its volume of blood, taken directly from the heart of a dog without defibrination, was added to the agar. The water of condensation was inoculated with a drop of blood from a white rat (No. 513) very rich in trypanosomes. On examining the tube six days later a few living trypanosomes were found, which appeared similar to the forms inoculated. On the

8th and 10th days no trypanosomes were seen in a loopful of fluid withdrawn from the tube. On the 15th day several active trypanosomes were seen in a sample. These trypanosomes were found singly and in groups of three or four. Dividing forms were also seen. These forms were distinctly larger than the trypanosomes originally inoculated, and on measurement were found in some cases to be as long as 54 μ. Besides being longer and broader than the trypanosomes in the blood of the rat, the position of the micro-nucleus was different (Plate 12, fig. 20). In the trypanosomes from the test-tube the micro-nucleus was situated at a considerable distance from the hinder end of the parasite and consequently nearer to the macro-nucleus. These trypanosomes closely resembled certain forms which we have found in the stomach and intestinal tract of tsetse-flies, 24 hours after being allowed to feed on infected monkeys. On the 17th day trypanosomes were still present in about the same numbers, but a few cocci were also found in the tube. Up to the 20th day trypanosomes were still found, but were sluggish in their movements and became fewer in number as the cocci increased. After this date the growth of cocci became profuse and the trypanosomes died off. Up to the present (seven days) no trypanosomes have been found in sub-cultures made from this tube, although the latter are free from bacteria.

As multiplication had commenced in the original tube, it is reasonable to expect that a successful culture would soon have resulted if it had not become contaminated by cocci.

The resemblance of the newly formed trypanosomes to forms seen in tsetse-flies after feeding on infected animals is of interest.

Appendix III.—*Some Notes on a* Herpetomonas *found in the Alimentary Tract of* Stomoxys (calcitrans ?) *in Uganda.*

By A. C. H. Gray, M.B., Lt. R.A.M.C.

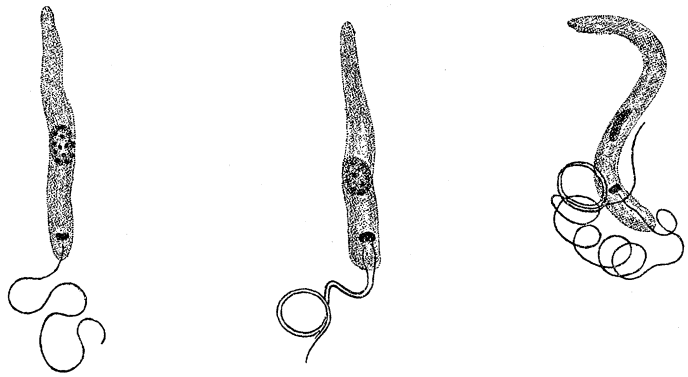
In the course of examining the contents of the alimentary tract of some specimens of *Stomoxys* (*calcitrans?*), which had previously been allowed to feed themselves on a monkey infected with the trypanosome of Sleeping Sickness, I found a species of *Herpetomonas* in the alimentary tract of three flies out of a total number of 280 examined.

In its movements, size, and general appearance, the flagellate seemed to closely resemble *H. muscæ-domesticæ* of the common house-fly.

In two flies this parasite was present in very large numbers. Those two flies were full of blood from the monkey they had fed on 24 hours previously, and in this blood practically unaltered *T. gambiense* were present in scanty

numbers. In the third fly this *Herpetomonas* was present in very scanty numbers and no trace of recently ingested blood could be found in it.

Films, fixed in osmic acid and stained with Borrel blue and eosin, showed that the commonest type of this parasite measures from 35 to 50 μ (figs. 2—4).

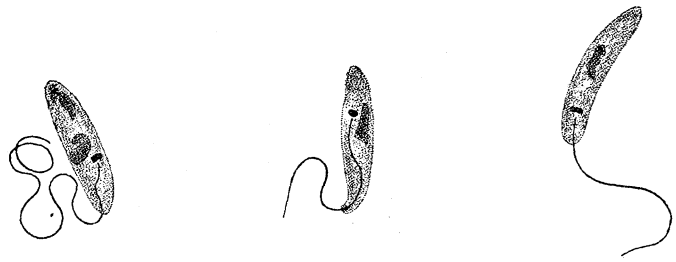


FIGS. 2—4.—*Herpetomonas* from the gut of *Stomoxys* (*calcitrans?*); fig. 2, common form with single flagellum, and with nucleus broken up into separate masses; fig. 3, commonest form, with double flagellum; fig. 4, form with compressed nucleus and very long flagellum.

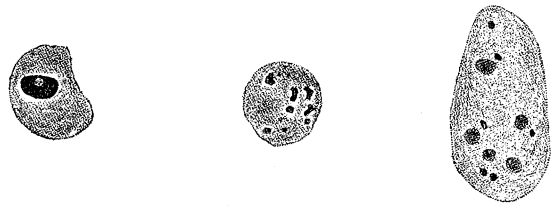
The body of the parasite is cylindrical, with a rounded anterior and more pointed posterior extremity. The protoplasm of the body stains rather deeply. A large rounded nucleus is placed at the centre of the body of the parasite. The chromatic substance of the nucleus is sometimes seen to be broken up into granules (chromosomes), apparently 14 in number, contrasting in this respect with *H. muscæ-domesticæ*, in the nucleus of which eight chromosomes are present (Prowazek). The blepharoplast is oval or kidney-shaped, of a large size and stains deeply. It is placed close to the origin of the flagella and to the anterior rounded extremity of the body of the parasite. The double flagellum arises close to the blepharoplast and may reach an enormous length in some individuals (fig. 4).

Besides these large forms, smaller individuals are present (figs. 5, 6, 7). The bodies of these parasites stain more faintly than the above and are often curved. The nucleus is more compressed. The blepharoplast is smaller and situated at a greater distance from the anterior extremity of the body. The single flagellum arises close to the blepharoplast and consequently has a somewhat longer course through the body of the parasite. It emerges as a short, thick, free flagellum.

Both these forms commonly undergo longitudinal division. In some cases the nucleus apparently divides before the blepharoplast.



FIGS. 5—7.—*Herpetomonas* from gut of *Stomoxys*; fig. 5, small form with dividing nucleus; fig. 6, small form showing the posterior position of the blepharoplast, and long intracellular course of the flagellum; fig. 7, small form, ordinary type.



FIGS. 8—10.—*Herpetomonas* from gut of *Stomoxys*, non-flagellated forms; fig. 8, mass of blue-staining protoplasm containing one large chromatin body; figs. 9 and 10, masses of protoplasm containing paired chromatin bodies.

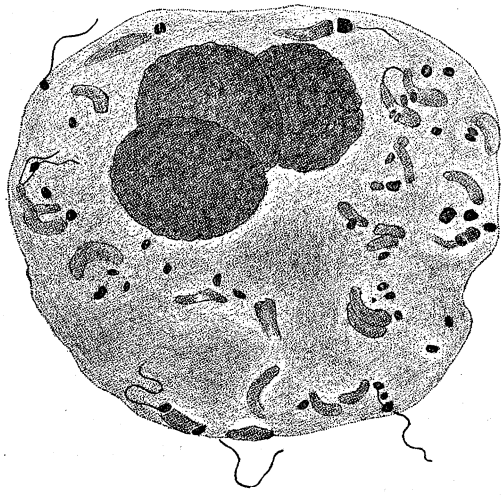


FIG. 11.—Large cell (probably a leucocyte) from contents of gut of *Stomoxys* (*calcitrans ?*), containing in its interior large numbers of disintegrated *Herpetomona* forms.

In the third fly non-flagellated forms were found to occur (figs. 8, 9). Masses of blue-staining protoplasm containing chromatin bodies in pairs (fig. 10) were also rarely found in this fly. Every such pair of chromatin bodies consisted of a larger and a smaller separate portion. The larger portion is circular and more faintly staining, the smaller is oval and more deeply staining. These paired masses of chromatin suggest a form analogous to Leishman-bodies.

On several occasions, in the first two flies, large cells (leucocytes ?) were found containing in their interior the broken-up remains of large numbers of the *Herpetomonas* (fig. 11).

The figures illustrating these notes are all × 2000 and drawn with the camera lucida from slides fixed in osmic acid and stained with Borrel blue and eosin.

DESCRIPTION OF PLATES.

Plate 12.—*Trypanosoma gambiense*, figs. 1—14, forms from the gut of the tsetse-fly (*Glossina palpalis*) one day after infection, (*i.e.* about 24 hours after being taken up by the fly. Fig. 15, two days (48 hours) in the fly. Figs. 16—19, forms from the blood of a monkey (*Cercopithecus* sp.) infected with the injection of cerebro-spinal fluid from a Sleeping Sickness patient. Fig. 20, culture form from blood of infected rat, 15th day. All except figs. 14 and 20 preserved wet with osmic vapour stain, Leishman or Geimsa. × 2000.

Figs. 1 and 2, male form with compressed nucleus. Fig. 3, male form with rounded nucleus. Figs. 4 and 5, similar forms with chromatin being given off from the nucleus. Fig. 6, male form dividing, chromatin being given off from both the daughter nuclei. Figs. 7—14, female forms, figs. 7 and 12 dividing. Fig. 15, indifferent form. Fig. 16, male form. Fig. 17, female form, very scarce (only one other was found). Figs. 18 and 19, indifferent forms which in the fly become female ; note the short, free flagellum. Fig. 20, female form from culture tube, 15th day.

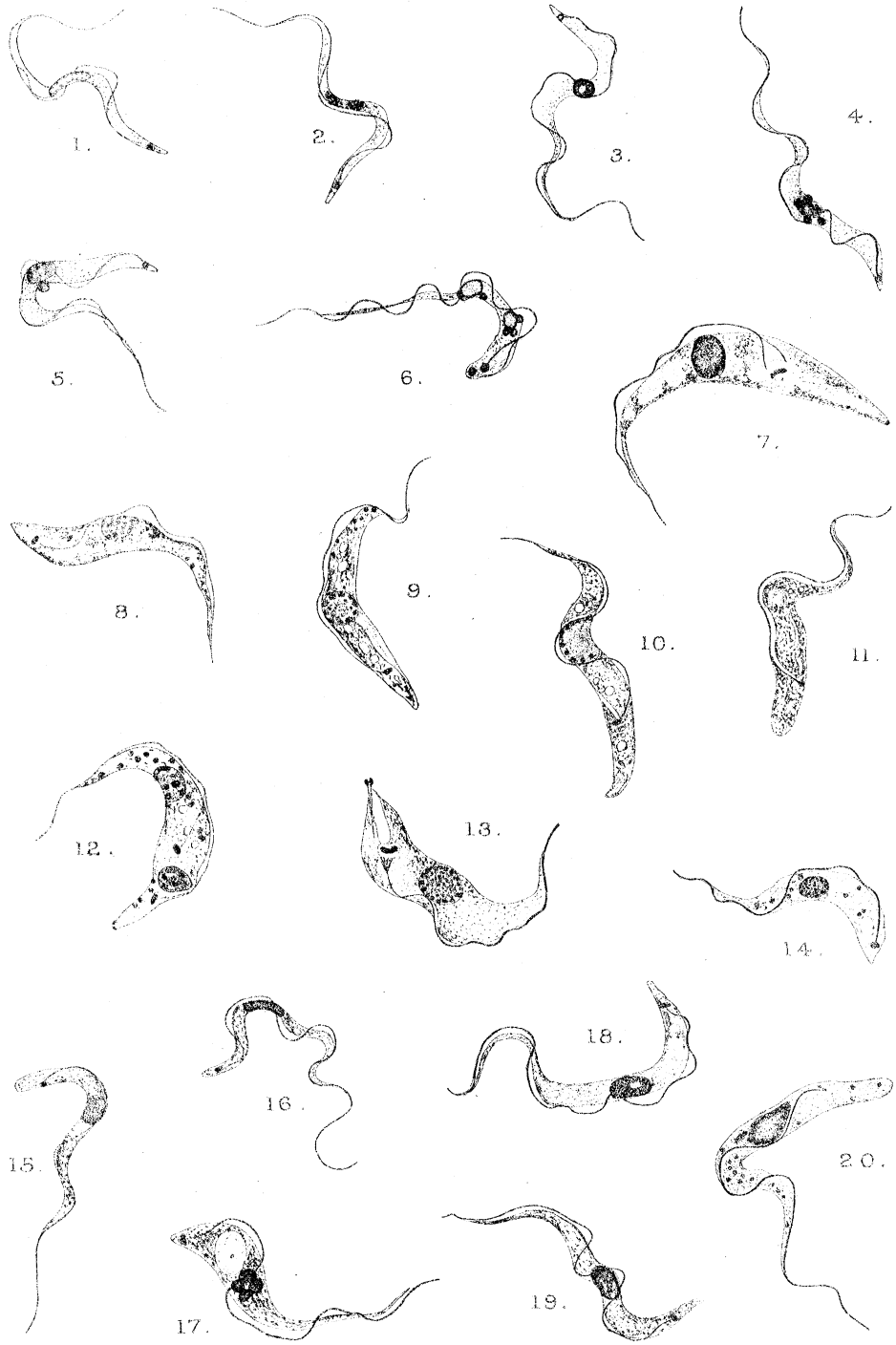
Plate 13.—*Trypanosoma grayi*, figs. 21—30, from the gut of the tsetse-fly (*Glossina palpalis*). The flies from which these trypanosomes were obtained had been fed regularly on the blood of neutral monkeys and were dissected after about 10 days of such feeding In all cases the whole gut of the fly swarmed with thousands of similar trypanosomes. Figs. 31 and 32, small forms ; flies dissected 24 hours after their first feed of blood generally contained forms such as these in very great abundance, many dividing forms were also present, larger forms, as above, were rare. Figs. 33—35, trypanosomes from fresh-caught tsetse flies, which had not fed on blood for a long while. In such flies, parasites were never very numerous and the types present were all of a large size. Figs. 36—40, various other types from flies which had fed on blood. Fixed in alcohol, stained with Leishman or Giemsa. × 2000.

Figs. 21 and 22, male forms with compressed nucleus and long free flagellum. Figs. 23, 24 and 25, female forms. Posterior extremity thickened, short free flagellum. Figs. 26, 27 and 28, stages of division showing the unequal size of the two resulting individuals. Fig. 29, division of a small form into two more or less equal-sized individuals. Fig. 30, aberrant dividing form, in this case the nucleus has already divided, whereas the blepharoplast has not yet done so. Figs. 31 and 32, young forms resulting from the unequal division of a large female form. Fig. 33, very long male form. Fig. 34,

very large female form. These two forms occur in flies which have not fed on blood for a long time. Fig. 35, very long form, with nucleus consisting of eight chromosomes. Figs. 36—40 are all from the same fly. Figs. 36, 37, and 38 show the nucleus broken up into separate chromatic granules, and the varying position of the blepharoplast. Fig. 39, young form showing regular division of the nucleus into eight chromosomes. Fig. 40, young form showing a separate mass of chromatin posterior to the nucleus.

Plate 14.—*Trypanosoma grayi*, figs. 41—52; *Trypanosoma tullochii*, figs. 53—60; all from the gut of the tsetse fly (*Glossina palpalis*). Fig. 41, very large female form, undergoing unequal division, from a fly which had not fed on blood. Fig. 42, rosette-like mass of similar young forms. Figs. 43—51 are all taken from the same fly, and show the various steps in the formation of circular non-flagellated forms from a common type, fig. 43. Figs. 44 and 45 show the flagellum becoming wrapped round the body of the parasite. Figs. 46, 47, and 48 show progressive stages in the absorption of the flagellum. Figs. 49, 50, and 51 are different types of the resulting non-flagellated bodies. Fig. 52, an uncommon type of parasite, with very large blepharoplast, and showing much chromatic granulation, from a fly which had not fed on blood.

Figs. 53—60, *Trypanosoma tullochii*. Fig. 53, small form, with minute circular blepharoplast and small vacuole. Fig. 54, larger form of a similar type. Fig. 55, small form showing the flagellum arising from a small granule of chromatin close to the blepharoplast. Fig. 56, large form. Figs. 57 and 58, large forms of trypanosome found in the proventriculus of an infected fly. Fig. 59, dividing form, common type. Fig. 60, dividing form, rare type showing division into three. Figs. 53—60 are all from the same fly. Figs. 57 and 58 are from the proventriculus, while the remainder are from the gut of the fly (*Glossina palpalis*).



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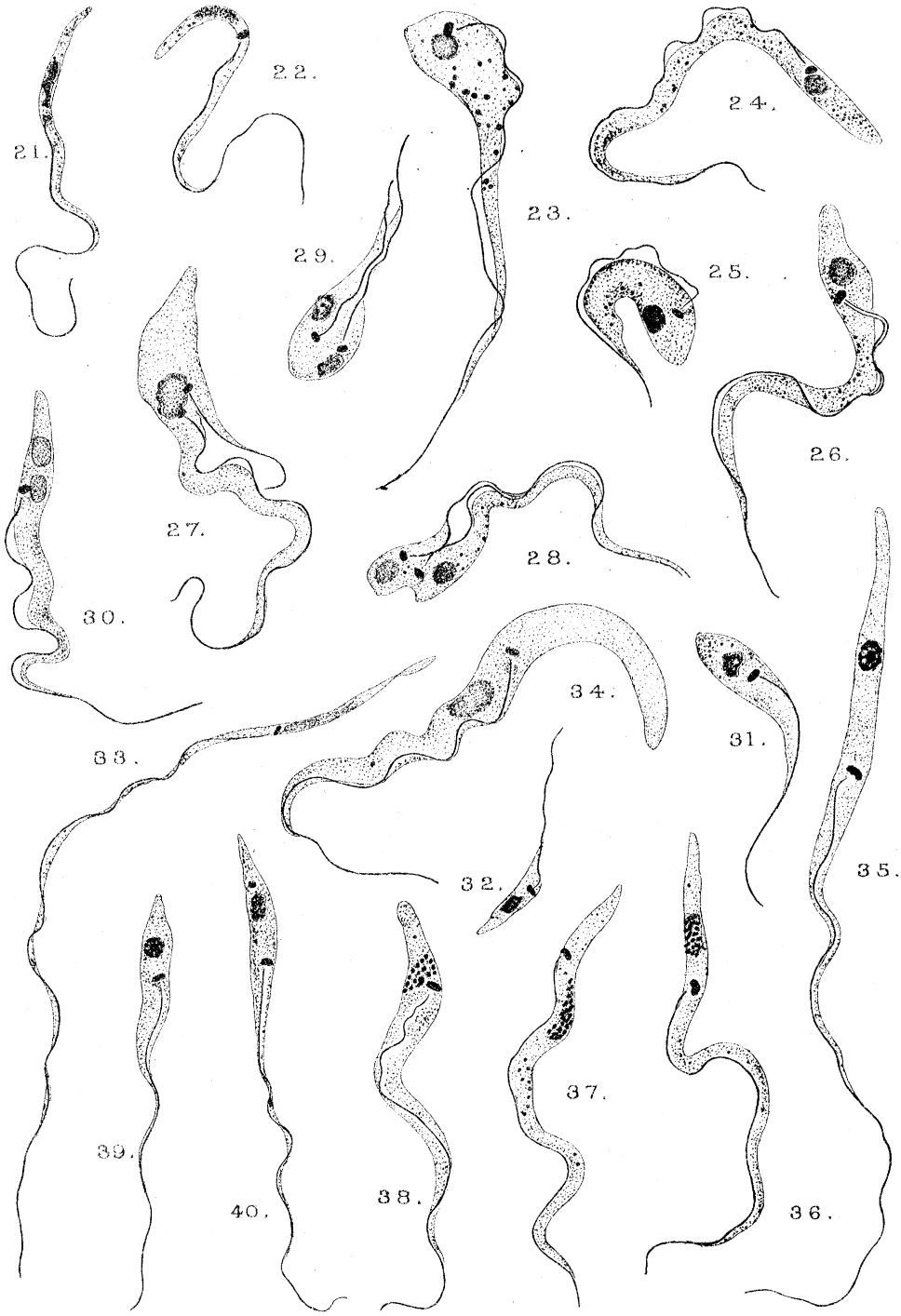

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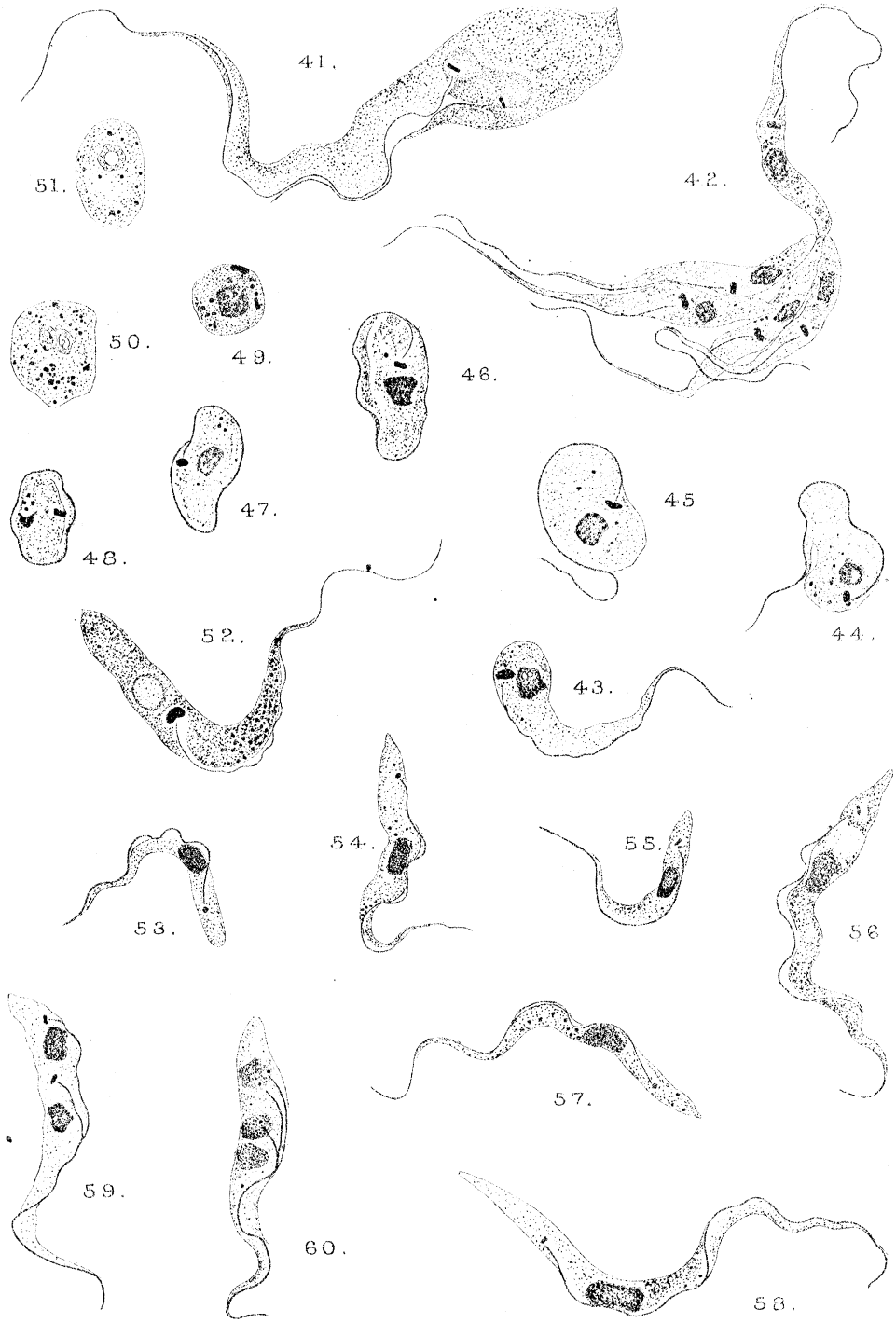

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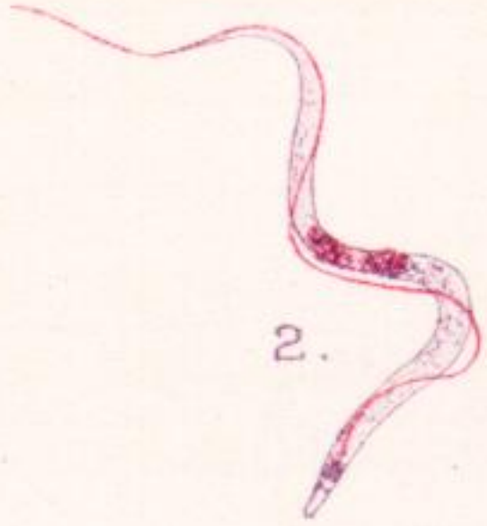

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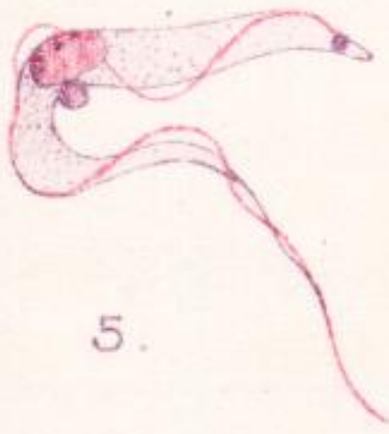

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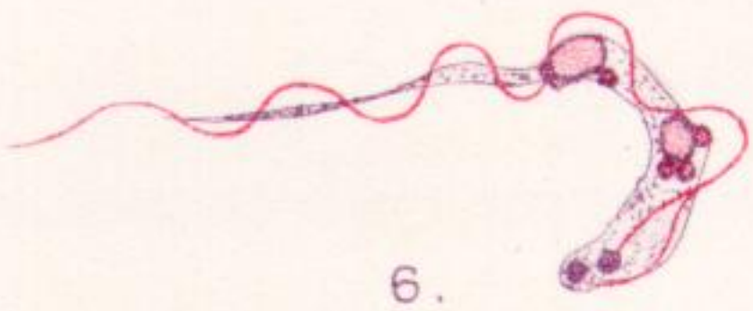

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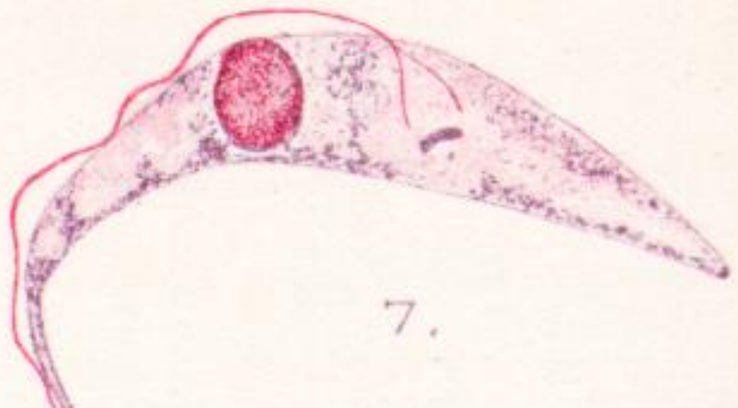

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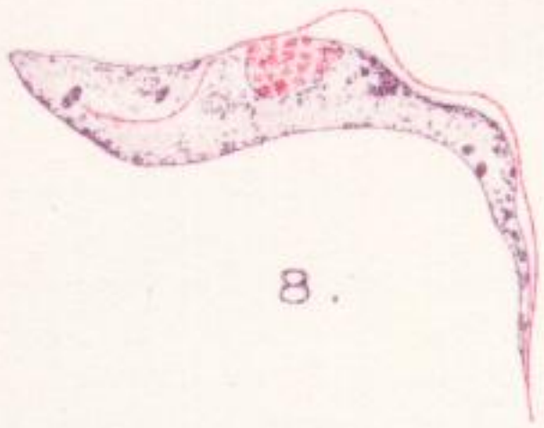

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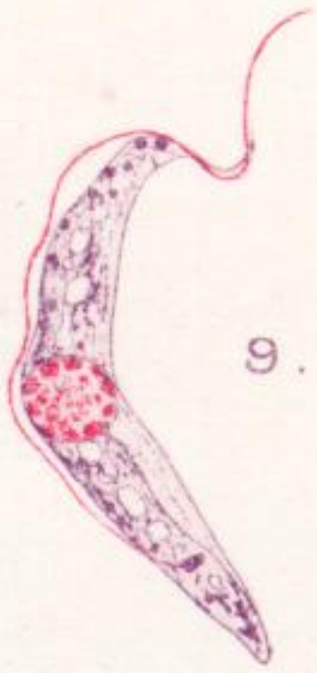

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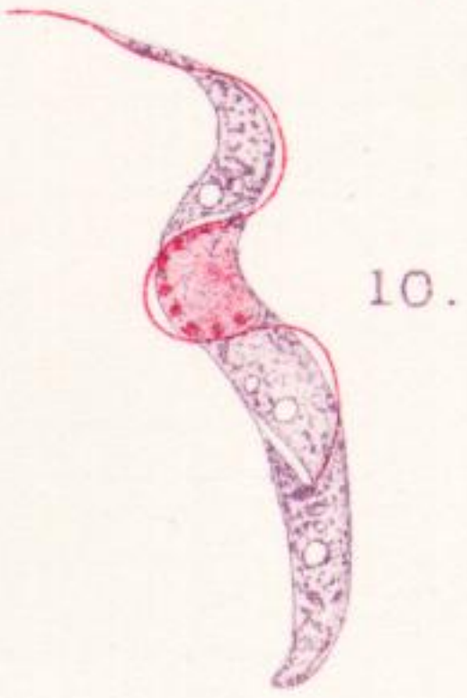

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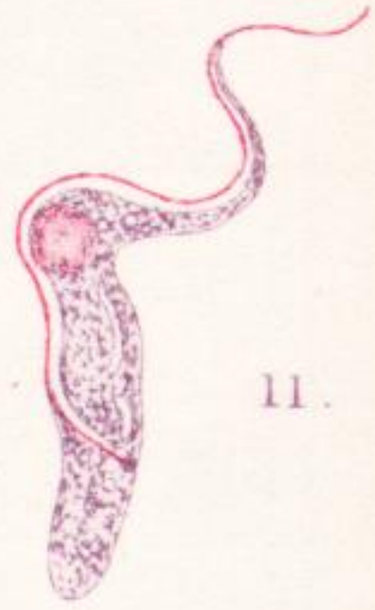

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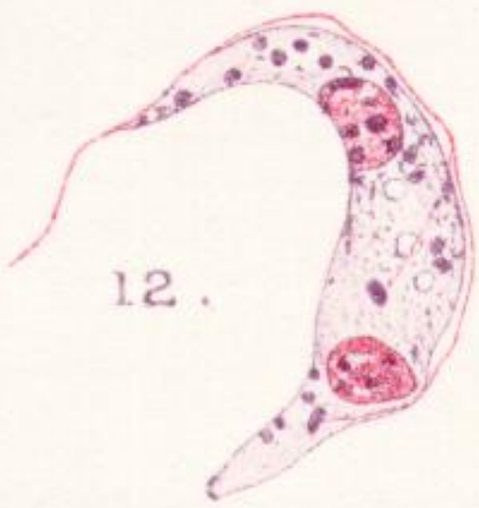

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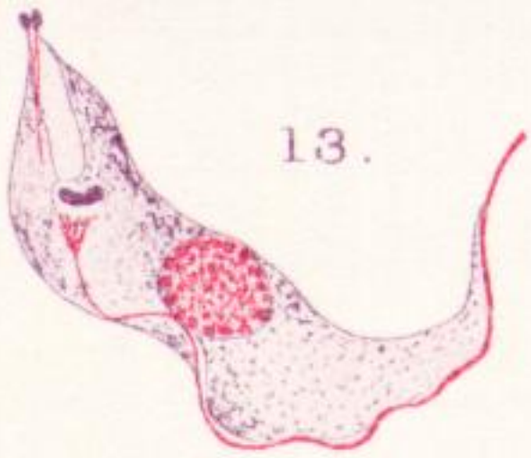

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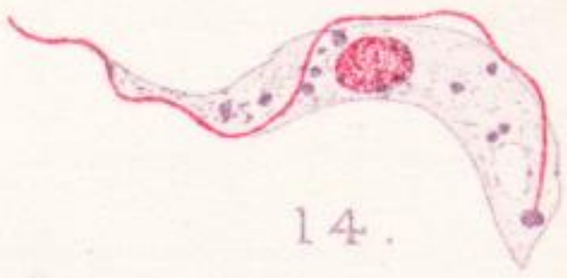

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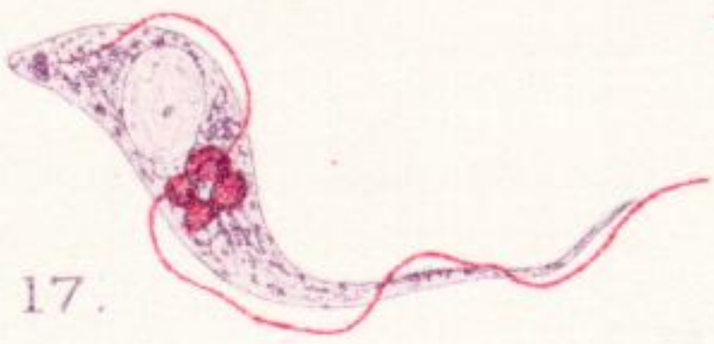

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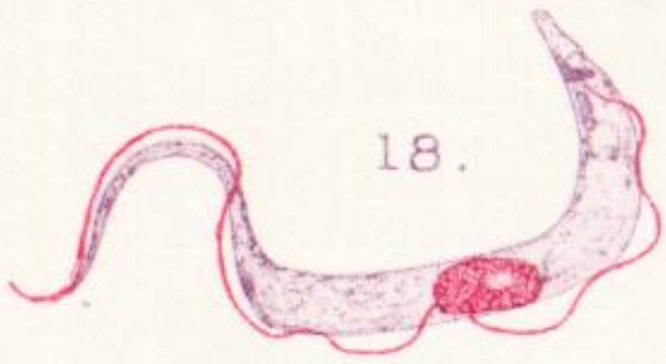

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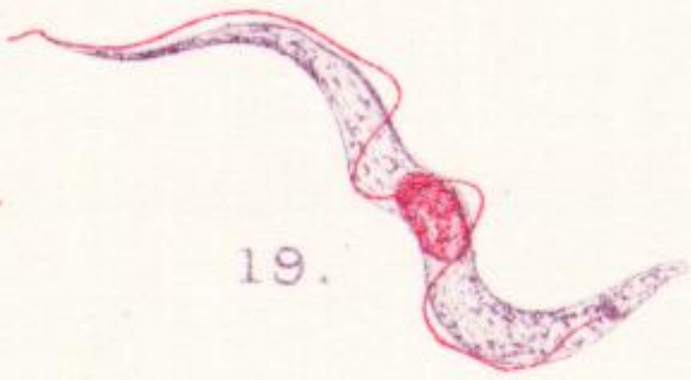

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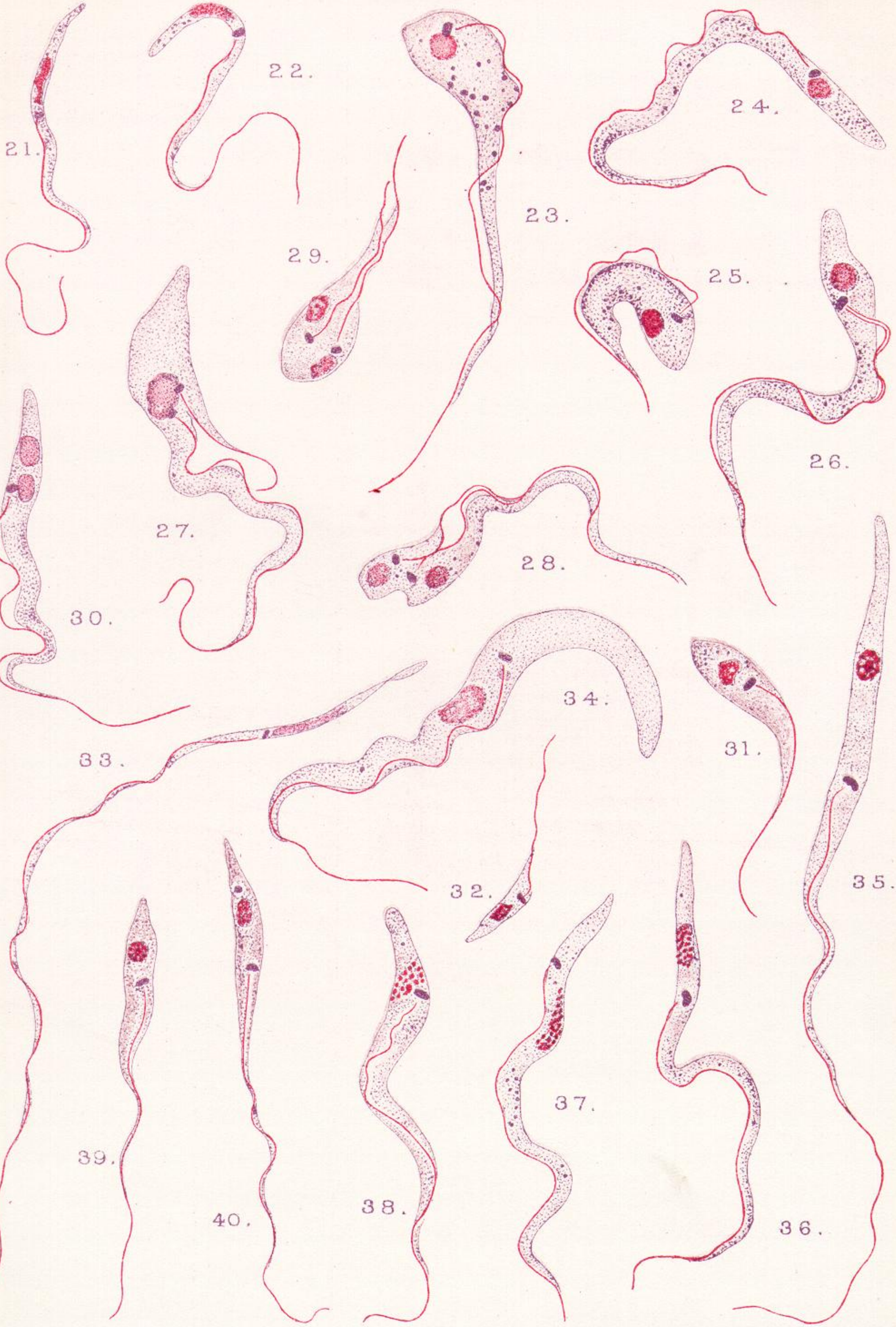

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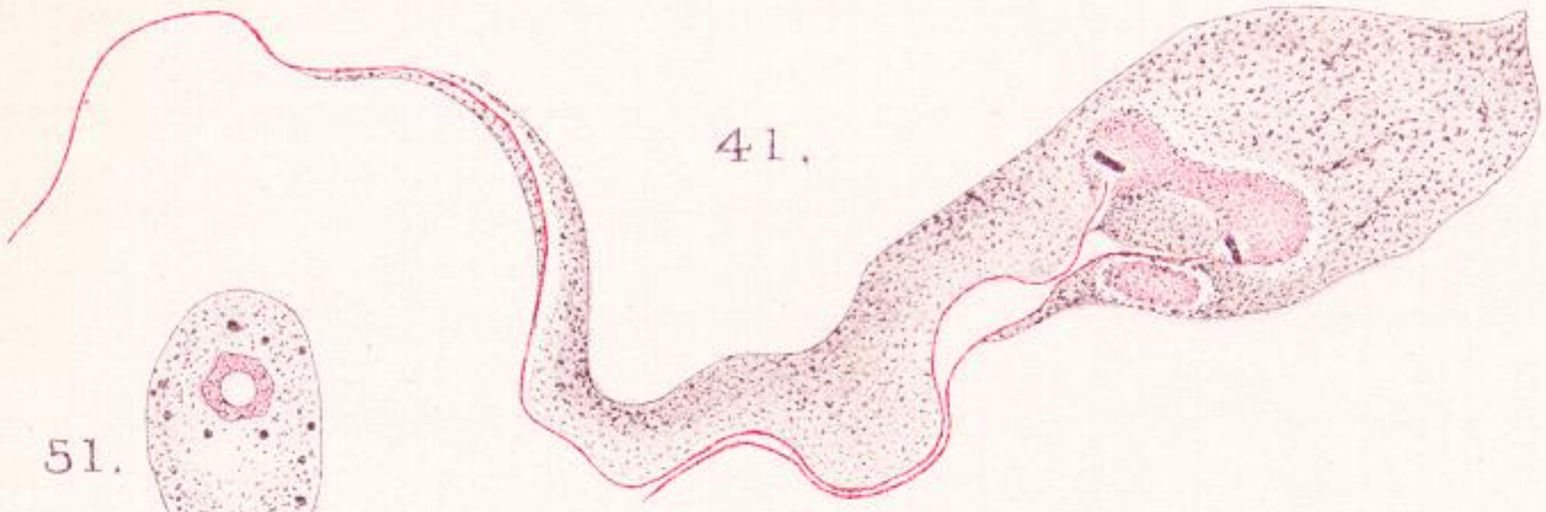
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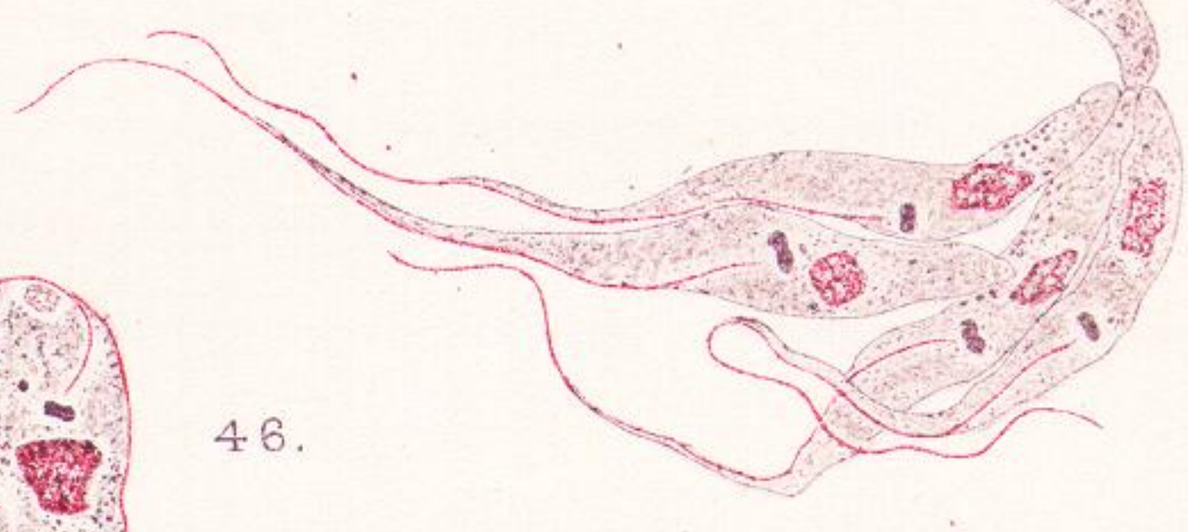
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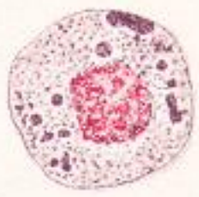
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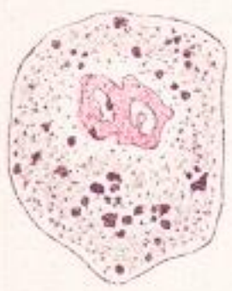
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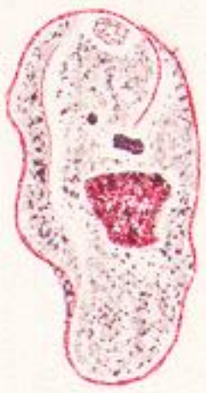
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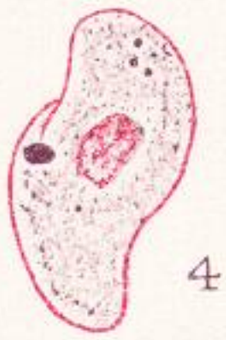
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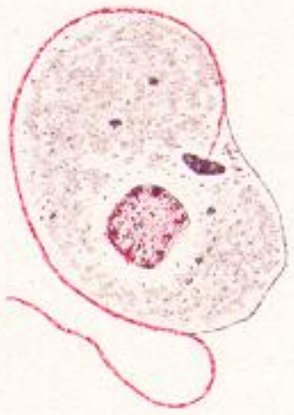
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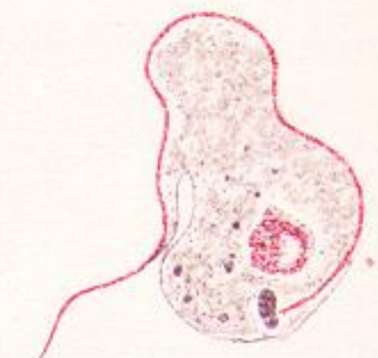
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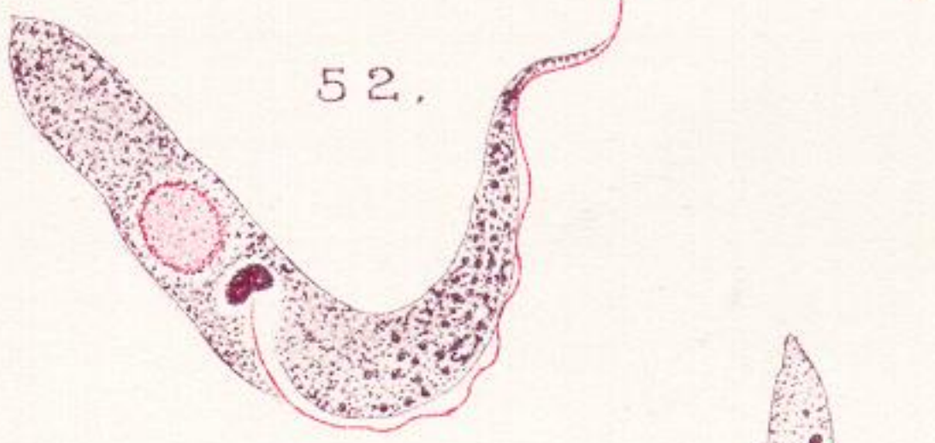
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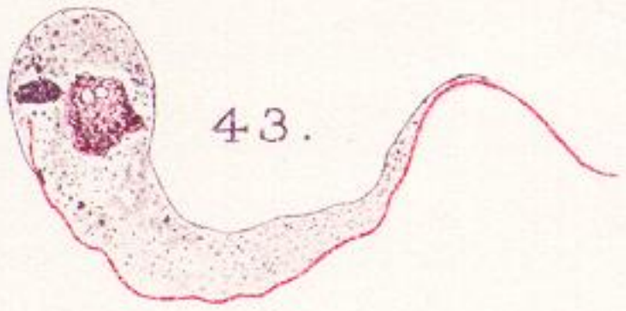
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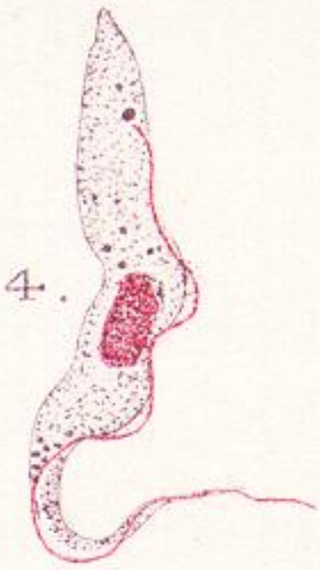
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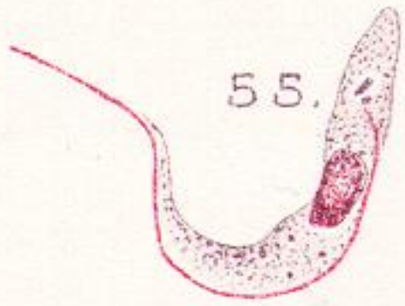
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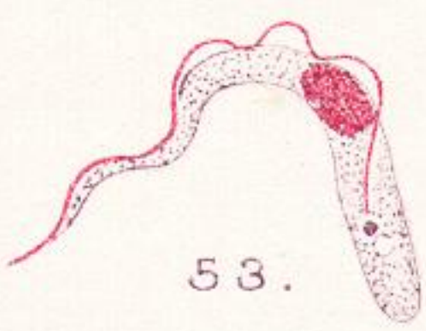
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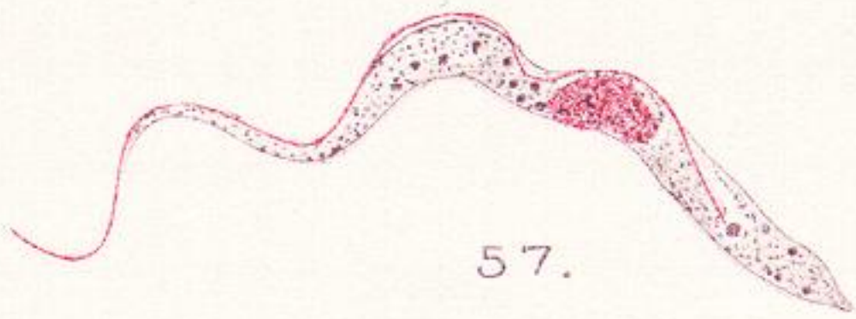
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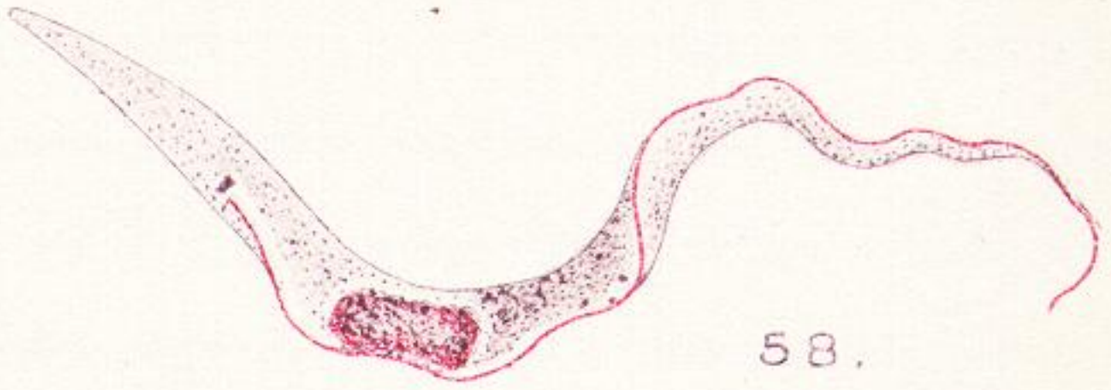
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TRYPANOSOMA GRAYI AND T. TULLOCHII × 2000.