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V. *Notes on the Life-History of* Trypanosoma gambiense, *with a Brief Reference to the Cycles of* Trypanosoma nanum *and* Trypanosoma pecorum *in* Glossina palpalis.

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[Plates 17–21.]

Introduction.

Among the mammalian trypanosomes carried by *Glossina palpalis*, *T. gambiense* may be taken as an example affording, as it were, a basis for the study of the group. In many ways the conditions are very clear, and no very serious difficulties on the score of technique or scarcity of parasites are met with at any point in the *Glossina* cycle.

In this paper I have considered the cycle of *T. gambiense* in detail, and have treated *T. pecorum* and *T. nanum* comparatively and much more briefly. I am indebted to Dr. H. L. Duke for the material of *T. pecorum* and *T. nanum* dealt with.

The material of *T. gambiense* was derived from a large number of experiments carried out in the Mpumu Laboratory in 1911 and 1912. The stages in *Glossina palpalis* were obtained from the study of the conditions in more than 200 infected flies, which were killed or died at different periods of the cycle. The advantage of so large a number is obvious, in that it eliminates casual variation. A considerable number of different strains of *T. gambiense* were used; thus, two separate strains derived from wild fly caught in Chagwe, a strain derived by direct injection into a monkey from an antelope infected with a human strain, this same strain after transmission by flies to clean monkeys, antelope strains of human origin passed by fly to clean monkeys, and a number of indifferent Uganda strains kept going among the Mpumu monkeys by means of *Glossina palpalis*, all served at one time or another as infecting material for the flies employed in the experiments.

I need not go into the very important question of the diagnosis of the strains derived from the Lake-shore flies, as that has already heen dealt with by Dr. H. L. Duke in a separate paper.

In all the experiments, the flies used belonged to the Uganda variety of *Glossina palpalis*,* and were hatched out in the Mpumu Laboratory from pupæ brought from

* See 'Sleeping Sickness Bulletin,' No. 38, vol. 4, for Dr. R. E. McConnell's account of *Glossina fuscipes* in Uganda.

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Damba Island in the Lake. Experiments had been carried out by Captain Fraser and Dr. Duke with several hundreds of these laboratory-bred flies, to ascertain if hereditary infection with flagellates of any kind occurred. The results were invariably negative. A similar conclusion had been reached by workers in other parts of Africa, notably by Dr. F. Kleine and his colleagues in German East Africa. No further experiments were made, therefore, in regard to this point, which seems now to be established beyond reasonable criticism.

Methods.

The method adopted in the study of the fly cycles was as follows: The newly hatched flies were starved for about 24 to 36 hours, and were then fed on the infecting monkey once, or in some cases twice. The infecting feed was the first blood ingested by the flies. After the infecting feed, the cage was starved for one or two days, and thereafter fed on clean monkey's blood every second or third day. Daily feeding is not essential to the welfare of the *Glossina*, and does not appear to occur in nature.

Late or provedly infective boxes were fed as a rule on cock's blood, but these cages were not used for gut stages, in order to eliminate all chance of accidental confusion with the trypanosome proper of the cock (*T. gallinarum*), which, as has been shown by Dr. H. L. Duke, not infrequently undergoes development in the *Glossina*.

Dissections were made in a drop of physiological salt-solution. The trypanosomes were studied both in the live state and in fixed and stained preparations. Unfortunately, trypanosomes do not live for any length of time in a normal condition outside the body of the fly, even when the fly has been dissected in monkey's serum.

The preserved material was fixed while wet by dropping the cover-slip film side downwards on to Schaudinn's corrosive-alcohol fixing solution. The preparation was subsequently stained by Heidenhain's iron hæmatoxylin method. This treatment of the films gave excellent results, and affords a more accurate account of the parasites than that obtained from the Giemsa method.

I. Endogenous Cycle in the Vertebrate.

The life-history of *T. gambiense* falls naturally into two parts, namely, the endogenous cycle in the vertebrate, and the exogenous cycle in the transmitting host. Certain aspects of the phases in the vertebrate have formed the subject of a previous paper, and they will therefore be treated very briefly here.

The course of the endogenous cycle as a whole is as follows: the description is drawn from the study of the conditions as found in monkeys (a species of *Cercopithecus*). In monkeys the incubation period is usually seven days. The organisms are very scarce in the blood at first, but increase rapidly in number. The multiplication takes place in the circulating blood, and intracellular multiplicative forms have not been observed at any time in the lung, liver, or spleen.

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The forms undergoing schizogony in the lung, described by VIANNA,* have never been seen, even in the earliest days of the infection in monkeys. VIANNA'S results are derived from guinea-pigs and white rats, and, as far as can be seen from the account given, there are no details concerning the origin of the strain used.

Intercellular forms have, however, been found in the lung and liver at certain periods, as will be seen hereafter, but they are involution-forms, and possibly latent forms, and no sign of multiplication has been observed.

The trypanosomes in the blood vary, as is well known, considerably in length and breadth, and the species shows the polymorphism characteristic of that set of trypanosomes, usually referred to as the "*brucei* group." From a long series of experiments, described in detail in the paper mentioned above, I have been led to the following interpretation of the cycle in the vertebrate. As here given, the account appears as a set of unsupported statements, and the reader must be referred to the earlier paper for the evidence upon which they are based:—

The short forms, 13 to 20 microns in length, are the "adult" blood type—this term being used to indicate that the form in question has the longest duration in time in the endogenous cycle, and appears to be the type from which the individuals capable of carrying on the cycle in the invertebrate host are derived. The blood of a monkey is infective to *Glossina* only so long as this form is present in sufficient numbers and in an appropriate physiological state—*i.e.*, not suffering from the exhaustion which seems to overtake the flagellates at certain times in very numerous infections. From these short forms there arise by growth the intermediate individuals, which are an ill-defined and quite artificial group, chiefly to be recognised by the fact that they have increased in length and have a longer free flagellum, but are of much the same average breadth as the short forms, namely, 2 to 2·5 microns. The long slender forms are derived from the intermediate type, and are simply the next step towards the division forms, which are the culminating stage of this whole process. The long slender forms are the individuals about to divide. It is incorrect to speak of three types of trypanosome; strictly speaking, it is a somewhat arbitrary attachment of names to the different stages by which the short forms proceed to division. The products of division give rise once more, directly or indirectly, as the case may be, to the adult type.

Another feature equally well known in the history of *T. gambiense* is the fluctuation in the number of trypanosomes to be seen in the blood. This has also been treated exhaustively elsewhere; it suffices to say here that a complex of circumstances, occurring at quite irregular and apparently incalculable intervals in the blood of an untreated vertebrate, brings about the disappearance of the vast majority of the parasites from the peripheral blood. The duration of the depressed period is very variable, and the reappearance of the flagellates is always accompanied by division in the peripheral blood. The percentage of divisions bears, moreover, an obvious relation

* 'Bulletin,' No. 38, vol. 4, p. 238.

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to the rapidity of the rise in number. An important feature of the depressed period is that, from the completion of the fall in numbers, which is usually rapid, until the first beginnings of the following rise, the blood is infective to fly, and the few surviving trypanosomes are of the short type.

The mechanism of the destruction is difficult to follow, but a certain amount of trypanolysis seems to occur in the blood. The liver and lungs have shown, in the case of a very teeming infection investigated at this period, both degenerative forms and what one is inclined to call involution-phases (fig. 39B). The liver of this monkey (633) showed many rounded-off individuals, possessing a trophonucleus and a kinetonucleus, but no flagellar apparatus. These forms seemed for the most part to be between the cells, and only in a few instances to be inside the cell—but this is a point very difficult to determine accurately in smear-preparations In addition to the rounded-off specimens, there were many trypanosomes of degenerate appearance. It is impossible to state what the fate of the rounded individuals may be. The infectivity of the blood during the depressed period, and the failure to find multiplicative intracellular stages, coupled with the gradual nature of the rise and the high percentage of dividing individuals in the peripheral blood during this increase, are all arguments against the form under discussion playing any serious part in the reappearance of the trypanosomes. These forms were not to be found in two late and chronic infections investigated, nor were they observed in a rather interesting case of an infection which had been a very teeming one, and which had just begun to take on the more chronic characters. This monkey died apparently from exposure during a severe storm, in which the animal had refused to stay in its box. It was observed when just dead, and the smears were taken at once. At the time of death, the infection was showing its first longer period of low numbers; it had been in a swarming condition only 10 days prior to the taking of the films. The infection had, of course, shown the usual short depressions, but these had been less frequent than is typical of the progress of affairs in monkeys.

It is impossible to dismiss altogether the consideration that the rounded forms may be latent individuals (capable of multiplication and further activity) lying in small numbers in the liver and lung, though their persistence in the latter seems less probable. Nevertheless, the three cases just cited do not bear this out, and the evidence, so far as I have been able to see, is all in favour of the rounded forms being destined to destruction. The point must, however, be borne in mind, as the possible survival of latent forms, however scarce, in contact with or enclosed in the cells of any part of the body is exceedingly important from the therapeutic point of view.

The question of a sexual interpretation of the polymorphism of *T. gambiense* has very frequently been raised, and has, indeed, already passed into the nomenclature adopted by many workers, so that one is constrained to give it a consideration it does not really deserve on the strength of its merits as a scientific hypothesis. The interpretation put upon the appearances is that the long slender forms are males,

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the short forms females, and the intermediate individuals are indifferent, or simply non-sexual forms. This is based primarily on a vague analogy with the life-cycle of the malarial parasite, and on the supposition that the long and short forms play the part of gametes or gametocytes in the transmitting host.

All the evidence brought to light by the study of the endogenous cycle is in absolute opposition to the sex-interpretation above sketched, and it may be pointed out that it is direct evidence of a nature easily observed by a careful study of the successive stages of the blood-cycle. The results of a long series of feeding experiments carried out on consecutive days with *G. palpalis* have further shown that it is on the presence of the short forms that the infectivity of the blood depends. It must be noted here that the distinction between the broad and slender forms is merely a physiological one, and is apparently entirely in respect to the process of division. The reason why it is from among the broad forms that the survivors in the fly are drawn is most probably that they are in a more stable condition, and therefore more capable of resisting the change of environment. Dismissing, then, the division of the long and short forms in the blood of the vertebrate into sex-categories, there remains to be considered a sexual differentiation among the short forms. I have not been able to detect any morphological distinction that could reasonably be attributed to sex. There is a certain amount of variation among the short forms in length and breadth, but it is not marked, and the nuclear features are very uniform. If the short forms do consist of male and female gametes or gametocytes, the differentiation is not expressed morphologically.

Cytology of T. gambiense *in the Blood of the Vertebrate.*

To consider very briefly the finer structure, and the process of division of *T. gambiense*, as studied on blood-films fixed by Schaudinn's corrosive-alcohol solution and stained by Heidenhain's iron-hæmatoxylin method. The body-form of trypanosomes is too well known to require description, and the species in question shows the typical relations. The kinetonucleus is small in size, and shows the usual two aspects, being either rounded or slightly rod-shaped. Close beside the kinetonucleus, at the actual origin of the flagellum, may be seen a small granule, the blepharoplast proper, or basal granule. The specimens derived from monkeys show a finely granular protoplasm, and larger inclusions are very rarely seen. The current view of the trophonucleus of *T. gambiense* is derived from dried Giemsa preparations, and bears only the slenderest relation to the real state of affairs. It is always figured as a large round or oval mass of granular chromatin.

With good illumination and a high-power system, any patient observer can see the nucleus in favourable specimens in the live state. This is, of course, more easily achieved in the larger species; in the trypanosomes of fishes and reptiles, an immersion-lens is not essential. The live picture is in all cases that of a circular, slightly refractile object, lying surrounded by a clear halo. The material fixed by the

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wet method, and never dried in air at any period during the preparation of the slide, shows conditions very closely resembling the live picture, and utterly different from the version given by the usual Giemsa method. All fixation is probably a choice of error, but it stands to reason that, if a delicate and highly flexible organism occupying three dimensions is swiftly flattened out into two, quite regardless of its attitude at the time, disasters are liable to occur. In dry preparations the nucleus has simply been burst. There is nothing wrong with this method so long as the observer remembers that he has destroyed this important structure, and does not proceed to describe it as though he had respected its integrity. The criterion of fixation must be the relation of the fixed material to the live picture, and, judged by this standard, the wet fixation and passage into some mounting fluid is greatly to be preferred. The criticisms that may be urged against the wet method are slight shrinkage of the protoplasm of the body, and the fact that, as the attitude in three dimensions has been very closely preserved, it is almost impossible in many cases to get an accurate microscopic measurement of the length of the animal. The ordinary methods of measurement supply no means whereby the elevation towards the eye of the observer can be accurately estimated in the case of small objects of irregular shape. Another drawback to the wet method is that all the parasites in a drop of fluid may not adhere to the cover-slip; it is clear that in certain kinds of work this is a very serious disadvantage.

The trophonucleus is of a type very common among flagellates, and is characterised by a large central karyosome, in which almost all the chromatic material is concentrated. This is surrounded by a clear space, which is in turn bounded by a faintly staining membrane or nuclear boundary—the word membrane is convenient, but its use here is not very clearly justified. Very delicate strands radiate out from the karyosome to the membrane; they are not always very clearly visible, but are to be made out in the vast majority of specimens. This condition of the nucleus is extraordinarily constant; practically no variation is found in any of the non-dividing blood-stages, and it is also found to persist through a large portion of the cycle in the fly. An important change does, however, take place in the latter part of the *Glossina* cycle; this will be noticed and discussed in treating of the forms in question (see Plate 17, figs. 1–4).

Division of the Trypanosomes in the Blood of the Vertebrate.

This process resembles very closely the division in the fly cycle, where the details are much clearer, so, to avoid repetition, I shall only draw attention to the most important features. The first sign of division is the doubling of the kinetonucleus. The behaviour of the blepharoplast is not clear, but fig. 6 suggests that it has the centrosomic function to be noted in the division in the fly cycle. The flagellum splits, but never throughout the whole length; it becomes free about two-thirds of the way down, and the flagellum of the daughter-individual is usually shorter than that of the

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parent. The trophonucleus shows one very interesting feature before division. When only the kinetonucleus and the flagellum have as yet begun to shown signs of re-duplication, two very well marked dark granules are to be observed on the membrane at opposite poles (figs. 5 and 6); they are generally joined by a fairly thick line to the karyosome. The other strands passing from the karyosome to the membrane persist for a time, but disappear when the division-figure begins to be formed. The two granules just mentioned apparently play the part of centrosomes. I have not been able to trace the exact place of origin of the granules. They seem certainly to be intranuclear, but I am quite unable to say if they arise from the karyosome. The division-figures are shown in figs. 5–8, Plate 17. There is no equatorial plate.

II. Exogenous Cycle in the Fly.

(1) *Conditions Obtaining in the Alimentary Canal of* G. palpalis *in Relation to the Development of* T. gambiense.

In considering the exogenous cycle, a few points bearing on the habits, etc., of the *Glossina* must be touched upon in passing.

The general anatomy of *Glossina palpalis*, and the structure of the proboscis, and the relations of the salivary glands, are so well known from the excellent work of Minchin, Stuhlmann, and others, that I shall not touch upon them. Following the practice of the Uganda Sleeping Sickness Commission of 1908, I have, in considering the infected flies, divided the portion of the gut which lies between the proventriculus and the origin of the Malpighian tubules, and which constitutes morphologically the mid-gut, derived from the embryonic mesenteron, into three parts, namely, the anterior or thoracic, the middle, and the hinder intestine.

From the dissection of a considerable number of wild tsetses from the Lake-shore, caught only three or four hours previous to examination, it appears that, under natural conditions, the majority of flies certainly do not feed daily. In most cases the whole gut is empty of food-material, except for a small quantity of pale greenish, completely digested fluid in the portion of the gut just anterior to the Malpighian tubules. This condition indicates a fast of at least two to three days, and possibly of a very much longer duration; in captivity, flies will live from nine to twelve days without food if the conditions of moisture are suitable. A large number of experiments of long duration were conducted, in which the cages were starved for two complete days between each feed, but there was nothing abnormal in the number of deaths. The fact that wild flies do not feed at such very close intervals of time as seems to be implied from the usual laboratory treatment of the flies has, as will be seen later, a somewhat important bearing on certain parts of the cycle.

The actual mechanism of feeding calls for attention, in that it affects the course of development. The sucking-stomach or crop leads out of the ventral side of the proventriculus by a long duct; in a full feed, the gut, up to a short distance behind

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the proventriculus, and the crop are both filled with blood. As digestion proceeds, the blood in the gut diminishes, and that from the crop is gradually passed, first forward along the duct, and then back down the gut. In a smaller feed, the gut may be completely filled up to the proventriculus without blood being taken into the crop at all. Flies are willing to feed before digestion is anything like complete, and it is important to know what occurs when the new blood is ingested. The experiments were made by feeding flies alternately on monkey blood and cock's blood.

The very first feed taken by a fasting *Glossina* (24 to 48 hours old) is usually a full feed, pretty well filling both crop and gut. The next feed, ingested 60 to 72 hours later, may behave in one of the following ways: The new blood may entirely replace all the material of the first feed from both crop and gut alike. In a large number of cases, however, some of the first feed is retained in the crop, and the second feed is taken in on top. In these cases one may also often find that the new blood has replaced all the first feed from the gut. There is thus a mixture in the crop, while the gut is, at the conclusion of the feed, full only of the new blood. In cases where digestion has been slow, the blood in both the crop and the gut may be mixed. Another state of affairs occurs, in which none of the new blood is taken into the crop, although the gut may be filled with the material of the second feed; moreover, in some cases, part of the original blood may be retained in the crop unmixed, and apparently unaltered, for as much as 10 days or more, although frequent feeds have intervened. A case of this kind was also observed in an experiment conducted by Dr. Duke and Captain Fraser.

The *rôle* of the crop in the economy of the fly appears to be simply that of a store-chamber; no digestion takes place there, and, in flies that have been allowed only one feed, and are thereafter starved entirely, one may find blood, the elements of which are optically unchanged after as much as 12 days. The crop can be completely emptied, and is, indeed, very often found in that state. It is also very frequently found, as is well known, to contain a bubble of gas.

Now, if a newly hatched fly has received trypanosomes with its first feed, it is interesting to see what has been the result of the subsequent clean feed.

This experiment was carried out with small groups of flies in glass jars. They were fed on blood containing numerous parasites; the greatest care was taken to see that all the flies had fed, there being, of course, no difficulty in ascertaining by inspection whether a fly has fed or not. The clean feed was of cock's blood. The results here noted are partially drawn in addition from some jars fed only once, and killed after a suitable interval without a second feed. Further evidence is derived, moreover, from the many early dead flies examined in the course of the general mass of experiments.

(1) The trypanosomes may be digested and disappear from the whole alimentary canal of the *Glossina* during the digestion of the first feed, *i.e.*, during the first 50 to 72 hours, without the intervention of a second feed.

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(2) The trypanosomes may survive in the gut in small numbers, but disappear during the early stages of digestion of the new blood.

(3) The trypanosomes may survive and multiply in the gut in the blood retained from the first feed, although a second feed has been taken in on top.

(4) Trypanosomes may survive and develop in the crop for as much as 12 days, provided the crop has never been entirely empty of blood during that period. The gut of these flies may often show no signs of trypanosomes. The trypanosomes in the crop seem unable to survive the emptying of this organ, and there is never a permanent infection in this situation.

(5) The trypanosomes may persist in greater or less numbers in the gut and in the crop of the same fly.

(6) The whole material of the first feed may be displaced from the gut by the second feed, and the trypanosomes still persist. The crop in these cases was either empty or filled with the new blood.

To consider which of the above conditions are likely to lead to an infected fly :—

(1) and (2) may be dismissed at once as negative.

(3) is a state of affairs that leaves the issue doubtful; if the trypanosomes are sufficiently established not to be swept out when the original blood is digested, a positive infection probably results.

(4) seems to suggest a negative result, as the condition of the gut implies that the trypanosomes are being digested and disposed of as they pass out from the crop into the gut.

(5) is a doubtful condition, almost identical with (3), though the chances of a positive result are obviously rather more favourable.

(6) seems to be pretty definitely a condition that will lead to a permanent infection of the fly. Once parasites are fairly well established in the new blood, the rate of multiplication is such that the chance of destruction at the next influx is small, and they have obviously been capable of withstanding the mere force of digestion by itself.

Clearly, two factors come into play here in the question of the establishing of an infection in the *Glossina*. First, the capacity of the trypanosomes to withstand the digestive processes; (1) and (2) show that this is not a property possessed by all the trypanosomes. The second factor is the clearing out of the trypanosomes by the new feed; that this is a very potent agent in the production of negative flies is shown by the very much higher percentage of infected flies found up to the 12th day in experiments where the flies had only one feed, and that the infecting one. These experiments were carried out with controls, fed every two or three days in the usual way; the starved flies lived up to about the 10th or 12th day. Out of 103 starved flies, 22 showed trypanosomes between the 6th and 12th day. Neglecting those flies which showed trypanosomes only in the crop, there were 16 left, which showed apparently well-established, though not very numerous, infections in the gut, *i.e.*,

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15·5 per cent. This is a very remarkably high percentage for the ordinary Uganda strain of *T. gambiense*. Under ordinary feeding conditions, the strain used for the starvation experiments just quoted produced 3 per cent. of + flies.

To quote a typical experiment. Jars Nos. 16, 17, and 18, each containing 15 flies, were fed on the same day and within a few minutes of each other on one monkey. Jars Nos. 16 and 17 were fed every third day, and dissected on the 22nd and 28th day of the experiment respectively; only one out of the 30 flies showed trypanosomes, and that was an individual which had died and been dissected on the 2nd day after the infecting feed. Jar No. 18 was starved outright after the infecting feed; between the 9th and 12th days three flies showed trypanosomes in the gut, and three showed trypanosomes in the crop—altogether, 6 out of the total 15 showed trypanosomes. It must, however, be noted that the trypanosomes may disappear entirely from all the flies in a starvation experiment, and fall under the same heading as negative experiments in general.

To obtain therefore a clear idea of the elements that come under observation in the confusing early days of the cycle in the alimentary tract of the fly, it must be borne in mind that there are several conditions which may show trypanosomes persisting.

(I) Mere persistence without multiplication. Here the parasites undergo the usual slight alteration in shape, to be discussed later, and degenerating specimens are also to be seen. This condition is not found after 72–84 hours, and is chiefly to be observed when the trypanosomes were numerous in the infecting blood.

(II) Cases where the trypanosomes persist in small numbers in some part usually of the mid- or hinder intestine and multiply, but are, nevertheless, unable to establish themselves permanently. These are mostly cases where the apparently adverse conditions of the new feed come upon the trypanosomes before they are able to withstand them. In this state dividing and degenerating specimens are to be met with as well as numbers that do not suggest either condition. The individual types will be treated later. Persistence and quite normal development may occur, as has already been stated, in the crop, and continue till the 10th or 12th day, after which time I have not observed trypanosomes in this situation until they reappear there occasionally at the end of the cycle. This persistence and development in the crop is very interesting and important for two reasons: first, in that it obviously allows of several chances of infecting the gut, as all the material in the crop, including the trypanosomes, is passed backwards down the alimentary canal. In the second place, it shows that the stimulus to development in the fly is not dependent upon the digestive action of the gut fluid upon the blood, *i.e.*, neither the digestive fluid nor its action upon the blood are essential factors in setting off the developmental processes.

(III) The third case found in the early days of the cycle is where the conditions admit of trypanosomes persisting and multiplying with sufficient vigour to establish themselves permanently in the gut. This may occur at any part of the middle or

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hinder intestine. I have never observed what one could with justice call established trypanosomes in the anterior intestine at an early period. This third condition leads to the production of a positive fly.

Out of a study of all these circumstances it may be deduced that, where no serious attempt at multiplication is made on the part of the trypanosomes, we have an expression of the incapacity of the parasites, and that we are dealing with a negative condition, that is to say, the large majority of the individual flagellates ingested are not in a fit state to carry on the cycle. This condition of the flagellates is, as has been already mentioned, a definite and recurring, though transitory, stage of the endogenous cycle in the vertebrate, and constitutes the negative period during which the short forms are not present in sufficient numbers, or are in an unsuitable state.

The very large number of cases where the attempted multiplication fails to establish an infection may be taken as representing the general inhibiting capacity of the *Glossina*, and appears to be a very fairly constant factor in all experiments dealing with newly hatched flies. The great value from the experimental point of view of the infecting feed being the first feed ingested lies just in the point that only in this way can any sufficient uniformity be ensured in the condition of the alimentary tract of a batch of, for instance, 50 flies. The inhibiting capacity just mentioned is responsible, given a suitable condition in the infecting material, for keeping down the percentage of infected flies. Individual strains of *T. gambiense* vary greatly in the general percentage of infected flies produced, this being due to the greater or lesser vigour of the trypanosomes interacting with the inhibiting forces of the fly. The negative blood periods are just as marked in very vigorous strains as in those of lesser vitality.

(2) *Development of* T. gambiense *in the Gut of* G. palpalis.

To consider now the typical course of the developmental cycle of *T. gambiense* in *Glossina palpalis*. Some general features may be disposed of at once. There does not appear to be an intracellular stage at any part of the cycle, either in the gut or in the salivary glands. Such a development was very carefully searched for, Prof. Minchin and Dr. Thomson's important discovery of this stage in *T. lewisi* naturally adding to the probability of an intracellular phase occurring in other cycles, though *T. lewisi* is obviously a very different type of trypanosome from any of those included in the "*brucei* group."

T. gambiense develops in the lumen of the gut from the very start and there is no gap or disappearance of the parasites from this situation at any period. The flagellates are never found to attach themselves in any way to the wall of the gut, but do attach themselves to the wall of the salivary gland when established there. In my experience the trypanosomes do not pass through the wall of the gut into the body cavity at any time.

While there is a very definite course of development in the fly before the

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trypanosomes are ready to infect another vertebrate, nevertheless the duration of this cycle of changes may vary to the extent of a full fortnight. A certain amount of experience with an individual strain will permit an observer to predict with considerable accuracy the state of affairs upon any given day; but it is quite impossible to say with a random strain that any type or condition is typical of a given date. A 10th or 12th day fly of one strain may correspond with a 20th day fly of another. Even a single strain may sometimes vary in regard to time as much as a week or ten days. This must be borne in mind in dealing with the stages of the cycle. Variation in the time-element of development is a well-known biological phenomenon, which has, however, not received much attention in dealing with Protozoa; the present instance is a very marked case. Flies may be infective already on the 17th day, while it is quite common for the cycle not to be complete until after the 30th day.

The earliest case obtained in which the trypanosomes could with certainty be considered as developing, and not merely persisting, is that of a fly of the 2nd day. That is to say, the trypanosomes had been in the gut 36 to 48 hours. A provedly developing infection of so early a date is merely obtained by a fortunate chance, and the evidence in favour of its authenticity is as follows :—Experiment 122 was fed on Monkey 597, an infected individual which was under close observation; both live and stained films were being examined daily. On the date of the infecting feed, March 5, 1912, 597 showed no trypanosomes in the course of the examination of the live film. Prolonged examination of a stained film, 3×1 in., showed very rare parasites. The fly in question (No. 8) died during the night of March 6–7 and was dissected at 9 A.M. on the morning of the 7th. It was found to contain partially digested blood and a very considerable number of trypanosomes, which were in an active and lively state, a number far in excess of those found on the stained film taken immediately before the cage was fed. It may be mentioned in passing that Experiment 122 afforded two more positive flies when the box was dissected on the 23rd day. Wet fixed films were made as usual from the contents of the gut of the fly and multiplying individuals were observed. Figs. 9–12 are from this fly. Although special attention was paid to this fly from Experiment 122, as being the earliest obtained, nevertheless the stages correspond very closely with those seen in the relatively large numbers of early positive flies dissected between the 3rd and 6th days. A reference to figs. 9–16 will make this clear.

No very striking or rapid changes occur when the trypanosomes are taken into the gut of the fly. Degenerating forms are present in greater or lesser numbers. The healthy-looking forms show a general but very slight difference from the blood type which is difficult to define in words; the membrane is slightly narrower; the kinetonucleus moves somewhat nearer to the trophonucleus, which itself in most specimens increases a little in size. There is no marked division into slender and broad forms; there is variation in respect to length and breadth, but the categories are not sharply marked off, and the extent of the variation is slight. One point,

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however, emerges, which is, I think, of real importance, namely, that the dividing individuals at this early stage are all relatively broad forms. Figs. 11 and 16 are typical of the process. A broadening of the individuals about to divide is not characteristic of the development in the gut of the fly as a whole, as a glance at figs. 20 and 30–33 will show, the deduction being that in the earliest days it is the broad individuals that have been ingested that are capable of division. This observation tends to confirm the impression received from purely external experimental sources that the broader, shorter forms are responsible for carrying on the cycle in the fly.

The finer details of division will be gone into in the case of the later days, where the cytology of the process is exquisitely clear. One thing is, however, worthy of notice, and that is the relations of the kinetonucleus of the daughter individuals in figs. 11 and 16. In both cases the young individual is still in a crithidial condition, a very transitory state, as it develops almost invariably into a trypanosome before it is set free. (See fig. 12, from 2nd day.) This is only characteristic of the earlier divisions, and (with the exception of a very slender form to be discussed shortly) is the only sign of a crithidial phase to be observed in the gut development. There is, as will be seen hereafter, a most marked crithidial stage in this, as in most trypanosome cycles, but it does not occur in the gut.

As multiplication proceeds there are produced a large variety of shapes and sizes, though the trypanosomes do not seem frequently to surpass the maximum length of the blood type, *i.e.*, 34–35 microns. By the 10th day or thereabouts there are very numerous trypanosomes exhibiting a wide range of form. The characteristic slender form so remarkable in the anterior intestine and proventriculus later in the cycle may begin to appear already, but only in small numbers.

A long thread-like form, such as that drawn in fig. 43, and which comes from a 12th–13th day fly (mid-gut), corresponds apparently to the "male" forms of KLEINE and TAUTE.* There is no evidence in favour of this being a sexual form at all; it has never been found in conjugation, in spite of much searching, either by KLEINE and TAUTE or myself. In dealing with flagellates, there is no support to be found, in any of the well worked-out cycles in which conjugation has undoubtedly been established, for the theory that markedly long slender forms are male gametes. In flagellates, all cases of conjugation involving hologametes (as must obviously be the case here) are practically isogamous.† There is no argument by analogy in favour of these long forms, such as fig. 43, being males. The use of analogy is obvious in working with an individual of a very well-known group; no one would hesitate to use this method in working with the Coccidia, for instance, but the analogy, to be sound at all, must be drawn from the group in question.

In the case of *T gambiense*, one must first establish clearly that these forms

* 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' vol. 31, Part II.

† See DOFLEIN'S 'Lehrbuch der Protistenkunde,' 1909, for a general account of the Flagellata.

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(fig. 43) do enter into conjugation before they can be held to be male gametes. In the case in point, the evidence is against these forms being gametes. They have never been seen in conjugation with broad or other forms. They are of rare occurrence, and appear in the middle of a period of very active multiplication, which is not a characteristic of gametes generally. I have never seen them earlier than the 12th day, even in the most rapid cycles. Moreover, their nucleus and protoplasm has almost always a pathological appearance, and one is inclined to consider them as degenerating slender forms (compare figs. 40–42).

A great deal of degeneration goes on in a well-infected fly, as is only to be expected when the tremendous numbers of parasites present are considered. These processes are easily recognised in the broader forms, but are much more obscure where the slender types are involved.

The posterior position of the nucleus sometimes seen in these forms seems to be due to the tendency so often shown in degenerating trypanosomes for all the protoplasm and its contents to aggregate at the posterior end (fig. 41). This condition does not seem to me to be really in any way comparable to the usual crithidial phases of trypanosome cycles, where the whole culture passes through a state in which the trophonucleus is definitely posterior to the kinetonucleus, and which is followed by a reassumption of the trypanosome-condition proper (compare the life-cycles of *T. lewisi*, *T. raiæ*, of the trypanosome of perch, goldfish, etc., the cycles of bird-trypanosomes, and the salivary gland phases of *T. gambiense*). Very slender individuals, without any trophonucleus at all, are seen at times; they are rare, and I do not consider that they are in any way normal forms. A limited vitality is preserved for a time in very degenerate specimens, and even in parts of a trypanosome, thus, actively motile flagella, with or without a portion of the membrane attached, are known to persist for some hours before disintegration sets in.

Cytology and Details of Division.

The cytological relations, and the details of division, are very well seen in the gut-forms, and the following account has been drawn from the middle period (10th to 12th day) of the cycle. The body-form, as already mentioned, is subject to the greatest variation. In the multiplicative types (figs. 30–35) the protoplasm is granular and somewhat dense in appearance, but does not usually show larger inclusions. The kinetonucleus is larger than in the blood types, and the blepharoplast (or basal granule) of the flagellum is very clearly visible. The trophonucleus contains a large karyosome, apparently very rich in chromatin; the relations are much as in the blood types, only the whole nucleus is generally rather larger, and the nuclear membrane is not quite so clearly marked.

At division, the blepharoplast divides before anything else, and is immediately followed by the first splitting of the flagellum. The two daughter blepharoplasts move to either side of the kinetonucleus, and very clearly and constantly play the

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part of centrosomes in its division (fig. 30). A centrodesmose is formed between the two blepharoplasts, and division takes place without the formation of an equatorial plate. In well-stained iron-hæmatoxylin preparations, this set of conditions can be seen with the most exquisite clearness and precision. The earliest appearances of division in the trophonucleus are seen in fig. 31, where the two centrosomes are lying on the slightly drawn-out membrane; they are joined by a centrodesmose, and the first signs of a division of the karyosome are to be observed. The nuclear membrane becomes very faint, but never seems to disappear, so that it seems highly doubtful in how far it is homologous to the nuclear membrane of the Metazoa. The chromatic material draws out along the lengthening centrodesmose into a somewhat irregular spindle, and the karyosomes ultimately re-form without the intervention of an equatorial plate phase. The remains of the spindle-figure, which is sometimes extraordinarily long (it may be much longer than that shown in fig. 34), are absorbed in the protoplasm. The fate of the centrosomes after division is obscure, and I cannot say whether they are incorporated in the daughter-karyosomes, or whether they disappear or lie on the membrane. The division of the protoplasmic body is very characteristic. There is no longitudinal splitting of the parent-organism, but the young specimen is really as it were pushed off (or grows off) at the posterior end (fig. 35), and the division is practically transverse. The two organisms never swing out so as to be arranged kinetonucleus to kinetonucleus, as in the majority of trypanosome-divisions. This behaviour is very constant; the young individual is usually somewhat smaller than the parent.

When the slender forms (figs. 36, 37, 39) which constitute the regular proventriculus type come to be developed (this occurs any time after the 10th to 15th day) they arise quite gradually from the broader forms and there is only one point of importance in which they differ from their predecessors (figs. 36 and 37). The body is long and slender, the protoplasm very finely granular and much less dense than in the broader forms, but the salient feature is the trophonucleus, which shows an interesting change. The karyosome has become very much smaller and the membrane has become much more marked and stains deeply, suggesting that it carries some quantity of chromatin; the fine rays can rarely be distinguished, but there are some indications that they nevertheless persist. There seems to be a reduction in the quantity of chromatic material in the whole nucleus, but it is impossible to say whether this is merely in relation to the lesser quantity of protoplasm, or whether it is some sort of a nuclear preparation for the subsequent development in the salivary glands. Division takes place in fairly slender types (fig. 38), but seems to be inhibited in individuals such as those shown in figs. 36 and 37.

The infection as a whole generally develops in the hinder intestine or the posterior part of the middle intestine. I have never found trypanosomes established in the rectum. The infection literally grows forward by sheer force of multiplication till it fills the whole of the middle and hinder intestine and the posterior part of the

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anterior intestine. The anterior portion of the anterior intestine and the proventriculus show the typical long slender forms (figs. 36 and 37) described in the preceding paragraph, and are not invaded till some time about the middle of the cycle, from the 10th to the 20th day, or thereabouts. There seems to be some difficulty in the trypanosomes reaching the proventriculus, and, once arrived there, they maintain themselves in that situation only so long as the fly is not exposed to too long a fast. If a fly has digested its meal and there is any considerable interval before the next is obtained, the trypanosomes ebb backwards to the posterior part of the anterior intestine, and only gradually recapture their position again. If, however, the new blood is taken in while the trypanosomes are still in the proventriculus, they retain their position and are apparently little affected by the influx of fresh blood.

These conditions can easily be observed from a series of simple experiments with boxes of the right age, in which the feedings have been suitably spaced and their relation to the state of the gut of the positive flies carefully observed.

This inability on the part of the parasites to maintain themselves in the proventriculus during a fast becomes of some importance in the cycle when the habits of the wild flies in regard to feeding, referred to earlier in this paper, are remembered. The majority of wild flies are obviously exposed to fasts of considerable duration, and it is this circumstance, combined with the peculiarity of the trypanosomes just mentioned, that seems to necessitate some seat of attachment for the trypanosomes anterior to the proventriculus in all cycles developing in *Glossina palpalis*. In *T. gambiense* the flagellates invade the salivary glands ; in *T. pecorum* and *T. nanum*, as will be shown later, they attach themselves in the proboscis after having gone through a gut-development not unlike that of *T. gambiense;* in *T. vivax* and *T. uniforme* the whole cycle takes place in the proboscis. There is at present, as far as I am aware, no case of a completely investigated gut form carried by *Glossina palpalis* being transmitted directly from the alimentary canal without some definite secondary focus occurring anterior to the proventriculus.

In very numerous infections the trypanosomes sometimes overflow from the proventriculus into the crop, and may be found there in considerable numbers ; they are typical slender proventricular forms. When injected into a clean monkey they are incapable of producing infection.

The gut-development may be said to culminate in the slender proventricular type. The hinder and middle regions of the intestine right up to the death of the fly present a medley of multiplying forms differing little from those produced in the first 12 days. There does not appear to be much, if any, multiplication among the very slender individuals, and the hinder and middle intestine seems to serve as a reservoir from which the more slender types are being constantly drawn.

In many trypanosome cycles, notably those of fish and reptiles, this slender form is the last phase of the development in the invertebrate, but this is not the case in *T. gambiense*. An essential development differing very markedly from that carried

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on in the gut still remains to be gone through in the salivary glands. Before, however, leaving the gut cycle it is necessary to consider two very important points: (*a*) The meaning of certain curious multiple and non-flagellate forms found in the gut from about the 5th to the 12th day; and (*b*) the question as to whether conjugation has taken place at any period in the gut cycle just sketched.

(*a*) In live preparations of the gut there may occasionally be seen confused motile forms with several flagella; others are somewhat amœboid, and the flagella are more or less adherent to the protoplasm; others have no flagella at all, and are quiescent; and still others are obviously wriggling masses of half-fused trypanosomes. Figs. 21–24 give some of these forms as they appear in stained preparations. They always lie right up against the peritrophic membrane. Now, there is little doubt from the fact that these forms can be seen in live films to be caused by the fusion of soft, rather unhealthy-looking trypanosomes under the unfavourable condition obtaining on the slide, that many of these appearances are cases of degeneration. Nevertheless, this is no proof that all the multiple individuals are degeneration products, to be dismissed without further consideration. I have not been able to get any evidence that the multiple forms play any progressive or essential part whatsoever in the cycle. They must, however, always be borne in mind, as these appearances may afford an unfortunate mask, obscuring the process of conjugation. Sometimes in live films from the early days in the fly cycle, and occasionally also on films from the monkey when these were prepared with a little water or with a 0·50-per-cent. salt solution, trypanosomes were observed to come together from opposite sides in pairs so as to overlap about one-third of the body length as in fig. 39A, which is from a freehand sketch of live individuals. The junction seems superficially to be extraordinarily close, suggesting fusion even under a high-power lens, nevertheless the specimens have always come apart again, the longest period of apposition observed being 35 minutes. Usually the individuals are quite similar to one another; on one occasion, however, in the case of a 3rd day fly, they showed a slight amount of differentiation, one being rather more slender than the other. The "male" type (fig. 43), discussed earlier in the paper, was not involved in any of these cases, in all of which the trypanosomes were of the blood type.

This is the sum of the direct evidence from live observation that I have been able to obtain in the course of more than a year's search, and it is obviously inadequate. The trypanosomes do not live long enough under the cover-slip to give satisfactory opportunities for this kind of observation. The fact that the divisions are so very characteristic and so constant in type would admit of valuable deductions being made from the stained material, were it not that the multiple degeneration and involution forms obviously rob these of all their value. It must also be considered whether conjugation might not take place in the salivary gland. Direct evidence there is none, and live observations are even more hopeless here, as the trypanosomes from the glands are extraordinarily sensitive to the unfavourable conditions of the slide.

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As will shortly be seen, there are important data for considering the salivary gland cycle as the really essential part of the whole development, and it is for that reason that one is inclined to entertain the idea that the sexual process might occur in this situation.

(*b*) Theoretically there is a good deal of evidence in favour of conjugation or some equivalent process occurring during the cycle in the transmitting host. The passage through the *Glossina* as a whole seems certainly to have the same biological significance. There is clear evidence in certain cases observed here at Mpumu—the case of Monkey 199 was cited in this connection in an earlier paper—that peculiarities acquired by a strain during its sojourn in a particular host are eliminated by passage through the fly, whereas these idiosyncrasies are retained when the strain is passed directly by injection. There can be no doubt that from a consideration of the life-history as a whole the part passed in the fly plays a definite and essential *rôle* in maintaining the integrity of the species, quite apart from its being a convenient method of transmission.

(3) *Stages of* T. gambiense *in the Salivary Glands.*

The penetration of the trypanosomes into the salivary glands occurs quite clearly from the hypopharynx, and the successive stages of the process can be seen very well in the live state, in careful dissections of the glands at the appropriate periods. An individual trypanosome cannot, of course, be watched through the process, but the study of a good number of flies leaves no shadow of doubt as to the sequence of development. The easiest way to get at the thin duct of the glands, which is the place where the first stages are to be found, is to starve the fly for full two days, then to cut off the posterior tip of the abdomen, snipping off a slightly larger portion than is usual for ordinary dissection, and to pull out the gut without breaking it off. This minimises the chance contamination of the gland with the contents of the gut. If the glands do not appear, stroke the abdomen gently with the flat of the needle until they do; when the tip of the gland emerges, a single rather slight stroke of the needle will bring it out right up to the narrow duct, and the same for the other side. The glands are transferred to a clean slide with a drop of normal salt solution or monkey serum; they are transferred again to a clean slide and examined. The pulling out without breaking the gland is an easily acquired knack and should be done under a low-power dissecting microscope.

The slender trypanosomes pass up into the hypopharynx from the proventriculus. The period at which this happens is in direct correlation with the vigour and number of the trypanosomes in the fly, and early infectivity is generally a character found in a strain which produces many positive flies. These slender trypanosomes may in rare cases be seen lying, in small numbers, free in the hypopharynx of flies whose salivary glands are not yet infected. They then come back along the narrow duct of the salivary glands, and can be seen there as free-swimming slender creatures.

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The gland, as is well known, is composed of (1) a narrow tubular part, which leads back to (2) a slightly broader cellular part, which in turn leads to (3) the glandular part, where the full width of the organ is attained.

The trypanosomes settle down and attach themselves in the second part of the gland, or at the entrance to the third part, the rest of the gland being quite free from trypanosomes. At first they are slender forms, which sway forwards and backwards, attached by their flagellum to the wall of the gland (figs. 44 and 45). Later on they become broad, round-ended, crithidial flagellates. They multiply, and gradually, what with divisions and fresh arrivals, finally fill up large portions of the gland with flagellates in all stages, from tadpole-shaped crithidias, attached by their flagellum, to free-swimming trypanosomes, closely resembling the blood type (figs. 44–60). These trypanosomes are usually below the length, but not below the minimum measurement, of the forms occurring in the blood. Division takes place among the trypaniform, as well as among the crithidial, parasites (figs. 49, 56, and 57). The cytology of the gland forms calls for no special notice, except that there seems to be an increase of chromatin within the nucleus, and there is a tendency for the karyosome to increase once more in size. The occurrence of marked crithidial, and in some cases almost herpetomonad, forms is very striking; they constitute a large proportion of all the gland types. Degenerating individuals are found in all stained films, but the great sensitiveness of the trypanosomes to the process of investigation leads one to imagine that some of these at least are due to manipulation. While the crithidial forms are mostly attached, they may, nevertheless, be found free also; it seems probable, however, that they attach themselves again.

The fly is, apparently, already capable of producing an infection in the vertebrate when only the proximal part of the gland close to the duct shows parasites. The *Glossina* seems to become infective about 2 to 5 days after the trypanosomes invade the gland, but, as one cannot both dissect and subsequently observe an individual fly, this time is naturally only an estimate made from the consideration of a number of only more or less similar cases. It appears to be clear, beyond question, that only when the salivary gland shows trypanosomes is the fly infective. Early positive transmissions are always accompanied by rapid virulent infections in the fly and an early invasion of the glands. In one case a salivary gland was found infected on the 12th day, but this is quite exceptional—on the 16th day infected glands are found in rare cases, and after the 20th day they become increasingly frequent.

Cycles of *T. nanum* and *T. pecorum* in *G. palpalis*.

It is interesting briefly to compare the general course of development in *T. nanum* and *T. pecorum* with that of *T. gambiense*.

The development of *T. nanum* in *Glossina palpalis* has many features that closely resemble the conditions in *T. gambiense*. The infection starts in the hinder

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intestine, and by the 10th day (no material was obtained from the very earliest days) numerous trypanosomes are to be found in the hinder and middle intestine. They show a considerable range of form, and have nuclear relations practically identical with those of *T. gambiense* (figs. 61–64). The method of division corresponds in such minute detail with that described in figs. 30–35 for *T. gambiense* that it is needless to recapitulate the description. Slender forms begin to be produced from the 10th to the 14th day of the cycle onwards, and the proventriculus comes to be invaded about the 20th day. The proventricular forms, while generally slender, do not show such a uniformity of type, nor are they so thread-like, as in the case of *T. gambiense*. Moreover, the characteristic change in the nucleus of *T. gambiense* shown in figs. 36 and 37 never takes place at all in the gut forms of *T. nanum*. About the 25th day trypanosomes begin to be found in the proboscis attached to the labrum, often lying in clusters. They assume the crithidial condition, many of them being extraordinarily long and slender (figs. 65–68). No reliable information was obtained concerning the nuclear detail of these types (the drawings are all from dried Giemsa preparations), as it was found that the trypanosomes could not be made to adhere to the cover-slip in the wet preparations, except in very rare instances. Besides the very long crithidial types, shorter forms, such as that shown in fig. 68, were observed, also crithidial in type. In live preparations, free trypaniform organisms were observed, sometimes in the hypopharynx and sometimes in the labrum.

As in *T. gambiense*, the gut forms do not attach themselves to the wall of the alimentary canal, nor are crithidial forms ever seen in the gut cycle. The salivary glands are never invaded in the case of *T. nanum*, the proboscis-infection apparently playing the *rôle* of the gland phases in the cycle of *T. gambiense*.

Trypanosoma pecorum develops in *G. palpalis*, but the cycle is so extraordinarily slow, and the transmission so difficult to effect, that it appears clear from Dr. H. L. Duke's experiments that this fly is at most only a facultative intermediate host for *T. pecorum*. For this very reason, the conditions of the cycle have some points of special interest, as will be seen later. The course of the development in the gut resembles that of *T. nanum* and *T. gambiense*; multiplication occurs, and numerous types, varying in length and breadth, are formed (figs. 69–71). The flagellates are more massive and larger than in either of the two forms just mentioned. The slender forms are produced as usual after the broad individuals, and are found in association with them; the long forms are extraordinarily attenuated, and the nuclear change remarked upon in the corresponding stages in *T. gambiense* occurs also here (fig. 72). The first invasion of the proventriculus was observed upon the 45th day of the cycle, and no proboscis-infection was found before the 76th day. The proboscis-infections were generally slight, and the usual difficulties were experienced in getting preparations from this situation. Figs. 73 and 74 show two individuals from a wet preparation, fixed in Schaudinn's fluid, and the nuclear

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relations recall those of the gland-stages of *T. gambiense*. The salivary glands never become infected.

General Considerations and Conclusions.

The most remarkable feature of the cycle of *T. gambiense* is the curious double development, first in the gut, culminating in the long slender form, and then in the salivary glands, where the crithidial stage so typical of all trypanosome cycles is so marked a feature. It seems difficult to escape the deduction that the gut-development is a somewhat indifferent multiplication, a mechanical device to enable the trypanosomes to establish themselves in sufficient numbers in contact with the salivary fluid, which in the fly seems alone capable of stimulating the trypanosomes to that apparently essential reversion to the crithidial type. In *T. pecorum* and *T. nanum* the gut-development follows in all essentials the scheme of *T. gambiense*, and here also, as already mentioned, the proventricular forms are not infective. While the double nature of the cycle is perhaps more obvious in *T. gambiense* than in *T. pecorum* or *T. nanum*, the cases are, nevertheless, close parallels. The parasitism in the case of *T. gambiense* is obviously of a more complete type, as the invasion of the salivary glands lends greater permanence and stability to the apparatus of infection than the more or less intermittent infection of the proboscis found in *T. pecorum* and *T. nanum*.

In accordance with the foregoing, it may be said that the gut-conditions of *G. palpalis* do not permit of the complete and essential development of any of the group of trypanosomes mentioned in this paper. So long as the parasites are established in the gut only, their presence is indifferent and negligible from the point of view of infection. This is very striking in those rather rare cases where flies infected with *T. gambiense* may show considerable numbers of flagellates as late as the 56th day without the salivary glands being infected. Such flies are invariably harmless. *T. pecorum* may also be considered in this connection; the culture in the gut persists for 70 days in a harmless state, only becoming infective when the parasites do at length succeed in establishing themselves in the proboscis, and going through the crithidial development.

In conclusion, it may be remarked that, if a sexual process is a necessary feature of the cycle passed in the transmitting host, it will probably occur at that stage which seems absolutely essential to the production of a trypanosome viable in the blood of the vertebrate, namely, the crithidial phase in the salivary glands or proboscis.

It is obvious that much of the foregoing work has been simply to carry somewhat further the researches of Minchin, Roubaud, Bruce, and Kleine, more especially of the last two workers. There are no serious discrepancies between the cycle in the fly sketched by Bruce, Hamerton, and Bateman, and that described above, except that I consider, as has already been said, the fly-history to be in reality a double development. In many points my work is also in agreement with that of Kleine

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and TAUTE, except that I do not consider that the "male" forms described by him play any important part in the cycle.

A further discrepancy consists in the view held by the German workers at the time of writing their paper, in regard to the salivary gland phases being a non-essential part of the cycle. My interpretation of the endogenous cycle in the blood of the vertebrate is at present, as far as I am aware, unconfirmed by other workers, largely, I imagine, owing to the fact that the interest has been concentrated for some time past on the appearances in the fly rather than on those in the vertebrate.

Summary.

1. *T. gambiense* undergoes an endogenous cycle of development in the vertebrate in the circulating blood. This cycle is of irregular duration and is repeated many times in the course of the disease.

2. The short forms may be regarded as the adult blood-types; the intermediate types are growth-forms, proceeding to the long individuals, which are those about to divide. The products of division give rise, directly or indirectly, to the adult forms. The adult forms appear to be alone responsible for carrying on the cycle in the transmitting host.

3. The multiplication occurs in the circulating blood.

4. Multiplication of the parasites was never found within the cells of the liver, spleen, or lungs in monkeys.

5. Rounded non-flagellate types were found on one occasion in the lung, liver, and spleen of a virulently infected monkey. They appear for the most part to be destined to destruction, but it is not excluded that they may survive in small numbers as latent forms.

6. In the fly the trypanosomes are first established in the posterior part of the mid-gut. Multiplication occurs and trypanosomes of very varying sizes are produced.

7. From the 10th or 12th day onwards slender long trypanosomes are to be found in increasing numbers. These finally move forward to the proventriculus and are the dominant, though not the only, type seen there. The proventriculus becomes infected as a rule between the 12th and 20th days.

8. The salivary glands become infected by the slender proventricular types. They reach the salivary glands by way of the hypopharynx; arrived in the gland, they become attached to the wall and assume the crithidial condition. Multiplication occurs and finally small trypanosomes are produced, closely resembling the blood type. The passage through the crithidial stage is the characteristic of the salivary development and the trypanosome forms just mentioned are derived from the crithidial types. The development in the salivary gland takes from two to five days before the forms are infective.

9. The fly is never infective until the glands are invaded. Trypanosomes from the proventriculus when injected into a monkey never produce infection. Trypanosomes

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may be found in the salivary glands as early as the 16th day of the cycle. An early infection of the salivary glands is always preceded by a very virulent and rapid gut infection.

10. The trypanosomes are never attached to the wall of the alimentary canal, and there is no intracellular multiplication in the gut cycle. A crithidial stage does not occur in the gut cycle. The trypanosomes are never found in the body cavity nor are they ever established in the rectum.

11. Conjugation has not been observed, nevertheless the fly cycle as a whole has the biological significance of conjugation.

12. The cycles of *T. nanum* and *T. pecorum* agree with that of *T. gambiense* in showing a gut development without a crithidial phase. The crithidial phase occurs in the proboscis, where the flagellates attach themselves. The salivary glands are never infected in the case of *T. nanum* and *T. pecorum*.

DESCRIPTION OF PLATES.

The figures are all drawn at an approximate magnification of 3000 diameters, with the aid of the drawing apparatus of Abbé.

Trypanosoma gambiense.

Figs. 1–4.—Trypanosomes from blood of monkey.
„ 5–8.—Division of blood-types.
„ 9–10.—Trypanosomes in the middle intestine of *Glossina*, 36–48 hours after ingestion.
„ 11–12.—Division in the middle intestine, 36–48 hours after ingestion.
„ 13–15.—Forms from the hinder intestine, 3rd to 4th day of cycle.
„ 16.—Division in the hinder intestine, 3rd to 4th day.
„ 17–19.—Forms from the middle intestine, 5th day.
„ 20.—Division from 5th day.
„ 21–23.—Multiple forms from the 6th day of cycle; 21 is obviously a degenerative appearance.
„ 24.—Involution form from 6th day.
„ 25–29.—Miscellaneous gut forms from the 12th to 20th day.
„ 30–35.—Details of division.
„ 36–37.—Slender proventricular types, final form of the gut development.
„ 38–39.—Slender forms in middle intestine.
„ 39A.—Sketch of live trypanosomes, from slide, from the middle intestine on the 3rd day.
„ 39B.—Non-flagellate form from liver smear of Monkey 653.
„ 40–45.—Early slender forms degenerating in the middle intestine; 43 seems to correspond to the "male" type of KLEINE.

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Figs. 44–45.—Specimens newly arrived in the salivary gland.
„ 46–55.—Typical salivary gland forms; note the crithidial condition.
„ 56–57.—Division figures in the salivary gland.
„ 58–60.—Final trypanosome types in the salivary glands, probably the infecting form.

T. nanum.

Figs. 61–63.—Gut types from 14th day.
„ 64.—From the proventriculus, 21st to 25th day.
„ 65–68.—From the proboscis, crithidial forms; note length of 65, stained by Giemsa, dry method.

T. pecorum.

Figs. 69–71.—Gut forms, 43rd to 49th day.
„ 72.—Proventricular type, 104th day.
„ 73–74.—Proboscis forms, crithidial types, 76th day.

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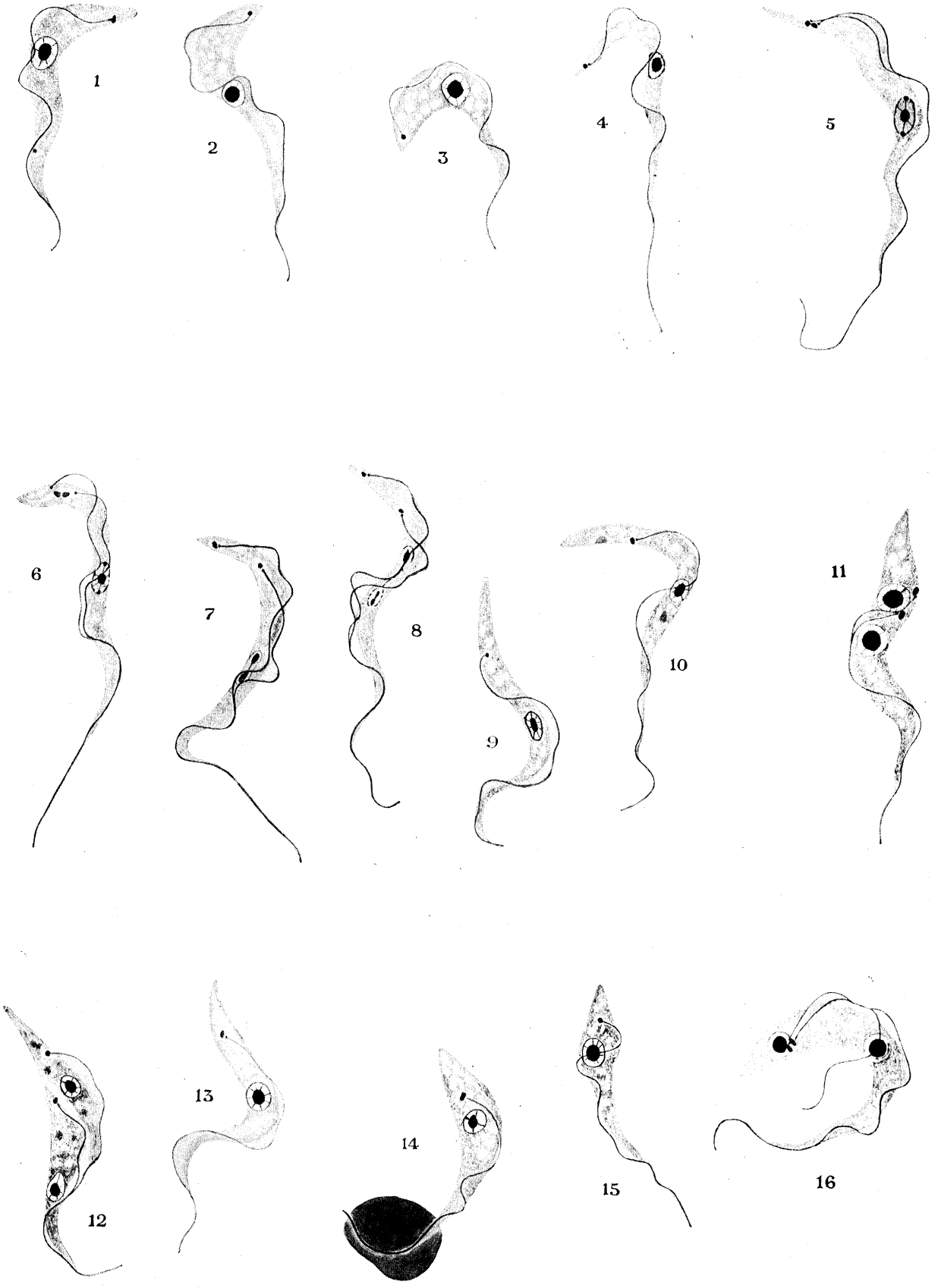

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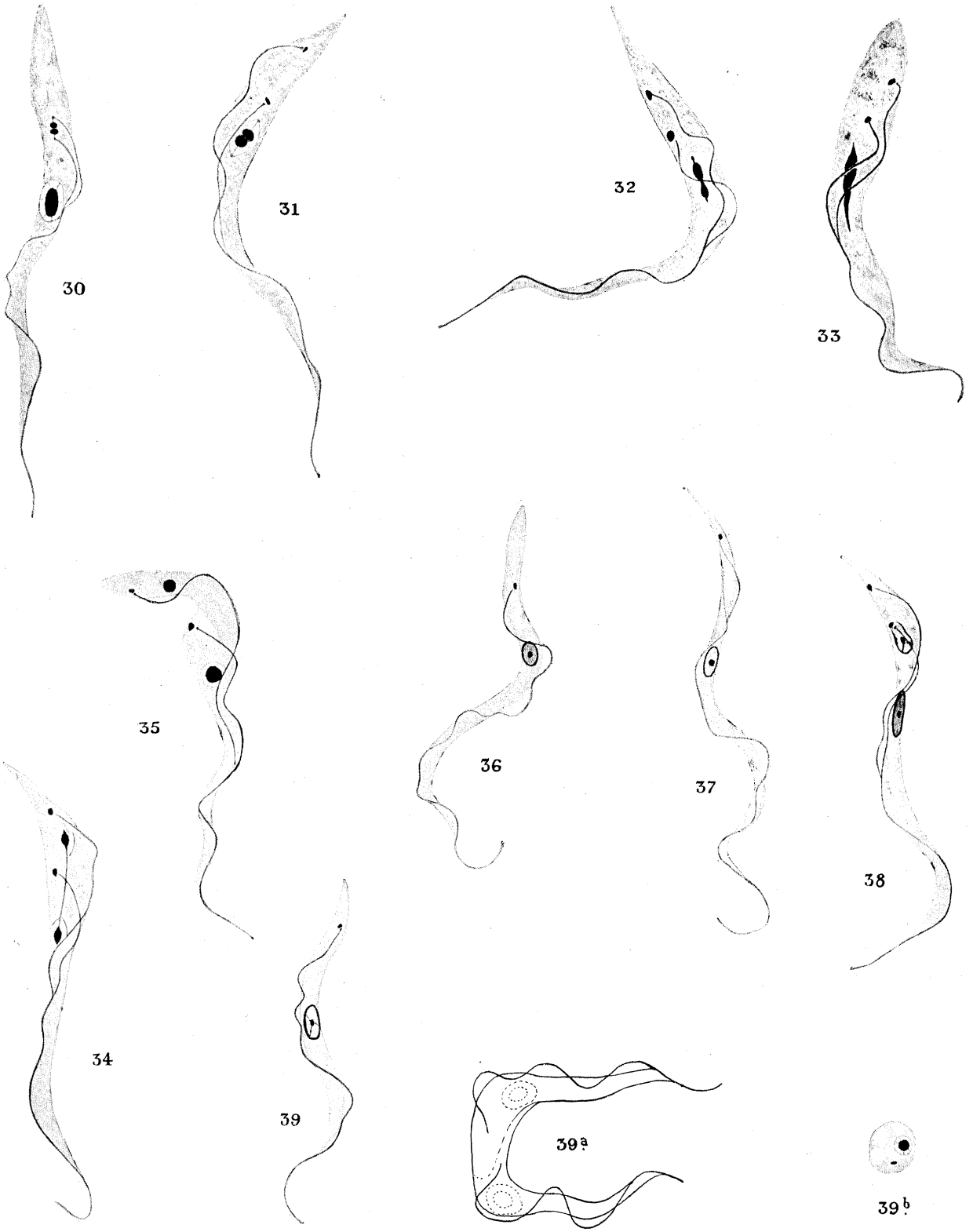

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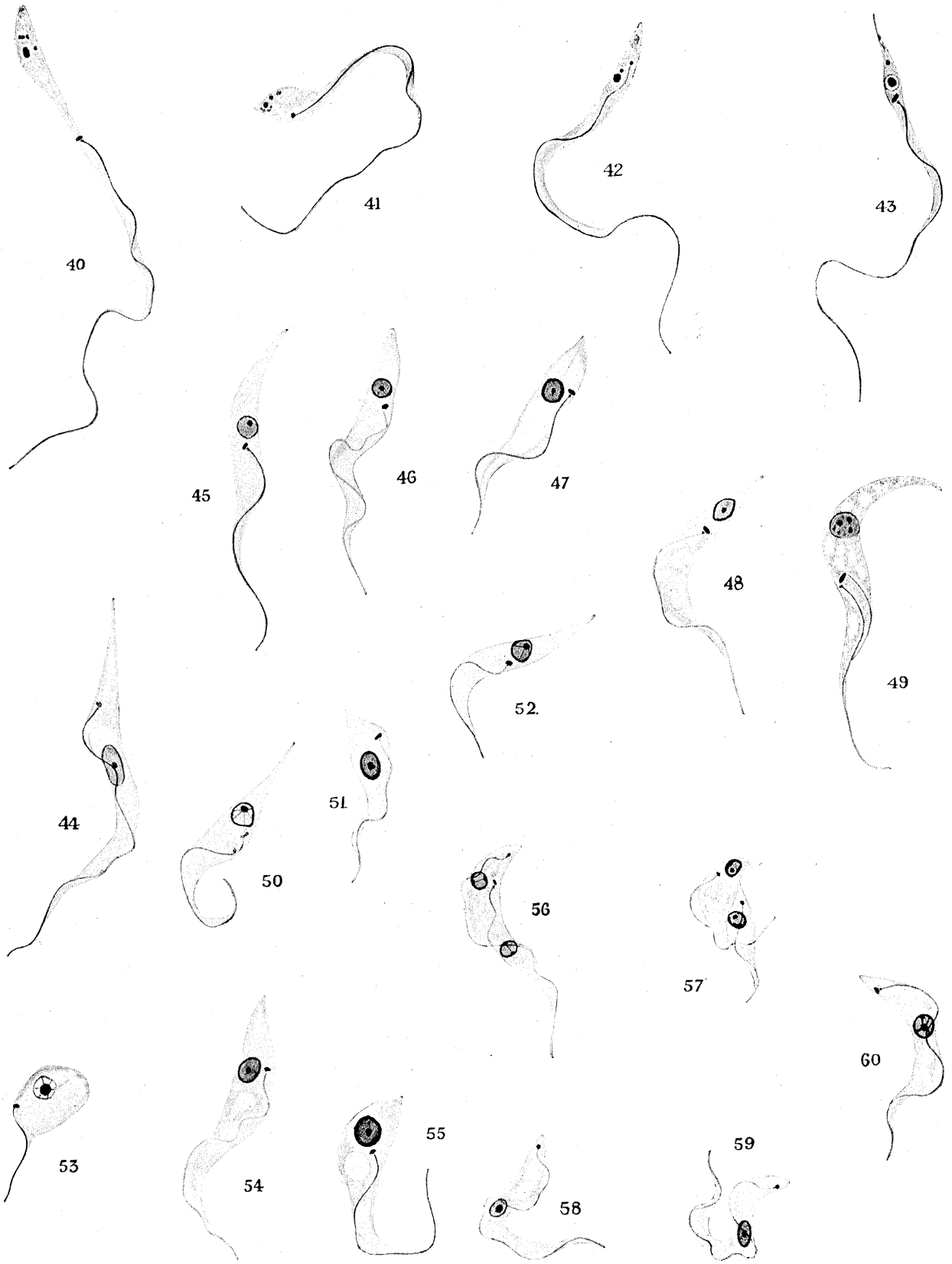

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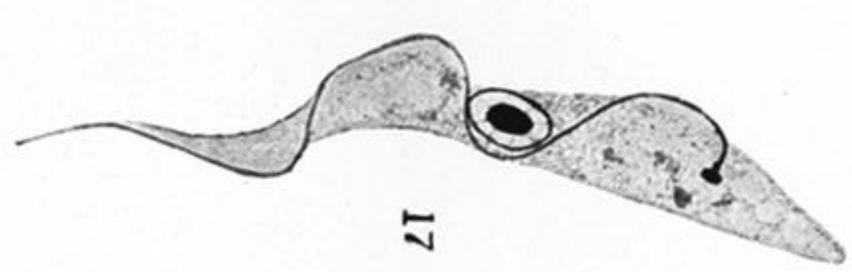
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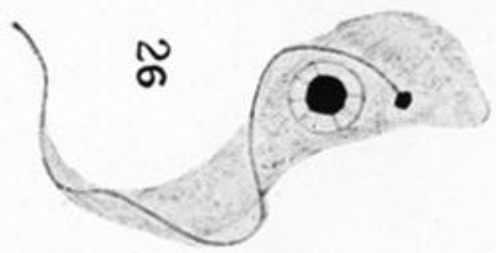
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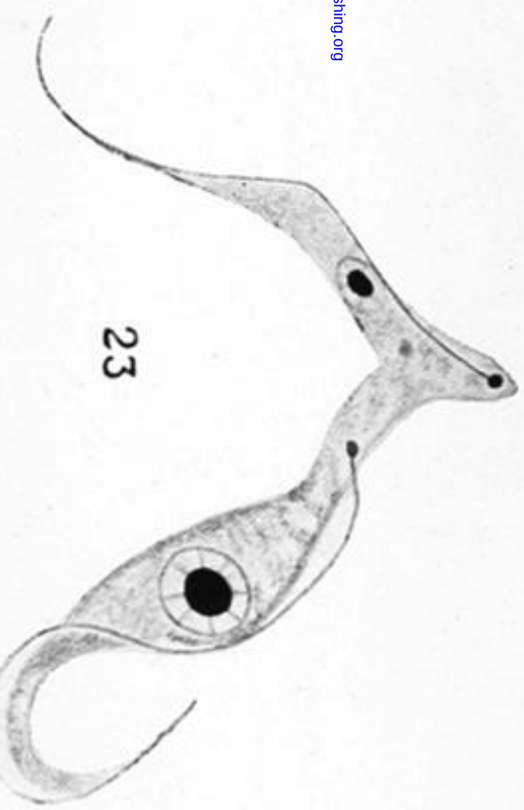
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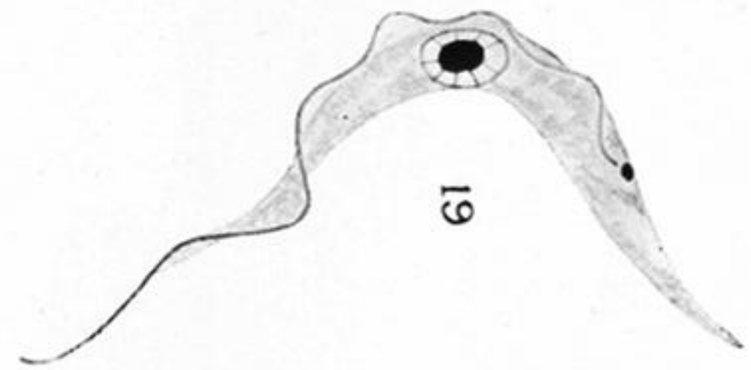
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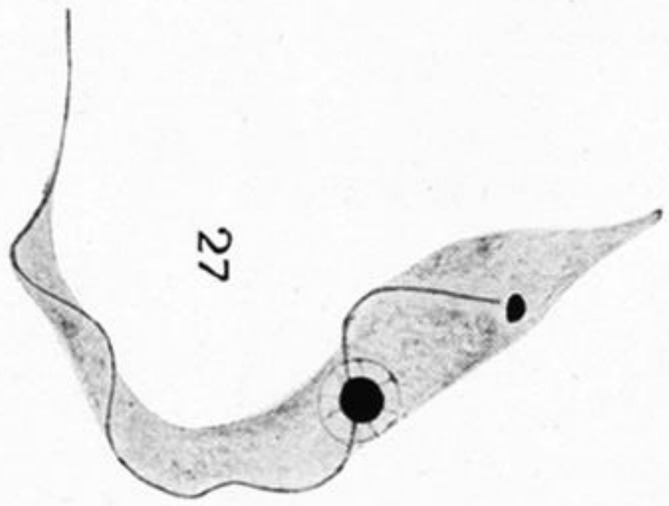
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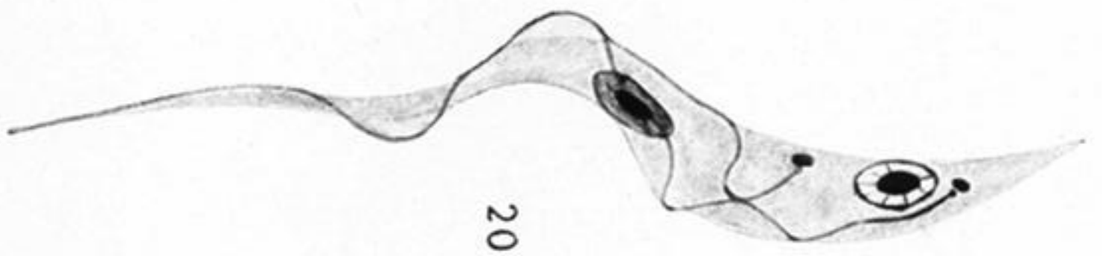
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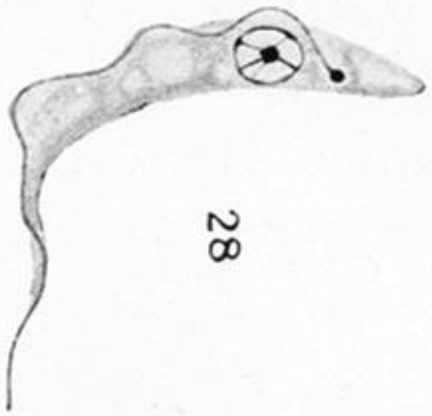
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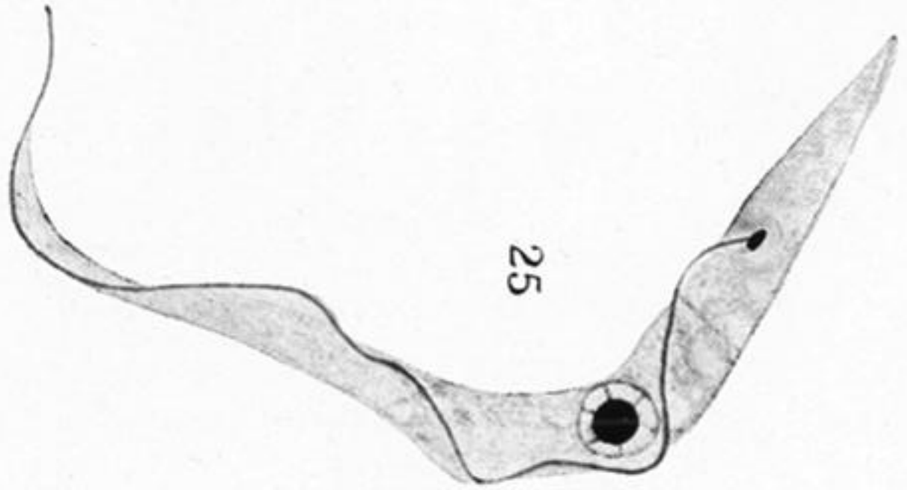
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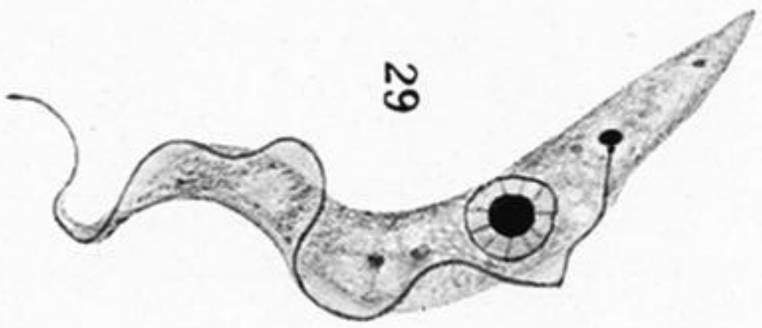
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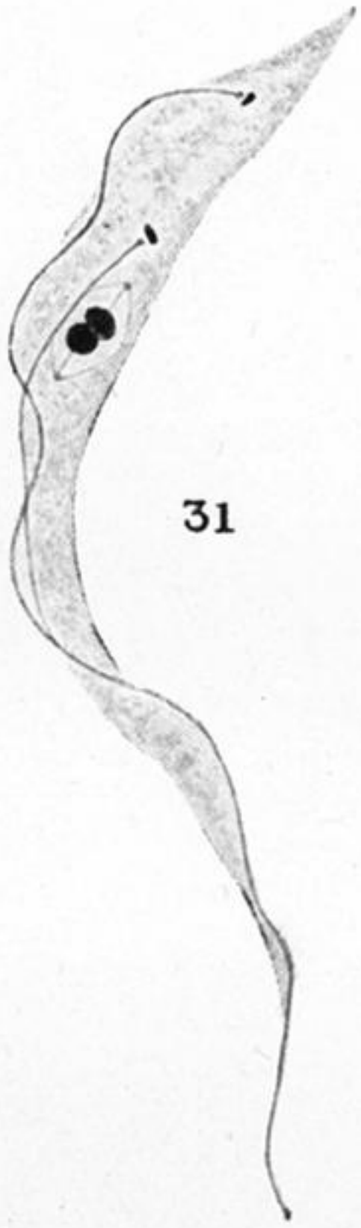

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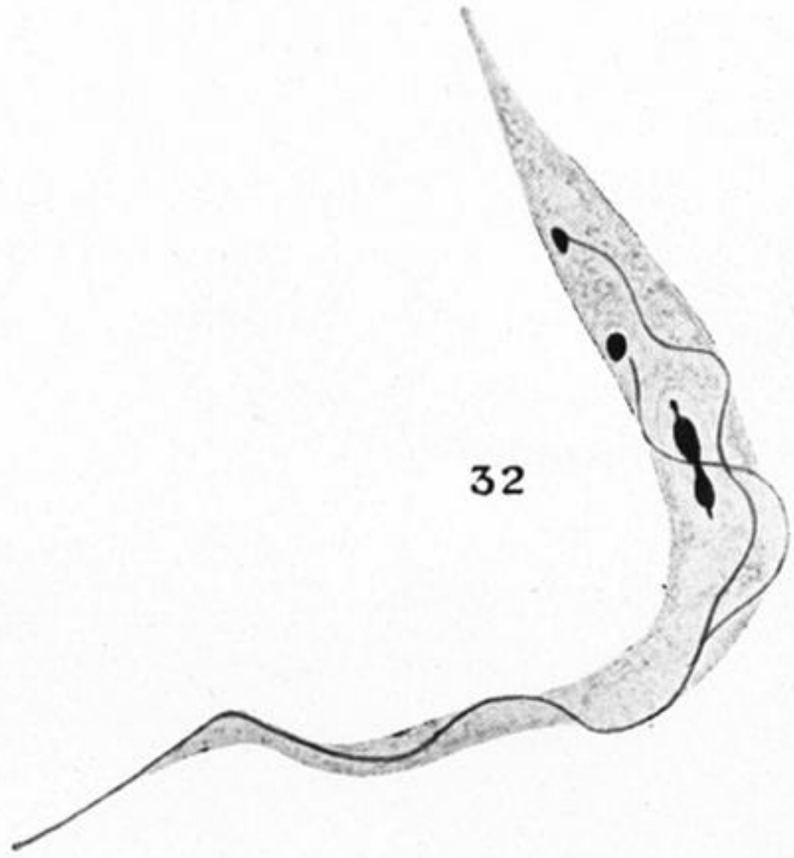

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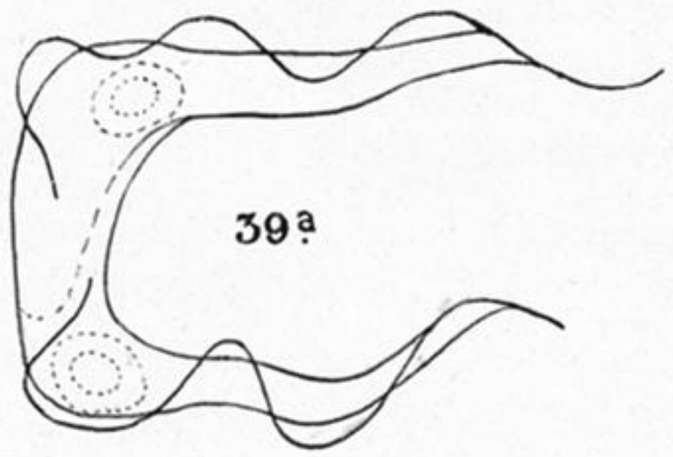

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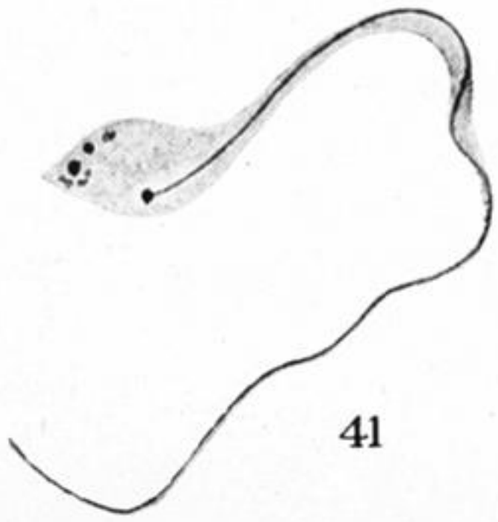

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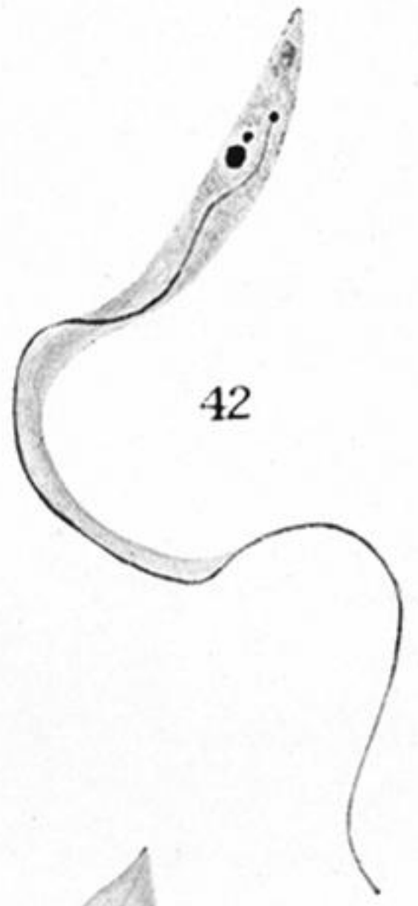

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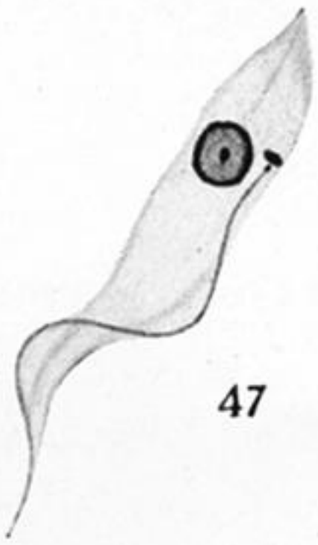

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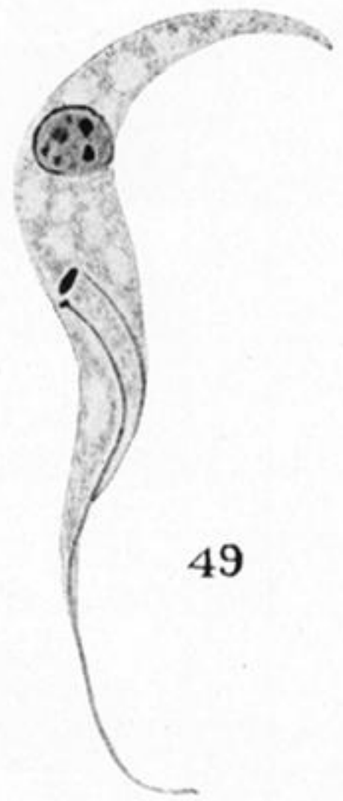

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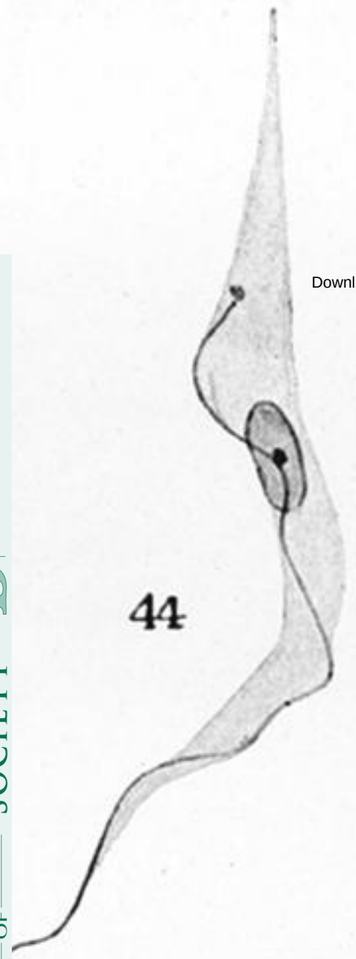

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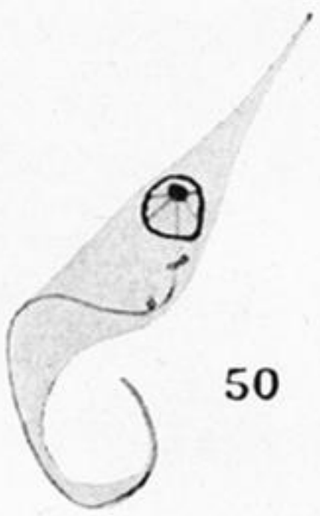

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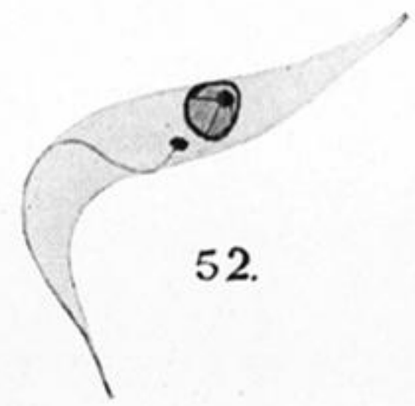

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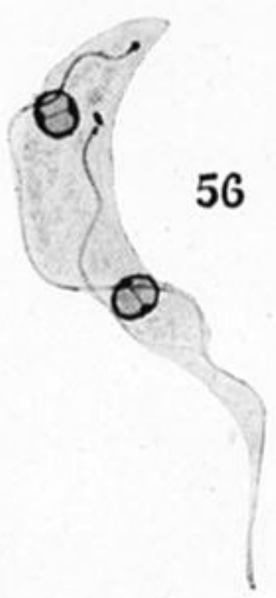

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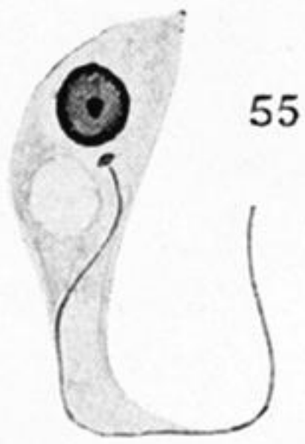

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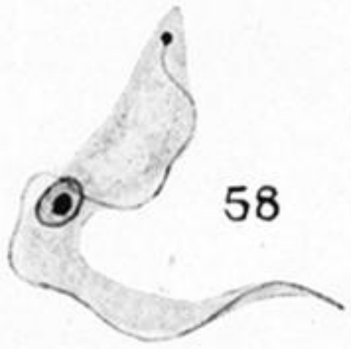

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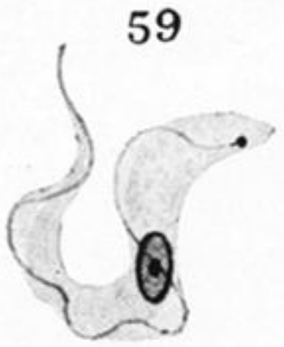

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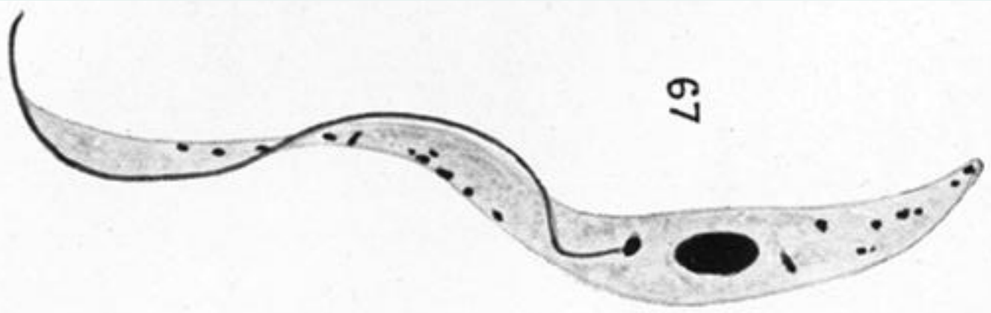

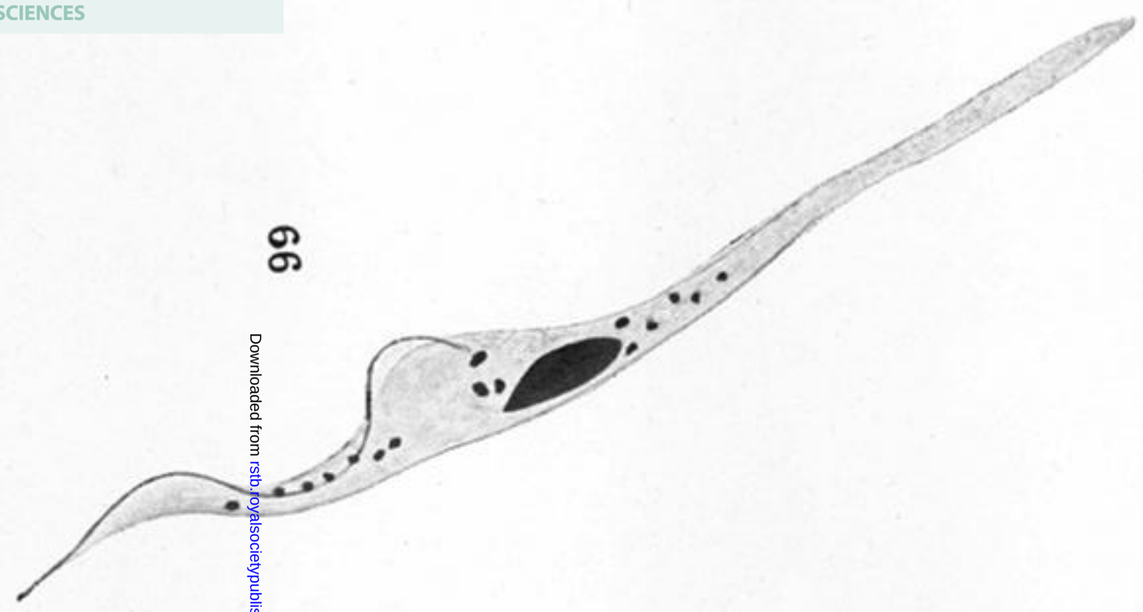

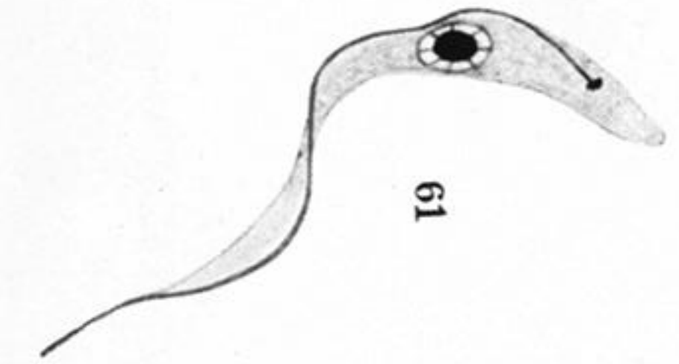
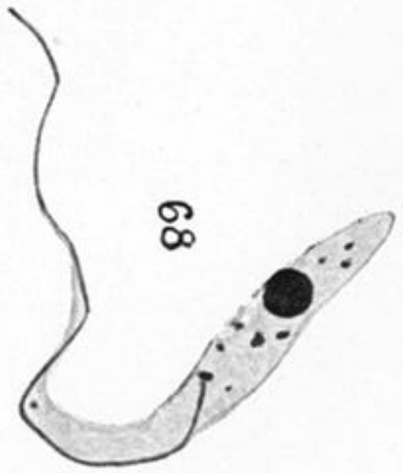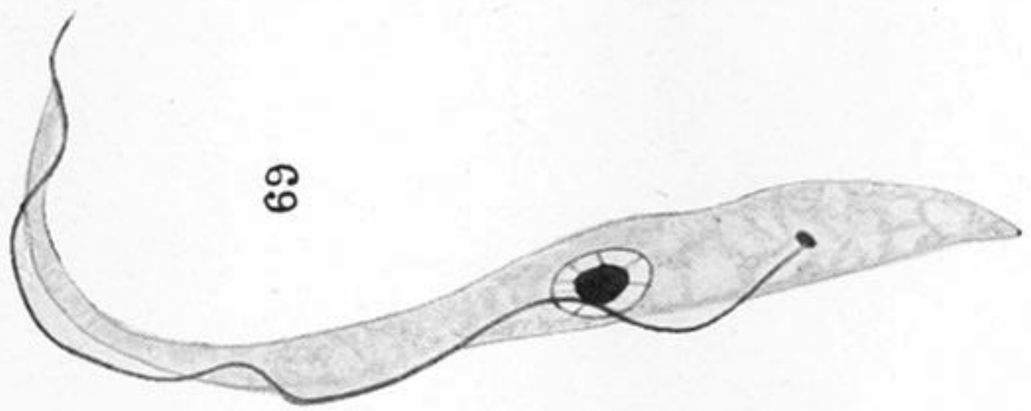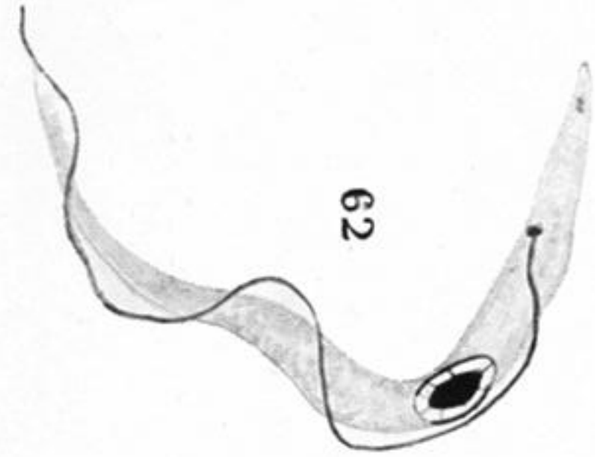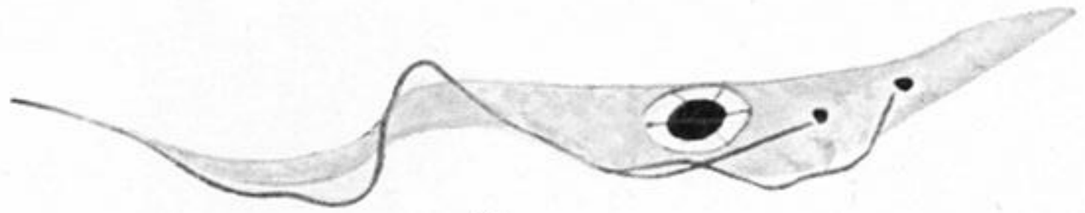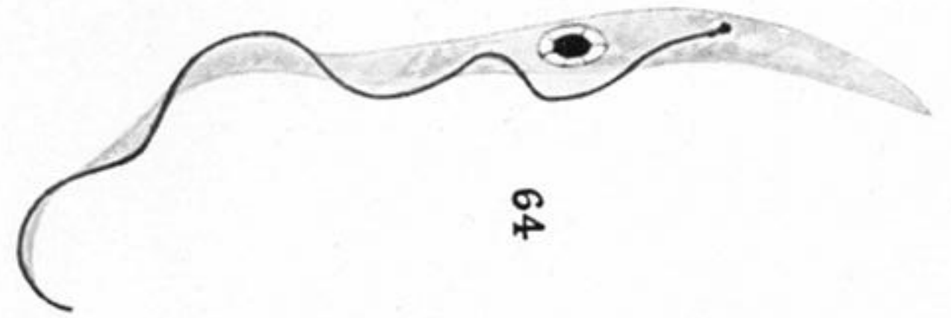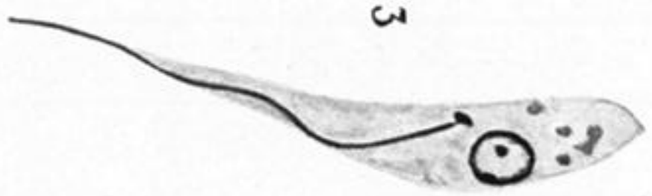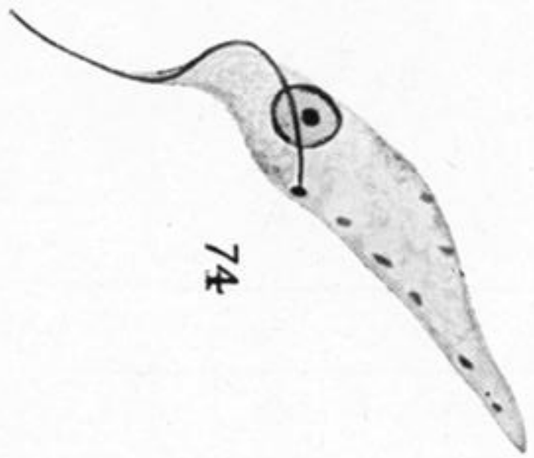
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